Oxidative Stress and Antioxidant Defence System: An Over view for practising Veterinarians

Philip W. Toll, Dennis E. Jewell, Bruce J. Novotny

Philip W. Toll, DVM, MS
Dennis E. Jewell PhD, Diplomate Acan
Hill's Science and Technology Centre Topeka, KS
Bruce J. Novotny, DVM
Helios Communications, LLC Shawnee, KS

Introduction

Oxygen usage represents one of the great biologic paradoxes. On one hand, aerobic organisms require oxygen to survive; on the other, oxygen is extremely toxic. For example, a small percentage of inspired oxygen is converted into reactive intermediates (also called free radicals, oxygen-derived free radicals, oxid ants, prooxidants, or reactive oxygen species [ROS]) as a part of normal metabolism. These ROS may interact with DNA, lipids, and proteins eventually causing mutation, neoplastic transfo rmation, loss of cellular function, and cell death.

Oxidants

Oxidant or ROS applies to any atom or molecule that contains one or more unpaired electrons. Most ROS are unstable and highly reactive. When a biomo lecule (lipid, nucleic acid, protein) reacts with a free radical, a new radical is created. The chain reaction may continue unabated, or the chain may be broken by an antioxidant.

Mitochondria use most of the oxygen an organism inspires for energy production (ATP). However, up to 5% of the oxygen used by mitochondria during normal energy metabolism is converted to powerful ROS–superoxide, hydrogen peroxide, and hydroxy 1 radical–the same ROS produced by radiation.

Peroxidation (autoxidation) of lipids exposed to oxygen is responsible) not only for deterioration of foods (rancidity) but also for damage to tissues in vivo, where it may be a cause of cancer, inflammatory disease, atherosclerosis, and aging. Lipid peroxidation is an extremely damaging chain reaction that markedly alters the structure and function of biologic membrances and generates highly toxic byproducts, including malondialdehyde (MDA) and 4-hydroxyalkenals and provides a continuous supply of free radicals that initiate further peroxidation. Methods now exist that allow investigators to measure free radical damage in the body by assaying byproducts of free radical damage to proteins, lipids and DNA.

Antioxidants

An antioxidant is a substance that has the ability to scavenge ROS and reduce the overall number of oxidants in a system, thereby inhibiting oxidation. Complex antioxidant defenses limit the effects of oxidant defenses limit the effects of oxidant molecules on tissues. The antioxidant defense system includes enzymes (superoxide dismutase, catalase, reductase, and glutathione peroxidase), free radical scavengers (vitamins A [carotenoids], C [ascorbic acid], and E [tocopherols]), and metal chelators (proteins). Antioxidants protect biomolecules by:

* Scavenging ROS
* Minimizing formation of ROS
* Binding metal ions that may be used to make poorly reactive species more dangerous
* Repairing damage to target tissues
* Destroying badly damaged target molecules and

replacing them with new ones.

Some antioxidants are made in the body (e.g., superoxide dismutase), whereas others such as X-tocopherol (vitamin E) are supplied by food. The major scavenger inside biologic membranes is X-tocopherol. In the body, vitamin E is transported in plasma lipoproteins and partitions into membranes and fat-storage sites, where it is one of the most effective antioxidants for protecting polyunsaturated fatty acids (PUFA) from oxidation. In vivo, vitamin E functions as a chain-breaking antioxidant that prevents propagation of free radical damage in biologic membranes. Tocopherols inhibit lipid peroxidation because they scavenge lipid peroxy 1 radicals much faster than these radicals can react with adjacent fatty acid side chains or with membrane proteins. Vitamin E, therefore, plays a dominant role in neutralizing oxidative damage. Antioxidant defenses are highly dependent upon adequate nutrition. Malnutrition can lead to oxidative stress. For example, inadequate ingestion of dietary antioxidants, such as vitamins C and E, mimics the effects of radiation exposure.

Antioxidants act synergistically and have sparing effects in which one antioxidant protects another against oxidative destruction. For example, vitamin C regenerates vitamin E after it has reacted with a free radical.

The balance between oxidants and antioxidants is crucial for health and an important determinant of immune function, particularly for maintaining the integrity and functionality of membrane lipids, cellular proteins, and nucleic acids, but also for controlling signal transduction and gene expression in the immune system. Cells of the immune system are particularly sensitive to changes in oxidant -antioxidant balance because of the high percentage of PUFA in their plasma membranes. Optimal amounts of antioxidants are needed for maintenance of the immune response.

Biomarkers can be used to investigate which foods and dietary constituents decrease levels of oxidative DNA and lipid damage in vivo. A recent study showed that normal dogs and cats experience oxidative stress and that increased distary levels of vitamin E can improve antioxidant status and modulate oxidative stress in vivo. The thresholds for significant reduction of one serun biomarker of oxidative damage in dogs and cats were 445 and 540 IU vitamin E/kg of food, respectively, on an as-fed basis.

Oxidative stress

Under normal physiologic conditions, the rate and magnitude of ROS formation is balanced by the rate of ROS elimination. An imbalance between ROS production and antioxidant defense results in oxidative stress, which is the pathogenic outcome of the overproduction of ROS that overwhelms the cellular antioxidant system. An increase in oxidative stress may occur as a result of an increase in ROS production, reduction in antioxidant defense, or both. Because generation of ROS and the level of antioxidant defense systems are approximately balanced, small changes may disrupt the balance in favor of the ROS and upset cell biochemistry. Most cells can tolerate a mild degree of oxidative stress because they have repair systems that recognize and remove oxidatively damaged molecules, which are then replaced. In addition, cells may increase antioxidant defenses in response to the oxidative stress.

Although free radicals are continuously generated in the body, most are intercepted by antioxidant defenses. some ROS perform useful metabolic and defense roles (e.g., bactericidal activity of white blood cells), whereas others escape the body's defense mechanisms to damage biomolecules. Damaged biomolecules are repaired and replaced; repair is especially important in maintaining the accuracy of the genetic information encoded in DNA. However, repair is not completely efficient. Detecting biomarkers of lipid peroxidation in biologic fluids and tissues indicates on going lipid peroxidation that is incompletely suppressed by antioxidant defenses, even in normal animals. Thus, the antioxidant defenses of the body do not completely protect host tissues against oxidative molecules produced by the immune system. Laboratory evidence suggests that oxidative stress, which occurs when free radical formation exceeds the body's ability to protect itself, forms the biologic basis of many chronic conditions.

Oxidatives stress: DNA Damage

DNA damage is considered to be one of the most important contributors to cancer. Much of this damage is oxidative in nature. Targets of ROS are now being characterized and quantified. It has been estimated that a typical human cell experience about 10,000 oxidative

"hits" to its DNA each day. Oxidative lesions to DNA accumulate with age, and so does the risk of cancer.

In 1956, Harmon originally proposed that normal aging results from random deleterious damage to tissues by free radicals produced and accumulated during normal aerobic metabolism. Today, some investigators believe that oxidative damage to DNA and other macromolecules appears to have a major role in aging and degenerative diseases associated with aging such as cancer. (See Aging and Longevity below.) Oxidative lesions accumulate with age in DNA and protein. A young rat has approximately 24,000 oxidative DNA lesions per cell and an old rat has approximately 66,000. DNA is oxidized in normal metabolism because antioxidant defenses, though numerous, are not perfect.

Oxidative stress: Chronic Diseases

Oxidative damage to biomolecules has been implicated in the development of many chronic diseases, in particular cardiovascular disease, cancer, and cataracts. After tissue injury by almost any mechanism, the body recruits and activiates phagocytes that make ROS. Catalytic metal ions are released when cells are disrupted, which hastens lipid peroxidation. Damaged mitochondria may become "leakier", releasing more electrons to oxygen to form superoxide. For these and many other reasons, tissue injury leads to oxidative stress. Growing evidence supports a role for peroxidation in the pathogenesis of inflammatory bowel disease. The activation of inflammatory cells, release of their mediators, and excessive production of free radicals may affect circulating lipids. Recent studies propose that some of the complications of diabetes are associated with increased activity of ROS and accumulation of lipid peroxidation products. Concentrations of MDA in patients with hypothyroidism or hyperthyroidism were significantly higher than in normal subjects. Patients with rheumatoid arthritis have significantly lower plasma levels of vitamin E and significantly higher lipid peroxidation products compared with control subjects.

The introduction and discussion sections of the following article in this monograph present more information about the role of ROS in chronic diseases.

Current literature review

A large body of experimental research indicates that oxidative stress contributes to the process of aging and the development of many degenerative diseases. The review that follows focuses on the pathophysiologic mechanisms involved with disease development and the benefits of endogenous and exogenous antioxidants. Much of the evidence is based on epidemiologic findings. prospective, randomized clinical trials need to be performed using specific antioxidants to prove benefits suggested by epidemiology.

Aging and Longevity

Aging is an extremely complex, multifactorial process. Several theories have been advanced to explain how and why animals age. During the last 10 years, the theory known as the "free radical theory of aging" has achieved prominence as one of the most compelling explanations for many of the degenerative changes associated with aging.

Some varieties of C. elegans have a greatly increased lifespan as a result of a genetic mutation that results in increased activities of the antioxidative enzymes superoxide dismutase and catalase. Other supporting evidence comes from people with Down's syndrome. Affected people have a significiantly shortened lifespan and are plagued by increased oxidative stress. which results in various free radical-related disturbances. Other indirect evidence comes from the myriad of age-related diseases that are affected by oxidative stress (e.g., cancer, heart disease, etc.) In addition, part of the free radical theory of aging involves the damaging role of ROS and various toxins on mitochondria, which lead to mitochondrial DNA mutations and a progressive decline in energy output and some of the signs of aging. Oxidative stress also inactivates critical enzymes and other proteins.

Several authors have reported a significant prolongation of survival of laboratory animals and dogs by long-term administration of deprenyl. Repeated administration of deprenyl for 2 to 3 weeks significantly increased the activities of various endogenous antioxidant enzymes.

Oxidative stress is one of the important mediators of vascular complications in diabetes including nephropathy. High glucose concentrations generate ROS as a result of glucose autoxidation, metabolism, and formation of advanced glycosylation end products.

Other investigators found direct evidence proving oxygen-derived free radicals contribute to impaired

endothelium-dependent coronary arteriolar dilation in diabetic dogs in vivo.

General Nutrition

Three experiments were conducted to study the uptake of oral B-carotene by blood plasma and leukocytes in domestic cats. Daily dosing of cats with B-carotene for 6 days resulted in a dose-dependent increase in circulating B-carotene. Investigators found that B-carotene accumulated mainly in mitochondria of white blood cells (40 to 52%), with lesser amounts in microsomes, cytosol, and nuclei. This study shows that cats readily absorb B-carotene across the intestinal mucosa and transfer it to peripheral blood leukocytes and their organelles.

Cardiovascular Disease

Investigators assessed the degree of oxidative stress and antioxidant concentrations in dogs with idiopathic dilated cardiomyopathy. A negative correlation was found between disease severity and plasma vitamin E concentration. The authors concluded that changes in glutathione peroxidase concentration and the correlation between vitamin E concentration and disease severity suggest that the oxidant-antioxidant system may play a role in the development of idiopathic dilated cardiomyopathy in dogs.

Although in-vitro studies have implicated oxygen-derived free radicals as possible mediators of inflammatory cytokine-induced cell injury, the role of radicals in the cytokine-induced myocardial dysfunction in vivo has been unclear.

Cancer

Oxygen free radicals have been implicated in many disease processes, including aging and carcinogenesis, and have been associated with a variety of complications resulting from treatment of cancer. Treatment of free radical-induced disease with antioxidants or free radical scavengers has become an important therapeutic modality. Ironically, the same oxygen free radicals also play a critical role in anti-cancer therapy. Antioxidants decrease the effectiveness of anti-cancer therapies, which depend on generation of free radicals for their action. In addition, tumor cells can use antioxidant activity to favor increased growth.

Exercise increases the generation of oxygen free radicals and lipid peroxidation. Strenuous exercise in a person who is unconditioned or unaccustomed to exercise will induce oxidative damage and result in muscle injury. Aerobic exercise, however, increases superoxide dismutase, strengthening the antioxidant defense system. Vitamin C. and, especially vitamin E, decrease the exercise-induced increase in the rate of lipid peroxidation.

Immunity and the Immune Response

Investigators fed beagle dogs increasing amounts of B-carotene to determine the role of this nutrient on the immune response. B-carotene supplementation increased plasma B-carotene concentrations in a dose-dependent manner, and heightened cell-mediated and humoral immune response.

Renal and Urinary Tract Disease

Taurine is an endogenous antioxidant and membrane-stabilizing agent. In a recent study involving rats, researchers concluded that taurine attenuates the accumulation of gentamicin within kidney tissue and counteracts the deleterious effect of gentamicin on renal tubular function.

Recent studies provided evidence that exposure of renal epithelial cells to oxalate and calcium oxalate crystals induces lipid peroxidation and injures cells. Cells of proximal tubular origin were more susceptible than those of distal tubules and collecting ducts. Free radical scavengers, catalase, and superoxide dismutase provided significant protection against the injury.

Shock

Sepsis, like other inflammatory conditions, results in a large increase in the production of ROS, including superoxide anions, within the body. The authors of a recent review proposed the use of superoxide dismutase mimetic for the treatment of septic shock. Removing superoxide anions protects exogenous and endogenous catecholamines from autoxidation, reversing hypotension and hyperactivity, reducing generation of potentially toxic adreonochromes, and improving survival.

Effects on Drug Metabolism

In one study, investigators challenged cats with acetaminophen after one of the challenge groups received bioflavonoid antioxidants for 2 weeks. Acetaminophen significantly increased

Carried over to page 21