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Review paper

CANINE COGNITIVE DISFUNCTION SYNDROME

Abstract

Alzheimer's disease (AD) is a devastating neurodegenerative disorder that usually affects individuals of an older age. Aged dogs spontaneously develop many features of human aging and Alzheimer's disease (AD) including cognitive decline and neuropathology. AD is accompanied by progressive dementia and the accumulation of senile plaques and neurofibrillary tangles. Plaques contain a toxic peptide called beta-amyloid (Aβ) which is produced from the longer Aβ precursor protein (APP) by sequential proteolytic cleavage by beta-secretase and gamma-secretase. Neurofibrillary tangles are composed of hyperphosphorylated tau protein that fills the cytoplasm of neurons, leading to degeneration. Moreover it is found that Aβ deposition may lead to oxidative damage or vice versa, oxidative damage may lead to Aβ. Canines are useful for aging research. The canine model has a rich literature in psychological and neurobiological research, dating back to the 1800s. Dogs also offer additional predictive validity when translating results to human clinical trials, as they absorb pharmaceuticals with similar if not identical pharmacokinetics. There are also cognitive testing procedures for canines that were developed to assess cognitive function in various domains and corresponding brain localization in dogs. Measures of canine function can also be assessed in a clinical setting. Clinical measures have been developed consisting of pet dog owner based evaluation of dog behavioral changes similar to those used in human clinical evaluations, such as the mini mental state exam (MMSE). The canine model has numerous advantages, so the aged dog may capture key features of human aging, making them particularly useful for studies of therapeutics that can be translated into human clinical trials.

Key words: *Alzheimer's disease (AD), beta-amyloid (Aβ), Canine Cognitive Dysfunction Syndrome (CCD), Dog behavioral changes*

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SINDROM KOGNITIVNE DISFUNKCIJE KOD PASA

Kratak sadržaj

Alchajmerova bolest (AB) je devastirajući neurodegenerativni poremećaj koji najčešće pogađa osobe starije dobi. Stariji psi mogu spontano razviti mnoge karakteristike ljudskog starenja i Alchajmerove bolesti (AB), uključujući pad kognitivnih funkcija i neuropatologiju. AB prati progresivna demencija i akumulacija senilnih plakova i neurofibrilarno zapetljavanje. Plakovi sadrže toksični peptid – beta-amiloid (Aβ) koji se proizvodi sekvencijacijom iz više AB prekursorskih proteina (APP) uz aktivnost proteolitičkih beta-sekretaza i gama-sekretaza. Neurofibrilarna klupka se sastoji od hiperfosforilisanih „tau" proteina koji ispunjavaju citoplazmu neurona, što dovodi do degeneracije. Osim toga je utvrđeno da AB taloženja mogu dovesti do oksidativnog oštećenja ili obrnuto. Psi su korisni za istraživanje starenja. Upotreba pasa kao modela ima bogatu istoriju u psihološkim i neurobiološkim istraživanjima, koja datiraju iz 1800. godine. Psi takođe nude dodatne informacije prilikom kliničkih ispitivanja na ljudima, jer oni apsorbuju lijekove i imaju sličnu ako ne i identičnu farmakokinetiku. Tu su i kognitivne procedure za testiranje pasa koje su razvijene za procjenu kognitivne funkcije u različitim oblastima i odgovarajućih lokalizacija u mozgu pasa. Mjere funkcije mozga pasa mogu se procijeniti u kliničkom okruženju. Razvijene su kliničke mjere koje se sastoje od vrednovanja baziranih na promjenama ponašanja pasa sličnim onima koje se koriste u kliničkim studijama ljudi, kao što su to „mini mental state exam" (MMSE). U modelu psa postoje brojne prednosti, tako da se starenjem kod psa mogu uočiti ključne karakteristike ljudskog starenja, što ih čini posebno korisnim za studije terapije koje se mogu koristiti u kliničkim ispitivanjima na ljudima.

Ključne riječi: *Alchajmerova bolest (AB), beta-amiloid (Aβ), kognitivni disfunkcija sindrom kod pasa, promjene ponašanja, pas.*

INTRODUCTION / UVOD

Dementia is defined as a clinical syndrome characterized by symptoms and signs such as changes in memory, orientation, behavior, language and other cognitive functions, and impairment in activities of daily living. Alzheimer's disease (AD), which is named after the German psychiatrist Alois Alzheimer, who first described this disorder in 1907, is the most common cause of dementia, accounting for up to 75 % of all dementia cases. AD is a progressive neurodegenerative disorder Canine Cognitive Dysfun-

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ction (CCD) is a term that was introduced to describe geriatric behavioral changes, not solely attributable to a general medical condition (Overall, 2000, Ruehl WW, 1998) such as infection, organ failure or neoplasm. Senile dementia and senile degeneration of the brain are terms also used in the literature.

EPIDEMIOLOGY / EPIDEMIOLOGIJA

It is estimated that about 46.8 million people worldwide are living with dementia in 2015. This number will almost double every 20 years, reaching 74.7 million in 2030 and 131.5 million in 2050.

A recent retrospective(Hart BL, 1997, Neilson et al., 2001) study examined 180 dogs (83 castrated males and 97 spayed females) representing 3 age groups, namely, dogs aged 11-12, 13-14, and 15-16 years old. Mild cognitive impairment was defined as signs in only one category whereas severe cognitive impairment was described as signs in 2 or more categories. The percentage of 11 to 12 year-old dogs with minor impairment was 17.5% and severe impairment was 10% (total number of dogs affected 27.5%). The percentage of 15 to 16 year- old dogs were 32% and 35% for mild and severe impairment respectively (total number of dogs affected 67%). Data were also collected to monitor progression of agerelated behavioral changes in old dogs over a period of 6-18 months. The increase in impairment was significant within all categories.

A large cross-sectional epidemiological study of older dogs (Salvin et al., 2010) aimed to estimate the prevalence of CCD amongst community based dogs (mean age 11.67 years; range 8–19.75) and to determine the rate of veterinary diagnosis amongst affected dogs. An 84-item questionnaire was used to obtain information across six behavioral domains. Of the eligible survey responses obtained (n = 957) a randomly selected one-half (n = 497) was used for this study. Using a provisional diagnosis based on 27 significant behavioral items, the prevalence rate of CCD was estimated to be 14.2%. This was in contrast with only 1.9% diagnosed with CCD by a veterinarian. There was an exponential increase in prevalence of CCD with age ($R^2 = 0.9435$), but prevalence did not differ by breed size or between longevity groups. The prevalence rate of CCD reported in this study is consistent with previous findings, and further supports the contention that the majority of these dogs do not receive a formal diagnosis.

ALZHEIMER DISEASE / ALCHAJMEROVA BOLEST

PATHOPHYSIOLOGY / PATOFIZIOLOGIJA

Just as canines can exhibit cognitive decline with age similar to aging humans and patients with AD, several human-type neuropathologies have been reported in dogs (Cotman and Head, 2008). The canine model has long been suggested as an excellent model of Aβ pathogenesis (Wisniewski et al., 1990). Dogs normally share environmental conditions with humans and present a sophisti-

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cated repertoire of complex cognitive behaviors. Furthermore, the brain in aged dogs shows many pathological changes common to humans and the neuropathological patterns are significantly associated with cognitive decline (Head et al., 2000b). Several changes observed in the aged canine brain are associated with cognition.

Individuals with AD show significant cortical and hippocampal atrophy relative to non-demented age matched controls (Alavi et al., 1993, Raz et al., 1998) and losses in brain volume correlate with cognitive decline (Du et al., 2005, Ezekiel et al., 2004). Similar events are seen in aged canines. On cross sectional MR imaging, aging canines show increased cortical atrophy and ventricular widening (Gonzalez-Soriano et al., 2001, Kimotsuki et al., 2005, Su et al., 1998). Ventricular widening over time was observed by MRI in a 3-year longitudinal study (Su et al., 2005). Canine cortical atrophy occurs earliest in the prefrontal cortex and later with age in the hippocampus (Tapp et al., 2004). As with humans, the more extensive the cortical/hippocampal atrophy seen in aged canines the more pronounced the cognitive deficits (Rofina et al., 2006). J. E. Rofina et. al. (Rofina et al., 2006) made a study, in which cognitive performance was correlated with brain pathology for 30 dogs of varying ages. Within these animals, two age-matched groups of old dogs with and without behavioral changes were compared. The behavioral changes were analyzed and scored with questionnaires and necropsy was performed to rule out any other cause for changed behavior. Measurements, (immuno)-histochemical staining and fluorescence microscopy were used to detect cortex atrophy, amyloid, rest-products of oxidative damage, demyelination and accumulations of macrophages in the brains of these dogs. Spearman rank correlation coefficients (r) were calculated and adjusted according to Bonferonni. In the whole group (young to very old dogs), the age of the animal showed a significant correlation with various behavioral changes (r = 0.7 to 0.9, P < 0.01). The dementia score correlated significantly (r = 0.6 to 0.8, P < 0.01) with all the brain lesions studied, except one, i.e. demyelination (r = −0.4, P=0.05). These results suggest that a questionnaire can be used to diagnose Alzheimerlike changes in canine practice. Oxidative damage on a cellular and a nuclear level plays an important role in behavior changes.

Neuronal loss occurs in human brain aging and could explain the brain volume loss seen in brain imaging (Simic et al., 1997). With normal brain aging, neuronal loss is only seen in the hilus (West et al., 1994), while neuronal loss is much more widespread in individuals with AD. Moreover, in individuals with AD there is neuronal loss in the CA1, CA2, CA4, and subiculum of the hippocampus (Bobinski et al., 1997, West et al., 2000). In aged beagles, the hilus of the dentate gyrus showed fewer neurons compared to younger dogs. Beagles with fewer neurons in the hilus made significantly more errors when performing the size discrimination task (Siwak-Tapp et al., 2008). Moreover, a loss of Purkinje cells in cani-

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nes correlate with data acquired by questionnaires quantifying behavioral deficits. However, neuronal loss may not account for all of the brain atrophy observed by MR as the loss of neuronal dendritic spines occurs with AD (Knobloch and Mansuy, 2008) but to our knowledge, there are currently no studies published evaluating similar changes with age in dogs.

The brain is also able to produce new neurons despite the fact that neuronal loss may occur with aging. The hippocampus, for example, grows new neurons in the sub-granular layer (Eriksson et al., 1998). Neurogenesis has been studied in aged beagles using BrdU and double cortin protein staining methods. Siwak-Tapp et al.(Siwak-Tapp et al., 2007) measured neurogenesis in aged beagles using BrdU and found that animals over the age of 13 experienced a significant loss of neurogenesis. Fewer newer BrdU positive neurons were associated with poorer cognitive function in learning and memory and learning ability.

Neuronal dysfunction could result in abnormal production of critical neurotransmitters in the brain. Neurotransmitter deficits have not been thoroughly explored in canines. In humans, decreases in specific neurotransmitter systems are associated with aging and AD (Meltzer et al., 1998, Rissman et al., 2007). Dogs with Aβ accumulation in the gyrus proreus possess fewer serotonergic neurons (Bernedo et al., 2009). A decrease in receptor binding of serotonin is seen with age in dogs over 8 years of age (Peremans et al., 2002).

Animals with high levels of Aβ in the prefrontal cortex experience a loss of noradrenergic neurons in the locus ceruleus, which is also associated with cognitive dysfunction (Insua et al., 2010). Acetylcholinesterase density is reduced in granule cells of the cerebellum with age (Pugliese et al., 2007). Aged canines experience a loss of gamma-aminobutyric acid interneurons in the prefrontal cortex (Pugliese et al., 2004), as well as the CA1 and dentate gyrus of the hippocampus. Additionally, a loss of glutamic acid decarboxylase neurons in CA1 of the hippocampus is seen in aged canines over 10 years of age (Hwang et al., 2008). The pathogenic mechanisms underlying neuronal dysfunction, neurotransmitter losses and death may include, e.g., the deposition of Aβ, cerebrovascular dysfunction, or oxidative damage.

AD is accompanied by progressive dementia and the accumulation of senile plaques and neurofibrillary tangles (Mirra, 1997). Plaques contain a toxic peptide called beta-amyloid (Aβ), which is produced from the longer Aβ precursor protein (APP) by sequential proteolytic cleavage by beta-secretase and gamma-secretase (Selkoe, 1994). Aβ forms either extracellular deposits or soluble assembly states (oligomers)(Haass and Selkoe, 2007, Walsh et al., 2002). Neurofibrillary tangles are composed of hyperphosphorylated tau protein that fills the cytoplasm of neurons, leading to degeneration (Iqbal and Grundke-Iqbal, 2008). As with most natural animal models of AD, dogs develop Aβ pathology and some evidence for tau abnormalities but not full blown ne-

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urofibrillary tangles. C.-H. Yu et. al. (Yu et al., 2011) investigated tau phosphorylation of neurons and astrocytes in the brain of aged dogs with progressive cognitive impairment. Changes in the brain of aged dogs with cognitive dysfunction were compared with those in the brain of patients with AD of Braak stage V. Immunohistochemically, Ab deposition, phosphorylated tau Ser396 (p-tau Ser396) and ubiquitin were observed in the parietal cortex and hippocampus of aged dogs with cognitive dysfunction. Astrocytes with expression of p-tau Ser396 and neurons with co-localization of p-tau Ser396 and ubiquitin were observed. Expression of p-tau Ser396 and accumulation of ubiquitin were significantly increased in the parietal cortex and dorsal part of the hippocampus of the brain of aged dogs when compared with expression of these molecules in human AD.

Beta-amyloid (Aβ) is derived from a longer precursor protein, the amyloid precursor protein (APP). The APP sequence of Canis familiaris has 98% homology with human APP2 and an identical amino acid sequence (Johnstone et al., 1991). Additionally, dog Aβ peptides may undergo the same posttranslational modifications as in humans (Azizeh et al., 2000). These similarities make canines a viable aging model without the need for genetic modification or overexpression of mutant human proteins(Selkoe et al., 1987). Geun-Shik Lee et. al. (Lee et al., 2014) generated transgenic canines that overexpressed the human amyloid precursor protein (APP) gene containing well-characterized familial Alzheimer's disease (AD) mutations. They successfully obtained five out of six live puppies by somatic cell nuclear transfer (SCNT). This was confirmed by observing the expression of green fluorescence protein in the body as a visual transgenic marker and the overexpression of the mutated APP gene in the brain. The transgenic canines developed AD-like symptoms, such as enlarged ventricles, an atrophied hippocampus, and β-amyloid plaques in the brain. Thus, the transgenic canines we created can serve as a novel animal model for studying human AD.

In another study, Giaccone et.al.(Giaccone et al., 1990) investigated the brains of 7 dogs aged 6 to 18 years histochemically and immunohistochemically at the light- and electron microscopy levels for preamyloid deposits and amyloid fibrils to verify the hypothesis that the accumulation of cleavage products of amyloid precursor protein is related not only to Alzheimer's disease but also to the normal aging of the brain. Preamyloid deposits were detected in the neuropil of the cerebral cortex and neostriatum, whereas amyloid fibrils were found in the walls of parenchimal and leptomeningeal vessels. The densities of preamyloid deposits in the neuropil and of deposits of amyloid fibrils in the vessel walls were higher in the brains of the most aged dogs. These findings suggest that aging of the canine brain is characterized by an accumulation of intermediate cleavage products of the amyloid precursor protein in both the neuropil and the vessel walls, and by processing of these products to amyloid fibrils in the vessel walls.

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The Aβ present in canines is ultra-structurally fibrillar and, though more compact deposits may form, it generally aggregates into diffuse plaques (Torp et al., 2000a, Torp et al., 2000b, Giaccone et al., 1990). This type of Aβ deposition most resembles early AD pathology(-Cotman and Head, 2008). In canines, the accumulation of Aβ begins in the prefrontal cortex (approximately 8 years at age of onset) and continues to develop with increasing age to include other regions such as the temporal and occipital cortex(Head et al., 2000a). Schmidt F et. al. (Schmidt et al., 2015) examined the brains of 24 dogs from various breeds. The frontal cortex, hippocampus, and entorhinal cortex were investigated. Deposits of β-amyloid (Aβ) and tau were analyzed phenotypically and quantified stereologically. In all dogs aged 10 years or older, plaques containing pyroglutamyl Aβ and Aβ8-17 were detected. Within the ventral hippocampus, significantly more pyroglutamyl Aβ plaques were deposited in small and medium dogs than in large dogs. Hyperphosphorylated tau with formation of neurofibrillary tangles was observed in 3 animals aged 13 to 15 years. This study provides the first investigation of pyroglutamyl Aβ in comparison with total Aβ (as shown by Aβ8-17 immunoreactivity) in dogs of different breeds, sizes, and ages. The results indicate that canine cognitive dysfunction syndrome is relatively common among aged canines, thereby emphasizing the relevance of such populations to translational AD research. Aβ peptide can also be measured in the cerebrospinal fluid (CSF) of dogs(Sarasa et al., 2013). Measuring CSF Aβ as a ratio of Aβ 42/Aβ 40 is a good predictor of Aβ in the brain in dogs (Head et al., 2010). While brain Aβ increases with age, CSF Aβ decreases with age reflecting the hypothesis that Aβ migrates from the periphery and deposits in the brain with age and AD. González-Martínez et. al.(-González-Martínez et al., 2011) made a study in order to assess plasma Aβ1-42 and Aβ1-40 levels in a blind study using pet dogs that were either successfully aging or exhibiting CDS (cognitive dysfunction syndrome). The severity of cognitive impairment was assessed using an owner-based questionnaire. On average, young dogs presented significantly higher plasma levels of Aβ1-42 and Aβ1-40 than aged, cognitively unimpaired dogs. Notably, among aged dogs, the levels of Aβ1-42 and the Aβ42/40 ratio were significantly higher in those showing mild cognitive impairment than in either cognitively unimpaired or severely affected dogs. These results suggest that increased plasma Aβ1-42 levels and Aβ42/40 ratio could be a biomarker for canine cognitive dysfunction, which is considered an excellent natural model of early AD.

Aside from the fibrillar Aβ found in diffuse plaques in AD, a smaller, more soluble and more toxic form of Aβ – oligomeric Aβ – is also seen in the aged dog brain, that affects synaptic function and can even be found in plaques(Selkoe, 2008). Similar to fibrillar Aβ, oligomeric Aβ can be measured in CSF, where levels are inversely related to levels in the brain (Head et al., 2010).

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Aβ can also aggregate in the cerebral blood vessel walls and cause cerebrovascular pathology (Prior et al., 1996). This type of deposition is referred to as cerebral amyloid angiopathy (CAA). Typically CAA is composed of the shorter Aβ 1-40 peptide (Attems et al., 2005). Vascular Aβ may compromise the blood brain barrier, and can cause microhemorrhages via vessel wall viability (Deane and Zlokovic, 2007). Much like humans, canines experience microhemorrhages with age. These cerebral hemorrhages are present in both animals with and without CAA, but are more common in those with the blood vessel pathology (Uchida et al., 1991). Further, the distribution of CAA is similar in both species, with the occipital cortex being predominantly susceptible.

Moreover it is found that Aβ deposition may lead to oxidative damage or vice versa, oxidative damage may lead to Aβ (Butterfield, 1997). Ultimately, oxidative damage accumulates with age and can lead to neuronal dysfunction and thus impact cognition(Butterfield et al., 2001). Oxidative damage occurs over time due to the overproduction of reactive oxygen species (ROS) produced primarily by mitochondria. ROS overproduction may exceed the levels or production rate of endogenous antioxidants and result in oxidative damage to proteins, lipids, and nucleotides. Oxidative damage can be measured by the amount of protein oxidation (carbonyl groups), 4-hydroxynonenal, lipofuscin, lipofuscin-like pigments, and malondialdehyde (lipid peroxidation). Further, 8hydroxy-2′-deoxyguanosine (8OHdG) can be measured to detect DNA/RNA oxidation.

While oxidative damage occurs with normal aging, it is more pronounced in AD (Lovell and Markesbery, 2008). In mitochondria isolated from aged canine brain, there is an increased production of ROS compared to mitochondria isolated from young animals. Canines also experience an accumulation of carbonyl groups with age(Skoumalova et al., 2003). Lipid peroxidation is exhibited in old dogs, measured by 4-hydroxynonenal (Papaioannou et al., 2001), lipofuscin (Rofina et al., 2006), lipofuscin-like pigments(Papaioannou et al., 2001), or malondialdehyde (Head et al., 2002). Increased 8OHdG in aged canines has also been reported (Rofina et al., 2006). In particular, increased protein oxidation and lipid peroxidation (lipofuscin-like pigment) correlates with cognitive decline in dogs(Rofina et al., 2006).

One hallmark AD pathology canines do not produce is neurofibrillary tangles (NFTs) (Russell et al., 1992). While no research to date has observed NFTs in the canine brain, the increased phosphorylation seen at some sites of tau in AD cases also occurs in cognitively impaired canines (Pugliese et al., 2006). This lack of NFT pathology could possibly be due to significant differences in the tau protein sequence between canines and humans. However, an advantage to dogs not accumulating NFTs is that they serve as a model that is selective for Aβ pathology and ideally suited for testing interventions that target this toxic protein. Dogs initially develop plaques between the ages of 8 and 9 years(Cotman and Head, 2008), a relatively young age, compared to many

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non-human primate models with naturally occurring AD-like pathology (Bons et al., 1992).

CANINE MODELS / PAS KAO MODEL

The canine model has a rich literature in psychological and neurobiological research, dating back to the 1800s. Canines are useful for aging research, have moderate lifespans of 12 to 20 years, depending on the breed (Brizzee, 1978, Mosier, 1989), and are easy to handle due to a long history of domestication (Cummings et al., 1996). Furthermore, canines are highly motivated to perform consistently on cognitive tests using simple food rewards, making food deprivation paradigms unnecessary. In contrast, mice are not readily cooperative in performing behavioral tasks, so physiological stressors including food restriction, water deprivation and immersion in water are often used (Blizard et al., 2003). Therefore measures of cognition may be engaging other processes involved in stress response, confounding performance scores. Importantly, the cognitive decline and progressive age-associated neuropathology observed in dogs parallels that of humans.

The dog model provides a complementary system in which to test various theories of aging and to develop therapeutics when used in combination with other models. Osella et. al. (Osella et al., 2007) conducted a study which had two purposes. The first purpose of the study was to investigate the prevalence of cli-nical signs of CDS in a general population of aged dogs. The second aim was to evaluate the use of a neuroprotective nutraceutical (Senilife1, Innovet Italia srl, Rubano, Italy) using an open-label clinical pilot trial. Dogs were recruited from a geriatric population not referred for behavioral consultations. A questionnaire with a checklist of behaviors was filled out to evaluate behavioral items grouped in the following categories: disorientation (D), socio-environmental interaction (I), sleep–wake cycles (S), house soiling (H), general activity (A)—(DISHA). Each owner was asked to rate the frequency of the behavioral signs: never, rarely, often, or always. One hundred and twenty-four dogs were assessed in the first survey; 22 of the 124 dogs tested in the survey were ruled out based on exclusion criteria (clinically and/or sensory severe impairment), 42 dogs had alterations in one category and 33 dogs had signs in 2 or more categories. Consequently 75 dogs had signs consistent with CDS. Among this population eight dogs affected by CDS were enrolled for the second step of the project, an open-label clinical pilot trial with the neuroprotective nutraceutical Senilife1. Senilife1 contains 25 mg phosphatidylserine, 50 mg of standardized Ginkgo biloba extract, 33.5 mg/d-alpha tocopherol and 20.5 mg pyridoxine per capsule and is dosed at one capsule per 5 kg body weight. The investigator asked the owners to rate the frequency of behaviours referring to DISHA using a four point frequency scale (never, rarely, often, always). Post-treatment, the owners were asked to evaluate all the si-

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gns in each category on a five point scale (much better, slightly better, the same, slightly worse, much worse). At the time of the first visit (V0) the owners were briefed verbally about the procedure; no behavioral advice was given throughout the study time and whenever appropriate therapy with Senilife1 (was started. At V0, V1 (28 3 days), V2 (56 3 days) and V3 (84 3 days) a control visit was performed and the owners were interviewed. Dogs treated with Senilife1 showed a highly significant difference at V3 compared to V0 (p < 0.001). Preliminary results from dogs on Senilife1 showed a marked improvement of CDS related signs, even if the dogs failed to show a complete remission of symptoms.

However, the use of dogs in aging studies provides some unique advantages, as dogs may share a common environment (including diet) with humans. Dogs also offer additional predictive validity when translating results to human clinical trials, as they absorb pharmaceuticals with similar if not identical pharmacokinetics. For example, due to similarities to humans in terms of responsiveness, drug tolerance and metabolism, the dog can be considered to be a useful model for chronic statin treatment(Alberts, 1990, Gerson et al., 1989). Barone et. al. (Barone et al., 2012) evaluated the effect of atorvastatin treatment (80 mg/day for 14.5 months) on Biliverdin reductase-A (BVR-A) in the parietal cortex, cerebellum and liver of a well characterized pre-clinical model of AD, the aged beagle. They found that atorvastatin significantly increased BVR-A protein levels, phosphorylati-

on and activity only in parietal cortex. Additionally, they found significant negative correlations between BVR-A and oxidative stress indices, as well as discrimination learning error scores. Furthermore, BVR-A up-regulation and post-translational modifications significantly correlated with β-secretase protein levels in the brain, suggesting a possible role for BVR-A in Aβ formation.

Further, an interesting new study suggests that in the process of domestication in dogs, genes associated with digestion have been selected that allow dogs to thrive on a diet rich in starch unlike wolves and more similar to humans (Axelsson et al., 2013), suggesting similar dietary absorption of nutrients. Middle aged beagles between 5 and 9 years are similar to humans between 40 and 60 years and beagles over 9 years are similar to humans over 66 years(Head, 2013). Head et.al. (Head et al., 2012) used a canine model of human aging and AD. Aged dogs naturally develop learning and memory impairments, human-type Aβ deposits and oxidative damage in the brain. Thus, 9 aged beagles (98-115 months) were treated with a medical food cocktail containing (1) an extract of turmeric containing 95% curcuminoids; (2) an extract of green tea containing 50% epigallocatechingallate; (3) Nacetyl cysteine; (4) R-alpha lipoic acid; and (5) an extract of black pepper containing 95% piperine. Nine similarly aged dogs served as placebo-treated controls. After 3 months of treatment, 13 dogs completed a variable distance landmark task used as a measure of spatial attention. As compa-

red to placebo-treated animals, dogs receiving the medical food cocktail had significantly lower error scores (t(11)=4.3, p=0.001) and were more accurate across all distances (F(1,9)=20.7, p=0.001), suggesting an overall improvement in spatial attention. Measures of visual discrimination learning, executive function and spatial memory, and levels of brain and CSF Aβ were unaffected by the cocktail. Treatment with a medical food cocktail in aged beagles, as a model of human aging and AD, resulted in an improvement in spatial attention, but not in other measures of cognition. Interestingly, the number of animals that stopped responding as the cognitive tests became more difficult was higher for controltreated dogs, suggesting a possible improvement in motivation level in treated animals. Neurobiological studies show no changes in either the extent of Aβ plaque accumulation or the biochemical measures of soluble and insoluble Aβ1-40 and Aβ1-42. Our results indicate that this medical food cocktail may be beneficial for improving spatial attention and motivation deficits associated with impaired cognition in aging and AD. This study included 18 beagles from Ridglan Farms, Inc. the ages of the animals ranged from 98-115 months (Mean=103.2 SD=5.3) or 8.2-9.6 years. All dogs were given a series of baseline tests and ranked on the basis of error scores. Animals were given tests to measure spatial attention (landmark discrimination learning), spatial memory (3-choice), oddity learning, discrimination learning and reversal learning.

Moreover, Heath S. (Heath et al., 2007) hypothesized that nutritional supplementation can be used in the management of the condition and their trial was designed to investigate the therapeutic effects of a specific supplement when compared to a placebo. The trial was conducted in a clinical context and involved 20 UK veterinary practices, giving geographical spread across the country. The duration of the trial was 56 days, including a baseline period of 7 days and a post-trial period of 7 days. There was a significant difference between the treated and the placebo groups in relation to improvement in their scores for disorientation, changes in interaction and house soiling behavior at day 21, day 28 and day 42. These results support the clinical practice of nutritional supplementation as a valuable component of the therapeutic approach in cases of canine cognitive dysfunction

COGNITIVE DEFICITS − FUNCTIONS / KOGNITIVNI DEFICIT - FUNKCIJE

Cognitive testing procedures for canines were initially developed by modifying nonhuman primate cognitive tests (Milgram et al., 1994). A variety of tests have been developed to assess cognitive function in various domains and corresponding brain localization in dogs. There are cognitive domains assessed in dog aging and how they compare with assessment tasks for non-human primate and humans. Many of these tests are analogous to cognitive tests used for nonhuman primates and humans. As in humans, canine individual variability and doma-

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in-specific cognitive vulnerabilities are key features of decline with age (Milgram et al., 1994). Beginning in middle age, individual variability in cognitive scores begins to increase, with the largest variability seen in aged dogs (Adams et al., 2000). Additionally, vulnerability to decline with age varies as a function of cognitive domain and the cortical circuits engaged. For example, size discrimination learning is sensitive to age, while simple object discrimination is not (Head et al., 1995), similar to monkey models of aging (Rapp, 1990). Further, prefrontal dependent reversal learning is more age sensitive than discrimination learning (Adams et al., 2000).

There are several measures of cognition that are age-sensitive and treatment-sensitive in dogs that can be used as intervention outcome measures to assess different cognitive abilities with analogous tasks in non-human primates and in humans. Much like humans, the aging canine shows cognitive decline with various cognitive domains and cortical pathways being differentially affected (Milgram et al., 1994). Dogs show cognitive deficits due to age in tests measuring complex learning, executive function, spatial learning and attention, and memory (Studzinski et al., 2006). In addition to cognitive domain variability, individual dogs also show variability in cognitive function as seen in humans (Adams et al., 2000). This variability becomes most apparent in old canines, and using spatial learning and memory tasks, we are able to distinguish three groups of variability: (1) successful agers, (2) impaired dogs whose scores fell two SD above the mean of the young animals, and (3) severely impaired dogs who failed to learn the task (Head et al., 2001). The availability of agematched animals with and without cognitive deficits allowed researchers to determine which types of neuropathology contribute to individual cognitive impairments in these animals.

Several tasks, similar to those used for testing cognition in non-human primates, have been developed to measure cognitive decline in the aging canine (Milgram et al., 2002). Such tasks include landmark discrimination, oddity discrimination, object, size and black/white discrimination and reversal tasks, and a spatial memory task. All testing occurs in a modified Wisconsin General Testing Apparatus such that the motor and sensory demands are consistent across tasks.

The landmark discrimination task, measures visuospatial function and allocentric learning. The oddity discrimination task measures complex learning, as well as prefrontal cortex function. Aged dogs show deficits in oddity discrimination learning (Cotman et al., 2002). Tests of object, size and black/white discrimination are administered to measure associative learning ability. Executive function can be evaluated immediately after discrimination learning has been completed by using the object, size or black/white reversal objects. Memory also declines with age in dogs. The most useful age-sensitive task used is a spatial memory task, in which dogs are required to recognize the location of a sample stimulus and then respond to a different location during the test trial (Head et al., 1995).

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In addition to cognitive outcome measures, researchers and veterinarians are interested in measuring functional outcomes. Further, laboratory-based cognitive testing as described above is labor intensive and requires many months to years to obtain data. An open field test can be used to observe the behavioral patterns of animals in an empty room for 10 min (Siwak et al., 2001).

Measures of canine function can also be assessed in a clinical setting (Landsberg et al., 2012). Clinical measures have been developed consisting of pet dog owner based evaluation of dog behavioral changes (Landsberg et al., 2012) similar to those used in human clinical evaluations, such as the mini mental state exam (MMSE), the MoCA and the CDR test. Although there are different versions of these questionnaires, all appear to be sensitive to the presence of canine cognitive dysfunction. The evaluation consists of items such as walking, posture/emotion of expression, elimination behavior, life rhythm, play behavior, exploratory behavior, learned specific behavior, adaptive capabilities, and interactions with other animals or with owners. The items of individual questionnaires can be used to derive scores that distinguish between normally and pathologically aging dogs. Adult and older dogs generally score worse with these types of evaluation tools, and old dogs show individual variability in terms of the amount of cognitive dysfunction reported (Bosch et al., 2012).

Salvine et. al. (2011) conducted a study, using data from a large crosssectional survey of older dogs (n = 957), which aimed to develop a clinical scale for assessing CCD (Canine Cognitive Dysfunction). Data-driven analytical techniques were used to distil 27 significant behavioral items (previously identified as relevant to CCD) into an assessment tool with maximal cognito-behavioural breadth whilst maintaining clinical utility. The resulting CCD rating scale (CCDR) comprised 13 behavioral items, of which three were sensitive to the severity of the disease stage. When tested on an independent survey sample, the CCDR had an overall 98.9% diagnostic accuracy with a 77.8% positive predictive value and a 99.3% negative predictive value. Test–re-test reliability of the CCDR over 2 months was also high (r = 0.73, P < 0.0001). In conjunction with veterinary assessment, the CCDR could be a valuable tool in research and clinical settings for both the assessment and longitudinal tracking of cognitive change. It can distinguish those neurobehavioral changes associated with cognitive dysfunction from normal ageing.

CONCLUSION / ZAKLUČAK

The aged dog naturally develops decline in many different cognitive domains and exhibits human-like individual variability in the aging process. The neurobiological basis for cognitive dysfunction may be related to structural changes that reflect degeneration. Data on the prevalence of CDS suggest that the phenomenon is underestimated in veterinary medicine. CCD shares several similarities with human AD, such as the progressive characteristics of the clinical syndro-

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me, neuropathological abnormalities and pharmacological responsiveness. CCD may therefore serve as a useful translational model for AD.

The canine model has numerous advantages, however, systematic cognitive testing can be a lengthy and costly process and requires significant technical support. Still, the canine model should be considered as an option since it is less involved and costly than a human clinical prevention study. Successful studies of human diseases require an appropriate animal model. Overall, using the dog as a pre-clinical model for testing preventive approaches for AD may be a useful step that complements work in rodents and non-human primates. Canines are more suitable for examining human disorders than mice, as canines have evolved physiologically and genetically in close proximity to humans. Taken together, the aged dog may capture key features of human aging, making them particularly useful for studies of therapeutics that can be translated into human clinical trials. Although a canine model is ideal for human disease studies, to our knowledge, no such model has been reported to date, due to the lack of canine embryonic stem cells, which are generally used for gene targeting, and proper protocols for producing mature oocytes in vitro.

Further, prevention studies could be accomplished to test the effects of an intervention on the development of cognitive decline and neuropathology.

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