TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2021, vol. 6, No 2(11): 121–129

OVERVIEW OF COGNITIVE DYSFUNCTION SYNDROME IN AGEING DOGS

Toni Todorov, Aikaterini Vathi

University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
E-mail: ttodorow@yahoo.com

ABSTRACT

The paper presents an outline of what cognitive dysfunction in dogs is and current approaches to its treatment. With improved living standards and personal care for pets these days, an increase in their life span has been observed making the consideration of dealing with cognitive dysfunction more prevalent, as it commonly occurs in older dogs. Understanding the different aspects of this syndrome including its pathogenesis and clinical manifestation is crucial, in order to obtain an efficient treatment regime that will assist in alleviating the symptoms of this disorder.

Key words: Cognitive Dysfunction Syndrome, brain pathology, dogs, nutrition.

Introduction

Cognitive dysfunction syndrome (CDS) is described as a progressive neurodegenerative disorder of senior dogs. The term was introduced to describe observed geriatric behavioral changes which were not solely attributable to a general medical condition. Although there is no cure for CDS, it has been determined that medication and changes in diet can slow its progress, while owner education in proper environmental management and behavior modification can also improve and prolong the life of geriatric patients.

Etiology

There has been noted a correlation between dogs developing behavioral and cognitive dysfunction with age. The ageing process represents a complex biological development, characterized by a progressive modification of tissues and cells with a gradual loss of adaptive capacity (Kiatipattanasakul et al., 1996). Features of this include brain atrophy, neuron loss, and accumulation of Alzheimer's disease like neuropathology, vascular pathology, oxidative damage, and inflammation.

Pathogenesis

Identifying neurodegenerative mechanisms underlying cognitive dysfunction in ageing dogs is the first step in fighting this disorder. The pathogenesis of CDS can be broken down into the different aspects of the brain integrity it affects.

Structural Changes

Progressive brain atrophy is a consistent feature in ageing animals. More specifically with the help of magnetic resonance imaging, generalized cortical atrophy and ventricular widening (Gonzalez-Soriano et al. 2001; Kimotsuki et al., 2005) can be observed. Tissue volume losses in the prefrontal cortex where found to develop in early ages in beagles eight to eleven years old, with this varying with breed and relative life span (Tapp et al., 2004). Another change that has been recorded is in the neuronal number or density specifically within the hilus of the hippocampus (Siwak-Tapp et al., 2008). This is hypothesized to be due to the slower rate of neurogenesis in the hippocampus. Neuron loss and cortical atrophy in vulnerable brain regions of the aged dog may be due to multiple neurodegenerative processes associated with the up- or down regulation of molecular pathways (Swanson et al. 2007).

Plaques and Aβ Accumulation

In dogs β-amyloid plaques which interfere with conduction, accumulate in the cerebral cortex and the hippocampus (Crowell-Davis et al. 2008). Plaques are extracellular deposits that contain the Aβ peptide. Aβ accumulation can occur in different types of plaques (e.g., diffuse, neuritic), but also can form structures called oligomers, which are particularly toxic to synapses (Haass and Selkoe, 2007). Aβ deposition occurs earliest in the prefrontal cortex of the dog and later in the temporal and occipital cortex (Head et al., 2000). Importantly, the extent of Aβ plaque deposition in the dog brain is linked to the severity of cognitive deficits (Colle et al., 2000). However not all studies show a correlation between Aβ and the presence of canine cognitive dysfunction (CCD) (Chambers et al., 2011; Ozawa et al., 2016) but studies that show a link between the extent of Aβ and cognition also indicate that the location of the deposition is important.

Vascular Neuropathology

A frequent pathology detected in ageing dogs is the presence of cerebral amyloid angiopathy (CAA), which is described as the accumulation of Aβ in the walls of cerebral vessels (Attems, 2005). Aged dogs are vulnerable to this vascular pathology with perivascular abnormalities and with CAA being frequently observed (Giaccone et al., 1990; Ishihara et al., 1991). The consequences of CAA accumulating in the brains of ageing dogs may be a compromise to the function of the blood-brain barrier and impaired vascular function (Prior et al., 1996). In turn, vascular dysfunction and BBB disruptions may lead to microhemorrhages (Deane and Zlokovic, 2007). In a systematic study of the extent of CAA in cognitively characterized pet dogs, CAA increased with age but did not correlate with cognition (Ozawa et al., 2016). Thus, aged dogs develop cerebrovascular abnormalities that may not contribute to cognitive decline.

Oxidative Damage and Mitochondrial Dysfunction

There are several studies on the potential role for oxidative damage and mitochondrial dysfunction on brain ageing in dogs (Cotman et al., 2002; Dowling and Head, 2012). The production of free radicals during the ageing process can lead to damaged proteins, lipids, and nucleotides, which may cause neuronal dysfunction and degeneration. The ageing dog brain accumulates carbonyl groups, a measure of oxidative damage to proteins (Head et al., 2002; Skoumalova et al., 2003). Typically, the activity of endogenous antioxidants balances the production of free radicals. However, several of these protective mechanisms decline with age. Carbonyl groups are associated with reduced endogenous antioxidant enzyme activity/protein levels, including those of glutamine synthetase and superoxide dismutase (SOD) (Head et al., 2002; Hwang et al., 2008). The consequences of increasing oxidative damage with age in dogs may be compromised neuronal function leading to deficits in cognition. In aged pet dogs, higher levels of oxidative end products are correlated with more severe behavioral changes (Rofina et al., 2004, 2006; Skoumalova et al., 2003).

Inflammation

Although not as well characterized as neuroinflammation in the human brain, there are several small studies in aged pet dog brains. In recent studies (Schutt et al., 2016) it was found that pro-inflammatory cytokines were generally at low levels and were not associated with the extent of cognitive dysfunction. However, using measures of glial activation (microglial cells and astrocytes), increasing numbers of both types of cells were associated with more extensive CCD (Ozawa et al., 2016). Similarly, the level of S100β astrocytosis, a putative measure of inflammation, is also correlated with cognitive deficits in pet dogs (Pugliese et al., 2006).

Clinical Presentation

As mention above CDS is a disease of aging dogs. A Web based survey with seven hundred and twenty-six valid responses reveals that the median age of the dogs was 12 years old, and the majority were neutered, housed inside and less than 10 kg (Ozawa et al., 2019). Typical behavioral changes in affected animals include signs of disorientation, a decrease in or alteration of social interaction, impairment of normal house training, and changes in both the usual sleep–wake cycle and general activity (Milgram et al., 1994); traditionally the clinical signs are described by the acronym DISHA (Landsberg et al., 2003). DISHA stands for disorientation, interaction changes, sleep/wake disturbances, house soiling and activity changes. Vision impairment and olfactory function failure and smell disturbances are also considered a common finding (Ozawa et al., 2019).

Diagnosis

Diagnosis of CDS is mainly based on the behavioral alterations; however there are more specific laboratory tests that can aid in its diagnosis.

Behavioral Changes

Laboratory tests are available to assess multiple domains that correlate to decreased capacity of learning and memory, spatial abilities, attention, psychomotor ability, and executive function. It is advisable that elderly pets be examined twice a year, for optimal screening of both medical and behavioral health. Once signs of CDS are identified, the diagnostic workup should include a thorough medical history including the use of cognitive screening questionnaire, complete physical and neurologic exam, and laboratory/diagnostic tests.

Physiologic Changes

It is necessary to obtain a complete blood count, serum biochemistry profile, thyroid level, and urinalysis as a minimum database. Additional diagnostic tests may include endocrine testing, radiographs, ultrasound, and advanced imaging may be needed depending upon presenting signs and physical examination findings. Biochemical markers related to CDS are underdeveloped. Biochemical diagnostics usually focuses on the body fluids as the most accessible sources of biological markers related to the disease. Cerebrospinal fluid and blood are the most commonly used, since the former should contain the highest concentrations of the biomarkers and the latter is the easiest to collect. Since CSF is in direct contact with the brain interstitial fluid (ISF) that soaks the neurons, biochemical changes in the brain are reflected in CSF. CSF has low protease activity, and most proteinaceous molecules do not change upon collection provided, of course, the collected CSF sample is not contaminated by blood. Therefore, CSF should be the best source of biomarkers that reflect the pathological changes of the brain (Blennow et al., 1993). Blood (plasma or serum) on the other hand is far more accessible than CSF. However any brain-derived proteins will be highly diluted and proteins and peptides might have a short half-life in plasma due to fast renal clearance. Blood contains relatively high proteolytic and other enzymatic activities causing most intracellular proteins released into the bloodstream to undergo degradation and/or modification by proteases and other enzymes, and, therefore, for most of the biomarker candidates, the half-life in the blood is unknown (Werle and Bernkop-Schnürch, 2006).

Differential Diagnosis

An elderly dog presenting with nonspecific signs compatible with cognitive impairment requires a complete physical examination, neurological evaluation, complete blood cell count, biochemistry profile and urinalysis to differentiate between primary CDS or other underlying medical conditions. The main medical differentials are musculoskeletal diseases, endocrine and metabolic disorders, sensory decline and neurological diseases. Even though in most medical issues there will be concurrent medical signs or abnormal findings, it is possible that behavioral signs be the first or only indication of underlying health problems. Imaging studies such as CT scan or MRI can assist in ruling out neoplastic causes of cognitive impairment.

Treatment

Treatment of cognitive dysfunction should be focused on three different but closely related targets: to improve the clinical signs, to slow the progression of decline, and to prevent or delay the onset of dementia. There are four treatment modalities that are presently available which can be used alone or in combination that target, improving brain metabolism, enhancing neuronal transmission, reducing oxidative damage, and helping to maintain neuronal integrity. These include drugs, natural supplements, therapeutic diets, and environmental enrichment.

Pharmacological Therapy

The main drugs marketed for treatment of cognitive dysfunction and mental confusion in senior dogs include selegiline, propentofylline, and nicergoline.

Selegiline has demonstrated an improvement of the clinical signs of CDS and an improvement in working memory in a laboratory model at an oral dose of 0.5–1 mg/kg in the morning (Campbell et al., 2001). Selegiline is categorized as a selective irreversible inhibitor of monoamine oxidase B (MAOB) although its mode of action in dogs is not entirely clear. Selegiline has been shown to increase 2-phenylethylamine in the cortex and hippocampus, a neuromodulator that enhances dopamine and catecholamine function. Selegiline may also alleviate CDS by increased release and decreased reuptake of norepinephrine. Its metabolites L-amphetamine and L-methamphetamine may further enhance cognitive function and improve behavior.

Propentofylline is a xanthine derivative which may improve microcirculation and inhibit platelet aggregation and thrombus formation to increase oxygenation to the brain and periphery. It is licensed for use in dogs at 5 mg/kg BID for signs of senility including mental dullness, lethargy, and tiredness when no other underlying medical cause is identified.

Nicergoline is an alpha 1 and alpha 2 agonist (given at 0.5 mg/kg SID), which may act to enhance cerebral circulation and enhance neuronal transmission and may have a neuroprotective effect (Siwak et al., 2000).

However, in one study both nicergoline and propentofylline resulted in no significant increase in locomotion in dogs compared to adrafinil and modafinil, which might enhance noradrenergic transmission. Therefore, these medications might be a consideration to increase mental alertness and daytime activity. Additional compounds that reduce neuronal damage and limit oxidation are continuously being studied in clinical trials. These include monoamine oxidase (MAO-B) inhibitors, anti-inflammatory agents (NSAIDs), antioxidants (vitamin E), estrogens, and others. Researchers have looked at the effect of gonadectomy on subsequent development of age-related cognitive impairment in dogs, sex differences in the effect of estrogens on learning and memory of dogs. Another thing to take into consideration is the cholinergic decline present in ageing dogs. Elderly pets have

a decline in cholinergic function, and anticholinergic medications further aggravate cognitive decline. In fact, use of anticholinergic drugs might potentially contribute to further cognitive impairment (Cai et al., 2013) so it is important to avoid their administration.

Nutritional Intervention

A primary therapeutic strategy for cognitive dysfunction in dogs is to reduce the risk factors that contribute to cognitive decline through nutritional intervention. Strategies for treatment include individual supplements and combinations or "cocktails" of ingredients, focused on reducing the effects of oxidative stress, correcting metabolic changes associated with cognitive decline, and improving mitochondrial function, neuronal health, and signaling. The importance of specialized diets, was proven in a recent study of dogs with cognitive dysfunction syndrome in Slovakia, when comparing controlled diets (quality commercial foods for size, breed, age, or health status) to uncontrolled diets (low-quality commercial foods or leftovers), it was observed that the risk for cognitive dysfunction was increased with the lower-quality diets (Katina et al., 2016).

Natural Supplements

In addition to pharmacological intervention, natural supplements may be used. Supplements have potentially less side effects and are not generally contraindicated with most drugs or disease processes (e.g., renal, hepatic, or cardiac dysfunction).

Phosphatidylserine an important building block of cell membranes that is purported to facilitate neuronal signal transduction and enhance cholinergic transmission (Tsakiris and Deconstantinos, 1985). Phosphatidylserine along with natural antioxidants, vitamins and neuroprotective molecules can be found in a number of nutraceutical products (*Senilife*®). It has been demonstrated to improve cognition in both a laboratory model and clinical studies in dogs (Osella et al., 2007). In addition to phosphatidylserine, the supplement contains Ginkgo biloba, vitamin E, and resveratrol for their potential antioxidant effects and also contains vitamin B6 (pyridoxine) which may have neuroprotective effect (Dakshinamurti et al., 2003). Phosphatidylserine combined with omega-3 fatty acids, vitamins E and C, L-carnitine, alpha-lipoic acid, coenzyme Q, and selenium (*Aktivait*®) has also demonstrated significant improvement over placebo in improving social interactions, disorientation, and house soiling in dogs with CDS (Heath, 2007).

S-adenosylmethionine is a natural supplement for dogs that may help to maintain cell membrane fluidity and receptor function, regulate neurotransmitter levels, and increase production of glutathione which may decrease oxidative stress (Rème et al. 2008).

Apoaequorin is a protein found in jellyfish that in two separate laboratory trials improved learning and attention in dogs. It is a calcium-buffering protein that may provide neuroprotection against ageing (Milgram et al., 2015). Dysregulation of intracellular calcium has been associated with increased age and there is some proof to suggest that it may be linked to cognitive dysfunction syndrome in dogs.

Docosahexaenoic acid There is some evidence to suggest that DHA might help in improving memory and general health.

Therapeutic diets

The first diet studied for the treatment of cognitive dysfunction was formulated by Hill's Pet Nutrition (**Prescription Diet b/d Canine**) with a focus on improving antioxidant defenses and reducing the effects of oxidative damage. It is supplemented with fatty acids, antioxidants (vitamins C and E, beta-carotene, selenium, flavonoids, carotenoids), and dl-alpha-lipoic acid and L-carnitine which are intended to enhance mitochondrial function. The diet was evaluated in 48 beagle dogs

with results indicating an improvement in visual discrimination, and reversal learning. The highest cognitive scores were seen in the dogs that received both antioxidant diet and added enrichment with spatial memory and reversal learning improving over 2 years. By contrast, there was no improvement in young dogs fed only with the cognitive diet (Scarmeas et al., 2006). The combined effect of the diet and enrichment acted, in a synergistic manner, to reduce oxidative damage and increase brain-derived neurotropic factor and neuronal health (Head et al., 2009). While enrichment protected against neuronal loss in the hippocampus (Siwak-Tapp et al. 2008), mitochondrial function and beta-amyloid pathology were significantly improved with diet and not with enrichment alone (Head et al., 2009).

A diet from Nestle Purina Research (**Purina Pro Plan Bright Mind**) is supplemented with botanic oils containing medium-chain triglycerides (MCT) to provide ketone bodies that might serve as an alternative source of energy for ageing neurons. In fact, studies have shown a significant reduction in cerebral glucose metabolism by 6 years of age compared to dogs at one year of age (London et al., 1983). Over an 8-month trial, the group supplemented with 5.5% MCT showed significantly better performance over a placebo diet in a variety of test protocols (Pan et al., 2010). The group given MCT supplement showed significantly elevated levels of the ketone body, β-hydroxybutyrate (Sullivan and Brown, 2005). Most recently in a double-blinded placebo-controlled clinical trial in dogs with CDS, a diet supplemented with 6.5% MCT and a brain protection blend (BPB) demonstrated significant improvement in all 6 categories of DISHAA over a 3-month trial (**ProPlan Veterinary Diets Neurocare**) (Pan et al., 2017). Another study by Nestle Purina utilized a proprietary brain protection blend (BPB) containing B vitamins, antioxidants including vitamins E and C and selenium, fish oil containing DHA and EPA for anti-inflammatory effects, and arginine to enhance nitric oxide synthesis to reduce blood pressure and improve circulation and cognition. Royal Canine, **Canine Mature Consult diet** is also formulated with a blend of phosphatidylserine, antioxidants, and L-tryptophan to help maintain cognitive health in senior pets.

Adjunctive Therapy

Together with diets, supplements, and drugs for the treatment for CDS, psychotropic medications may be required concurrently to manage underlying stress and address those signs such as night waking, agitation, and anxiety. As the health and behaviour of senior pets may necessitate the use of multiple medications, caution must be exercised to insure that there are no incompatibilities or contraindications when combining medications and supplements. For example, selegiline should not be used in combination with MAO inhibitors such as amitraz or drugs that might increase serotonin. Since anticholinergic drugs should be avoided, fluoxetine, sertraline, or buspirone might be considered as ongoing therapeutics and clomipramine, amitriptyline, and paroxetine avoided. While benzodiazepines could contribute to further cognitive deficits, sedation, or incoordination, they may be useful in managing signs of anxiety and sleep disturbances. Lorazepam and oxazepam would be preferred since they have no active intermediate metabolites. Adjunctive use of propranolol or clonidine or dexmedetomidine oromucosal gel may inhibit release or block the effects of noradrenaline. Gabapentin might reduce reactivity and neuropathic pain (Landsberg et al., 2011). Natural products might also aid in the control of anxiety including pheromones, L-theanine, alpha-casozepine, melatonin, or supplements containing GABA, phellodendron and magnolia, or souroubea.

Behavioral and Environmental Management

Together with medical and nutritional therapy, providing both physical stimulation and mental enrichment are important aspects of maintaining behavioral and physical health and slowing decline

(Head et al., 2009). However, since senior pets may develop an increasing number of medical health issues with increasing age including sensory decline, endocrinopathies, organ dysfunction, increasing pain, and decline in mobility, the amount, level, and type of enrichment will need to be tailored to the needs and limitations of the individual. Meeting the individual needs of the pet is the primary consideration when designing an optimal enrichment plan. These needs include feeding, sleeping, grooming and self-hygiene, elimination, and attention (affection and interaction) from the owner. Behavioral enrichment should include social interactions with the owner such as play, scent work, and training. Behavioral and emotional stability in senior pets is achieved via owners' positive interactions, predictability, and structured daily routine. By adopting these measures owners provide an optimal environment necessary to combat their pet's cognitive decline.

Conclusion

CDS in dogs is characterised by mental decline due to loss of brain integrity associated with ageing. This interferes with the daily functioning of pets and their owners' lives. There are various medical treatments available which are being continually tested. In addition it has been indicated that early diagnosis in combination with owner education and appropriate dietary modifications can alleviate the relatively rapid progression of this disorder thus improving the quality of the pet's life. Scientific studies in this field are ongoing offering promising results.

References

1. Attems J. (2005). *Sporadic cerebral amyloid angiopathy: pathology, clinical implications, and possible pathomechanisms*. Acta Neuropathol., 110 (4):345–359.
2. Blennow K, Fredman P, Wallin A, Gottfries CG, Skoog I, Wikkelsö C, Svennerholm L. (1993). *Protein analysis in cerebrospinal fluid. II. Reference values derived from healthy individuals 18–88 years of age*. Eur. Neurol., 33:129–133.
3. Cai X, Campbell N, Khan B, Callahan C, Boustani M. (2013). *Long-term anticholinergic use and the aging brain*. Alzheimer's Dementia, 9(4):377–385.
4. Campbell S., Trettien A., Kozan B. (2001). *A noncomparative open-label study evaluating the effect of selegiline hydrochloride in a clinical setting*. Veterinary Therapeutics, 2 (1):24–39.
5. Chambers JK, Mutsuga M, Uchida K, Nakayama H. (2011). *Characterization of AβpN3 deposition in the brains of dogs of various ages and other animal species*. Amyloid, 18 (2):63–7.
6. Colle MA, Hauw JJ, Crespeau F, Uchihara T, Akiyama H, Checler F et al. (2000). *Vascular and parenchymal Aβ deposition in the aging dog: correlation with behavior*. Neurobiol Aging 21(5):695–704.
7. Cotman, C. Head E., Muggenburg B. A., Zicker C., Milgram N. M. (2002). *Brain aging in the canine: a diet enriched in antioxidants reduces cognitive dysfunction*. Neurobiology of Aging, 23(5), 809–818.
8. Crowell-Davis, S. L. (2008). *Cognitive Dysfunction in Senior Pets. Compend*. Contin. Educ. Vet.; 30 (2):106-8, 110.
9. Dakshinamurti K, Sharma SK, Geiger JD. (2003). *Neuroprotective actions of pyridoxine*. Biochim Biophys Acta (BBA)-Proteins Proteomics, 1647(1):225–229.
10. Deane R., Zlokovic B.V. (2007). *Role of the blood-brain barrier in the pathogenesis of Alzheimer's disease*. Curr. Alzheimer Res., 4(2):191–197.
11. Dowling AL, Head E. (2012). A*ntioxidants in the canine model of human aging*. Biochim. Biophys. Acta (BBA)-Mol. Basis of Dis., 1822(5):685–689.

12. Frank, D., *Cognitive Dysfunction in Dogs. In: Hill's European Symposia on Canine Brain Ageing (en línea) 2002*. (fecha de acceso 27 de mayo de 2009). URL disponible en: http://www.ivis.org/proceedings/Hills/brain/frank.pdf?LA=1.
13. Giaccone G, Verga L, Finazzi M, Pollo B, Tagliavini F, Frangione B, Bugiani O. (1990). *Cerebral preamyloid deposits and congophilic angiopathy in aged dogs*. Neurosci. Lett. 114(2):178–183.
14. Gonzalez-Soriano J, Garcia PM, Contreras-Rodriguez J, Martinez-Sainz P, Rodriguez-Veiga E. (2001). *Age-related changes in the ventricular system of the dog brain*. Ann Anatomy- Anatomischer Anzeiger 183(3):283–291.
15. Haass C, Selkoe DJ. (2007). *Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid β-peptide*. Nat. Rev. Mol. Cell. Biol., 8(2):101–112.
16. Head E, McCleary R, Hahn FF, Milgram NW, Cotman CW. (2000). *Region-specific age at onset of β-amyloid in dogs*. Neurobiol. Aging, 21(1):89–96.
17. Head E, Liu J, Hagen TM, Muggenburg BA, Milgram NW, Ames BN, Cotman CW. (2002). *Oxidative damage increases with age in a canine model of human brain aging*. J. Neurochem., 82(2):375–381.
18. Head, E. et al. (2009). *Effects of age, dietary, and behavioral enrichment on brain mitochondria in a canine model of human aging*. Experimental Neurology, 220(1), pp.171–176.
19. Heath, S.E., Barabas, S., Craze, P.G. (2007). *Nutritional supplementation in cases of canine cognitive dysfunction – A clinical trial*. Applied Animal Behaviour Science, 105(4), 284–296.
20. Hwang I.K., Yoon Y.S., Yoo K.Y., Li H., Choi J.H., Kim D.W., Yi S.S., Seong J.K., Lee I.S., Won MH. (2008). *Differences in lipid peroxidation and Cu, Zn-superoxide dismutase in the hippocampal CA1 region between adult and aged dogs*. J. Veterinary Med. Sci., 70 (3):273–277.
21. Ishihara T., Gondo T., Takahashi M., Uchino F., Ikeda S.I., Allsop D., Imai K. (1991). *Immunohistochemical and immunoelectron microscopical characterization of cerebrovascular and senile plaque amyloid in aged dogs' brains*. Brain Research, 548 (1):196–205.
22. Katina S, Farbakova J, Madari A, Novak M, Zilka N. (2016). *Risk factors for canine cognitive dysfunction syndrome in Slovakia*. Acta Veterinaria Scandinavica 58(1):1.
23. Kiatipattanasakul, W. et al. (1996). *Apoptosis in the aged dog brain*. Acta Neuropathologica, 92(3), 242–248.
24. Kimotsuki T, Nagaoka T, Yasuda M, Tamahara S, Matsuki N, Ono K. (2005). *Changes of magnetic resonance imaging on the brain in beagle dogs with aging*. J. Veterinary Med. Sci., 67(10):961–967.
25. Landsberg,G.M.,Hunthausen, W., Ackerman, L. (2003).*The effects of aging on the behavior of senior pets*. In:Landsberg, G.M., Hunthausen, W., Ackerman, L. (Eds.), Handbook of Behavior Problems of the Dog and Cat. 2nd ed. Saunders, Edinburgh, 269–304.
26. Landsberg GM, DePorter T, Araujo JA. (2011). *Clinical signs and management of anxiety, sleeplessness, and cognitive dysfunction in the senior pet*. Veterinary Clin. N. Am.: Small Animal Practice, 41(3), 565–590.
27. Landsberg, G.M., Maďari Aladár & Žilka Norbert. (2017). *Canine and feline dementia: molecular basis, diagnostics and therapy*. Cham, Switzerland: Springer.
28. London ED, Ohata M, Takei H, French AW, Rapoport SI. (1983). *Regional cerebral metabolic rate for glucose in beagle dogs of different ages*. Neurobiol. Aging, 4(2), 121–126.
29. Milgram NW, Head E, Weiner E, Thomas E. (1994). *Cognitive functions and aging in the dog: acquisition of nonspatial visual tasks. Behav*. Neurosci. 108(1), 57–68.
30. Milgram NW, Landsberg G, Merrick D, Underwood MY. (2015). *A novel mechanism for cognitive enhancement in aged dogs with the use of a calcium-buffering protein*. J. Veterinary Behav. Clinl. Appl. Res., 10(3):217–222.
31. Osella, M.C. et al. (2007). *Canine cognitive dysfunction syndrome: Prevalence, clinical signs and treatment with a neuroprotective nutraceutical*. Applied Animal Behaviour Science, 105(4), 297–310.
32. Ozawa M., Chambers J.K., Uchida K., Nakayama H.Q. (2016). *The relation between canine cognitive dysfunction and age-related brain lesions*. Journal of Veterinary Medical Science, 78 (6):997–1006.

33. Ozawa M., Inoue M., Uchida K., Chambers J. K., Takeuch Y., Nakayama H. (2019). *Physical signs of canine cognitive dysfunction*. Journal of Veterinary Medical Science, 81(12): 1829–1834.

34. Pan Y, Larson B, Araujo JA et al. (2010). *Dietary supplementation with medium-chain TAG has long-lasting cognition-enhancing effects in aged dogs*. Br. J. Nutr., 103:1746–1754.

35. Pan Y, Landsberg G, Mougeot I. (2017). *Efficacy of a therapeutic diet on dogs with signs of cognitive dysfunction syndrome (CDS); a prospective double blinded placebo controlled clinical study*. Front nutr., 5:127.

36. Prior R, D'Urso D, Frank R, Prikulis I, Pavlakovic G. (1996). *Loss of vessel wall viability in cerebral amyloid angiopathy*. Neuroreport, 7(2):562–564.

37. Pugliese M., Geloso M.C., Carrasco J.L., Mascort J., Michetti F., Mahy N. (2006). *Canine cognitive deficit correlates with diffuse plaque maturation and S100β (−) astrocytosis but not with insulin cerebrospinal fluid level*. Acta Neuropathol., 111(6):519–528.

38. Rème CA, Dramard V, Kern L, Hofmans J, Halsberghe C, Mombiela DV. (2008). *Effect of S-adenosylmethionine tablets on the reduction of age-related mental decline in dogs: a double- blinded, placebo-controlled trial*. Veterinary Therapeut: Res. Appl. Veterinary Med., 9(2):69–82.

39. Rofina JE, Singh K, Skoumalova-Vesela A, van Ederen AM, van Asten AJ, Wilhelm J, Gruys E. (2004). *Histochemical accumulation of oxidative damage products is associated with Alzheimer-like pathology in the canine*. Amyloid, 11(2):90–100.

40. Rofina JE, Van Ederen AM, Toussaint MJM, Secreve M, Van Der Spek A, Van Der Meer I, Van Eerdenburg FJCM, Gruys E. (2006). *Cognitive disturbances in old dogs suffering from the canine counterpart of Alzheimer's disease*. Brain Res., 1069(1):216–226.

41. Scarmeas N, Stern Y, Tang MX, Mayeux R, Luchsinger JA. (2006). *Mediterranean diet and risk for Alzheimer's disease*. Ann. Neurol., 59(6):912–921.

42. Schutt T., Helboe L., Pedersen L.O., Waldemar G., Berendt M., Pedersen J.T. (2016). *Dogs with cognitive dysfunction as a spontaneous model for early Alzheimer's disease: a translational study of neuropathological and inflammatory markers*. J. Alzheimer's Dis., 52(2):433–449.

43. Siwak C.T., Gruet P., Woehrlé F., Muggenburg B.A., Murphey H.L., Milgram N.W. (2000). *Comparison of the effects of adrafinil, propentofylline, and nicergoline on behavior in aged dogs*. Am. J. Veterinary Res., 61(11):1410–1414.

44. Siwak-Tapp CT, Head E, Muggenburg BA, Milgram NW, Cotman CW. (2008). *Region specific neuron loss in the aged canine hippocampus is reduced by enrichment. Neurobiol*. Aging, 29(1):39–50.

45. Skoumalova A, Rofina J, Schwippelova Z, Gruys E, Wilhelm J. (2003). *The role of free radicals in canine counterpart of senile dementia of the Alzheimer typ*e. Exp. Gerontol. 38(6):711–719.

46. Sullivan P.G., Brown M.R. (2005). *Mitochondrial aging and dysfunction in Alzheimer's disease. Progr*. Neuro-Psychopharmacol. Biol. Psychiatry, 29(3):407–410.

47. Swanson K.S., Vester B.M., Apanavicius C.J., Kirby N.A., Schook L.B. (2007). *Implications of age and diet on canine cerebral cortex transcription*. Neurobiol. Aging 30(8):1314–1326.

48. Tapp P.D., Siwak C.T., Gao F.Q., Chiou J.Y., Black S.E., Head E., Muggenburg B.A., Cotman CW, Milgram NW, Su MY. (2004). *Frontal lobe volume, function, and β-amyloid pathology in a canine model of aging*. J. Neurosci., 24(38), 8205–8213.

49. Tsakiris S., Deliconstantinos G. (1985). *Phosphatidylserine and calmodulin effects on Ca 2+-stimulated ATPase activity of dog brain synaptosomal plasma membranes*. Int. J. Biochem. 17(10), 1117–1119.

50. Werle M., Bernkop-Schnürch A. (2006). *Strategies to improve plasma half life time of peptide and protein drugs*. Amino Acids, 30,(4), 351–367.