Oxidative Stress in Applied Basic Research and Clinical Practice

Editor-in-Chief
Donald Armstrong

For other titles published in this series, go to
http://www.springer.com/series/8145

Note from the Editor-in-Chief

All books in this series illustrate point-of-care testing and critically evaluate the potential of antioxidant supplementation in various medical disorders associated with oxidative stress. Future volumes will be updated as warranted by emerging new technology, or from studies reporting clinical trials.

Donald Armstrong
Editor-in-Chief

Lester Mandelker · Peter Vajdovich
Editors

Studies on Veterinary Medicine

Humana Press

Editors
Lester Mandelker
Fellow, American Academy of Veterinary
Pharmacology and Therapeutics
Community Veterinary Hospital
Largo, FL 33770, USA
lestervet2@aol.com

Peter Vajdovich
Faculty of Veterinary Sciences
Department of Internal Medicine and Clinics
Szent Istvan University
Istvan u. 2, 1143, Budapest, Hungary
Vajdovich.Peter@aotk.szie.hu

ISBN 978-1-61779-070-6 e-ISBN 978-1-61779-071-3
DOI 10.1007/978-1-61779-071-3
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2011921347

Printed on acid-free paper

Humana Press is part of Springer Science+Business Media (www.springer.com)

Preface

The production of free radicals (ROS) is an unavoidable consequence of life in an aerobic environment. Free radicals produced from the metabolic activities of oxygen attack biological membranes and lipoproteins via oxidation in a process called lipid perioxidation. This attack damages cells and lipids often in a chain reaction with carbon-based molecules such as polyunsaturated fatty acids (PUFA) in a reaction with molecular oxygen. This creates oxidative stress and damage to tissues.

Free radicals also damage chromosomal DNA. It is more likely that damage to DNA occurs from external sources rather than mitochondrial-produced free radicals. Synthetic compounds, pollutants, radiation, xenobiotics (drugs), and food components make up the most likely factors that damage DNA. Proteins are another target of free radicals as proteins and their amino acids can be modified and degraded through free radical mediated reactions. Oxidized proteins then become the target of specialized proteases that turn them into less biologically active smaller peptides and amino acids.

Growing evidence supports a major role of oxidative stress in aging and disease. It has been almost a half century since Denham Harman first proposed the free radical theory in relation to disease. This hypothesis simply states that oxidative stress is the most important determining factor for aging and age-related diseases. Evidence continues to suggest that oxidative stress limits the chronological lifespan which is consistently shortened when there is a reduction of antioxidant enzymes. Furthermore, there is also ample evidence to indicate that reducing oxidative stress is both important and necessary for an extended lifespan. However, even though there appear to be beneficial effects of antioxidant treatment against pathological disease, major preventative clinical trials of dietary antioxidants have failed to prove benefits in increasing longevity. It may be that oxidative stress is more like an active bystander instead of an active component in increasing longevity. No assessment of the free radical theory of aging and pathogenesis of age-related diseases would be complete without an up-to-date account of the major impact oxidative free radicals have in the pathology of disease.

There are various disorders with clear signs of oxidative damage (i.e., paracetamol (Tylenol®) toxicosis, hypoxia reperfusion injury, etc.), and there are some in which oxidative stress is a side effect (i.e., diabetes mellitus, inflammation, liver failure, etc.). Our opinion is that the appropriate way to treat a disorder is multimodular and

antioxidant therapy is one important member among the options. In cases of clear oxidative damages we still have to use other treatment therapies (e.g., fluid therapy, antimicrobial therapy, etc.) with antioxidants. There are also diseases in which oxidative stress is a secondary problem, such as diabetes mellitus where the primary treatment is insulin, but antioxidant therapy appears beneficial as well in improving insulin sensitivity.

The purpose of this book is to inform clinicians, students, and others of the vast effects of free radical damage on various cells, tissues, and organs and in different species of animals. In addition, the effects of oxidative stress are analyzed in aging and various disease states such as diabetes, cognitive dysfunction, and heart disease. Each author presents his or her interpretation of the effects of oxidative damage in disease and in several species and the challenges in controlling oxidative damage with antioxidant therapies. We have compiled ideas and scientific information from scientists, veterinarians, and the medical community from around the world. This is surely a universal effort to promote further understanding of oxidative stress and its effects on various animals and organ systems. We would like to commend the authors for their vision and undertaking such a daunting task.

Largo, FL — Lester Mandelker
Budapest, Hungary — Peter Vajdovich

Contents

Contributors

Krisztián Balogh, Ph.D.
Research Group of Animal Breeding and Hygiene, University of Kaposvár,
H-7400 Kaposvár, Guba S. u. 40, Hungary
balogh.krisztian@mkk.szie.hu

Wendy Baltzer, D.V.M., Ph.D.
Small Animal Surgery, College of Veterinary Medicine, Oregon State University,
Corvallis, OR 97331-4801, USA
wendy.baltzer@oregonstate.edu

Pietro Celi, D.V.M., Ph.D.
University of Sydney, C04 MC Franklin Lab, PMB 3, 425 Werombi Rd,
Camden, NSW 2570, Australia
pietro.celi@sydney.edu.au

Stefano Comazzi, D.V.M., Ph.D., dipl. ECVCP diabetes
University of Milan, Department of Veterinary Pathology,
Hygiene, and Health, Section of Veterinary General Pathology
and Parasitology, Via Celoria 10, Milan 20133, Italy
stefano.comazzi@unimi.it

Elizabeth Head, Ph.D.
Sanders-Brown Center on Aging, University of Kentucky,
800 S. Limestone St, Lexington, KY 40536, USA
Elizabeth.head@uky.edu

John Kuck, D.V.M.
Aspen Animal Hospital, Nutrient Selection in the Management of Canine Atopic
Dermatitis, Aspen, CO, USA
john_kuck@yahoo.com

Lester Mandelker, D.V.M., Dipl. ABVP
Fellow, American Academy of Veterinary Pharmacology and Therapeutics,
Community Veterinary Hospital, Largo, FL 33770, USA
lestervet2@aol.com

Dr. Kevin J. McGraw, M.S., Ph.D.
School of Life Sciences, Arizona State University, Tempe,
AZ 85287-4501, USA
kevin.mcgraw@asu.edu

Miklós Mézes, Ph.D.
Department of Nutrition, Szent István University, H-2103 Gödöllő, Hungary
Mezes.Miklos@mkk.szie.hu

Gordon Moe, M.Sc., M.D., F.R.C.P.(C.), F.A.C.C.
Li Ka Shing Knowledge Institute, St. Michael's Hospital, University of Toronto,
Toronto ON, Canada M5B1W8
moeg@smh.toronto.on.ca

Mustafa Nazıroğlu
Department of Biophysics, Süleyman Demirel University, Morfoloji Binasi,
TR-32260 Isparta, Turkey; Neuroscience Research Center,
Suleyman Demirel University, Isparta, Turkey
mnaziroglu@med.sdu.edu.tr

Patricia A. Schenck, D.V.M., Ph.D.
Endocrine Diagnostic Section, Diagnostic Center for Population and Animal Health,
Michigan State University, 4125 Beaumont Rd, Lansing, MI, USA
schenck5@msu.edu

Peter Vajdovich, D.V.M., Ph.D. Dipl. ECVCP
Faculty of Veterinary Sciences, Department of Internal Medicine and Clinics,
Szent Istvan University, Istvan u. 2, 1143, Budapest, Hungary
Vajdovich.Peter@aotk.szie.hu

Gustavo L. Zapata, D.V.M.
Servicio Central de Laboratorio, Facultad de Ciencias Veterinarias,
Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina
zapatagu@fcv.unlp.edu.ar

Steven C. Zicker, D.V.M., Ph.D., Dipl. ACVIM, Dipl. ACVN
Pet Nutrition Center, Hill's Pet Nutrition, Inc,
P.O. Box 1658, Topeka, KS 66601, USA
steven_zicker@hillspet.com

Oxidative Stress, Free Radicals, and Cellular Damage

Lester Mandelker

Abstract Oxidative stress is a term relative to the elevated levels of reactive free radicals in an organism. Oxidative stress can occur from diminished antioxidants and/or increased production of reactive free radicals such as reactive oxygen species and/or reactive nitrogen species (ROS/RSN). The increased production of free radicals is more relevant to disease and frequently the attempted target of supplementation intervention. In many instances the body can adapt to an increase in oxidative stress by upregulation of antioxidant defense systems. If the oxidative stress can be neutralized, there is often no adverse contribution to disease pathology. If the antioxidant defense induction is inadequate or nonexistent then accompanying cellular and tissue damage often occurs. Some diseases can be caused directly by oxidative stress, however, in most diseases oxidative stress is a consequence and may often only be a secondary event. It does, however, play an important role in promoting additional tissue injury in most diseases. On the other hand, oxidative stress may have beneficial effects in activating biological pathways that alter antioxidant defenses and allow an organism to adapt. Oxidative stress is also considered necessary to promote healing and repair of tissues. Therefore, not all cases of oxidative stress are damaging. It is only when oxidative stress is excessive and inappropriate should we address it with supplementation and antioxidant therapy that reduces oxidative damage to cells, tissues, proteins, cellular membranes, and mitochondria.

Keywords Oxidative stress • Cell homeostasis • Nuclear factor kappa B (NF-kB) • Mitochondrial permeability pore transition (MPT) • Gap junctional intercellular communication (GJIC) • Glutathione

L. Mandelker (✉)
Fellow, American Academy of Veterinary Pharmacology and Therapeutics,
Community Veterinary Hospital, Largo, FL 33770, USA
e-mail: lestervet2@aol.com

L. Mandelker and P. Vajdovich (eds.), *Studies on Veterinary Medicine*,
Oxidative Stress in Applied Basic Research and Clinical Practice 5,
DOI 10.1007/978-1-61779-071-3_1,

Oxygen

Earth is the only planet in our solar system known to have sufficient oxygen to maintain life as we know it. As the O_2 content of the atmosphere rose, it exposed living organisms to oxygen toxicity. In its ground state (its normal configuration; O_2) molecular oxygen is relatively inert. Molecular oxygen (O_2) is the premier biological electron acceptor that serves vital roles in fundamental cellular functions. However, during normal metabolic activity, and as a consequence of various environmental disturbances such as extreme temperatures, radiation, xenobiotics, toxins, air pollutants, and various stresses and diseases, O_2 is capable of giving rise to reactive excited states such as free radicals and their derivatives [1]. With the beneficial properties of O_2 comes the inadvertent formation of reactive oxygen species (ROS) such as superoxide ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($OH^{\bullet}$). If unabated, ROS pose a serious threat, possibly causing the death of aerobic cells. Aerobic metabolism using oxygen (oxidative phosphorylation) in mitochondria is highly efficient in producing energy from organic compounds and remains the major form of energy production in virtually all animals [1]. Despite its beneficial effects there remains a fundamental problem: how to protect cells and specifically mitochondria from oxygen toxicity. To minimize the damaging effects of ROS, aerobic organisms evolved nonenzymatic and enzymatic antioxidant defenses. The latter include catalases, peroxidases, superoxide dismutases (SOD), and glutathione S-transferases. Thus, oxygen is a "double-edged sword" in that it makes life on earth possible, but in its radical form (ROS), it is highly toxic and lethal.

Oxidative Stress

Oxidative stress occurs when there is an increase in oxidant production and free radical formation that exceeds the body's ability to neutralize and eliminate these reactive radical forms. There is a great deal of evidence that oxidative stress is involved in many animal and human diseases. However, in many cases it may be that oxidative stress is a complicating factor of the pathological process and not necessarily the primary cause. The involvement of oxidative stress often depends on the nature of the disease. Every cell undergoes oxidative stress at some point in its existence and this is especially true in disease and aging tissues. All organ systems are involved with oxidative stress but when the oxidative stress is excessive it can lead to organ damage, which has a direct effect on the health of the body. The organ systems especially affected include kidneys, liver, pancreas, heart, CNS/nervous tissue, intestines, and adrenal, bone marrow, lungs, and thyroid tissues. Therefore, oxidative stress not only has an impact on cells and cellular components but on most organ systems especially the neuroendocrine system as well as the immune status and aging [2]. Common causes of oxidative stress include: infections, toxins, hypoxia/ischemia, hyperglycemia, xenobiotics (drug metabolism), hyperlipidemias,

hyperproteinemias, cancer, inflammation, phagocytic and immune reactions, and elevated metabolic rates. Additionally, aging tissues often undergo oxidative stress because mitochondria fail to produce enough ATP (energy) to sustain optimum health.

Oxidative Stress and Free Radicals

In healthy animals, production of free radicals is approximately balanced by antioxidant defenses. The balance is not perfect, therefore continual damage occurs when antioxidant defenses are inadequate. Free radicals are molecules that contain one or more unpaired electrons and are capable of independent existence. Most free radicals are derivatives of oxygen (reactive oxygen species) or ROS and derivatives of nitrogen (reactive nitrogen species) or RON. They are formed wherever there is disease and cell damage and are especially produced in large quantities during infection [3]. They are even produced under normal conditions from the cell's mitochondria during energy production from the intake of oxygen. Thus, even in healthy animals up to 25% of the oxygen they breathe forms free radicals. However, in sick animals up to 75% of the oxygen may form free radicals. The presence of free radicals is not always a bad thing; in fact free radicals can act to fight infection and disease. This is the primary purpose of bacterial phagocytosis which involves an oxygen-dependent system. In bacterial phagocytosis, neutrophils show a burst of metabolic activity characterized by an increase in oxygen consumption and a subsequent increase in free radical production such as superoxide, hyroxyl radical, and hydrogen peroxide (O_2^-, OH^-, H_2O_2). This process has been termed the "respiratory burst" of phagocytosis [4]. In addition, mild elevations of free radicals drive many physiological and metabolic pathways [5] and only when there are excessive and inappropriate levels of free radicals that may accumulate during cell injury and disease do they cause oxidative damage to protein molecules, cell membranes, mitochondria, and DNA [6].

Hypoxia and Oxidative Stress

Hypoxic tissues and tissues that undergo ischemia–reperfusion cycles cause an increase in free radical production and increase in oxidative damage. The severity of the damage depends on the tissues involved and the length of ischemia. Tissues respond in several ways: the early response includes glycogen depletion causing energy levels to decrease, which depletes ATP. This causes mitochondria to dysfunction. Damaged and aging tissues also undergo oxidative stress when the mitochondria fail to produce enough energy (ATP) to sustain maximum health. This also results in mitochondrial dysfunction [7]. Mitochondrial dysfunction encompasses all the clinical diseases associated with the defective energy metabolism primarily

of oxidative phosphorylation (production of ATP). These diseases also include multiple neurodegenerative conditions, cancer, and the skeletal muscle disorders and are termed mitochondrial cytopathies.

Cell Homeostasis

Cell homeostasis is defined as cells in oxidative balance. This occurs when there is a balance of antioxidants (known as reducing agents) and oxidants (known as oxidizing agents). This balance is referred to cell redox. When the balance of antioxidants to oxidants shifts to high levels of oxidants the cell undergoes oxidative stress. Oxidative stress then can also be defined as the disruption or alteration of cell redox or cell homeostasis that favors oxidants. A progressive increase in oxidative stress because of altered redox homeostasis is important in processes that regulate gene transcription in both normal and abnormal health [8]. Therefore, ROS/RSN serve as signaling molecules for the initiation and perpetuation of the inflammatory process that occurs with conditions of oxidative stress. This involves genetic regulation. Transcription factors that are directly influenced by reactive species (redox sensitive) and proinflammmatory signaling include nuclear factor kappa B (NF-kB), activated protein (AP-1), and hypoxia-inducible factor −1@ (HIF-1@) [9]. Hence, sustained oxidative stress leads to inflammation by means of upregulation of transcription factors such as NK-kB that alter genetic function and induce inflammation. Following cell injury NF-kB, which is the primary transcription factor, translocates into the nucleus and targets genes primarily concerned with cellular repair and proliferation and this often promotes inflammation [10].

Significance of NF-kB

Often referred to as the master regulator of genetic function, this signaling protein molecule is activated by cell damage and free radicals and hence is redox sensitive. It is so potent that it is contained in a protein called IkB or inhibitory kB. Before NF-kB can be activated in the cell the inhibitory protein IkB must be inactivated. The effects of stimulation of genes by NF-kB is very profound and is the driving force in cellular repair, growth, and cellular inflammation. NF-kB plays a central role in regulating genetic transcription and encoding of inflammatory cytokines, growth factors, acute phase proteins, adhesion molecules, other transcription factors, and cell death regulators. These NF-kB regulated genes are important in regulating genetic activity during critical illness, inflammatory diseases, and cancer [11]. One can clearly see the significance of the activity of NF-kB and how modulating this activity would have profound effects on inflammation, cell growth, and cancer. There are many supplements, proteins, antioxidants, minerals and vitamins, and drugs that can modulate NF-kB activity. Some work by increasing inhibitory actions of IkB,

some work by inactivation of NF-kB, and some antagonize binding of NF-kB, but in the end they decrease the actions of NF-kB which has profound effects in reducing inflammation and cancer [12]. The activation of NF-kB is not always a detriment. It is the primary method that mediates cell growth and repair and protection from TNF-alpha activation-induced cell death. Many cytokines act directly or indirectly through NF-kB including transforming growth factor-B (TGF-beta), ROS, prostaglandins (PGs), leukotrienes, nitric oxide (NO), protein kinases, and certain hormones and growth factors [13].

Measure Oxidative Stress

We can measure oxidative stress in many ways: one way is to measure GSH/GSSH (reduced glutathione/oxidized glutathione). Reduced glutathione (GSH) is the primary antioxidant found in cells. Most cats and dogs that have liver and kidney disease or other chronic disease have reduced levels of glutathione. It is estimated that over 75% of cats with chronic disease have depleted levels of cellular glutathione. By supplying agents (antioxidants) that improve glutathione levels in the cells we often see a beneficial impact on the progression of many feline diseases. For example, in cats that are poisoned with acetaminophen (Tylenol®) glutathione depletion occurs in RBCs. This condition is best treated with glutathione inducers (thiol antioxidants) such as N-acetylcysteine, SAM-e, alpha lipoic acid, and/or taurine [14]. Another indicator of oxidative stress in the feline is elevation of the acute phase protein produced by the liver called serum amyloid A. This can be measured as there are assay kits available [15]. This indicator of oxidative stress and inflammation is similar to C-reactive protein that is more often used in people and dogs to measure inflammation. There are several other inflammatory chemicals that can be measured in blood or urine to confirm excessive oxidative stress. Nitric oxide and lipid perioxidation products such as malondialdehyde (MDA) are such examples.

Managing Oxidative Stress

Cells can usually tolerate mild oxidative stress, which often results in the upregulation of antioxidant synthesis in an attempt to restore cell redox (cell homeostasis) or the antioxidant–oxidant balance of the cell. Mild oxidative stress upregulates defenses so as to protect against more severe oxidative stress. This mechanism includes cellular adaptation which often involves changes in gene expression that result in elevated antioxidant defenses. However, if adaptation does not improve the antioxidant defenses, permanent cell injury occurs. In many cases, permanent injury alters the homeostasis of the cell and the cell enters a temporary prolonged altered injurious state that may or may not lead to cell death [15].

Cell Death

Cells die by necrosis or apoptosis. Necrosis is the result of cellular damage that cannot be repaired and is the result of internal or external forces or chemical or physical events that destroy cells. The progression of events as a result of necrosis is that cells swell, rupture the outer cell membranes, and eventually die and release biochemical mediators of inflammation into the systemic circulation. The cellular death that follows often induces a well-orchestrated series of cellular and biochemical events that commonly provoke a strong inflammatory response. Inflammation consists of a series of physiological reactions by the body that brings cells and molecules of the immune system to the site of cell injury [16]. The net result might appear in the form of increased blood supply, increased migration of leukocytes, and increased vascular permeability. Immediate biological mediators include PGs, leukotrienes, serotonins, histamine, platelet-activating factor, and others [17]. These are released during cell death and may also act as signaling molecules in the acute phase of inflammation. During sustained injury, other biological mediators perpetuate cellular inflammation by additionally activating chemical mediators such as TNF-alpha, substance P, acute phase proteins (C-reactive protein, Serum Amyloid A), interferons (i.e., IL-1, IL-4, etc.), adhesion molecules (VEGF), colony stimulating factors such as TGH beta, and so on [18]. This chronic response involves a more extensive commitment to inflammation. This inflammation phase can occur from continued, acute, nonspecific stimulation or from sustained immunologic stimulation. In addition, enzymes are produced by genetic stimulation (upregulation) due to chronic inflammation such as MMPs (matrix metallo-proteinases) that act to destroy the intercellular matrix between cells which promotes more inflammation and may perpetuate carcinogenesis [19].

Apoptosis

Programmed cell death (apoptosis) is different from necrosis in that it is a restrained cell death. It can be a normal physiological function of growth and development or can occur from oxidative stress to the cell and/or loss of energy production by the mitochondria. The activation of apoptosis in individual cells is based on its environment, internal metabolism, genetic information, or other various external or internal forces. One such mechanism involves the cell death protein P-53 which acts as an internal signal to induce cells to die. During this process, the cell shrinks, the nucleus condenses, and DNA fragmentation occurs [20]. However, there is no disruption of the outer membrane in contrast with necrosis and no inflammatory mediators are released into the circulation.

Apoptosis is a quiet suicide with little or no inflammation involved. Research has revealed that the mitochondria's integrity is the determining factor of whether cells live or die. Changes in both the inner and outer mitochondrial membrane lead to a disruption of the membranes, opening of the mitochondrial pores, and

release of cytochrome C into the cytoplasm. This process initiates cell death by activation of the death proteins called caspases [21]. Caspases are very sensitive to the redox status of the cell and reduced levels can block their activity. Thus, alterations of intracellular redox status might trigger or block the apoptotic death proteins.

Significance of Cell Death

Knowing how cells die might allow us to find better ways of treating cell injury before cell death becomes apparent. For example, we know cell injury just prior to cell death often causes the mitochondrial pores to open (mitochondrial permeability pore transition or MPT) and this allows Ca^{2+} to enter. This starts the death pathway first beginning with mitochondrial release of cytochrome C. We have drugs that can block the pore opening such as cyclosporine. The use of cyclosporine reduces cell death by inhibiting MPT. Inhibiting MPT explains the effectiveness and why it is effective in transplant rejection disease (graft vs. host disease). Some supplements such as melatonin also inhibit MPT and are therefore beneficial in reducing many forms of mitochondrial dysfunction and cell death.

Chronic Oxidative Stress

Chronic oxidative stress can also lead to cancer. The upregulated or prolonged production of cellular oxidants has been linked to DNA mutation, cell proliferation, and cellular growth. All these are precursors to carcinogenesis [22]. Oxidative stress often damages DNA. DNA is found in both the nucleus and the mitochondria. The mitochondria is the only cell structure other than the nucleus to contain DNA (mtDNA). Nuclear DNA contains two copies of DNA, one inherited from the father and the other from the mother. On the other hand, mtDNA is different in that virtually all the mitochondria DNA mutations occur only from the mother's DNA. The free radicals and oxidative stress that damage mtDNA often reduce the ability for mitochondria to replicate, reduce the energy output by the mitochondria (ATP production), and lead to deletions, mutations, and ways of treating cell injury before cell death becomes apparent [23].

Response to Oxidative Stress

The body handles excessive oxidative stress by using both enzymatic and nonenzymatic antioxidants to neutralize damage from free radicals. A network of antioxidants is best able to handle excessive oxidative stress. So it is better to offer multiple antioxidants vs. just one or two. SOD supplements act to replenish antioxidant

enzymes in the body. These are often depleted in chronic oxidative stress conditions and supplementation often helps reduce oxidative damage to tissues and cells [24]. Nonenzymatic supplementation takes the form of oral or injectable antioxidants such as reduced glutathione, Vitamin E, alpha lipoic acid, N-acetylcysteine, SAM-e, Vitamin C, flavanoids/polyphenols, and minerals including zinc and selenium. Some of these antioxidants replenish intracellular glutathione (i.e., alpha lipoic acid, N-acetylcysteine, SAM-e), which is the most important and the only available antioxidant available to the mitochondria. The body responds to oxidative stress through complex signaling pathways [25]. In this regard, these tissues are considered to be redox-sensitive as they respond to imbalances in the oxidant–antioxidant levels. Many times the body responds in a positive way to oxidative stress and in fact, this physiological mechanism drives vital cellular functions. Oxidative stress can often cause the body to adapt to the stress in a positive manner. For instance, by increasing mitochondrial numbers in tissue via genetic stimulation the body can adjust to oxidative stress in an appropriate manner. This often occurs in myocardial muscle tissue from which a cell modulates the intracellular redox state of the cell by increasing energy production.

Oxidative Stress and Cellular Communication

Cells carry on their daily activities by using signaling molecules, which are specialized proteins that act on receptors inside cells. This is referred to as intracellular communication. Intracellular communication occurs in cells via signaling molecules such as NF-kB. NF-kB is the primary intracellular signaling molecule that activates genes inside the cell and is considered the master regulator of numerous genes that involve the immune and inflammatory response. It is activated by ROS/RSN, toxins, hormones, hyperglycemia, and various inflammatory cytokines. Cells must communicate with neighboring cells to survive. This intercellular communication occurs between same cells of the same tissue. The communication between such cells occurs through very small junctions. This type of cellular communication is called gap junctional intercellular communication (GJIC). Cells share ions and electrolytes through these gap junctions made up of connexin molecules [26]. These protein molecules also contain genes. Connexin genes account for many of the cellular responses to inflammation and disease and are influenced by many of the inflammatory mediators such as PGs, leukotrienes, kinins, serotonin, platelet activating factor (PAF), TNF-alpha, adhesion molecules, interleukins, and colony growth factors (i.e., VEGF, vascular endothelial growth factor) [27]. The importance of GJIC cannot be understated. In many diseases, toxins, and physical events such as a burn GJIC are compromised. When this occurs independent cells either die or eventually become cancerous. For example, phenobarbitol is a known liver toxin. It acts in the liver to disrupt and destroy normal GJIC. This is the same mechanism of action in the brain that reduces seizures. However, in the liver the use of phenobarbitol which causes interruption of GJIC can promote carcinogenesis.

Significance of GJIC

GJIC is disrupted especially where there is extensive inflammation, oxidative stress, and cellular damage as seen with extensive injury and chronic diseases such as liver disease, kidney disease (glomerulonephritis), pancreatic disease, heart disease, bladder inflammation, and other similar conditions. Abnormal intercellular gap junctional communication has been implicated in tumor promotion, neuropathy, and angiogenesis [28]. Oxidative stress has also been implicated in similar diseases such as cancer. This appears to be a direct link to oxidative stress and GJIC as increasing oxidative stress causes more interruption of GJIC. Because there are genes located in the gap junctional areas, oxidative stress acts to stimulate many genetic functions involving inflammation and promotion of disease. There are many nutraceuticals and antioxidants that restore or improve GJIC. Carotenoids especially improve and repair GJIC [29]. DMSO is a chemical solvent that repairs cell membranes and also acts to improve GJIC. Their use in such diseases offers a novel method of altering disease progression and in this manner are considered disease-modifying agents.

Glutathione and Oxidative Stress

Glutathione is the major intracellular antioxidant by which oxidative stress is measured. It is the only antioxidant available to mitochondria. The imbalance of antioxidants to pro-oxidants can be quantified in plasma as the redox state of reduced glutathione (GSH) to oxidized glutathione (GSSG) or GSH/GSSG [30]. In health, the high efficiency of GSSG reductase often can maintain the cellular GSH pool in a predominately reduced state (intracellular GSH/GSSH >98%). However, this reduction of oxidized glutathione (GSSH) to reduced glutathione occurs only in the presence of nicotinamide adenine dinucleotide phosphate (NADPH). By supplying needed levels for this cellular reaction necessary cellular NADPH stores may be diminished [30]. NADPH is vital for scavenging toxic free radicals in the mitochondria and cytosol where it is produced. Hence, in certain situations NADPH availability might be the rate limiting factor in GSH regeneration. These complex factors reaffirm the complex interaction of vital nutrient chemicals in maintaining the balance in cell redox. GSH-Px (glutathione perioxidase) is an antioxidant enzyme that plays an essential role in stabilizing the cell redox. To achieve maximum benefits in modulating excessive oxidative stress certain nutrients, cofactors, enzymes, and vitamins should be supplied to the patient in times of physiological need.

Oxidative Stress and β-Cell Dysfunction

It has been well established that there has been a recognized link between the presence of chronic hyperglycemia and the progressive deterioration in β-cell function seen in patients and animals with diabetes. More recently, however, studies have indicated

that this progressive β-cell dysfunction is a result of tissue damage induced by oxidative stress resulting from this hyperglycemia. β-cells are thought to be particularly vulnerable to oxidative stress because they contain very low levels of antioxidant enzymes [31]. In support of the hypothesis that chronic oxidative stress might play a role in the progressive β-cell dysfunction seen in type-2 diabetes are the findings that the pancreatic β-cell undergoes oxidative stress when exposed to supraphysiological concentrations of glucose and that this process can be prevented by an antioxidant. The results of a number of studies in vivo support these findings. In one study, antioxidant treatment was found to normalize plasma glucose levels and to restore insulin secretion in a diabetic rat model [32]. During the generation of oxidative stress, prolonged elevations in blood glucose levels lead to, among other things, the activation of various intracellular metabolic pathways, promoting the formation of advanced glycation end-products (AGEs), auto-oxidation, and an increase in the activity of the sorbitol pathway [33]. A number of important proteins also undergo glycation, such as the Cu,Zn-SOD, one of the most important antioxidant enzymes. Erythrocytes in patients with type-1 diabetes have been found to contain a higher percentage of glycated Cu,Zn-SOD, which is inactivated under hyperglycemic conditions compared with controls, thus leading to oxidative stress [34].

Oxidative Stress and Hypertension

Research has determined that oxidative stress plays an important role in the pathogenesis of hypertension. Several research scientists [35] showed that in response to hypertension the free radical superoxide accumulated in the extracellular space and contributed to the impairment of a normal vascular response by inhibiting the endothelial-derived relaxing factor now known as nitric oxide. In addition, it was shown by adding SOD, an antioxidant enzyme, the impaired vascular response could be restored. These findings undoubtedly prove the impact of changes in NO bioavailability and that increases in ROS reduce the bioavailability of NO resulting in increases in vascular tone. Thus, increases in ROS would be suspected to have a significant influence on blood pressure. There also appears to be a link between oxidative stress and angiotensin II. Angiotensin has been shown to generate ROS and beneficial results occur when using agents that reduce ROS such as ACEI (angiotensin-converting enzyme inhibitors) and/or angiotensin receptor blockers. These results confirm the role of angiotensin II-derived ROS in hypertension [36].

Another major source of ROS in vasculature is NADPH oxidase. Pharmacological or molecular inhibition of NADPH oxidase lowers blood pressure, reduces oxidative stress, and improves vascular response in hypertension. It has been determined that ACEI and angiotensin receptor blockers reduce expression of NADPH oxidase as does supplementation with niacinamide, a precursor to NADPH. By supplying the antioxidant niacinamide one can overcome excessive enzymatic destruction by the enzyme NADPH oxidase [37]. Antioxidant treatment in organ systems such as kidneys and CNS also have beneficial blood pressure lowering effects in hypertension.

This reaffirms that renal and central mechanisms that regulate blood pressure are also affected by oxidative stress. For example, SOD protects against increases in superoxide and endothelial dysfunction produced by angiotensin II. Glutathione peroxidase has also been shown to be of benefit in reducing hypertension and reducing vascular tone [38].

Oxidative Stress and Iron

Unbound transition metals such as iron have long been recognized as a potent source of ROS. This occurs as a result of their reaction with the superoxide ion via the Fenton reaction. The Fenton reaction promotes free iron to react with the superoxide ion, the by-product of energy production from the mitochondria to form the dangerous free radical OH– molecule [39]. In the presence of adequate SOD the reaction is reduced and less harmful H_2O_2 is formed and upon further degradation with the antioxdant enzyme catalase forms H_2O and O_2. The iron binding agent, desferrioxamine, has also been shown to decrease ROS stress from iron accumulation in mitochondria undergoing oxidative stress. These findings support the notion that therapeutic intervention with mitochondrial targeted antioxidants can reduce mitochondrial dysfunction caused by free iron and oxidative stress.

Oxidative Stress and Inflammatory Mediators

Oxidative stress aids in the breakdown of arachidonic acid (AA) in response to inflammation and cell injury. This inflammatory reaction activates phospholipases which then act on phospholipids to form AA. AA is an inflammatory molecule and the processes associated with inflammatory responses are complex and often involve ROS. There are many mediators that initiate and amplify the inflammatory response such as histamine, bradykinin, serotonin, proinflammatory cytokines [interleukin-1B (IL-1b) and tumor necrosis factor (TNF-alpha)], inflammatory cells (eosinophils, macrophages) and metabolic products of AA (thomboxane A(2), PGs, and leukotrienes) [40]. The PGs, thromboxanes, and leukotrienes constitute a rapidly growing family of compounds, all of which are oxygenated derivatives of certain polyunsaturated fatty acids, such as AA. Most of these metabolites are biologically very potent substances, displaying a wide variety of actions in many different biological systems. In addition, isoprostanes are PG-like compounds formed in vivo from the free radical-catalyzed peroxidation of essential fatty acids (primarily AA) without the direct action of cyclooxygenase (COX) enzyme. COX activity produces H_2O_2 which may nonenzymatically produce isoprostanes. A large body of evidence indicates that measurement of F2-isoprostanes, specific PG F2-like compounds derived from the nonenzymatic peroxidation of AA, is a reliable biomarker of oxidant stress in the body [41].

Oxidative Stress and Sepsis

In sepsis, there are several potential sources of ROS, including the mitochondrial respiratory electron transport chain, xanthine oxidase activation as a result of ischemia and reperfusion, the respiratory burst associated with neutrophil activation, and AA metabolism. Activated neutrophils produce superoxide as a cytotoxic agent as part of the respiratory burst via the action of membrane-bound NADPH oxidase on molecular oxygen. Neutrophils also produce the free radical nitric oxide, which can react with superoxide to produce peroxynitrite, itself a powerful oxidant, which may further proceed to form the hydroxyl radical. Under ischemic conditions followed by subsequent reperfusion, the enzyme xanthine oxidase catalyzes the formation of uric acid with the coproduction of superoxide. Superoxide release results in the recruitment and activation of neutrophils and their adherence to endothelial cells, which stimulates the formation of xanthine oxidase in the endothelium, with further superoxide production [42]. During oxidative stress, damage mediated by ROS can occur. Oxidation of DNA and proteins may take place, along with membrane damage, because of lipid peroxidation, leading to alterations in membrane permeability, modification of protein structure, and functional changes. Oxidative damage to the mitochondrial membrane can also occur, resulting in membrane depolarization and the uncoupling of oxidative phosphorylation, with altered cellular respiration [43]. This can ultimately lead to mitochondrial damage, with release of cytochrome C activation of caspases and finally apoptosis.

Oxidative Stress and Aging

Accumulating evidence supports a role of oxidative stress in aging. In as much as mitochondria are the main source of free radicals produced during energy, it was theorized some 30 plus years ago that their dysfunction could be responsible for aging due to progressive decline from molecular oxidative damage. However, the role of mitochondria in the model of senescence has been largely discarded because earlier research has failed to confirm this theory. Instead, the discovery of telomeres, the ends of chromosomes that shorten with repeated cell division, suggests that senescence is the result of the number and length of telomeres and that determines the biological clock and as such is not compatible with ROS/RSN-derived molecular damage as a cause of aging [23]. On the other hand, senescent cells display mitochondrial dysfunction characterized by lower mitochondrial membrane potential, mitochondrial DNA damage (mtDNA), and increased superoxide production [23]. This is evident by improvement of mitochondrial function by antioxidant intervention and extension of lifespan. It appears that targeting antioxidants directly to the mitochondria counteracts telomere shortening in cells undergoing mild oxidative stress [23]. Mitochondrial damage has also been shown to decrease the replicative life span of cells. From this information it appears that mitochondrial ROS/RSN do play a part in telomere-dependent replicative senescence although the level and

importance have yet to be defined. Oxidative stress in aging remains one of the most popular theories of aging at the cellular levels. The imbalance of pro-oxidants to antioxidants causes excessive destructive free radical chemistry. Thiol systems (sulfur-type antioxidants) are important in the control of these processes, both by protecting against damage and serving in redox signaling mechanisms to sense danger and repair the damage. Studies by a number of research groups in collaboration with the Emory Clinical Biomarkers Laboratory show that the redox state of the central tissue antioxidant, glutathione (GSH), can be measured in plasma and provides a quantitative systemic indicator of oxidative stress [44].

Other Theories of Aging

1. Wear and tear; cell wear is a significant factor in aging tissues.
2. Waste accumulation during aging in cells disrupts normal cell functions.
3. Error catastrophe occurs from aging and damage to transcriptional and translational pathways.
4. Somatic mutations from repeated DNA damage alter genetic information.
5. Cross-linking and glycation of proteins with glucose alter their functions.
6. Dysfunction and impaired regulation of gene activation and repression mechanisms.
7. Increased metabolic rate as lifespan inversely correlates with it in mammals.
8. Neuroendocrine dysfunction controls normal physiological homeostasis.
9. Immune dysfunction; decline in immune function leads to decreased resistance to disease and infections and increases in autoimmunity [45].

Benefits of Oxidative Stress

Exposure to mild oxidative stress causes an increase in synthesis of antioxidant enzymes and other defenses. These responses help protect the cell against cell damage and more oxidative damage. This is accomplished by an increase in regulation of transcription and redox regulation of protein binding to mRNA which may increase antioxidant enzymes such as catalase activity. This is accomplished through redox-sensitive transcription factors such as NF-kB [46]. Benefits of oxidative stress include adaptation and promotion of cellular repair. Adaptation occurs when oxidative stress signals transcription factors such as NF-kB and AP-1 to upregulate antioxidant defenses and/or to increase cellular resistance to oxidative stress. This may take the form of increases in the numbers of mitochondria which would have the effect of increasing the total energy of a tissue. This commonly happens in cardiac muscle when more energy is needed to maintain muscular contractions. Hypertrophy is another adaptation that occurs in muscular tissue (skeletal, cardiac) which again

allows the tissue to respond to oxidative stress by increasing the strength of contractions. The same reaction occurs when exercise is used to build muscle tissue. Skeletal muscular contraction, growth, differentiation, and adaptation all occur as a result of mild oxidative stress [47].

Cellular replacement and wound repair are other indications where transcription factors and oxidative stress are necessary. In the intestinal tract NF-kB acts to control the maintenance of tissue-immune homeostasis. In normal cells, this is very important. In pathological states where there is excessive oxidative stress, suppression of NF-kB is important in controlling excessive inflammation and disease. This dual action of NF-kB demonstrates the importance it has in regulating the immune response in both normal and disease conditions.

Summary

Despite the benefits of oxygen there remains the fundamental problem of how to protect cells and specifically mitochondria from oxygen toxicity and oxidative stress. Oxidative stress occurs during disease and the extent to which it causes significant damage depends on the antioxidant status of the animal and the disease process. When persistent and inappropriate levels occur, oxidative stress can damage cells and alter various physiological processes including an increase in aging. The body responds to oxidative stress in many ways, including induction of intracellular and intercellular signaling pathways, genetic alterations, and changes in physiological function and adaptation. These changes are necessary for cells and animals to evolve. The use of antioxidants to reduce excessive and inappropriate oxidative stress is often beneficial but not in all cases. Inappropriate and excessive antioxidant supplementation may impede the body's ability to adapt; thus during cell repair and tissue growth, excessive antioxidant supplementation may be counterproductive. Where antioxidant therapy appears beneficial is in diseases aggravated by excessive and inappropriate oxidant production.

It is much more appropriate to use multiple antioxidants than single ones. These should include both enzymatic antioxidants such as SOD and supplemental nonenzymatic antioxidants such as vitamins A, B, C, D3, and E at supraphysiological doses. Minerals such as selenium, magnesium, and zinc, along with thiol (sulfur) amino acid supplements that increase cellular glutathione (i.e., SAM-e, alpha lipoic acid, taurine, N-acetylcysteine, reduced glutathione) also benefit cells from oxidant damage. Additional benefits can be obtained with coenzyme Q10, and numerous other proteins including l-carnitine, l-carnosine, chondroitin, and glucosamine targeted for specific tissues. In addition, hormones such as melatonin, DHEA, iron binders such as lactoferrin, and omega-3 fatty acids all show the ability to reduce the damaging effects of oxidative stress on cells. Recently, it has been verified that diets high in omega-3 fatty acids reduce aging and increase longevity by altering the shortening of telomeres, a known contributor to aging. Other beneficial supplements include many polyphenols and flavonoids such as green tea extract (catechins ECGC), grape seed

extract (proanthocyanidins), quercetin, reservatrol, tumeric, bilberry, and silymarin which all have various antioxidant and anti-inflammatory effects. For a more complete listing of supplements that reduce or alter oxidative stress please consult the table at the end of the book.

Adaptogens, herbal extracts are nontoxic substances that improve the body's ability to handle stress have also demonstrated the ability to reduce oxidative stress. Such adaptogens include Gingseng (eleutherococcus), licorice (Glycyrrhiza), Goldenseal (Hydrastis), Astragalus, Hawthorn, Golden Root (Rhodiola), Ashwandgha, and Schizandra and are often formulated in a combination of products but none presently exists for use in animals such as there is for people (i.e., Prime One). For a complete summary of supplements that alter oxidative stress please consult the table at the end of the book.

References

1. Miwa S, Beckman KB, Muller FL. Oxidative stress in aging. Totowa: Humana Press; 2008: pp. 4–9
2. Halliwell B, Gutterridge JMC. Free radicals in biology and medicine. Oxford: Oxford University Press; 2003: pp. 62–66
3. Southern PA, Powis G. Free radicals in medicine. 1. Clinical nature and biological reactions. Mayo Clin Proc 1988;63(4): 381–389
4. Riley PA. Free radicals in biology: Oxidative stress and the effects of ionizing radiation. Int J Radiat Biol 1994;665(1): 27–33
5. Vajdovich P. Oxidative stress: The role of mitochondria, free radicals and antioxidants. Vet Clin North Am 2008;1(1): 50–54
6. Dröge W. Free radical in the physiological control of cell function. Physiol Rev 2002;82(1): 57–89
7. Mates JM, Segura JA, Alonso J et al. Intracellular redox status and oxidative stress: Implications for cell proliferation, apoptosis and carcinogenesis. Arch Toxicol 2008;82(5): 273–299
8. Haddad JJ. Antioxidant and prooxidant mechanism in the regulation of redox(y)-sensitive transcription factors. Cell Signal 2002;14(11): 879–897
9. Chen F, Castranova V, Shi X. New Insights into the role of nuclear factor-kappa B in cell growth regulation. Am J Pathol 2001;159(2): 387–397
10. Klaunig JE, Kamendulus LM. The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol 2004;44: 239–267
11. Breithaupt TB, Vazquez A, Baez I et al. The suppression of T-cell function and NF (kappa) B expression by serine protease inhibitors is blocked by N-acetycysteine. Cell Immunol 1996;173(1): 124–130
12. Conner EM, Grisham MB. Inflammation, free radicals and antioxidants. Nutrition 1996;12(4): 274–714
13. Center SA. Metabolic, antioxidant, nutraceutical, probiotic and herbal therapies relating to the management of hepatobiliary disorders. Vet Clin North Am Small Anim Pract 2004;34(1): 67–132
14. Sasaki K, Ma Z, Khatlani TS et al. Evaluation of Serum Amyloid A (SAA) as an inflammatory marker. J Vet Med Sci 2003 Apr;65(4): 545–548
15. Milesi MA. Oxidative, diseases and antioxidants. Agro Food Ind Hi Tech 2006;25(7): 4–9
16. Halliwell B, Gutterridge JMC. Free radicals in biology and medicine. Oxford: Oxford University Press; 2003: pp. 247–251

17. Zimmerman KC, Gree DR. How cell die: Apoptosis pathways. J Allergy Clin Immunol 2001;108(4 suppl): S99–S103
18. Tsudo M. Cytokines and disease. Rinsho Byori 1994;42(8): 821–824
19. Lovschall H, Mosekilde L. Apoptosis: Cellular and clinical aspects. Nord Med 1997;112(4): 133–137
20. Susin SA, Zamzami N, Kroemer G. Mitochondria as regulators of apoptosis: Doubt no more. Biochem Biophys Acta 1998;1366(1–2): 151–165
21. Klaunig JE, Kamendulus LM. The role of oxidative stress in carcinogenesis. Annu Rev Pharmacol Toxicol 2004;44: 239–267
22. Lopez-Torres M, Barja G. Oxidative stress in aging. Totowa: Humana Press; 2008: pp. 149–158
23. Vierra HL, Kroemer G. Pathophysiology of mitochondrial cell death. Cell Mol Life Sci 1999;56(11–12): 971–976
24. Blackwell TS, Christina JW. The role of nuclear factor-kappa B in cytokine gene regulation. Am J Respir Cell Mol Biol 1997;17(1): 3–9
25. Lo CW. Genes, gene knockout, and mutations in the analysis of gap junction. Dev Genet 1999;24(1–2): 1–4
26. Yamaski H, Krutovshikh V, Mesnil M et al. Role of connexin (gap junction) genes in cell growth and carcinogenesis. CR Acad Sci III 1999;322(2–3): 151–159
27. Trosk JE, Ruch RJ. Gap junctions as targets for cancer chemoprevention and chemotherapy. Curr Drug Targets 2002;3(6): 465–482
28. Stahl W, Sies H. The role of carotenoids and retinoids in gap junctional communication. Int J Vitam Nutr Res 1998;68(6): 354–359
29. Halliwell B, Gutterridge JMC. Free radicals in biology and medicine. Oxford: Oxford University Press; 2003: pp. 140–151
30. Poitout V, Robertson RP. Minireview: Secondary beta cell failure in type 2 diabetes – a convergence of glucotoxicity and lipotoxicity. Endocrinology. 2002 Feb;143(2): 339–342
31. Martino L, Novelli M, Masini M et al. Dehydroascorbate protection against dioxin-induced toxicity in the beta-cell line INS-1E. Toxicol Lett 2009;189(1): 27–34
32. Osawa T, Kato Y. Protective role of antioxidative food factors in oxidative stress caused by hyperglycemia. Ann N Y Acad Sci 2005 Jun;1043: 440–451
33. Du X, Edelstein D, Du XL et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycemic damage. Nature 2000;404: 787–790
34. Wei EP, Komtos HA, Christman CW et al. Superoxide generation and reversal of acetylcholine-induced cerebral arteriolar dilation after acute hypertension. Circ Res 1985;57: 781–787
35. Laursen JB, Rajagopalan S, Galis Z et al. Role of superoxide in angiotensin II induced but not catocholamine induced hypertension. Circulation 1997;95: 588–593
36. Vaziri ND, Wang XQ, Oveisi F et al. Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. Hypertension 2000;36: 142–146
37. Halliwell B, Gutterridge JMC. Free Radicals in biology and medicine. Oxford: Oxford University Press; 2003: pp. 129–140
38. Mandelker L. The natural activities of cells, the role of reaction oxygen species and their relation to antioxidants, nutraceuticals, botanicals and other biological therapies. Vet Clin North Am Small Anim Pract 2004;34(1): 39–60
39. Heller, Koch T, Schmeck J et al. Lipid mediators in inflammatory disorders. Drugs 1998;55(4): 487–496
40. Halliwell B, Gutterridge JMC. Free Radicals in biology and medicine. Oxford: Oxford University Press; 2003: pp. 217, 307, 411
41. Macdonald J, Galley HF. Oxidative stress and gene expression in sepsis. N.R. Webster Academic Unit of Anesthesia and Intensive Care, Institute of Medical Sciences; Abstract: Medline Br J Anaest 2003;90(2): 221–232
42. Dare AJ, Phillips AR, Hickey AJ et al. A systematic review of experimental treatments for mitochondrial dysfunction in sepsis and multiple organ dysfunction syndrome. Free Radic Biol Med. 2009;47(11): 1517–1525

43. Rinaldi S, Landucci F, De Gaudio AR. Antioxidant therapy in critically septic patients. Curr Drug Targets 2009;10(9): 872–880
44. Passos J, Von Zglinicki T. Oxidative stress in aging. Totowa: Humana Press; 2008: pp. 39–50
45. Jones DR. Extracellular redox state: Refining the definition of oxidative stress in aging. Rejuvenation Res 2006 Summer;9(2): 169–181
46. Miwa S, Muller FL, Beckman KB. Oxidative stress in aging, Introduction. Totowa: Humana Press; 2008: p. 7
47. Halliwell B, Gutterridge JMC. Free radicals in biology and medicine. Oxford: Oxford University Press; 2003: pp. 619–624
48. Dröge W. Free radical in the physiological control of cell function. Physiol Rev 2002;82(1): 45–97
49. Hakim J. Pharmacologic control of intracellular signaling pathways: From research to therapy. J Cardiovasc Pharmacol 1995;25 (suppl 2): S106–S113