Systemic Illness: The Role of Oxidative Stress and Antioxidant Supplementation

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Abstract

In humans, antioxidant depletion and lipid peroxidation are correlated with disease severity and associated with poor outcomes. Antioxidant supplementation during illness may reduce mortality. Illness in dogs and cats is associated with oxidative stress, however, species differences exist. Dog and cat populations that may benefit from antioxidant supplementation include patients with acute or chronic hepatopathies, drug-associated toxicosis, heart failure, chronic kidney disease, chronic inflammatory disease, and diabetes mellitus. This review highlights the need for species and disease-specific clinical studies to further assess the role of oxidative stress during illness and the therapeutic role of antioxidant supplementation.

Glossary of Abbreviations

CCNU: Lomustine
CKD: Chronic Kidney Disease
DM: Diabetes Mellitus
FIV: Feline Immunodeficiency Virus
GPx: Glutathione Peroxidase
GSH: Glutathione
IMHA: Immune-Mediated Hemolytic Anemia
MDA: Malondialdehyde
NAC: N-acetylcysteine
ROS: Reactive Oxygen Species
SAMe: S-adenosylmethionine
SOD: Superoxide Dismutase
TAC: Total Antioxidant Capacity
TBARS: Thiobarbituric Acid Reactive Substance

Introduction

Reactive oxygen species (ROS) are a continuous byproduct of oxidative metabolism generated as part of both physiologic and pathologic cellular processes. These transient, highly reactive ROS oxidize cellular components including lipids, proteins, carbohydrates, and DNA. It is the endogenous antioxidant network that functions to modulate or quench ROS and maintain cellular homeostasis and redox balance.[1] When ROS are produced in excess of the capacity of the endogenous antioxidant network, increased cellular oxidative products are generated leading to a state of oxidative stress.[1,2]

The antioxidant network is made up of thiol antioxidants (i.e., glutathione [GSH], cysteine), scavenger enzymes (i.e., glutathione peroxidase, glutathione reductase, superoxide dismutase, catalase), lipids, water-soluble vitamins (i.e., A, C, E, beta-carotene), and trace minerals (i.e., selenium, zinc, iron, manganese, copper). The endogenous antioxidants function independently and synergistically to maintain cellular redox balance. GSH is the main intracellular antioxidant that is intimately linked to the thiol enzymes and other essential antioxidants, cysteine, vitamin E and selenium.

All components of the endogenous antioxidant network, as well as the cellular oxidation byproducts produced by ROS, are used as biomarkers of oxidative stress. Some of the more common indirect biomarkers of oxidative stress quantified and reported in the literature are GSH, vitamins E and C, selenium, and 8-isoprostane concentrations. Due to the diversity in study design, analytical methods used, and biomarker stability, there is no consensus as to which biomarker is the most specific or sensitive in the assessment of oxidative stress.[3] In most studies, multiple biomarkers are used to provide a more complete assessment of cellular redox status and its impact on the endogenous antioxidant network. However, antioxidant concentrations are often evaluated, as antioxidant supplementation may be a logical next step in the treatment of ill patients with disease-associated oxidative stress.

Oxidative stress plays a role in the pathogenesis of many acute and chronic systemic diseases in humans.[2-4] ROS induce cellular injury directly via oxidation and indirectly through cytokines and proinflammatory gene induction contributing to progressive systemic inflammation, tissue injury, cellular oxidative damage, and mitochondrial dysfunction.[5] In critically ill humans, antioxidant depletion (e.g., glutathione,[5,6] ascorbate[7,8]) correlates with severity of illness and survival, is associated with systemic decompensation, and increases susceptibility to infections.[6,9-11] GSH deficiencies have been reported in many systemic disease[9,10] states including diabetes mellitus,[12] trauma,[13] kidney failure,[14] retroviral infections,[15,16] sepsis,[17] liver disease,[18] and acute pancreatitis.[19] In critically ill patients, decreased GSH concentrations have been associated with decreased survival.[4,20] Urine or plasma 8-isoprostane concentrations, a stable marker of lipid peroxidation, correlate with disease severity in humans,[21,22] are

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significantly increased in animal models of oxidative injury,[22] and are modulated by antioxidants.[23]

Oxidative Stress: Dogs and Cats

The data reported in humans supports that oxidative stress contributes to the clinical outcome in some patient populations, including the acute and chronically ill as well as the critically ill. The available published studies and case reports in dogs and cats support the state of oxidative stress in association with various systemic diseases. However, extrapolations across species are difficult based on differences in diet, metabolism, and disease etiologies and pathophysiology. In comparing endogenous antioxidant concentrations in healthy dogs and cats,[24] healthy dogs have significantly higher antioxidant concentrations (i.e., GSH, cysteine and ascorbate), suggesting species differences in antioxidant homeostasis. These interesting but not well-understood differences in healthy dogs and cats exemplify the need for additional research in understanding the role of oxidative stress during illness and the therapeutic role antioxidant supplementation plays during illness.

Systemic Illness

Hospitalized ill dogs experience systemic oxidative stress including decreased endogenous antioxidants (erythrocyte GSH and serum vitamin E) and increased urinary isoprostane concentrations, a marker of lipid peroxidation.[24,25] Compared to healthy cats, systemically ill cats have increased ascorbate concentrations, suggesting a different endogenous antioxidant profile or response during illness compared to that of dogs or humans.[24]

Liver Disease

Similar to humans, both dogs and cats with spontaneous liver disease have decreased hepatic GSH levels.[26-28] Approximately 50% of dogs and cats with inflammatory liver disease have low liver GSH concentrations as well as dogs with copper toxicosis and extrahepatic cholestasis.[26,27] Studies in humans describe decreased liver GSH concentrations in naturally occurring liver disease and animal models of induced hepatopathies.[29-32] Multiple mechanisms likely contribute to the low GSH levels including limited GSH precursors, impaired hepatic synthesis of S-adenosylmethionine (SAMe), increased GSH hepatic efflux, increased GSH utilization, and reduced redox recycling of oxidized GSH.

Kidney Disease

In humans, chronic kidney disease (CKD) leads to a pro-oxidant environment and oxidative stress.[33] In dogs with CKD, vitamin concentrations (decreased 25-hydroxycholecalciferol and folate and increased ascorbate and vitamin A) are altered relative to healthy dogs.[34] Azotemic dogs have increased urinary markers of oxidative stress and increased urinary malondialdehyde (MDA)/creatinine ratio, which correlated with plasma creatinine concentration and urinary protein/creatinine ratio.[35] Cats with CKD, IRIS stage I-IV, do not have significant changes in erythrocyte glutathione peroxidase(GPx), serum selenium or plasma total antioxidant capacity (TAC) concentrations compared to healthy cats.[36] However, the IRIS stage IV cats have increases in plasma GPx compared to healthy cats, suggesting enzyme upregulation in cats with more advanced disease. Dogs and cats, like humans with CKD, have evidence of oxidative stress that may contribute to disease pathology, including renal interstitial fibrosis, glomerulosclerosis, glomerular hypertension, renal inflammation, and a decline in kidney function.[37]

Cardiovascular Disease

Dogs in congestive heart failure due to both chronic valvular disease and dilated cardiomyopathy have significant increases in plasma oxidized GSH, ascorbate and isoprostane levels; providing evidence of an oxidative state associated with congestive heart failure in dogs.[38] In addition, vitamin E concentrations negatively correlate with disease severity in dogs with idiopathic dilated cardiomyopathy.[39] These altered antioxidant levels are speculated to be a compensatory adaptation in dogs with cardiac dysfunction and suggests a population of dogs that may benefit from antioxidant supplementation. Studies designed to assess the effects of antioxidant supplementation in dogs with naturally occurring cardiac disease or with congestive heart failure are lacking.[40]

Neoplasia

The role of ROS in cancer is complex and multifactorial. Recent studies not only support the role of ROS in contributing DNA damage and tumorigenesis, but also the impact of ROS on tumor biology through their role in modifying cellular signaling to promote tumor growth and contributing to metastisis.[41] Studies in humans support increased circulating markers of oxidative stress in cancer patients.[42] For example, patients with breast, lung, or oral cancer have increased circulating markers of lipid peroxidation.[43,44]

Markers of lipid peroxidation, specifically serum MDA, are increased in association with many types of cancers in dogs relative to healthy dogs; consistent with a redox imbalance or oxidative stress in tumor-bearing dogs.[45] In this heterogeneous group of dogs diagnosed with cancer, the most common tumor types included mammary gland carcinoma, mast cell tumor and osteosarcoma. Others have reported evidence of oxidative stress in dogs with cancer including mammary gland tumors,[46] mast cell tumors[47] and lymphoma.[48] Dogs with mammary gland tumors have unchanged serum markers of lipid peroxidation but have increased thiobarbituric acid reactive substance (TBARS) and decreased vitamin E concentrations within the excised neoplastic tissue.[46] At the time of diagnosis, dogs with lymphoma have reduced vitamin E concentrations and increased levels of oxidative markers, including isoprostanes and glutathione peroxidase activity, which return to normal following chemotherapy and disease remission.[48]

In cancer patients, the benefits and risks of antioxidant supplementation remain controversial. The role of antioxidant therapy

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in cancer patients is complicated as traditional chemotherapy and radiation therapy are, in part, successful in killing tumor cells via the generation of ROS. This raises the question whether concurrent antioxidant therapy in cancer patients reduces the efficacy of treatment.[49-51]

Infectious/Inflammatory Diseases

In the few published studies available, both infectious and inflammatory diseases of dogs and cats are associated with oxidative stress. Dogs experimentally infected with *Ehrlichia canis* had increased serum concentrations of nitric oxide, TBARS and glutathione reductase activity, supporting a redox imbalance post-infection.[52] Acute gastroenteritis in dogs due to canine parvovirus is associated with increased markers of lipid peroxidation (i.e., erythrocyte MDA).[53] Relative to healthy dogs, dogs with immune-mediated hemolytic anemia (IMHA) are reported to experience oxidative stress (i.e., increased plasma MDA levels) and reduced antioxidant reserve (i.e., decreased vitamin E serum concentrations) during illness.[54]

An experimental study in cats infected with feline immunodeficiency virus (FIV) reported increased whole blood superoxide dismutase (SOD) and glutathione peroxidase concentrations over the first 9 to 12 weeks of an acute FIV infection with values returning to baseline 16 weeks postexposure.[55] In another study, a group of naturally exposed FIV-positive client-owned cats with no clinical signs of illness were compared to healthy cats. Despite no significant change in erythrocyte GSH or plasma cysteine concentrations, these chronically infected FIV-positive cats had increased plasma ascorbate concentrations.[24] These small studies independently support that FIV-positive cats experience oxidative stress during the acute as well as the chronic stage of infection. However, FIV-positive cats have a very different antioxidant pattern compared to human HIV-positive patients.[56]

Endocrinopathies

Human endocrinopathies are associated with oxidative stress. A few published studies support a shift toward an oxidative state in dogs and cats with diabetes mellitus (DM) and feline hyperthyroidism. Cats with DM experience a decrease in plasma SOD concentrations when compared to healthy control cats; consistent with an increase in oxidative stress in cats with DM.[57] Following a diet change to a high-protein/low-carbohydrate diet, despite an increase in glutathione peroxidase, there was no significant change in the plasma SOD concentration over the eight-week period of dietary intervention. Diabetic dogs, both well-controlled and poorly regulated dogs, have increased erythrocyte catalase activity, which relates to disease severity and supports that oxidative stress occurs in dogs in association with DM.[58]

Feline hyperthyroidism is an endocrine disorder of geriatric cats that is most commonly associated with a functional thyroid adenoma. Return of the euthyroid state is attained following radioiodine therapy. During the hyperthyroid state, cats experience lipid peroxidation identified by increased urinary isoprostane concentrations that normalize following radioiodine therapy.[59] Interestingly, endogenous antioxidants, erythrocyte GSH, plasma ascorbate, and plasma vitamin E are unchanged in hyperthyroid cats.

Toxicosis

Acetaminophen is a common analgesic and antipyretic used by owners that is sometimes inappropriately dosed or accidentally ingested by dogs and cats leading to toxicity. The mechanism for acetaminophen toxicosis in dogs and cats is complicated by differences in metabolism between species, but in both species decreased GSH concentrations contribute to toxicity.[60-62] The primary antidote for the treatment of acetaminophen toxicosis is supplementation with a GSH source. Clinically N-acetylcysteine (NAC) is most commonly used in dogs and cats.[63]

Antioxidant Supplementation

Increasing evidence supports the role of oxidative stress in the pathogenesis of many systemic diseases in humans,[2,11] as well as in dogs and cats,[24] making antioxidant supplementation a rational therapy to block the formation of ROS, scavenge ROS and augment endogenous antioxidants. Many clinical studies in humans have evaluated antioxidant supplementation during illness with mixed results, in part, due to the lack of standardization of methodologies, including the type, dose and route of the antioxidant intervention and the biomarkers evaluated.[64,65] By comparison, much less is known about oxidative stress and antioxidant supplementation in ill veterinary patients. Most of what is known is limited to experimental studies, case reports and small clinical studies.

Glutathione Supplementation

GSH supplementation is a logical approach to treating patients with liver disease. The results of human clinical studies assessing antioxidant supplementation in liver disease patients are difficult to compare as most trials consist of a low number of patients and have a high risk of bias and heterogeneity. However, some reported clinical trials and meta-analyses support the use of GSH precursors (SAMe, NAC and silibinin) to replenish hepatic GSH concentrations. Extrapolated from human studies, the use of antioxidant supplementation in dogs and cats with liver disease is common despite the paucity of species-specific controlled clinical trials or meta-analyses.

S-adenosylmethionine (SAMe): A methyl donor and also a precursor to GSH, SAMe is essential for cellular metabolic processes, detoxification and antioxidant pathways. Adequate concentrations of SAMe and GSH are normally produced in the liver, but in patients with liver disease low GSH concentrations contribute to hepatocyte damage and disease progression.[26]

Studies in both dogs and cats support that oral SAMe administration increases GSH concentrations. SAMe administration increased both hepatic and erythrocyte GSH concentrations in dogs treated with prednisone[66,67] and was successful in increasing GSH levels in a dog with acetaminophen toxicosis.[68] In healthy cats, SAMe administration increased hepatic GSH concentrations

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and decreased markers of systemic oxidative stress.[69] SAMe has been reported to be protective in limiting Heinz body formation and erythrocyte destruction, as well as increasing hepatic GSH concentrations, in cats with acetaminophen toxicosis.[70] In humans, supplementation with SAMe is recommended for the treatment of hepatotoxicity and cholestatic liver disease.[71] Clinically, SAMe supplementation in dogs and cats parallels that of humans.

Silibinin (milk thistle): A natural product of the flowering plant of the aster family, silibinin functions as an antioxidant, anti-inflammatory and antifibrotic. Silibinin is used as an adjunctive therapy in humans with chronic hepatitis, alcoholic or viral hepatitis, nonalcoholic steatohepatitis, and cirrhosis.[72] No randomized clinical trials have evaluated the use of silibinin in dogs and cats with spontaneous liver disease. Veterinary use of silibinin has primarily been extrapolated from its use in the treatment of hepatopathies in humans and based on a limited number of experimental studies in dogs and cats.

Experimental studies in dogs support the use of silibinin in the prevention and/or treatment of acute toxicities, including Amanita mushroom toxicosis and gentamicin-induced nephrotoxicity. Silibinin prevents the phalloidin toxin (the toxic principle in Amanita mushroom toxicosis) from binding hepatocytes, minimizing hepatotoxicity and Amanita mushroom toxicosis.[73] In another study, dogs treated with gentamicin for nine days were concurrently supplemented with silibinin or saline; the silibinin-supplemented dogs had a reduction in gentamicin-induced nephrotoxicity relative to the dogs treated with saline.[74]

In healthy cats, granulocyte GSH concentrations increase following silibinin administration, and silibinin is reported to be well-tolerated when administered orally with a bioavailability of 6-7%.[57] Cats experimentally exposed to acetaminophen and prophylactically administered silibinin were protected from the development of acetaminophen-induced toxicosis.[63]

Denamarin®: A combination product of silibinin and SAMe, Denamarin is often used empirically as a GSH source in dogs and cats with liver disease. No published prospective clinical studies are available to assess the efficacy of Denamarin in dogs and cats with spontaneous liver disease.

Recently, a heterogeneous group of dogs diagnosed with neoplasia that were treated with lomustine (CCNU) were randomized to treatment with CCNU alone or in combination with Denamarin. Only 68% of the Denamarin-treated dogs experienced increases in their liver enzymes associated with CCNU administration versus 85% of the dogs treated with CCNU alone.[75] The results of this study suggest a population of dogs that may benefit from prophylactic GSH supplementation to prevent iatrogenic hepatoxicity associated with CCNU administration.

N-acetylcysteine (NAC): An acetylated variant of L-cysteine, NAC is used as a thiol source to replenish intracellular cysteine and GSH levels. In humans, NAC is used in the treatment of acute hepatoxicity,[76] nonalcoholic steatohepatitis[77,78] and alcoholic hepatitis.[79] Studies evaluating the use of NAC in the treatment of dogs and cats have primarily focused on acetaminophen toxicosis. NAC remains the standard of care for the treatment and prevention of the oxidative damage associated with acetaminophen-induced hepatoxicity in dogs[62,80] and methemoglobinemia in cats.[62]

Recently, a randomized placebo-controlled clinical study supplemented GSH-deficient ill dogs with NAC for 48 hours. The NAC-supplemented dogs stabilized their erythrocyte GSH concentrations compared to the control dogs, which had a further decline in erythrocyte GSH. However, short-term NAC supplementation did not resolve the systemic oxidative state, improve serum vitamin E concentrations, or impact long-term outcome. Further studies are needed to investigate whether longer duration or combination antioxidant therapy would benefit ill dogs.

Vitamin E

Decreased vitamin E concentrations are associated with chronic liver disease of different etiologies in humans. The overall clinical benefits of vitamin E in the treatment of hepatobiliary disease in humans remains controversial.[81] However, a recent study supports the use of vitamin E as part of a combination protocol in the treatment of nonalcoholic steatohepatitis.[82] Decreased vitamin E concentrations and the use of vitamin E in the treatment of spontaneous liver disease in dogs and cats is not well-documented. Despite the paucity of data, vitamin E supplementation is often pursued empirically in dogs and cats with liver disease. The published studies or case reports evaluating vitamin E in dogs with liver disease are summarized below.

Experimental studies suggest that dogs with ischemia-induced liver disease may benefit from vitamin E supplementation. Anesthetized dogs with experimentally induced hypoxia experience a time-dependent depletion in plasma and liver vitamin E concentrations.[83] A single clinical study that used vitamin E in dogs with spontaneous liver disease has been published. In a small group of dogs with chronic inflammatory liver disease, dogs were randomized to treatment with or without vitamin E. The vitamin E-supplemented dogs had a significant increase in serum and liver vitamin E concentrations, increased GSH concentrations and decreased ALTs relative to the controls.[84]

Vitamin E supplementation may also have a role in treating or preventing acute drug toxicities (e.g., acetaminophen, tetracycline, gentamicin). Vitamin E combined with cysteine was used in an experimental study in cats to successfully reduce the oxidative state induced by acetaminophen.[85] In a single case report, vitamin E combined with selenium was used therapeutically in a cat with a marked increase in ALT suspected to be secondary to a tetracycline-induced hepatotoxicity.[86] In an experimental study, dogs treated with gentamicin for nine days were supplemented with vitamin E during therapy, and, similar to silibinin administration, the vitamin E-supplemented dogs had a reduction in gentamicin-induced nephrotoxicity relative to dogs treated with saline.[39]

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Dietary Antioxidants/Vitamins

The rationale for the use of vitamins/dietary antioxidants (e.g., vitamins A, C, E, beta-carotene, essential fatty acids, and polyphenols) is to provide a balanced dose of antioxidants that directly functions as free-radical scavengers to limit oxidative injury but also induces endogenous antioxidants and repair enzymes.[40,87,88] A recent meta-analysis of clinical studies evaluating antioxidant supplementation during illness in humans concluded that trace element and vitamin supplementation not only supports antioxidant function but also may reduce mortality.[89] As in humans, dogs and cats may benefit from dietary antioxidant/vitamin supplementation during illness. Patient populations that experience chronic oxidative stress, including dogs and cats with CKD,[90-92] dogs in heart failure,[40] dogs with chronic inflammatory disease (e.g., IMHA), diabetic dogs or cats, and FIV-positive cats, may benefit the most from dietary antioxidants.

Many antioxidant trials in human CKD patients have produced equivocal results.[93] However, in a few small randomized trials, antioxidant supplementation (i.e., vitamins A, C, E and beta-carotene) in predialysis CKD patients suggests a slower progression to end-stage disease based on an overall reduction in serum creatinine concentrations.[94] Interestingly, diets supplemented with vitamins A, C and beta-carotene reduced markers of oxidative stress in cats with chronic kidney disease.[91] In dogs, vitamin E in combination with omega-3 polyunsaturated fatty acids have been used to reduce glomerular hypertension, proteinuria and minimize proinflammatory mediators.[90,91] Based on limited studies, dogs and cats with CKD are one population of veterinary patients that would likely have a clinical benefit from dietary antioxidant/vitamin supplementation.

Summary

As in humans, an altered redox state is associated with illness in dogs and cats. In dogs, endogenous antioxidant concentrations during illness more closely resemble the alternations reported in humans. Unexpectedly endogenous antioxidant concentrations in healthy cats and the changes in endogenous antioxidants in cats during illness appear to be unique to that species, making cross-species comparisons and extrapolations difficult.

Antioxidant supplementation has not been extensively studied in veterinary medicine, making therapeutic recommendations difficult. Glutathione supplementation in dogs and cats with liver disease or acetaminophen toxicosis seems to have the most evidence to support its use. However, the duration of supplementation remains empirical. Other systemic diseases of dogs and cats that may benefit from antioxidant supplementation include chronic kidney disease, cardiovascular disease, chronic inflammatory disease, and diabetes mellitus. However, the use of antioxidant supplementation in patients with neoplasia remains controversial.

Our understanding of oxidative stress and antioxidant supplementation during health and disease is in its infancy. Antioxidant supplementation in ill dogs and cats remains to a large extent extrapolated from humans and based on a limited number of experimental studies, small clinical studies and case reports. The available evidence-based data in dogs and cats highlight the need for species and disease-specific clinical studies to further assess the role of oxidative stress during illness and the therapeutic role of antioxidant supplementation.

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