Cognition and Optimal Brain Function in Older Dogs

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Introduction

The number of geriatric pets has increased considerably in the last 10 years. In 1995 in the United States 24% of pet cats were over 6 years. Today it is estimated to be approximately 47%. In Europe between 1983 and 1995 the number of geriatric cats increased by over 100% while the number of geriatric dogs increased by approximately 50% in that time period. This growing subset of the pet population has received very little scientific research and publications on geriatric critical care are extremely sparse.

In humans, older age alone does not predict mortality. The main determinants of mortality are prior health status and severity of current disease process. Although we do not have studies of this nature in veterinary medicine it seems prudent to offer aggressive treatment to the older dog and cat once comorbid diseases, quality of life for the pet, and the owner's desires are taken into consideration.

This presentation will begin with a brief overview of geriatric pets, then delve into the main non-cognitive comorbid conditions that can mimic cognitive decline in older dogs, specifically dehydration, medications, nutrition, vision and hearing. Cognition, cognitive decline and the progression of it will be discussed next. We will conclude with actionable, practical options for older pets that have significant potential in our patient populations.

Definition of Geriatric in Veterinary Medicine

The term geriatric is difficult to define in veterinary medicine because it differs between dogs and cats and between different breeds (i.e., Great Dane has a much shorter

lifespan than a Chihuahua). In general, animals older than 7 year of age are considered to be geriatric. A more specific definition has recently been proposed. Giant and large breed dogs are senior at 6-8 years and geriatric at 9 years or older. Medium and small breed dogs are senior at 7-10 years of age and geriatric when 11 years or older. Cats can be considered senior at 12-14 years of age and geriatric when 15 years or older.

Fluid Therapy

Dehydration, common in older dogs, can have significant effects on cognition. Significant changes in multiple organ systems in geriatric animals should be taken into account when selecting the type, dosage, and rate of fluid choices in this age group.

Myocardial fibrosis, valvular malfunction, and myocardial fiber atrophy seems to increase with age in geriatric pets. The decrease in ventricular compliance limits the volume that the geriatric animal can tolerate while paradoxically increasing its dependency on volume. Geriatric animals are highly dependent on end-diastolic volume to increase cardiac output and therefore do not tolerate volume depletion very well during times of stress (i.e., illness, anesthesia, etc.).

Renal changes such as the decreased ability to concentrate or dilute urine, decreased renal blood flow, and the limited ability to conserve sodium all limit the geriatric animal's ability to handle either volume depletion or volume overload.

Balanced isotonic crystalloids (i.e., LRS, 0.9% NaCl) are ideal for the dehydrated geriatric patient. Both natural (i.e., fresh frozen plasma) and artificial colloids (i.e., Hetastarch®) are additional options for hypovolemia but should be administered at a slower rate in geriatric animals due to their propensity for volume overload. Supplements such as potassium chloride, vitamin B complex, and dextrose are added as needed.

A thorough search for any underlying or chronic disease processes (i.e., chronic valvular disease, renal failure) is essential when planning fluid therapy. It is imperative that fluid therapy in geriatric animals be monitored both diligently and frequently. Monitoring for optimal perfusion includes frequent checks of pulse quality, extremity temperature, venous lactate, urine output, body weight and mentation. Monitoring for fluid overload includes frequent checks of thoracic auscultation, body weight, urine output, central venous pressure, arterial blood gases or pulse oximetry, and thoracic radiographs.

Pharmacology

Medications, particularly in the very young or very old, can have major effects on cognition since optimal dosing is unknown and overdosing may occur more easily than in younger animals . Aging imposes several changes in the absorption, distribution, metabolism and elimination of many drugs. Oral absorption may be decreased due to decreased GI function as the animal ages. The loss of lean body mass can alter IM route absorption.

If fluid retention is present (such as with congestive heart failure, cirrhosis, or renal failure) drugs that are distributed to extracellular water (e.g., penicillins, NSAIDS, aminoglycosides) will be altered in their distribution. Albumin, the protein to which many drugs bind, also decreases with age.

Drug metabolism may change as the geriatric patient experiences a decline in hepatic function. The mass of the liver decreases with age and decreases hepatic function. This could cause increased plasma half-life of drugs that depend on hepatic excretion, metabolism, or conjugation. Decreased function of phase I metabolism reactions in the

liver appear to occur with age and cause decreases in oxidation, reduction, dealkylation, and hydroxylation reactions. Phase II reactions do not appear to be altered with age.

Drug elimination may be affected by a progressive decline in renal function with age. In geriatric people, there is a steady decline in renal function with approximately 40% of the nephrons becoming sclerotic by the age of 85 and renal blood flow and GFR decreasing by almost half. Due to the loss of lean body mass creatinine may remain normal (decreased production and decreased clearance). In dogs and cats approximately 15-20% are thought to suffer some degree of renal insufficiency as they enter the geriatric years.

In geriatric people, there is a progressive decline in the number of cardiac myocytes and in ventricular compliance. Autonomic tissue is replaced by fat and connective tissue and shows decreased responsiveness to autonomic drugs. It is likely that some decline of cardiac function occurs with age in animals and careful monitoring for specific endpoints is essential when prescribing cardiac drugs to geriatric animals.

Options for appropriate drug dosing in geriatric animals include measurement of renal function, therapeutic drug monitoring with frequent dosage adjustments, and dosage or interval reduction according to creatinine concentrations. The most practical and cost efficient of these options is dosage or interval reduction. Dosage and interval adjustments based on creatinine use the following formulas;

Adjusted dosage = Normal dosage X (Normal serum Cr/Patient's serum Cr) OR

Adjusted interval = Normal interval (1 ÷ {Normal Serum Cr/Patient's Serum Cr})

It is essential to take any co-morbid diseases (e.g., congestive heart failure, chronic renal failure, hepatic fibrosis) into account when considering dosage adjustments

for geriatric small animals. For example, if a dog with chronic renal insufficiency requires therapy with angiotensin-converting enzyme inhibitors the clinician must be aware of the significant likelihood of decreased renal clearance in this animal due to both its chronic renal disease and the older age.

Nutrition in Older Pets

Maintenance energy requirements (MERs) decrease with age in dogs but appear to increase after the age of 12 years in cats. There may also be a decrease in the ability to digest fat and protein as cats age. These changes can lead to either weight gain (i.e., if an older dog is fed food with the same caloric content as it ages) or weight loss (i.e., if an older cat is fed food with the same caloric content as it ages) in the older pet. The reduced ability to digest fats can lead to deficiencies in fat soluble vitamins (e.g., vitamin E) along with water soluble vitamins (e.g., B vitamins) and electrolytes. In older dogs with a limited ability to digest fats due to a diminished ability to secrete pancreatic lipase or bile acids medium chain triglycerides may be beneficial as a concentrated and highly absorbable energy source.

Adequate protein intake is essential for optimal immune function and is critical in geriatric animals. Protein requirements actually increase in older dogs and the old dogma of protein restriction for kidney protection has been discounted.

Antioxidants are essential to combat oxidative stress, which has been shown to increase with age in many species. The “Free Radical/Oxidative Stress Theory of Aging” suggests that levels of reactive oxygen species increase with age and amelioration of this increase can retard the aging process. Anti-oxidants can be administered exogenously and are thought to contribute to decreased levels of oxidative stress and perhaps to increased

quality of aging. Some specific antioxidants that can be easily added to the treatment regimen include Vitamin B complex added to IV fluids (@4 ml per liter when given at a maintenance rate), SAMe given orally (@ 20mg/kg PO q12h), and N-acetylcysteine given intravenously (@ 50mg/kg IV over 1 hour diluted 1:4 with 0.9% NaCl q 8h) or orally (@ 50mg/kg PO q8-12h). Oral N-acetylcysteine can be found in health food stores in the amino acid section.

Anorexia in the older critically ill patient is common and should be aggressively treated after a thorough search for underlying causes. Midazolam (cats), propofol (dogs) and probiotics (e.g., Fortiflora; dogs and cats) may improve appetite. Smell is an important appetite stimulant in both dogs and cats and clogged nasal passages (i.e., bilateral nasal catheters for delivery of oxygen) may cause a decreased appetite. Warming the food and placing a small amount on the tongue may help stimulate eating.

Vision Changes

Vision changes are common in older pets and may mimic cognitive decline. Nuclear sclerosis (lenticular sclerosis) due to increased density of the lens, although common, usually has no effect on vision. However, loss of night vision is common as well as decreased tear production with age. Cataracts are more common with age with a mean onset of ~7-8 years and corneal deposits (e.g., calcium, lipids, cholesterol) also increase with age. Retinal degeneration is also common with age with a decrease in cones and rods seen. With diminishing vision comes reluctance to venture out or the get lost in the year and this may mimic cognitive decline. Adding a vaporizer, especially, during cold weather, may help mitigate the dry eyes that are common in this group.

Hearing Changes

Hearing loss is common in older humans and cochlear degeneration is seen in older dogs. Hearing is extremely important in dogs and loss of hearing is often the straw that broke the camel's back. When hearing loss becomes apparent it usually unmasks vision abnormalities as well since the pet cannot compensate. They may appear to ignore commands, or respond inappropriately when hearing is compromised and this may mimic cognitive decline. N-acetylcysteine is used for hearing loss by the Navy when divers' hearing becomes damaged.

Cognition Dysfunction

Cognitive dysfunction syndrome (CDS) and Disorientation/Dysfunctions in Interaction, Sleep and Housetraining (DISH) are thought to occur in over 50% of dogs over 15 years of age. DISH resembles advanced dementia in humans and our patients may benefit from the new research in people. The recent discovery of the glymphatic (glial + lymphatic) system offers an exciting opportunity to optimize brain health in pets.

Why do we sleep?

Sleep is an extremely vulnerable state that can increase mortality so it must serve an important purpose. Recently, essential brain cleansing, has been documented to occur during certain phases of sleep. It has been shown that during deep sleep the brain "shrinks" up to ~60% in volume to allow for CSF "flushing" and clearance.

Glymphatic System

The glymphatic system uses an aquaporin (AQP4) dependent flow to clear debris (solutes) from the brain. Respiration moves CSF through the aqueduct and this occurs during the deep phase of sleep. Accumulation of these solutes is associated with

neurodegeneration in humans and laboratory animals. Elimination of the waste does not occur in daylight hours (the off switch is thought to be mediated by norepinephrine). Sleep disturbances are associated with dementia in humans and are common in aged animals. There was an 80% decline in glymphatic clearance in aged mice versus young mice and this is thought to be, partially, due to melatonin decreases with age. Sleep disturbances are also very common in hospitalized pets due to noise, stress/pain, loss of circadian rhythm, and lights. Blue light (high in LED and fluorescent lights) depress melatonin synthesis and should not be used after ~12 noon or so. In a traumatic brain injury model, CSF clearance was impaired for 28 days after injury.

Taurine, Not Just for the Heart!

Interestingly, one of the main determinants of success of CSF clearance is taurine, in the form of tauro-cholic acid. This bile acid is essential for the clearance of the solutes via conjugation and the limiting factor is taurine, an amino acid that we usually think of only for the heart.

Essential Strategies for Older Dogs

Vitamin B12, in the form of methylcobalamin or hydroxycobalamin, absorption declines with age in humans and is associated with cognitive decline. Providing subcutaneous injections may help to support cognitive health by providing this essential vitamin. Taurine supplementation, natural anti-inflammatory strategies (e.g., turmeric, low carbohydrate diet, etc.) and melatonin supplementation (e.g., 3-6 mg half an hour before bedtime) can all help to mitigate cognitive decline in older dogs and will be discussed in more detail during the presentation.

REFERENCES are available upon request

Microbiome Update: How it changes with age and how to optimize it for your older pet population

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All Disease Begins in the Gut
Hippocrates 3rd Century BCE

Death begins in the colon
Elie Mechnikov (1845-1916)

Why the MB Matters

Changes in the MB are associated with cancer (colorectal, gastric, lung, etc.), Neurological (e.g., MS, epilepsy), Behavioral (e.g., anxiety, depression), Endocrine (e.g., diabetes, hepatitis), and Autoimmune conditions among others. In humans, 1 in 12 Americans, 1 in 9 women are affected with autoimmune conditions.

Autoimmune conditions are increasing in prevalence with 23-50 million Americans affected. There are approximately 120-140 different autoimmune conditions, many of these seen in veterinary medicine (ITP, IMHA, IBD, polyarthritis, etc.). What is driving this increase?

Chronic inflammation seems to be a central nidus and the systemic inflammation appears to stem from leaky gut. Leaky gut is driven by pathologic alterations in the microbiome, many of which occur with age.

What is the Microbiome?

Microbes in/on mammals include Bacteria, fungi, archaea, viruses, parasites Each niche has own MB. Skin, mouth, ears, lungs, eyes, etc. Importantly, these are functional! For instances, Atopic dermatitis affects 10-15% canine population. It has been shown that during flare ups there is a decreased diversity in skin bacteria leading to a decreased protective barrier (*Grice; J Invest Derm 2016)*

The Gut Microbiome contains approximately ~100 trillion microbes human gut, which is ~10X more than # of human cells, ~100X more genes than human genome, and ~90% of human illness attributed to MB to some degree This means that ~99% genetic material in humans is bacterial or viral and that we can change our DNA! Rapidly, 3-4 days

Quantifying the MB

Huge difference between SI, colon. Segments near epithelium vs stool, Inside vs outside stool, Inner vs outer end of stool. Clinical improvement matters most!

Formation of Microbiome

At birth neonate inoculated Microbes from vaginal canal
C-section microbes from skin. Very different microbiome with higher rates asthma, autism in humans. Animals?? Human birth centers “inoculate” C-Section baby with mother’s vaginal microbiome. Bulb syringe secretions are removed then the oral cavity is swabbed. During formation there are significant detrimental effects from antibiotics – especially oral! Humans

show higher incidence of asthma when exposed to antibiotics at birth. This critical window is when the MB directs formation of the Immune system and the gut epithelium. Newborns resemble microbiome of mother, siblings until weaning and then MB affected by diet. There is a high variation in MB due to diet, geography, and culture.

Structure of Microbiome

Adult Dogs and Cats have mostly Firmicutes, Bacteroidetes, Proteobacteria Fusobacteria, and Actinobacteria.

Mucous lines intestines, particularly the colon and this mucous layer is very important. It renews every hour and is critical to protect the gut lining. Layers of microbes are in the lumen as well as on the outer and inner mucus layer. The bilayered mucous layer allows the luminal layer to exit with feces. The mucous layer provides lubrication and escorts microbes out. Approximately 50% stool weight = bacteria!

Pathogenic bacteria are sensed by MB, which then increases peristalsis with the goal that the pathogenic bacteria are removed with the mucous. The mucous protects the epithelium of the colon as well. Colonic bacteria eat fiber, if they run out of fiber (low fiber diet) they begin to eat the mucous. When they run out of mucous they eat through the epithelial wall of the colon.

Fiber is essential for bacteria survival and for the formation of short chain fatty acids (SCFAs), which are anti-inflammatory compounds produced by the colonic bacteria. All carbohydrates are absorbed in the small intestine

and high carbohydrate diets starve the colon. Sugar loving bacteria communicate with the brain via the vagus and give the signal to eat more sugar!

Low fiber diets lead to degradation of the mucous (protective) layer by bacteria. After they eat the mucous, bacteria then eat through the epithelium. This is called bacterial translocation and because the entire GI tract, from the esophagus to anus, exists as a single layer of epithelium, extensive damage can occur in a short period of time.

Bacterial Translocation

Bacterial translocation is associate with chronic inflammation due to the inflammatory cascade effects of translocating bacteria. Since the bacteria cannot make SCFAs without enough fiber, there is a loss of that anti-inflammatory effect as well (e.g., SCFAs are anti-inflammatory). Short Chain Fatty Acids are an essential energy source for colonocytes. They also maintain epithelial tight junctions, and produce anti-inflammatory compounds. SCFA administration limits colitis in experimental studies, haven been shown to regulate sodium, water absorption, and increase mineral absorption. The lower pH in gut inhibits pathogens and PPIs are associated with increased pneumonia in humans.

The gut MB also has essential roles for immune regulation. There are tight junctions between epithelial cells that, in health, allow for an anti-inflammatory environment. The GALT, T&B cells, dendrites are all poised to initiate response depending on the microbe sensed. A signal tells a naïve T cell to become Pro-inflammatory or Anti-inflammatory (via T regulatory

cells;Tregs). The immune system also performs surveillance with dendritic cells sitting behind the epithelium and sending dendrites into the lumen to sample bacteria. Dendrites are the only cells that can capture live bacteria to determine the appropriate response (e.g., Pathogenic vs commensal). During pregnancy the dendrites transport beneficial microbes to breast milk.

There is a strong association between mental state and the MB via the Gut brain axis. The vagus nerve affects mentation via direct connections with the MB and the gut neurons are called the 2nd brain. Approximately, 80-90% of serotonin (e.g. the feel good chemical) is made in the gut.
The gut is also home to much vitamin production including cobalamin (B12), biotin, thiamine, and others.

Dysbiosis

What used to be called SIBO is now called dysbiosis and is thought to be due to loss of commensals, excessive growth of harmful organisms and reduction in overall diversity. Bacteria can induce chronic inflammation (from too little fiber) and pathogenic bacteria set off chronic inflammatory cascade that can induce cancer. Beneficial microbes are associated with decreased cancer, mainly due to decreased inflammation.

There are significant links between dysbiosis and autoimmune conditions in humans, such as RA, Asthma, Atopy, ITP, IMHA. Similar links occur with neurodevelopmental and neurodegenerative conditions such as Alzheimer's, and Parkinson's. It is unknown whether such links occur with cognitive dysfunction in animals.

There is also a strong correlation with dysbiosis and obesity, which is more prevalent as pets age. In mice, a MB transplant from obese mice to lean mice resulted in the lean mice becoming obese. The opposite occurred when lean mice MB was transplanted to obese mice. This suggests that the MB may be contributing the obesity in the aged pet. Proposed mechanisms include an increased dietary energy harvest as well as microbe induced changes in host glucose & lipid metabolism. Obesity is also associated with chronic low grade inflammation which can lead to insulin resistance via chronic LPS exposure. Probiotics (bifidobacteria) have been shown to lower LPS, improve glucose tolerance and reduce inflammation.

Dysbiosis in Older Pets

The top three causes of dysbiosis in dogs and cats are antibiotics, Proton Pump Inhibitors (& H2 blockers) and diet. Antibiotics are common and approximately 4/5 humans in US take at least one course of antibiotic every year. A single course of ciprofloxacin was shown to alter the MB for >1 year The number of days of antibiotic use is associated with increased risk breast cancer fatality (JAMA 2004, Lancet 2012).

Long term (over 7 days) use of Proton Pump Inhibitors (PPIs) is associated with Osteoporosis, C. Difficile, Pneumonia, B12 deficiency, AKI, Dementia, V fibrillation – magnesium deficiency in humans.

Diet, particularly a high carbohydrate, low fiber diet, is very detrimental to the MB. There are specific concerns as well to select ingredients such as glutenin and gliadin, the 2 main proteins associated with gluten. Gliaden triggers zonulin production, which results in break down of the gut tight

junctions resulting in leaky gut and chronic inflammation. Research at Harvard (Dr. Alessio Fasano) has shown that many autoimmune conditions show high levels of zonulin. When mice are exposed to zonulin they make get a leaky gut and make antibodies to beta cells.

Prebiotic fiber, non-digestible polysaccharide and oligosaccharide are fermented by colonic bacteria, generating SCFAs. Resulting in lower inflammation, more anti-inflammatory mediators, and a lower pH. Prebiotics (Bifidobacterium, Lactobaccili) protect the gut epithelium, increase the mucous layer, elongate the microvilli, and prevent adherence of pathogenic organisms. Prebiotic fiber acts like fertilizer, for every 100g consumed, 30 grams of bacteria produced. Food sources of prebiotic include Inulin – in chicory, garlic, onion, leaks, jicama, chicory, Jerusalem artichoke, raw dandelion greens, raw asparagus, green banana and potato (cook, then refrigerate, then warm to increase prebiotic fiber). In dogs, chicory root supplementation resulted in improved fecal scores in healthy dogs.

Probiotics have been documented since 1909, when they improved clinical signs in autoimmune arthritis. Supplementation with live cultures (Streptococcus lacticus, Bacillus bulgaricus) were used. Live organisms confer the best benefit, maintain tight junctions, up regulate tight junction proteins and increase mucin secretion by globlet cells. Probiotics increase defensins, prevent pathogen colonization, produce SCFAs, stimulate IgA secretion and decrease luminal pH. Even non-viable organisms may confer health benefits as they adhere to the mucous layer and stimulate immune function. In a study in shelter dogs a probiotic (*E.faecium* SF68; Fortiflora®) with metronidazole iImproved fecal scores versus metronidazole alone.

Foods rich in probiotics include all properly fermented foods such as kimchi, Sauerkraut, kefir, lassi, kombucha tea, tempeh, and pickles.

Other options for favorably altering the MB include giving a probiotic enema, which dates back to ancient Egypt and the Mayans. When making these it is important not to use chlorinated water and approximately 3-6 probiotic capsules are used. Ideally, probiotics with a high percent of Bifidobacteria, which are predominant in the colon, would be used.

Fecal microbial transplant (FMT)

Other options include FMT, which has been approved in US for recurrent *C.difficile.* There is >90% cure rate for this deadly condition after FMT. In Europe FMT is used for autoimmune conditions and there are multiple options for delivery including oral capsule, nasogastric tube, nasoduodenal, colonoscopic, or enema. Reports of clinical improvement with IBD, MS, myoclonus dystonia, Refractory ulcerative colitis, Autism, Parkinson's and Rheumatoid arthritis.

One of the most dramatic studies was in 17 children with Autism Spectrum Disorder. Researchers wiped out the endogenous flora with Vancomycin and then administered FMT. They reported a 80% decrease GI signs, 24% decrease core ASD symptoms, and even greater improvement at 2 years!

In dogs with eosinophilic IBD there were improvements after FMT with the dogs being symptom free for 3 months. Another example of 8 dogs with refractory C. perfringens that were given FMT showed the diarrhea resolving in all dogs afterward.

Conclusion

The microbiome is now known to be an essential player in health and disease. Microbes, chiefly from the gastrointestinal tract, are critical players in immune function, vitamin production, utilization of nutrients, detoxification, inflammatory and autoimmune disorders, and neurotransmission among others. In 2008 the NIH launched the human microbiome project and a plethora of new research is highlighting the importance of the maintenance of balance in the 100 trillion microbes residing on and in the mammalian body. Cutting edge treatment options include probiotic enemas, fecal capsules and fecal microbial transplants (FMT) and these are being used for infectious diarrhea (C difficile), autoimmune conditions (rheumatoid arthritis, multiple sclerosis, Crohn's disease) and obesity. The Harvard Medical School teamed up with MIT to create the OpenBiome Project to make FMT more widely available.

Hypercoagulable Changes in Older Pets
Mitigation Strategies

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Introduction

The coagulation system becomes more prone to clotting (prothrombotic) with age in humans and, likely, pets. A combination of toxin exposure (e.g., pollution, pesticides, chlorinated water, etc.), stress, poor diet, and oxidative stress contribute. This talk with start with a brief review of physiologic hemostasis, including an update on the new models of coagulation. We will discuss the aging changes that contribute to prothrombosis, detrimental effects (including hidden and micro-thrombosis) and will conclude with practical strategies to mitigate prothrombotic states in our pet patients.

Endothelial Review

Our research into the endothelium has evolved from the belief that it was an inert tube that carried blood all the way to today where it is a living, breathing (internal mitochondrial respiration), organ that is extremely heterogeneous, highly intelligent (endothelial cells sense what other endothelial cells upstream or downstream are reacting to), and absolutely essential for the health of every cell in the body.

The endothelium in health is a powerful antithrombotic surface. First, the glycocalyx, a hairy like covering of the endothelium, has a negative charge that repels cells towards the center preventing platelets from rolling on the endothelium. There is also constitutive expression of numerous substances including thrombomodulin, endothelial protein C receptor, heparan sulfate proteoglycan, tissue factor pathway inhibitor, nitric oxide, prostacyclin and ADPases (enzymes that break down ADP). These are all antithrombotic. Lastly, the endogenous prothrombotic substances (e.g., collagen, von Willebrand Factor, tissue factor) are sequestered from the flowing blood being contained in the subendothelium (collagen, vWF) and adventita

(tissue factor).

Normal hemostasis is local – very important. We never want systemic clotting! During the process of physiologic hemostasis these three protections are removed – locally. For instance, when a needle penetrates the endothelium (e.g., phlebotomy) multiple mechanisms remove/inactivate thrombin diffusing away from a local clot. A breach in the endothelium does several things; it removes the negative charge, removes the anti-thrombotic substances, and exposes pro-thrombotic substances that are normally sequestered from flowing blood. This allows the creation of a local fibrin clot.

Prostacyclin & Prostaglandin

Prostacyclin (predominantly large vessels), and PGE_2 (predominantly small vessels) are constituitively expressed on the endothelium and are potent vasodilators. Importantly, these anti-thrombotics are inhibited via blocking of the COX enzyme system (e.g., NSAIDS), which is one way NSAIDS can lead to thrombosis.

Ectonucleotidases

Ectonucleotidases break down ADP (ADP; adenosine diphosphate) after it is released from platelets after platelet activation. ADP that diffuses away can lead to systemic platelet activation and these scavenge any ADP that diffuses away from a local clot.

HSPGs and HCII

Heparan sulfate proteoglycans repel platelets and are an integral part of the glycocalyx

HSPGs are markers of GLX degradation

Tissue Factor Pathway Inhibitor

Very important inhibitor of coagulation

Inhibits initial steps in coagulation

TFPI-F10a complex inhibits TF-7a

Antithrombin

Must bind to cofactor, Pentasaccharide sequence, On ~30% pharmaceutical grade heparin And on endothelial bound HSPG, AT inhibitory activity enhances >1000 fold By binding to heparin

Protein C pathway

Thrombomodulin expressed on endothelium and any that diffuses away from local clot becomes bound in TM. Complex of TM-Thrombin activates protein C and becomes an antithrombotic powerhouse. Activated protein C (APC) inactivates F5&8
Endothelial protein C receptor (EPCR) acts similar to APC but in different vessels.

Nitric Oxide Gas Diffusion

Because NO is a gas it has the ability to diffuse through the endothelial wall to the lumen, where it inhibits platelet reactivity and to the abluminal side, where it relaxes vascular smooth muscle and inhibits cell proliferation. This gas is crucial for blood vessel health! The precursor to NO is arginine and cats have a specific need for arginine in their diet. A study found that cats with thromboembolism were low in arginine compared with normal cats. (JVIM McMichael 2001).

Thrombosis

Thrombosis is defined as an unwanted clot (e.g., no breach in endothelium) that may be obstructive. Thromboembolism occurs after either dislodging of a local clot or the occurrence of systemic clotting (DIC – disseminated intravascular coagulation).

Hemostasis vs Thrombosis

The Inter-relationship between immunity and coagulation can be traced back to the beginning of evolution where one cell, the hemocyte, controlled immunity and hemostasis (invertebrates). This basic survival strategy walls off damaged, infected tissues in an attempt to limit infections. The coagulation system inhibits pathogens and the immune system sets off

clotting. Platelets wall off pathogens, neutrophils extrude DNA in nets to limit pathogens and platelets extrude NETS too! Polyphosphate (PolyP) in bacteria and viruses sets off the contact pathway to trigger systemic clotting.

Thrombosis and Immunity

There is a clear crosstalk between the coagulation and the immune system and there is a significant morbidity from thrombosis with infection. Newer treatments address both

Virchow's Triad

The components of clot formation occur due to changes in blood flow (stasis), blood constituents (retention of procoagulant factors), and the endothelial wall (stretching or damage).

Blood flow changes that are associated with prothrombotic states in veterinary patients include stasis in the Left Atrium (cats with HCM) which often leads to arterial thromboembolism (e.g., saddle thrombus). Blood changes that can lead to prothrombotic state include increased viscosity (e.g., Polycythemia, Hypergammaglobulinemia).

Alterations in Endothelium

Damage leading to down regulation and/or elimination of antithrombotics along with up regulation or exposure of prothrombotic substances. Inflammation, hyperglycemia, oxidative stress, LA stretching, physical damage (e.g., Tumor), Infections (e.g., ticks, bartonella) and Dilation (e.g., DCM, enlarged LA) all contribute.

Antithrombin

Inhibits F10a, F9a, F11a, thrombin (2a), only free thrombin, not bound in clot
Inhibits leukocyte activation –rolling and adhesion. Blocks expression of proinflammatory cytokines. Exogenous heparin eliminates anti-inflammatory effects of AT.

Once thrombin is made it sets off coagulation & inflammation. This system is dysregulated in sepsis.

Tissue Factor

TF is a key initiator of hemostasis in vivo. It is likened to a hemostatic envelope surrounding blood vessels & surfaces – fibroblasts, pericytes, keratinocytes. It is bound to F7a/F7 in dermal vasculature. High TF lungs, brain (astrocytes), pancreas, heart, uterus/placenta/testes
Influenza A increases TF expression in mouse lungs. Should we consider anti-thrombotics with pneumonia? TF is not thought to flow freely in blood because it is highly prothrombotic. Studies of stagnant blood – no free TF but Studies of flowing blood – free TF found
Flow may "activate" TF and this is an active area of research. TF in blood likely inhibited or encrypted and needs activation or key. Blood-borne TF may contribute to thrombosis not hemostasis.

What Do Platelets Do? Maintain tight junctions, especially post-capillary venules. When platelet numbers go down capillary endothelial junctions open.

Anti-Platelet Medications to be discussed include aspirin and clopidogrel.
Thromboxane A2 - aspirin
P2Y12 receptor - clopidogrel

Hemolytic Uremic Syndrome (Alabama Rot)
What Does von Willebrand Factor Do? Protection of Factor VIII
ADAMTS13 & Thrombotic Thrombocytopenia will be discussed in the context of the recent cases in the UK.
JA Kremer Hovinga, JN George. N Engl J Med 2019;381:1653-1662.

Conditions Associated with Thrombosis

IMHA – platelet reactivity, TF expression, MPs, inflammation, free heme scavenges NO

Cardiac – stasis, turbulence, endothelial injury, inflammation

PLN, PLE – loss of AT, endogenous anticoagulants, inflammation

Neoplasia – platelet reactivity, TF expression, inflammation, release TF + MV into circulation

Pancreatitis – inflammation, TF expression

Trauma – inflammation, TF expression

Thrombosis and Disease

Sepsis –activated monocytes major source of TF, Activates coagulation during endotoxemia

Surgery –triggers inflammatory response, increase monocyte TF

Pretreatment of monocytes with ubiquinol (reduced CoQ10) leads to decreases of TF expression and decreases oxidative stress

DIC - Definition

Disseminated intravascular coagulation

From a strong activator of coagulation. Could be PolyP, or Blood Borne TF. DIC is characterized by presence of widespread thrombi with deposition in the microvasculature. This occurs concurrently with a bleeding tendency.

DIC - Sequelae

Thromboses impede perfusion, may lead to multi-organ dysfunction syndrome (MODS).

Consumption of clotting factors & platelets leads to bleeding after the hypercoagulable phase.

DIC independent, powerful predictor of mortality but is always a complication of another disease.

DIC – Common Causes

Sepsis and systemic infections

Widespread inflammation

Cancer

Severe trauma – exposure of TF to multiple sites

Summary – Thrombosis associated with…

Inflammation, cytokines, increased platelet reactivity

Increased TF expression monocytes & ECs

Alterations in blood flow (turbulence)

Vessel wall changes

Endothelial damage, ROS, inflammation, hyperglycemia

Coagulation changes in blood leading to Increased prothrombotic substances, decreased antithrombotic substances.

Mitigation

Options for mitigation will be discussed in order of;

Lease likely to harm, low cost, may work

To More likely to harm, high cost, may work

Options to be discussed include arginine, methylcobalamin, N-acetylcysteine, dietary changes, microbiome enhancements, low dose aspirin, clopidogrel, factor X inhibitors, and others.

References available upon request

Oxidative Stress and Select Antioxidants in Aging Pets

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Objectives

This talk will focus on what you can do for your aging small animal patients to optimize health using anti-oxidants. Specifically, ubiquinol (heart failure, neuropathy, muscular degeneration, aging), N-acetylcysteine and SAMe (liver disease, detoxification, lung disease), curcumin (ingredient in turmeric; neurological diseases, aging, inflammation, brain health), alpha-lipoic acid (general health, cognitive decline), and DMSA (much less smell than DMSO; joint disease, arthritis) will be discussed and dosages given.

Measurement of Oxidative Stress is Problematic

Oxidative stress (OS) is a continually evolving state that is associated with numerous diseases and conditions in humans and animals. It is defined as reactive species (RS) in excess of antioxidant defense mechanisms. Antioxidants (AO) are defined as substances that can delay or prevent oxidation of a target molecule. OS can occur due to an excess of RS, a reduction in AO or both. Physiological levels of RS interact with the redox state and play essential roles in cell signaling and may be necessary to induce adaptive responses through antioxidant defense. Pathological levels of RS result in oxidative damage and activate cell death pathways. Elucidating the specific damage caused by RS and measuring the effect of treatment with exogenous substances is challenging. There is a significant drawback to *in vitro* testing in that cell culture is exposed to significantly more oxygen (environmentally) than most cells *in vivo. In vivo* testing has also been associated with significant issues including a lack of sensitive & specific, non-invasive, standardized tests to evaluate damage done by RS. Because of this lack of standardization, the clinical pharmacology of AO has not been effectively studied. In addition, much of the research on OS involves methodologies that are either not directly applicable or are not practical in clinical situations. Newer diagnostics that are more sensitive & specific are promising. Treatment of OS has not been rewarding and it is thought to be due to most studies using a single antioxidant. Newer treatment modalities include targeting the mitochondrial (MT) AO and using multiple AO that are synergistic. This review will cover new findings in OS in relation to specific disease states, the mitochondrial theory of aging, and normal antioxidant defense mechanisms.

Normal anti-oxidant defense mechanisms

In health, the major source of RS formation in cells is electron leakage from electron transport chains with ~90-95% of the oxygen converted to water and the remaining 5-10% is reduced, creating RS. The generation of RS is kept to a minimum by the high efficiency of electron transfer and sequestration of metal ions. Separate microenvironments exist for the MT, the lysosome, and the peroxisome; each contains a RS-generating system coupled to immediately adjacent antioxidant defense mechanisms. Three extra-MT sources of RS are the xanthine oxidase system, NADPH oxidase, and uncoupled nitric oxide. Once formed, RS can either react with another radical to form a covalent bond or, more commonly, react with a non-radical. When a free radical reacts with a non-radical, the non-radical loses an electron, transforming into a free radical. This is the essence of the chain reaction that propagates extensive damage to cell membranes. When the

radical combines with another radical the product can be more damaging than the original radical. An example is when nitric oxide (NO) combines with superoxide ($O^{\bullet -}$) creating peroxynitrite ($OONO^-$), which is 2,000 times more damaging than hydrogen peroxide (H_20_2). Alternatively, the reaction of two radicals can result in a termination of the cascade. The interaction of RS with lipids in the presence of free iron results in lipid peroxidation. Production of RS is balanced with endogenous AO defenses with the AOs controlling *levels* of RS, not eliminating them. RS appear to play essential roles in vivo including redox regulation of gene expression. Cells exposed to RS may undergo proliferation, senescence, apoptosis, or necrosis. The level of RS that causes cells to change from proliferation to any of theses appears to be cell type specific.

Types of Antioxidant Defense

In general, there are three lines of AO defense against damage caused by RS. AO proteins, such as albumin, haptoglobin, ferritin, and ceruloplasmin are abundant in plasma. Intracellular enzymatic AO include superoxide dismutase (SOD), catalase, and glutathione peroxidase. These are expressed in most mammalian cells and prevent the generation of RS. Small molecule AO are divided into water-soluble and lipid-soluble categories. Water-soluble AO include ascorbic acid (vitamin C), uric acid, bilirubin, glutathione (GSH), zinc and selenium. Lipid soluble AO include tocopherols (vitamin E), β-carotene, ubiquinol (co-enzyme Q; CoQ), and lycopene. Cell membranes contain tocopherols and β-carotene within their lipid layer and these can act to quench chain reactions of lipid peroxidation. Extracellular fluids contain molecules with AO properties (ascorbic acid, bilirubin, transferrin, haptoglobin, albumin, urate).

Glutathione peroxidase, synthesized in mammalian cells, is generally considered the first line of defense against RS formation. It is a sulfur-containing tripeptide (glycine, cysteine, glutamine) that reduces H_20_2 to water, using GSH as a substrate. OS has been shown to be associated with a depletion of GSH and this has been shown to induce apoptosis of hepatocytes. Vitamin E, the 2nd line of defense, inhabits the lipophilic interior of the cell membrane, where the PUFAs are located, and is a chain-breaking scavenger, halting lipid peroxidation. When a wave of lipid peroxidation reaches vitamin E it is oxidized to a free radical, sparing any adjacent PUFAs from oxidation. Vitamin C then combines with the E radical forming a poorly reactive, water-soluble, vitamin C radical, and regenerating vitamin E. Vitamin C is the most abundant water-soluble antioxidant and it can directly scavenge RS or regenerate vitamin E. Superoxide dismutase (SOD) is an oxido-reductase that contains copper, zinc or manganese at the active site. It catalyzes the dismutation of superoxide to oxygen and H_20_2. It is present in the cytosol (requires copper and zinc), the mitochondria (requires manganese), and on the extracellular surface (requires copper and zinc). Mitochondrial SOD is believed to play a major role in AO defense mechanisms. Catalase is a heme protein located in peroxisomes, which converts H_20_2 to water and oxygen. Catalase functions in conjunction with SOD; SOD converts superoxide to H_20_2 and catalase then converts the H_20_2 to water and oxygen

Mitochondrial Theory of Aging

The mitochondria (MT) play a central role in the generation of RS and OS has been shown to damage mitochondrial DNA (mtDNA). This may lead to lower numbers of mitochondria per cell with age or more dysfunctional MT. MT, the chief source of ATP, are considered the energy centers of the cell. MT damage is thought to contribute to the negative effects of

aging. Numerous experimental studies correlate increased MT AO with prolonged lifespan. In mice overexpression of MT catalase extended lifespan, a MT targeted AO (SkQ1) prolongs lifespan, and a mutation associated with decreased MT RS generation increased lifespan. A premature aging phenotype mouse model correlated aging with mtDNA deletions and MT respiratory chain failure occurred with high loads of mtDNA deletions. Age related conditions such as muscle and hearing loss have been associated with increased levels of MT OS. Mice with increased MT RS had accelerated hearing loss while mice with increased Mt AO had improved hearing compared with aged controls.

Oxidative Stress and the CNS

The CNS, due to its high oxygen demand, high level of polyunsaturated fatty acids, high levels of iron, and low level of endogenous AO, is quite vulnerable to OS. Increased MT OS is well documented in numerous CNS conditions including Alzheimer's, Parkinson's and Huntington diseases as well as multiple sclerosis. The amyloid plaques seen in Alzheimer's inhibit MT function by inhibition of the electron transport chain which leads to increased RS production. Overexpression of catalase in mice MT decreased amyloid toxicity and extended lifespan in a mouse model of Alzheimer's. There is substantial evidence for a central role of MT in the pathogenesis of Parkinson's, again with overexpression of MT catalase showing a protective role.

Oxidative Stress and Coagulation

There is strong evidence that OS leads to a hypercoagulable state in people and research animals. Nitric oxide (NO) is rapidly inactivated by RS, specifically superoxide anion. NO is essential for the maintenance of vascular flow (prevents vasoconstriction) and also prevents platelet aggregation. Preservation of NO may be one method that AO use to modulate coagulation and AO status is an important determinant of platelet function. Addition of specific AO leads to increased bleeding time in many studies. Alpha tocopherol is a platelet inhibitor and decreased platelet AO content in people is associated with platelet hyperactivity.

Oxidative Stress and Neoplasia

Mice lacking or low in specific AO systems show increased rate of hepatic carcinoma, lymphoma, adenocarcinoma, and pituitary adenomas later in life. Some malignant cells use RS to increase metastasis, angiogenesis, and proliferation and to suppress apoptosis. One mechanism that RS may use to promote angiogenesis is by increasing cell production of VEGF. RS have been shown to modulate integrin expression and suppress anoikis. Metastasis may be enhanced by RS via changes in intercellular communication, cell mobility, and increased vascular permeability. Matrix metalloproteinases (MMPs), which are involved in degradation of the extracellular matrix, can be activated by RS. Some RS (e.g., H202) have been shown to stimulate the proliferation and migration of human prostate cancer cells. In mice, non-metastatic cancer cells became invasive after depletion of AO and the effect was eliminated after instilling an AO rich preparation. Interestingly, RS appear to have a bi-phasic effect in cancer with significant excesses of OS impairing angiogenesis. The loss of MT cytochrome oxidase is associated with colonic dysplasia and MT catalase expression reduces tumor burden in mice with mammary carcinoma and hematopoietic tumors. Reducing MT RS in mice reduced their propensity to develop thymic lymphoma.

Quantifying Oxidative Stress

A common research method for studying OS is cell culture but this has significant drawbacks including the lack (or severe deficiency) of many AO (vitamins E, C, selenium) and the addition of free iron. Perhaps most importantly is the fact that most cells in culture are exposed to 95% air and 5% C02, which is equivalent to approximately 152 mmHg of oxygen, an extremely hyperoxic environment, compared to in vivo (in which most cells are exposed to less than 10 mmHg oxygen). There is likely increased RS formation due to the hyperoxia alone. Oxidation in cell culture can lead to false results in some studies. The excess exposure to RS in cell culture has been discussed as one reason why cellular proliferation is so common in laboratory settings.

In vivo testing of OS is problematic, time consuming, and less convenient than cell culture but has some inherent advantages. The most widely accepted in vivo method of testing for OS is the measurement of isoprostanes. When RS attack arachidonic acids on cell membranes isoprostanes are formed. They are produced *in vivo* independently of the cyclooxygenase enzyme by free radical-catalyzed peroxidation of arachidonic acid. The isoprostanes most commonly studied are the αF_2-isoprostanes. Much evidence now exists indicating that the F_2- isoprostanes are a reliable, non-invasive way to measure lipid peroxidation *in vivo* compared with other methods. Administration of AO has been shown to inhibit the formation of F_2-isoprostanes in both animal models of oxidant injury and in humans. Isoprostanes can be detected in all types of biological fluids and tissues; the free form can be measured in urine and plasma, esterified complexes can be measured in tissue, or metabolites can be measured in urine. Auto-oxidation can occur in lipid-containing samples during processing and storage which is why many researchers prefer urine. CNS specific isoprostanes appear to form with damage and some appear to be effective markers of damage. F2-dihomo-isoprostanes are derived from damage to myelin and may be a selective marker of white matter injury in vivo. Urine isoprostanes were 6 fold higher in humans with MS compared with controls and CSF levels of isoprostanes were significantly higher in another study. Urinary isoprostanes were higher in ALS patients compared with healthy adults.

Measurement of Endogenous Antioxidants

Measurement of specific AO in blood or tissues can be used as an indicator of OS if the levels of AO are low. Glutathione peroxidase is an essential part of the endogenous AO defense system. Glutathione, the substrate, exists in 2 forms; reduced glutathione (GSH) and the oxidized form, glutathione disulfide (GSSG). During OS, GSH is oxidized to GSSG. Measurement of the ratio of GSH to GSSG can be used to assess oxidative damage via depletion of GSH and has been reported in dogs and cats. This method is also susceptible to spontaneous oxidation *ex-vivo* and artificially elevated GSSG levels. In sled dogs α-tocopherol was shown to decrease significantly after an exercise run, suggesting that the endogenous AO capacity may not be adequate for the challenges of vigorous racing. In another study aimed at decreasing OS in racing sled dogs, Baskin, et al., reported that supplementation with α-tocopherol, β-carotene, and lutein increased plasma concentrations of these AO significantly in Alaskan sled dogs.

Treatment of oxidative stress

Most treatment of OS involves blocking the formation of RS, scavenging RS after they are formed or augmenting AO. Alpha lipoic acid is protective for diabetic neuropathy. Coenzyme Q (CoQ) reduced diastolic dysfunction in children

with cardiomyopathy. The reduced form of CoQ10 is called ubiquinol and is associated with greater OS reduction. Resveratrol prevented LV hypertrophy, diastolic dysfunction, and interstitial fibrosis in a mouse model of the metabolic syndrome. Quercetin reduced systolic BP and oxidized LDL in overweight humans and improved cardiac function in rats. It is likely that the best treatments will encompass a combination of drugs that target several steps in the OS injury cascade.

Blocking Formation of RS

Glutathione can act both as a chain breaking antioxidant, inhibiting lipid peroxidation, and as a metal chelator, preventing formation of the hydroxyl radical. It is synthesized in all mammalian cells with the rate of synthesis dependent upon cysteine stores in most organs except the liver. In the liver, GSH can be synthesized from either cysteine or methionine and the liver is the primary site for GSH synthesis, supplying up to 90% of circulating GSH. When GSH is given exogenously it cannot penetrate cell membranes. Cysteine is the rate-limiting amino acid in the formation of GSH and treatment with N-acetylcysteine (NAC) enables continued production of GSH. NAC is also a powerful scavenger of both the hydroxyl radical and hypochlorous acid. The protective effects of NAC are believed to be associated with the sulfhydryl groups trapping electrophilic intermediates by acting as a nucleophile. Treatment with NAC is protective against endotoxin challenge, radiation induced injury, and lung injury from toxic gas. In a rat model of IR injury, NAC blocked NFkB activity in addition to scavenging RS. It has attenuated IR injury during cardiac catheterization and has shown cardioprotective effects during ischemia. NAC has also shown some benefit in both sepsis and ARDS patients.

Vitamin E, composed of 4 tocopherols and 4 tocotrienols, is a lipid-soluble vitamin that antagonizes the peroxidative injury of membrane lipids and inhibits propagation of cell membrane destruction. It converts the alkylperoxyl radicals to hydroperoxides and then to tocopheroxyl radicals. The tocopheroxyl radicals are then reduced by vitamin C. Vitamin E, C and ubiquinol destroy RS involved in the "chain reaction" of lipid peroxidation**.** Vitamin C (ascorbic acid) is a water-soluble vitamin that allows regeneration of vitamin E for continued antioxidant effects. Ascorbic acid reduces the tocopheroxyl radical back to the antioxidant tocopherol. Vitamin C reduces ferric iron to ferrous iron, which under normal conditions improves absorption of iron from the GI tract. Under conditions of ischemia or increased availability of free iron, vitamin C can function as a pro-oxidant by providing more ferrous iron for the generation of hydroxyl radical (via the Haber-Weiss reaction). Several clinical trials reported disappointing results with supplementation of vitamin E. Unfortunately, most of these studies used either the synthetic form (no antioxidant properties) or only $1/8^{th}$ of the natural form. In patients with coronary artery disease, endothelial dysfunction was attenuated by administration of vitamin C and this appears to be due to superoxide scavenging by vitamin C. However, it appears that vitamin C must be given in very high concentrations to compete effectively with NO for superoxide. Ubiquinol (CoQ) appears to act as an antioxidant but the exact mechanisms are not clear. It appears to prevent both the initiation and propagation of lipid peroxidation.

Scavenging RS

SOD exists on the extracellular surface, in cytosol and MT. It scavenges superoxide anion and converts it to H_20_2. If there is not sufficient catalase available to convert the H_20_2 to water then H_20_2 will accumulate and contribute to the formation of the hydroxyl radical. In this case, SOD can be considered a pro-oxidant. Exogenous SOD has been shown to be protective in many models of IR injury. Its short half-life may be a factor in the studies that showed no

improvement. In renal transplants, it has been shown to decrease acute rejection and improve 4 year graft survival. Catalase converts H_20_2 to water and oxygen. It is essential that catalase be present along with SOD to convert the H_20_2 produced by SOD to water and oxygen. The paired administration of SOD and catalase conjugate has been shown to be effective in attenuating OS in several models.

Since free iron is central to the formation of the hydroxyl radical many treatment strategies attempt to block iron. However, iron is essential to many biological processes and iron chelation therapy can have potentially toxic side effects when they interfere with normal iron metabolism. Most strong chelating agents remove ferric iron from proteins (i.e. transferrin) and can interfere with iron incorporation into hemoglobin. Deferoxamine chelates ferrous iron and has been shown to reduce RS injury in several models. Several studies evaluating deferoxamine have been unrewarding most likely due to the toxic side effects and its short half-life in circulation in humans (~5minutes). DMSO scavenges the hydroxyl radical and the metabolite that is formed traps other RS. It permeates cell membranes to get to intracellular sites of RS formation and is also thought to inhibit platelet aggregation and increase vasodilation. It can lead to the formation of the methyl radical, which can then react with PUFA's to form methane gas or can react with oxygen to form methyl peroxyradicals. It is believed that the levels of DMSO needed to scavenge the hydroxyl radical may be high enough to cause damage to healthy cells. During the breakdown of ATP during ischemia, there is a buildup of adenosine. Adenosine is also released by neutrophils, endothelial cells, and myocytes. Interestingly, adenosine, in high concentrations, is believed to be responsible for the benefit seen with ischemic pre-conditioning. In addition to stimulating A1, A3, and potassium ATP channels, adenosine may inhibit conversion of XD to XO during ischemic periods. If this is true, then the accumulation of adenosine during ischemia would result in decreased RS formation. Adenosine is believed to decrease release of superoxide radical by neutrophils, to decrease leukocyte adhesion, and to increase the synthesis of NO via A2 receptor binding. Adenosine can cause hypotension and AV block when given IV.

Simultaneously administering several AO that work synergistically has shown promising results. A human product, Protandim®, combines 5 active ingredients: Silybum marianum (milk thistle), Bacopa monnieri, Withania somnifera (ashwaganda), Camellia sinensis (green tea), and Curcuma longa (turmeric extract). A new veterinary product, Canine Health®, by the makers of Protandim, adds joint protection and comes in a chewable tablet. This combination has been shown to work synergistically to increase SOD and catalase and to induce heme oxygenase-1 (an enzyme that breaks down heme) in humans.

Curcumin, the active ingredient in turmeric, is a potent anti-inflammatory agent with over 200 published studies and will be discussed in more detail in the talk.

DMSA has potent anti-inflammatory properties and is also inexpensive and less offensive (to the olfactory system) than DMSO.

References available upon request

Update on Anti-Aging Research
Applications for the Geriatric Pet

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Introduction

There has been a plethora of anti-aging research in the last few years, some of which is applicable to our veterinary patients. The main benefits of these strategies are their ability to halt the negative effects associated with aging such as arthritis, cognitive decline, frailty, sensory loss (e.g., hearing, vision loss), and energy level. The area that has received the most attention is caloric restriction and this area has very promising results.

Review of Essential Repair Mechanisms

Autophagy

This is the body's ability to clear damaged organelles and to clean up damaged DNA by clearing excised genomic fragments. When an organelle is targeted for recycling (e.g., mitochondria) the organelle is isolated within a vesicle called an autophagosome which subsequently fuses with a lysosome. The new, autolysosome, is then degraded and the organelle parts are recycled. This essential mechanism allows the body to maintain healthy cells. Autophagy is inhibited in early stage cancer, allowing tumor formation and growth. Autophagy occurs during periods of low to no energy input (e.g., fasting, low protein ingestion).

Sirtuin Signaling

Sirtuins (SIRTs) are a family of NAD+ dependent deacylases that prevent disease and may reverse some aspects of aging. Sirtuins delay cellular senescence and extend lifespan through age related telomere attrition and promoting DNA damage repair. Sirtuins regulate DNA repair, fat differentiation, glucose output, insulin sensitivity, fatty acid oxidation, inflammation and aging. There are different SIRTs in the cytoplasm, mitochondria, nucleus of each cell. There is an

association between loss of SIRTs in cancer cells and accumulation of mutations and genomic instability.

mTOR

The mammalian (or mechanistic) target of rapamycin (mTOR) are kinases that play important roles in autophagy, protein synthesis, mitochondrial biogenesis, cell growth & proliferation, cell survival & motility, and transcription. Decreased mTOR is associated with increased life span in mice and worms. A simplified description is that when mTOR is turned on mammals are in growth mode (e.g., cells divide, all energy goes towards growing) and when mTOR is turned off we are in preservation and clean up mode (e.g., autophagy). Amino acids, particularly methionine and leucine, are potent activators of mTOR and this "abundant energy" signal tells the body to grow. When mTOR is off (no energy or amino acids coming in) it tells the body to switch to quiescent mode. In quiescent mode the body switches to autophagy and the clean up begins.

Caloric restriction

Calorie restriction acts, in part, by inhibition of mTOR. When no protein is coming in, mTOR shuts off, and autophagy (clean up, recycle) begins. This allows removal of damaged organelles, cleaning up debris, and removal of damaged DNA. Calorie restriction (fasting) reverses chemotherapy induced DNA damage. Cycles of fasting delay progression of melanoma, glioma, breast cancer and improve effectiveness of chemotherapy.

In 2006 researchers published findings from a study in dogs. There were 48 Labrador Retriever puppies from 7 litters that were split into pairs (matched for sex and weight). All were fed the exact same diet but the calorie restricted group were fed 25% less than the control group starting at 8 weeks of age. The calorie restricted group lived 1.8 years longer, and, importantly had a significant delay in age related diseases such as osteoarthritis. Newer research into this study suggests that signals from the microbiome of the calorie restricted group may be involved in the longevity and health of the dogs.

https://pubmed.ncbi.nlm.nih.gov/18062831/

Studies in humans are a bit more difficult and the CALERIE study attempted to mimic the dog study. The humans, however only cut down their intake by 11.9% in the calorie restriction group. Despite this, after 2 years there was a decrease in cholesterol, systolic and diastolic blood pressure, insulin sensitivity, metabolic syndrome score and C-reactive protein.

Promising Therapies

There is so much new research on anti-aging. Some are not practical in pets (e.g., significant decrease in all cause mortality from sauna use) while others are (e.g., cold therapy –jumping into a cold lake or ocean has been shown to improve immune function. Here we will discuss some of the more promising ones.

Diet

First, caloric restriction has the most solid research behind it. Cutting a dog's calories by slowly decreasing the amount fed over time to ~20% less calories is likely to be very helpful over the course of the dog's life.

As we discussed in the microbiome talk, the gut is the workhorse of the body, and likely where health or disease begins. Inflammation that begins in the gut spreads to all areas of the body wreaking havoc, pain, and dysfunction. There is a delicate balance between health producing microbes and disease producing microbes that is easily disrupted leading to disease. All of these conditions are significantly worsened as dogs age and many of them can be addressed with simple and safe strategies.

Essentials of a healthy microbiome start with high quality fiber. The best sources of this good fiber are often lacking in many K9 diets. These include asparagus, Jerusalem artichoke, dandelion greens, banana, leeks and garlic and onions. These last two (garlic and onions) can cause problems in dogs if ingested in large amounts so it is best to limit these to small amounts

or skip them. Intestinal gas (flatulence) can occur if the fiber is increased too quickly. It is best to go slow and build up.

There are changes to the microbes in the gut as canines age. Depending on the state of health there can be loss of balance with a higher percentage of more harmful microbes that can induce inflammation. This inflammation is not limited to the gut but affects the entire body. That means the joints, the brain, the heart, these all feel the effects of inflammation. Fermented foods such as pickled veggies, miso, sauerkraut, and kombucha are very helpful in replacing some of the good microbes that may have been lost due to antibiotic administration, illness, or even from chlorinated water. Chlorinated water, which we all drink, is one reason to continue to supplement the diet with fermented food. Many dogs love fermented veggies and these make a wonderful snack.

The absorption of fats, fat soluble vitamins, and some B vitamins becomes harder and harder as dogs get older**.** Supplementation with select vitamins that are very difficult for the gut to absorb in older dogs (e.g., B12) should be considered. Additionally, small amounts of healthy fats (e.g., Medium chain triglyceride oil, coconut oil, grass fed pastured butter, omega 3 fatty acid supplements) may be helpful. Vitamin B12 is essential for gut health and, at the same time, are harder for the gut to absorb with age. Absorption of B12 is quite complicated as first it has to be present in the food, then get bound up to intrinsic factor (a carrier) in the stomach, then travel all the way down the intestinal tract and then get absorbed much lower down in the intestines by specialized receptors. As dog's age, all of the systems that help absorb B12 can become less efficient, making it harder and harder to absorb. Not having enough B12 can lead to decreased absorption of nutrients from food, low RBC counts (and low energy), difficulty fighting off infections (e.g., immune function compromise), mental decline (e.g., forgetting or not responding to commands, acting confused), decreased energy, balance problems (seem wobbly or unstable), difficulty sleeping at night, and many other issues. In order to get around the difficulty of absorbing this orally in older dogs many veterinarians prescribe injections of

B12. The Texas A&M GI Lab has published dosages for dogs on their website. The website can be found here. https://vetmed.tamu.edu/gilab/research/cobalamin-information/#dosing

Gastric Acid

The gastric acid and prostaglandins in the stomach decrease with age. The combination of these make digesting food take longer and will increase the chance of ulcers in the stomach if the dog is on any anti-inflammatory medications (e.g., NSAIDs, Rimadyl®, Ibuprofen, etc.). Alternative options for inflammation include turmeric, ginger, Epsom salt soaks, cold laser therapy, and acupuncture.

In older humans (and likely dogs and cats) the taste buds decrease. It is likely that this contributes to a diminished appetite. The combination of nasal changes (decrease in olfactory receptors, drying out of nasal mucous) and decreased taste buds likely contributes to appetite changes. A simple vaporizer may help improve both taste and smell functions a bit.

There is decreased movement of food through the GI Tract due to lower gastric acid, lower numbers of neurons and decreased blood flow to the gut. This slows the movement of the intestines so that it takes food longer to move through the GI tract. Allowing older dogs more time to digest (e.g., before vigorous exercise) may be helpful.

Constipation if common. Constipation should be considered a serious condition as it is related to cognitive decline in humans (and likely dogs). There is a clear association between constipation and onset of Parkinson's in humans. Interestingly, the only substance that is associated with absence of Parkinson's is coffee consumption, which has a beneficial effect on constipation. Decreased movement of the GI tract leads to constipation, which is quite common in older dogs. Two issues that can worsen constipation are dehydration and lack of fiber. Older dogs have less efficient kidneys (they cannot optimally dilute or concentrate urine) and are predisposed to dehydration. In addition, the thirst response is diminished in older dogs so they will often become dehydrated *before* they become thirsty and have a hard time catching up. As

dogs age, it is essential that they have access to water more frequently to prevent dehydration. Healthy fiber options include raw carrots, raw beets, sweet red peppers, pumpkin, squash, sugar snap peas, green beans, cauliflower, broccoli. Trying multiple fresh veggies is worth it as many dogs will happily chew on a sweet red pepper or a sugar snap pea but refuse other options.

Gastrointestinal Bleeding is Common. Anemia is very common in older humans and leads to lots of complications and poor outcomes after illness or injury. It is likely common in our geriatric patients as well. It is not completely clear why older humans are anemic but it is likely from loss of blood in the gut. Chronic inflammation of the gut from lack of high quality nutrition, significant lack of healthy fiber, and ingestion of toxins in the foods (e.g., pesticides) contribute to the chronic inflammation. Optimizing the diet with high quality fiber (e.g., fresh veggies), fermented foods, organic bone broth (source of collagen), and high quality fat in small quantities to start (e.g., coconut oil, grass fed butter, cod liver oil, etc.) can lead to gut healing.

Immune Function

There is decreased immune function with age. The largest part of the immune system is housed in the gut. As dogs age, there is a decrease in immune function that can lead to a greater number of infections. The immune system is also less responsive with age so it is slower to respond to an infectious challenge. Keeping the immune system in top shape can significantly increase the healthspan (healthy lifespan) in dogs. Keeping the gut healthy is essential in building healthy immunity. Gut healing options include anti-inflammatories (e.g., turmeric, ginger), supplementation of gut healing vitamins (e.g., B12), and replacement of healthy collagen to heal the gut lining (e.g., organic bone broth).

Energy Requirements

In dogs a 20% decrease in energy requirements was documented to occur after the age of 7 years. It is likely that in pet dogs the decreased activity (e.g., partially from arthritis) contributes to lack of exercise and this contributes to decreased food requirements. Aging is associated

with decreased muscle mass (sarcopenia). The loss in muscle leads to a host of problems including instability in the joints (progressing to arthritis), weakness, and lack of desire for exercise. The vicious cycle then leads to more muscle loss (from lack of activity). High quality protein is essential for muscle strength.

Melatonin

Melatonin, a hormone that is essential for sleep, is produced in the gut and the brain (80% is made in the gut). There is a documented decrease in melatonin with age in people and it is likely that at least some of this comes from a dysfunctional gut. Keeping the gut healthy may help keep melatonin levels normal but supplementing with melatonin (3-6 mg/dog given 30 minutes before bed) can help older dogs sleep. Since the detrimental solutes in the brain are cleared by the glymphatic system during the deep phase of sleep, melatonin may help prevent cognitive decline.

Promising Strategies to be discussed

Fasting Mimicking Diet

This diet has been shown to mimic a fast due to the very low protein nature of the diet. There is significant research on this diet in humans with cancer and the diet will be discussed in the context of preventing aging.

Curcumin

There are over 200 studies documenting the powerful anti-inflammatory effects of curcumin, the active ingredient in turmeric. It is best consumed with a little bit of fat and black pepper. We will discuss the options for curcumin in aging pets.

Ubiquinol (COQ10)

Ubiquinol, the reduced form of CoQ10, is essential during several points in the electron transport chain in the mitochondria. Ubiquinol decreases with age and there is a large body of research on supplementation for cardiac health.

Nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN)

These two precursors to NAD+ are very important for functioning of the cell, particularly the mitochondria. Research on supplementation will be discussed along with options for treatment.

Crocin

This is the active ingredient in Saffron, the deep yellow spice. Current research suggests it is anti-inflammatory, anti-cancer, anti-anxiety, and anti-diabetic. It has been shown to inhibit reactive oxygen species (e.g., free radicals) and advanced glycation end products (AGEs).

And more….

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