Timely Topics in Nutrition

Oxidative stress, antioxidants, and assessment of oxidative stress in dogs and cats

Maureen A. McMichael, DVM, DACVECC

Oxidative stress is defined as ROS in excess of antioxidant defense mechanisms. Oxidative stress can result from an excess of ROS, a reduction in antioxidants, or both. Damage attributable to oxidative stress is widespread, and oxidative stress is recognized as a prominent feature in numerous disease processes, including neoplasia and heart disease, as well as trauma and burns.[1-3] Reactive oxygen species are produced endogenously and exogenously, and elaborate antioxidant defense mechanisms have developed in aerobic organisms to limit the damage caused by ROS.

It is debatable whether ROS are causative or an epiphenomenon of a specific disease process. Determination of causation depends on elucidating the specific damage caused by ROS and measuring the effect of treatment on the degree of oxidative stress and the progression of disease. Assessment of oxidative stress includes measurements of endogenous antioxidants or ROS, evaluation of damage caused by ROS, or a combination of these. Because there are numerous ways to assess oxidative stress but no standardization for these measurements, it is difficult to evaluate the clinical pharmacologic effect of antioxidants.[4] In addition, much of the research on assessment of oxidative stress involves methods that are not directly applicable or are not practical in clinical situations. Newer diagnostic methods, such as measurement of urinary concentrations of isoprostanes, are sensitive, specific, noninvasive, and clinically applicable.[4]

Treatment for oxidative stress includes augmenting endogenous antioxidants, decreasing ROS generation, or scavenging existing ROS. Research on antioxidant supplementation has yielded conflicting results, mainly because of a lack of standardization in testing methods and for dosages and routes of administration.[5] The information provided here is intended to review the pathophysiologic processes of oxidative stress, physiologic defense mechanisms against antioxidants, and assessment of oxidative stress in dogs and cats.

ROS

Free radicals are molecules with 1 or more unpaired electrons in the outer shell. Because not all of the species that cause oxidative injury are free radicals (eg, hydrogen peroxide), a more appropriate term is ROS. The ROS are capable of reacting with all biological molecules, including nucleic acids, proteins, carbohydrates, and lipids.

Abbreviations

ROS	Reactive oxygen species
NADPH	Reduced form of nicotinamide adenine dinucleotide phosphate
PUFA	Polyunsaturated fatty acid
SOD	Superoxide dismutase

The major source for ROS formation in cells is electron leakage from electron transport chains. It is estimated that 90% to 95% of the oxygen passing through mitochondria is converted to water, and the remaining 5% to 10% is reduced, which creates ROS.[6] The generation of ROS is kept to a minimum by the high efficiency of electron transfer and sequestration of metal ions. Separate microenvironments exist for mitochondria, lysosomes, and peroxisomes; each contains an ROS-generating system coupled to immediately adjacent antioxidant defense mechanisms. It has been suggested[7] that this compartmentalization may be the most important endogenous defense mechanism against oxidative stress. Other endogenous sources of ROS are cytochrome P450 in the endoplasmic reticulum, peroxisomes, lipoxygenases, cyclooxygenases, xanthine oxidase, and NADPH oxidase.[8] In addition, during pathophysiologic states, inflammatory cells (neutrophils, macrophages, and eosinophils) contribute substantially to ROS production. Exogenous sources of ROS include UV light, drugs (especially chemotherapeutic drugs), cigarette smoke, and ozone.

Interestingly, an age-related increase in mitochondrial formation of ROS is the basis for the mitochondrial theory of aging.[6] Mitochondrial DNA is continually exposed to a steady stream of ROS that are generated as by-products during the transfer of electrons to molecular oxygen. The rate of mitochondrial production of ROS increases with age, and concentrations of mitochondrial antioxidants decrease with age. The result is oxidative damage to mitochondrial DNA, with the extent of the damage increasing exponentially with age. The damaged mitochondrial DNA leads to impaired electron transport and increased leakage of ROS from the electron transport chain, which creates a vicious cycle of destruction.[9]

Controversy exists regarding whether the effects of ROS on lipids or on DNA constitute the bulk of ROS-induced damage.[1] Reactive oxygen species can initiate

From the Department of Small Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843-4474. Dr. McMichael's present address is the Department of Veterinary Clinical Medicine, College of Veterinary Medicine, University of Illinois, Urbana, IL 61802.

lipid peroxidation by abstracting a proton from PUFAs in the cell membrane. This causes the formation of a new radical, which can then attack other PUFAs in the membrane and propagate a chain reaction of lipid peroxidation, which ends when the substrate (ie, lipids of cell membranes) is eliminated or the radical encounters a chain-breaking antioxidant (such as vitamin E).[10] Lipid peroxidation severely damages cell membranes, which causes alterations in enzyme systems and receptors, alterations in ionic channels, and increased permeability to calcium and other ions and may initiate inflammation and apoptosis.[4]

However, ROS-induced damage to proteins and DNA may have more far-reaching effects leading to mutations and eventually carcinogenesis.[1] In fact, ROS are now considered an important class of carcinogen and have been implicated in the initiation, promotion, and progression of cancer.[1] The association between chronic inflammation and cancer may be mediated via release of ROS from leukocytes during inflammation with subsequent DNA damage. Hepatitis B and C virus–associated hepatocellular carcinoma and *Helicobacter pylori*–associated gastric carcinoma are examples of chronic infectious processes that are believed to lead to cancer.[11]

Endogenous Defense Against Antioxidants

Antioxidants are substances that can delay or prevent oxidation of nucleic acids, proteins, lipids, or carbohydrates. In the scavenging process, antioxidants produce a more stable compound after they react with a radical. The basic concept of antioxidant scavenging of ROS involves the antioxidant donating a single electron to a free-radical species.[5]

During steady-state conditions, ROS play important regulatory roles in cellular function. The homeostatic balance of ROS is modulated by effective endogenous defense mechanisms against antioxidants (**Figure 1**).[12] In general, there are 3 categories of defense: antioxidant proteins, enzymatic antioxidants, and small molecule antioxidants. Antioxidant proteins, such as albumin, haptoglobin, ferritin, and ceruloplasmin, are abundant in plasma.[11] Enzymatic antioxidants include SOD, catalase, and glutathione peroxidase.[11] Enzymatic antioxidants are expressed in most mammalian cells and prevent the generation of ROS. Small molecule antioxidants are further characterized into water-soluble and lipid-soluble categories. Water-soluble antioxidants include ascorbic acid (vitamin C), uric acid, bilirubin, glutathione, zinc, and selenium. Lipid-soluble antioxidants include tocopherols (eg, α-tocopherol [vitamin E]), β-carotene, ubiquinol-10 (coenzyme Q10), and lycopene.[11] The lipid layer of cell membranes contains tocopherols and β-carotene, and these antioxidants can act to quench chain reactions of lipid peroxidation.[11]

Albumin appears to be the most important of the antioxidant proteins, mainly because of its abundance in plasma.[13] In 1 study,[14] experimental exposure of plasma to challenges with exogenous oxidants revealed the following total plasma antioxidant activity: albumin, 43%; uric acid, 33%; vitamin C, 9%; vitamin E, 3%; bilirubin, 2%; and other, 10%. Albumin acts as an antioxidant via a free thiol group that is readily oxidized by ROS; however, in humans, the proportion in the reduced form (ie, the form that can act as an antioxidant) varies and is altered by disease states.[15] Interestingly, most commercial sources of human albumin have a lower percentage of thiol groups in the reduced form, compared with the percentage of the reduced form in healthy adult males.[16]

Enzymatic antioxidants typically work synergistically and require cofactors. Superoxide is the major ROS produced during physiologic and pathophysiologic states and is scavenged by the enzyme SOD. Superoxide dismutase is found in the cytosol (requires copper and zinc) and mitochondria (requires manganese) and on extracellular surfaces (requires copper and zinc). Mitochondrial SOD is believed to play a major role in defense mechanisms against antioxidants.[6,9] The reaction of SOD and superoxide anion creates hydrogen peroxide, another ROS. Hydrogen peroxide is then detoxified by glutathione peroxidase or catalase.[13]

Catalase is a heme protein located in peroxisomes. Catalase uses iron located at its catalytic center to convert hydrogen peroxide created in the cytosol or peroxisomes to water and oxygen.[13]

Mitochondria do not appear to possess catalase activity, except for mitochondria located in cardiac tissues. In mitochondria, hydrogen peroxide is scavenged by glutathione peroxidase, which is a sulfur-containing tripeptide that reduces hydrogen peroxide to water by use of glutathione as a substrate.[6] Glutathione peroxidase differs from catalase in that it is protective against

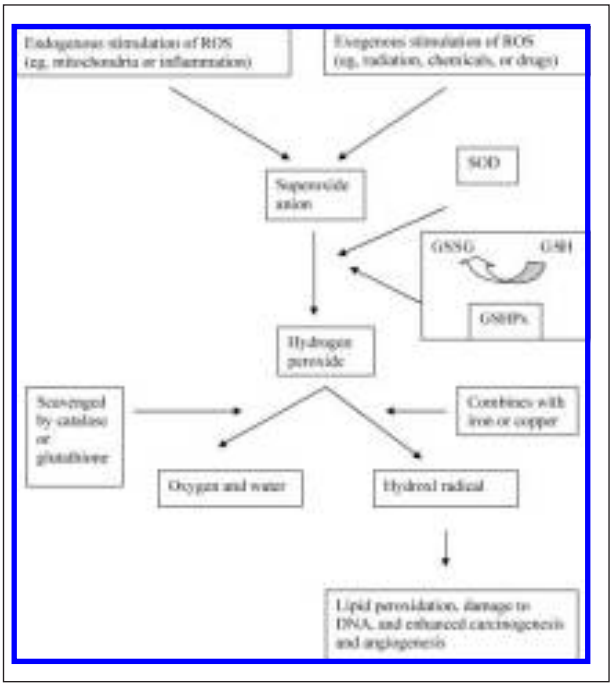

Figure 1—Schematic illustration depicting the creation of a superoxide anion, scavenging of the superoxide anion by SOD or glutathione to form hydrogen peroxide, and subsequent scavenging of hydrogen peroxide by catalase or glutathione to form oxygen and water. When hydrogen peroxide is not scavenged by catalase or glutathione, it can react with free metals to form hydroxyl radicals, which can initiate lipid peroxidation and damage to macromolecules. GSSG = Oxidized glutathione. GSH = Reduced glutathione. GSHPx = Glutathione peroxidase.

lipid peroxides (which protect cell membranes) and is effective at low concentrations of hydrogen peroxide.[13]

Much of the current research on small molecule antioxidants has focused on vitamin E, vitamin C, and glutathione. Vitamin E is located in the lipophilic interior of cell membranes, where the PUFAs are located, and is a chain-breaking scavenger that halts lipid peroxidation.[17] When a wave of lipid peroxidation reaches vitamin E, it is oxidized to form a free radical, which spares adjacent PUFAs from oxidation. Vitamin C then combines with that radical to form a poorly reactive, water-soluble, vitamin C radical, which results in regeneration of vitamin E. Vitamin C is the most abundant water-soluble antioxidant, and it can directly scavenge ROS or regenerate vitamin E.

Glutathione, a tripeptide that contains glutamate, cysteine, and glycine, is a cosubstrate for the enzyme glutathione peroxidase, which detoxifies hydrogen peroxide. Glutathione can also act as a metal chelator that prevents formation of hydroxyl radicals.[18] Glutathione is synthesized in all mammalian cells. The rate of synthesis is dependent on cysteine stores in most organs, except the liver. The liver, which is the primary site for glutathione synthesis, can synthesize glutathione from cysteine or methionine, and it supplies up to 90% of circulating glutathione.[18] Oxidative stress can be associated with a depletion of glutathione that leads to apoptosis of hepatocytes.[19]

Interestingly, glutathione can conjugate with carcinogens, which hastens their removal. This is believed to be an important component of endogenous detoxification.[12] Cellular depletion of glutathione has been implicated in the susceptibility of specific tissues (colon and rectum) to develop neoplasms.[12] Glutathione is believed to be crucial in the protection of the mitochondrial complexes in the respiratory tract, and hepatic amounts of glutathione are decreased in humans during critical illness.[13]

Assessment of Oxidative Stress

Traditional assessment of oxidative stress includes measurement of reaction products, measurement of ROS, or quantification of endogenous antioxidants. Newer testing, called oxidative stress profiling, encompasses assessment of several markers of oxidative stress as well as markers of inflammation and is believed to be a more thorough evaluation of oxidative stress.

Measurement of reaction products—Lipids are a common substrate that are attacked by ROS and are the most extensively studied compounds that can serve as markers of oxidation.[20] Breakdown products of lipid peroxidation include malondialdehyde, conjugated dienes, short-chain alkanes, and lipid hydroperoxides. Malondialdehyde can form when endoperoxides combine with unsaturated fatty acids and free iron. The test most commonly used to assess malondialdehyde concentrations is the thiobarbituric acid reactive substances test. There are numerous problems with this test. Up to 98% of malondialdehyde that reacts with thiobarbituric acid reactive substances is formed after collection (ie, during the incubation period of the assay).[21] Malondialdehyde can also form as a result of thromboxane synthesis, which is important when serum or plasma samples are used because there is likely some degree of platelet activation.[22]

Isoprostanes form when ROS attack arachidonic acid on cell membranes.[23] Isoprostanes are produced in vivo independently of cyclooxygenase enzymes by free radical–catalyzed peroxidation of arachidonic acid.[23] Measurement of the F_2-isoprostanes can provide reliable, noninvasive quantification of lipid peroxidation in vivo, compared with results for other methods.[23] In 1 study,[24] F_2-isoprostane concentrations increased dramatically in animals with experimentally induced oxidant stress, and the concentrations were correlated with the degree of tissue damage. Administration of antioxidants can inhibit the formation of F_2-isoprostanes in humans and other animals with experimentally induced oxidant injury.[25,26] Isoprostanes can be detected in all types of biological fluids and tissues.[21,23] In a study[27] conducted by our laboratory group, increased concentrations of F_2-isoprostanes were detected in the urine of dogs with intervertebral disk disease, compared with concentrations in the urine of healthy surgical control dogs. Measurement of isoprostanes may provide a practical, noninvasive approach to establish the connection between oxidative stress and various disease processes in veterinary medicine.

Measurement of ROS—Because of their reactivity and brief half-life, ROS are extremely difficult to measure in biological systems. Electron paramagnetic resonance spectroscopy is a technique that detects unpaired electrons and appears to be a direct method of detecting free radicals. The unpaired electron in free radicals gives rise to a typical absorbance spectrum when placed in a magnetic field. A special technique, called spin trapping, forces free radicals to react with a scavenger (or spin trap) to produce a more stable free radical that has a longer half-life and a spectrum that can then be identified by use of electron paramagnetic resonance spectroscopy.[28] In a study[29] on muscle flaps of dogs exposed to ischemic conditions for 4 hours, electron paramagnetic resonance signals characteristic of free-radical adducts were only detected in 5 of 9 dogs.[29]

Measurement of endogenous antioxidants—Measurement of specific antioxidants in blood or tissues of some species can be used as an indicator of oxidative stress when the amounts of antioxidants are low. Species variation is substantial, with SOD having the lowest activity in mice and the highest activity in humans.[30] It also appears that SOD activity is increased in proportion to the amount of ROS produced in many species.[31] Extrapolating concentrations of antioxidants among species may be inaccurate, and it is essential to have baseline data for each species.

Glutathione, the substrate used by the enzyme glutathione peroxidase, exists in 2 forms: reduced glutathione and the oxidized form, GSSG. During oxidative stress, reduced glutathione is oxidized to GSSG. Measurement of the ratio of the content of reduced glutathione to the GSSG content can be used to assess oxidative damage via depletion of reduced glutathione in dogs and cats.[32] This method is also susceptible to spontaneous oxidation ex vivo and artificially increased amounts

of GSSG. Depletion of reduced glutathione can cause irreversible cell damage and death.[33] In 1 study,[32] investigators reported decreased concentrations of reduced glutathione in liver biopsy specimens of dogs and cats with necroinflammatory disorders and extrahepatic bile duct occlusion and cats with hepatic lipidosis, compared with values in healthy dogs and cats.

In sled dogs, serum concentrations of α-tocopherol decrease significantly after an exercise run, which suggests that the endogenous antioxidant capacity may not be adequate for challenges of vigorous racing.[34] In another study[35] aimed at decreasing oxidative damage in racing sled dogs, dietary supplementation with α-tocopherol, β-carotene, and lutein significantly increased plasma concentrations of these antioxidants.

Because many substances (eg, albumin) function as antioxidants, measurement of a single antioxidant may be misleading. It has been suggested[31] that a much more extensive battery of tests is needed to evaluate oxidative stress and antioxidant status. Unfortunately, the cost of this extensive oxidative stress testing, which includes tests for 13 antioxidants, 22 trace elements, an iron profile, 9 markers of inflammation, and 11 markers of oxidative damage, is likely to be prohibitive to most researchers and clinicians.[31]

Treatments for Oxidative Stress

During the past 20 years, numerous studies have been conducted to examine > 1,000 types of interventions to ameliorate oxidative damage. Some of the current treatment strategies appear promising. Most of the treatment strategies used for oxidative stress involve blocking the formation of ROS, scavenging existing ROS, or augmenting endogenous antioxidants. Numerous other treatment modalities, which include endothelin receptor antagonists,[36] angiotensin receptor antagonists,[37] and hyperbaric oxygen,[38] have yielded mixed results. It is likely that the most effective strategies will encompass a combination of treatments that target several steps in the oxidative stress injury cascade.

Blocking formation of ROS—Exogenously administered glutathione cannot penetrate cell membranes.[39] Cysteine is the rate-limiting amino acid in the formation of glutathione, and treatment with N-acetylcysteine enables continued production of glutathione. N-Acetylcysteine is also a powerful scavenger of hydroxyl radicals and hypochlorous acid.[40] Treatment with N-acetylcysteine protects against endotoxin challenge,[41] radiation-induced injury,[42] and pulmonary injury from toxic gases.[43] In a study[44] of rats with experimentally induced ischemia-reperfusion injury, N-acetylcysteine blocked nuclear factor kappa B activity and scavenged ROS. N-Acetylcysteine can attenuate ischemia-reperfusion injury during cardiac catheterization and has cardioprotective effects during ischemia.[45] Finally, N-acetylcysteine has provided some benefit in humans with sepsis,[46] acute respiratory distress syndrome,[47] and contrast-induced nephropathy.[48]

S-Adenosylmethionine is synthesized from L-methionine and ATP and plays a major role in 3 key pathways (ie, transmethylation, amino propylation, and transsulfuration). Cysteine is generated via a series of enzymatic steps during SAMe transsulfuration. Cysteine synthesis augments glutathione synthesis. Because many of the acute-phase proteins require cysteine, SAMe and N-acetylcysteine may be useful for treatment of animals with inflammatory diseases as well as those with oxidative stress.[13]

Vitamin E, composed of 4 tocopherols and 4 tocotrienols, is the vitamin most commonly supplemented in most clinical trials. Negative effects of vitamin E supplementation on the progression of cardiovascular disease were reported in 1 clinical trial.[49] Unfortunately, investigators in that clinical trial used a synthetic form of vitamin E (dl-α-tocopheryl) that is devoid of antioxidant properties.

Vitamin C can act as a pro-oxidant during times of increased amounts of free iron, such as those resulting from a blood transfusion or inflammation. Vitamin C reduces ferric iron to ferrous iron, which, in physiologic conditions, improves absorption of iron from the gastrointestinal tract. When conditions of ischemia or increased availability of free iron exist, vitamin C can provide more ferrous iron for the generation of hydroxyl radicals via the Haber-Weiss reaction.[50] In patients with coronary artery disease, endothelial dysfunction is attenuated by administration of vitamin C; this effect appears to be attributable to scavenging of superoxide by vitamin C.[51]

Ubiquinone (also known as coenzyme Q10) enhances mitochondrial production of ATP, acts as an antioxidant, and stabilizes cell membranes.[52] Myocardial function is improved, and a meta-analysis[53] revealed a significant improvement in ejection fraction and cardiac output with ubiquinone treatment in patients with cardiac disease.

Allopurinol is a structural analogue of hypoxanthine that competitively inhibits xanthine oxidase, which prevents the formation of superoxide. Treatment with allopurinol attenuates microvascular permeability and decreases neutrophil infiltration in cats with experimentally induced ischemia-reperfusion injury.[54] Allopurinol improves kidney function after transplantation,[55] and it is now a component of the preservation solutions used in most transplant centers.

Scavenging ROS—It is believed that administration of SOD will be protective during ischemia-reperfusion injury. In situations in which use of SOD did not result in improvement,[56] its short half-life may have been a factor. Use of SOD in renal transplant patients can decrease acute rejection and improve graft survival at 4 years after transplantation.[57]

Catalase works in conjunction with SOD. Because of the iron content (iron is a cofactor for catalase), catalase may have the propensity to act as a pro-oxidant. Treatment of cats prior to ischemia-reperfusion injury of the small intestine decreases neutrophil infiltration.[58] Administration of a combination of SOD and catalase conjugate can be effective in attenuating oxidative stress in animals with experimentally induced ischemia-reperfusion injury.[59]

Free iron is central to the formation of hydroxyl radicals. Thus, many treatment strategies attempt to block iron. However, iron is essential to many biological processes, and iron chelation treatment can have poten-

tially toxic effects when it interferes with normal iron metabolism. Most strong chelating agents remove ferric iron from proteins (ie, transferrin) and can interfere with iron incorporation into hemoglobin.[60] Deferoxamine chelates ferrous iron and can reduce ROS injury in animals with experimentally induced conditions.[61,62] Use of deferoxamine may be unrewarding, most likely because of the toxic effects and its short half-life in circulation (approx 5 minutes) in humans.[63]

Dimethyl sulfoxide scavenges hydroxyl radicals. The metabolite that forms then traps other ROS. Dimethyl sulfoxide permeates cell membranes to reach intracellular sites of ROS formation. It is also believed that dimethyl sulfoxide inhibits platelet aggregation and increases vasodilation. Treatment with dimethyl sulfoxide prior to ischemia-reperfusion injury decreases microvascular permeability and neutrophil infiltration in cats[64] and rats.[65] It is believed that the concentrations of dimethyl sulfoxide needed to scavenge hydroxyl radicals may be so high that it may cause damage to healthy cells.[66]

Food-based antioxidants and ROS scavengers—Flavonoids are a subclass of polyphenols that have important antioxidant, vasodilatory, blood pressure–regulating, anti-inflammatory, and anticoagulating properties.[67,68] They are found primarily in fruits, vegetables, minimally processed tea (eg, green tea), and cocoa. Flavonoids scavenge ROS through rapid donation of electrons.[67] Some flavonoids also possess substantial abilities to suppress cyclooxygenase-2. One study[69] revealed significant protection of endothelial cells via ROS scavenging and increased expression of nitric oxide. Flavonoids in cocoa lower blood pressure via inhibition of angiotensin-converting enzyme and inhibit platelet aggregation via increased production of nitric oxide or prostacyclin.[67] There is a large increase in plasma total antioxidant capacity after consumption of relatively small amounts of flavonoids.[68] One possible theory for this effect is that flavonoids induce a major increase in uric acid, and uric acid is itself a powerful antioxidant. According to this theory, it is the uric acid that provides the antioxidant protection.

Resveratrol is a polyphenolic phytoalexin produced in plants, presumably as an antifungal compound.[70] Although found in grapes, berries, and peanuts, most commercially processed resveratrol is obtained from Japanese knotwood.[70] Resveratrol possesses antioxidant properties and can extend the life span of yeast, worms, and fruit flies and improve survival of obese mice.[70]

Decreased concentrations of antioxidants and increased amounts of ROS are believed to contribute to oxidative damage to lipids and proteins in the brains of older dogs, and these changes apparently increase with age of the dogs.[71] Dietary supplementation with antioxidants, whole fruits, and vegetables is associated with less cognitive impairment in dogs, compared with the cognitive impairment in dogs fed a conventional diet.[71]

Nutritional additives that involve antioxidants in foods formulated for dogs have been used successfully in laboratory settings and clinical treatment of older dogs.[71] There will continue to be a high degree of interest in antioxidants because of the significant effects they can have on signal transduction and modulation of enzyme and genomic systems.[67,72] A few of the more common antioxidants and their dosages in dogs and cats have been determined (**Table 1**).[73-77]

Conclusion and Clinical Summary

Oxidative stress is an important contributor to morbidity for many diseases. Multiple factors influence the interplay between oxidative stress and disease progression. It is a challenge to predict the appropriate type and dosage of antioxidant needed to ameliorate the complex phenomenon of oxidative stress. Standardization of the assessment of damage attributable to oxidative stress and response to treatment is an essential first step for evaluating the success of clinical trials. Isoprostane, a product of ROS action on arachidonic acids, has been a good indicator of oxidative stress in several laboratory studies and clinical trials and offers the benefits of cost effectiveness and noninvasive testing.

Table 1—Recommended dosages for antioxidants in dogs and cats.

Antioxidant	Dogs	Cats
SAMe[73]	20 mg/kg/d [9.1 mg/lb/d] as 1 dose/d or divided into 2 doses/d; administer orally 0.5 hours before a meal	20 mg/kg/d as 1 dose/d or divided into 2 doses/d; administer orally 0.5 hours before a meal
N-acetylcysteine[74]*	50 mg/kg [22.7 mg/lb]; dilute 1:4 with 0.9% NaCl solution and administer IV over a 1-hour period; can administer 1 dose every 6 hours	50 mg/kg; dilute 1:4 with 0.9% NaCl solution and administer IV over a 1-hour period; can administer 1 dose every 6 hours
Vitamin E[75]†	400 units, PO, q 24 h	30 units, PO, q 24 h
Vitamin C (ascorbic acid)[76]‡	500 to 1,000 mg, PO, q 24 h; decrease dosage if patient develops soft feces	125 mg, PO, q 12 h; decrease dosage if patient develops soft feces
Ubiquinone (coenzyme Q10)[77]	2.0 mg/kg [0.9 mg/lb], PO, q 24 h‡	2.0 mg/kg, PO, q 24 h‡

*Use the form approved for IV administration (sterile preparation) or administer through a filter needle. †Mixed tocopherols and tocotrienols; only purchase d-form (the dl-form is synthetic). ‡Extrapolated from dosages recommended for humans.

Because of the enormous complexity of oxidative stress, treatment that encompasses only 1 target is unlikely to yield substantial results. Currently, no single agent has been able to completely ameliorate oxidative stress. The best strategies will, in all likelihood, include a combination of treatments that target several steps in the oxidative stress pathway. Treatments that are promising in small animals include allopurinol administered to cats before oxidative stress and deferoxamine and dimethyl sulfoxide administered to dogs with experimentally created gastric dilatation-volvulus. Nutritional intervention is likely to be a popular method for delivery of antioxidants to pets in the future and offers the benefits of whole-food supplements that contain several synergistic antioxidants.

Because oxidative stress is not detectable clinically, it can be frustrating to monitor treatment. Obvious improvement in commonly measured variables is rarely evident after treatment with antioxidants. Objective improvement by use of standardized tests in several studies indicates the potential for the role of antioxidants in veterinary medicine. In addition, the lack of toxic effects reported with many of these supplements provides clinicians a greater sense of confidence when prescribing their use. A number of antioxidants (eg, SAMe and coenzyme Q10) have already appeared on the veterinary market. In addition, there are several additives for pet foods. It is likely that large-scale clinical trials will be performed to evaluate the potential beneficial effects of various antioxidants.

References

1. Karihtala P, Soini Y. Reactive oxygen species and antioxidant mechanisms in human tissues and their relation to malignancies. *APMIS* 2007;115:81–103.
2. Korantzopoulos P, Kolettis TM, Galaris D, et al. The role of oxidative stress in the pathogenesis and perpetuation of atrial fibrillation. *Int J Cardiol* 2007;115:135–143.
3. Berger MM. Antioxidant micronutrients in major trauma and burns: evidence and practice. *Nutr Clin Pract* 2006;21:438–449.
4. Montuschi P, Barnes P, Roberts LJ. Insights into oxidative stress: the isoprostanes. *Curr Med Chem* 2007;14:703–717.
5. Hermans N, Cos P, Maes L, et al. Challenges and pitfalls in antioxidant research. *Curr Med Chem* 2007;14:417–430.
6. Bulteau AL, Szweda LI, Friguet B. Mitochondrial protein oxidation and degradation in response to oxidative stress and aging. *Exp Gerontol* 2006;41:653–657.
7. Rangan U, Bulkley GB. Prospects for treatment of free radical-mediated tissue injury. *Br Med Bull* 1993;49:700–718.
8. Curtin JF, Donovan M, Cotter TG. Regulation and measurement of oxidative stress in apoptosis. *J Immunol Methods* 2002; 265:49–72.
9. Wei YH. Oxidative stress and mitochondrial DNA mutations in human aging. *Proc Soc Exp Biol Med* 1998;217:53–63.
10. Radi R, Beckman JS, Bush KM, et al. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* 1991;288:481–487.
11. Halliwell B. Antioxidant defence mechanisms: from the beginning to the end (of the beginning). *Free Radic Res* 1999;31:261–272.
12. Opara EC. Oxidative stress. *Dis Mon* 2006;52:183–198.
13. Eaton S. The biochemical basis of antioxidant therapy in critical illness. *Proc Nutr Soc* 2006;65:242–249.
14. Miller NJ, Rice-Evans C, Davies MJ, et al. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clin Sci (Lond)* 1993;84:407–412.
15. Soejima A, Matsuzawa N, Hayashi T, et al. Alteration of redox state of human serum albumin before and after hemodialysis. *Blood Purif* 2004;22:525–529.
16. Bar-Or D, Bar-Or R, Rael LT, et al. Heterogeneity and oxidation status of commercial human albumin preparations in clinical use. *Crit Care Med* 2005;33:1638–1641.
17. Meydani M. Vitamin E. *Lancet* 1995;345:170–175.
18. Sciuto AM. Antioxidant properties of glutathione and its role in tissue protection. In: Baskin S, Salem H, eds. *Oxidants, antioxidants, and free radicals*. Washington, DC: Taylor & Francis, 1997;171–191.
19. Kurose I, Higuchi H, Miura S, et al. Oxidative stress-mediated apoptosis of hepatocytes exposed to acute ethanol intoxication. *Hepatology* 1997;25:368–378.
20. Meagher EA, FitzGerald GA. Indices of lipid peroxidation in vivo: strengths and limitations. *Free Radic Biol Med* 2000;28:1745–1750.
21. Moore K, Roberts LJ. Measurement of lipid peroxidation. *Free Radic Res* 1998;28:659–671.
22. McMillan RM, MacIntyre DE, Booth A, et al. Malonaldehyde formation in intact platelets is catalysed by thromboxane synthase. *Biochem J* 1978;176:595–598.
23. Morrow JD, Roberts LJ. The isoprostanes: unique bioactive products of lipid peroxidation. *Prog Lipid Res* 1997;36:1–21.
24. Morrow JD, Roberts LJ. The isoprostanes: their role as an index of oxidant stress status in human pulmonary disease. *Am J Respir Crit Care Med* 2002;166:S25–S30.
25. Roberts LJ, Morrow JD. The generation and actions of isoprostanes. *Biochim Biophys Acta* 1997;1345:121–135.
26. Reilly M, Delanty N, Lawson JA, et al. Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation* 1996;94:19–25.
27. McMichael MA, Ruaux CG, Baltzer WI, et al. Concentrations of $15F_{2t}$ isoprostane in urine of dogs with intervertebral disk disease. *Am J Vet Res* 2006;67:1226–1231.
28. Brisson BA, Miller CW, Chen G, et al. Effects of adenosine pretreatment on detection of free radicals in ischemic and reperfused canine gracilis muscle flaps by use of spin-trapping electron paramagnetic resonance spectroscopy. *Am J Vet Res* 2002;63:175–180.
29. Brisson BA, Miller CW, Chen G, et al. Detection of free radicals in ischemic and reperfused canine gracilis muscle flaps by use of spin-trapping electron paramagnetic resonance spectroscopy. *Am J Vet Res* 2001;62:384–388.
30. Cutler RG. Oxidative stress profiling: part I. Its potential importance in the optimization of human health. *Ann N Y Acad Sci* 2005;1055:93–135.
31. Cutler RG, Plummer J, Chowdhury K, et al. Oxidative stress profiling: part II. Theory, technology, and practice. *Ann N Y Acad Sci* 2005;1055:136–158.
32. Center SA, Warner KL, Erb HN. Liver glutathione concentrations in dogs and cats with naturally occurring liver disease. *Am J Vet Res* 2002;63:1187–1197.
33. Reiter R, Wendel A. Chemically-induced glutathione depletion and lipid peroxidation. *Chem Biol Interact* 1982;40:365–374.
34. Hinchcliff KW, Reinhart GA, DiSilvestro R, et al. Oxidant stress in sled dogs subjected to repetitive endurance exercise. *Am J Vet Res* 2000;61:512–517.
35. Baskin CR, Hinchcliff KW, DiSilvestro RA, et al. Effects of dietary antioxidant supplementation on oxidative damage and resistance to oxidative damage during prolonged exercise in sled dogs. *Am J Vet Res* 2000;61:886–891.
36. Szabo G, Bahrle S, Fazekas L, et al. Endothelin-A receptor antagonist BQ123 protects against myocardial and endothelial reperfusion injury. *Thorac Cardiovasc Surg* 1998;46:232–236.
37. Harada K, Sugaya T, Murakami K, et al. Angiotensin II type 1A receptor knockout mice display less left ventricular remodeling and improved survival after myocardial infarction. *Circulation* 1999;100:2093–2099.
38. Buras JA, Stahl GL, Svoboda KK, et al. Hyperbaric oxygen downregulates ICAM-1 expression induced by hypoxia and hypoglycemia: the role of NOS. *Am J Physiol Cell Physiol* 2000; 278:C292–C302.

39. Marino P. The threat of oxidant injury. In: Marino P, ed. *The ICU book*. Baltimore: The Williams & Wilkins Co, 1998;32–50.
40. Scott BC, Aruoma OI, Evans PJ, et al. Lipoic and dihydrolipoic acids as antioxidants. A critical evaluation. *Free Radic Res* 1994;20:119–133.
41. Bernard GR, Lucht WD, Niedermeyer ME, et al. Effect of N-acetylcysteine on the pulmonary response to endotoxin in the awake sheep and upon in vitro granulocyte function. *J Clin Invest* 1984;73:1772–1784.
42. Revesz L, Malaise E. Significance of cellular glutathione in radioprotection and repair of radiation damage. In: Larsson A, ed. *Function of glutathione: biochemical, physiological, toxicological, and clinical aspects*. New York: Raven Press, 1983;163–173.
43. Sciuto AM, Strickland PT, Kennedy TP, et al. Protective effects of N-acetylcysteine treatment after phosgene exposure in rabbits. *Am J Respir Crit Care Med* 1995;151:768–772.
44. Carroll JE, Howard EF, Hess DC, et al. Nuclear factor-kappa B activation during cerebral reperfusion: effect of attenuation with N-acetylcysteine treatment. *Brain Res Mol Brain Res* 1998;56:186–191.
45. Ferrari R, Ceconi C, Curello S, et al. Oxygen free radicals and myocardial damage: protective role of thiol-containing agents. *Am J Med* 1991;91:95S–105S.
46. Henderson A, Hayes P. Acetylcysteine as a cytoprotective antioxidant in patients with severe sepsis: potential new use for an old drug. *Ann Pharmacother* 1994;28:1086–1088.
47. Suter PM, Domenighetti G, Schaller MD, et al. N-acetylcysteine enhances recovery from acute lung injury in man. A randomized, double-blind, placebo-controlled clinical study. *Chest* 1994;105:190–194.
48. Marenzi G, Assanelli E, Marana I, et al. N-acetylcysteine and contrast-induced nephropathy in primary angioplasty. *N Engl J Med* 2006;354:2773–2782.
49. Lonn E, Bosch J, Yusuf S, et al. Effects of long-term vitamin E supplementation on cardiovascular events and cancer: a randomized controlled trial. *JAMA* 2005;293:1338–1347.
50. Halliwell B, Gutteridge JM. Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol* 1990;186:1–85.
51. Heitzer T, Just H, Munzel T. Antioxidant vitamin C improves endothelial dysfunction in chronic smokers. *Circulation* 1996;94:6–9.
52. Sander S, Coleman CI, Patel AA, et al. The impact of coenzyme Q10 on systolic function in patients with chronic heart failure. *J Card Fail* 2006;12:464–472.
53. Soja AM, Mortensen SA. Treatment of congestive heart failure with coenzyme Q10 illuminated by meta-analyses of clinical trials. *Mol Aspects Med* 1997;18(suppl):S159–S168.
54. Granger DN. Ischemia-reperfusion: mechanisms of microvascular dysfunction and the influence of risk factors for cardiovascular disease. *Microcirculation* 1999;6:167–178.
55. Toledo-Pereyra LH, Simmons RL, Najarian JS. Effect of allopurinol on the preservation of ischemic kidneys perfused with plasma or plasma substitutes. *Ann Surg* 1974;180:780–782.
56. O'Farrell D, Chen LE, Seaber AV, et al. Efficacy of recombinant human manganese superoxide dismutase compared to allopurinol in protection of ischemic skeletal muscle against "no-reflow". *J Reconstr Microsurg* 1995;11:207–214.
57. Negita M, Ishii T, Kunikata S, et al. Prevention of posttransplant acute tubular necrosis in kidney graft by perioperative superoxide dismutase infusion. *Transplant Proc* 1994;26:2123–2124.
58. Zimmerman BJ, Grisham MB, Granger DN. Role of oxidants in ischemia/reperfusion-induced granulocyte infiltration. *Am J Physiol* 1990;258:G185–G190.
59. Jolly SR, Kane WJ, Bailie MB, et al. Canine myocardial reperfusion injury. Its reduction by the combined administration of superoxide dismutase and catalase. *Circ Res* 1984;54:277–285.
60. Galey JB. Recent advances in the design of iron chelators against oxidative damage. *Mini Rev Med Chem* 2001;1:233–242.
61. Menasche P, Antebi H, Alcindor LG, et al. Iron chelation by deferoxamine inhibits lipid peroxidation during cardiopulmonary bypass in humans. *Circulation* 1990;82:IV390–IV396.
62. Haraldsson G, Sorensen V, Nilsson U, et al. Effect of pre-treatment with desferrioxamine and mannitol on radical production and kidney function after ischaemia-reperfusion. A study on rabbit kidneys. *Acta Physiol Scand* 1995;154:461–468.
63. deLemos RA, Roberts RJ, Coalson JJ, et al. Toxic effects associated with the administration of deferoxamine in the premature baboon with hyaline membrane disease. *Am J Dis Child* 1990;144:915–919.
64. Zimmerman BJ, Guillory DJ, Grisham MB, et al. Role of leukotriene B4 in granulocyte infiltration into the postischemic feline intestine. *Gastroenterology* 1990;99:1358–1363.
65. Sekizuka E, Benoit JN, Grisham MB, et al. Dimethylsulfoxide prevents chemoattractant-induced leukocyte adherence. *Am J Physiol* 1989;256:H594–H597.
66. Schiller HJ, Reilly PM, Bulkley GB. Tissue perfusion in critical illnesses. Antioxidant therapy. *Crit Care Med* 1993;21:S92–S102.
67. Engler MB, Engler MM. The emerging role of flavonoid-rich cocoa and chocolate in cardiovascular health and disease. *Nutr Rev* 2006;64:109–118.
68. Lotito SB, Frei B. Consumption of flavonoid-rich foods and increased plasma antioxidant capacity in humans: cause, consequence, or epiphenomenon? *Free Radic Biol Med* 2006;41:1727–1746.
69. Steffen Y, Schewe T, Sies H. Epicatechin protects endothelial cells against oxidized LDL and maintains NO synthase. *Biochem Biophys Res Commun* 2005;331:1277–1283.
70. Baur JA, Pearson KJ, Price NL, et al. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 2006;444:337–342.
71. Roudebush P, Zicker SC, Cotman CW, et al. Nutritional management of brain aging in dogs. *J Am Vet Med Assoc* 2005;227:722–728.
72. Mutoh M, Takahashi M, Fukuda K, et al. Suppression by flavonoids of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells: structure-activity relationship. *Jpn J Cancer Res* 2000;91:686–691.
73. Denosyl SD4 [package insert]. Edgewood, Md: Nutramax Laboratories Inc, 2006.
74. Cakir O, Kamalettin E, Oruc A, et al. Neuroprotective effect of N-acetylcysteine and hypothermia on spinal cord ischemia-reperfusion injury. *Cardiovasc Surg* 2003;11:375–379.
75. Williams D. Exocrine pancreatic disease. In: Ettinger S, Feldman E, eds. *Textbook of veterinary internal medicine*. 6th ed. Philadelphia: WB Saunders Co, 2005;135–167.
76. Plumb D. Ascorbic acid/Vitamin C. In: Plumb D, ed. *Veterinary drug handbook*. 4th ed. Ames, Iowa: Iowa State University Press, 2002;69.
77. Langsjoen PH, Langsjoen A, Willis R, et al. Treatment of hypertrophic cardiomyopathy with coenzyme Q10. *Molec Aspects Med* 1997;suppl 18:S145–S151.