Oxidative stress

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In this issue of the *Southwest Respiratory and Critical Care Chronicles*, Meda and coauthors review oxidative stress in critically ill patients.[1] They provide a detailed summary of oxidation reactions and the cellular damage associated with oxidative stress, discuss the importance of balance between endogenous and exogenous oxidants and antioxidants to maintain physiological processes and to prevent injury, review glutathione metabolism, and summarize recent studies on the enzyme RalA Binding Protein 1 (RALBP1, also called Rlip) involved in the transport of glutathione adducts out cells for metabolism and excretion.

The term oxidative stress refers to self-amplifying free radical chain reactions that damage biomolecules. Free radicals contain unpaired electrons, usually in outer orbitals, and have important functions in normal cellular physiology, including oxidative phosphorylation and cellular signaling.[1] One to two percent of the oxygen consumed by mitochondria normally results in the formation of reactive oxygen species; mitochondrial dysfunction results in an increase in reactive molecules within the mitochondria.[2,3] Oxidative stress develops when electron deficient molecules which are reactive enough to remove electrons from adjacent molecules accumulate inside cells or in extracellular spaces. Exogenous oxidants and acute stress, such as sepsis, tissue hypoxia, and ischemia-reperfusion, initiate the formation of free radicals. This chain reaction is propagated by the formation of lipid hydroperoxides and usually involves lipid bilayers. Free radicals damage intracellular membranes, proteins, and nucleic acids, and these complex biochemical events contribute to the development of multiorgan failure in critically ill patients.

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Free radical scavenging antioxidants, such as vitamin D, vitamin C, vitamin K, and vitamin D, are present at varying levels in human tissues. Metals are an important source of unimpaired electrons, and metal binding proteins are excellent antioxidants. Critical issues in oxidative stress include an imbalance between the levels of oxidants and antioxidants and the possibility that under certain conditions antioxidants can become oxidants. Increased oxidative stress can be identified by the detection of biomarkers, such as reactive oxygen species, free radicals, and inflammatory cytokines.[1]

Glutathione is a sulfhydryl containing peptide which is the dominant nonprotein thiol in eukaryotic cells. The SH group on glutathione has electrons which can react with electron poor biochemical structures, such as lipids, in the cell and thus limit lipid, DNA, and protein damage. The compounds (glutathione adducts) formed by this reaction accumulate inside cells and have the potential to become toxic molecules.[1] These chemicals must be transported out of the cell, and this is accomplished through ATP dependent plasma membrane transporters. The extracellular compounds are then metabolized by cell surface enzymes and excreted in the kidneys as mercapturic acid. The Ral-binding protein 1 functions as a membrane transporter for these compounds, and mice without this gene have increased levels of lipid hydroperoxides and 4-hydroxynonenal.[1]

Several acute disorders, including sepsis, in critically ill patients are associated with oxidative stress. The initial events in sepsis involve leukocyte migration into sites of infection, the ingestion of bacteria, and the formation of phagolysosomes which kill bacteria with enzymes and free radicals.[4] Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase,

an enzyme complex bound to the neutrophil membrane, produces superoxide free radicals by transferring electrons from NADPH to oxygen during oxidative bursts associated with phagocytosis. The superoxide moves into the cell into phagolysosomes to provide microbiocidal activity. However, some free radicals are released into the extracellular space, and this can lead to the formation of other reactive oxygen species, including hydrogen peroxide, hydroxyl radicals, and hypochlorite (HOCL). In addition, neutrophils release granules which contain enzymes, such as elastase, myeloperoxidase, and lactoferrin, into the inflammatory foci. Some neutrophils undergo apoptosis or necrosis following these interactions with pathogens. Other neutrophils lose the integrity of their nuclear membranes which allows nuclear DNA (chromatin) and protein in granules in the cytoplasm to mix.[5] These chromatin "threads" form complex webs, and after the cellular membrane deteriorates are released as extracellular traps (or NETS). These traps bind to and kill bacteria and fungi.

Neutrophils adherent to the endothelium in vessels can cause direct injury to endothelial cells through oxidation; endothelial cells in turn also produce reactive oxygen species. This process can cause gaps in the endothelium resulting in increased permeability and extracellular edema. Neutrophils also release microparticles which contain active enzymes, such as myeloperoxidase that produces reactive molecules which damage both bacteria and other nearby structures.[6] Neutrophils adjacent to erythrocytes can cause significant changes in erythrocyte structure and metabolism.[7] These erythrocytes have a more spherical morphology with spikes in the cellular membrane and have decreased transit through small vessels. Erythrocytes can also form reactive oxygen species secondary to auto-oxygenation of hemoglobin. These complex events occur early in sepsis and are associated with a heterogeneous microvasculature, decreased flow in some vessels, and increased permeability with edema formation. There are complex morphological and functional changes in leukocytes and erythrocytes. Sepsis causes mitochondrial dysfunction in multiple (or all) tissues. This decreases the formation of ATP and increases the formation of reactive oxygen species because of decreased electron flow through the mitochondrial chain. The formation and "leakage" of intracellular reactive oxygen species, such as super oxide anion and hydroxyl radicals and reactive the nitrogen species, such as peroxynitrite or nitric oxide, can cause tissue injury and trigger apoptosis.

In summary, multiple events occur during the initial host defense response in patients with sepsis. Infectious foci contain bacteria, bacterial products such as enzymes and lipopolysaccharides, cytokines, neutrophils, enzymes from neutrophil granules, extracellular traps, reactive oxygen and nitrogen species, abnormal erythrocytes, and abnormal endothelial cells. This complex response to infection makes it difficult to determine the initial crucial event(s) needed to clear the bacterial pathogens and to determine whether or not the response to infection is causing "normal" or excessive tissue injury. Oxidative stress is an expected response to an ongoing infection and the proper balance between oxidant and antioxidants presumably drives beneficial host defense responses. Clinical studies with antioxidants have largely been unsuccessful. Meda and coworkers provide the information needed to understand the pathophysiology of acute oxidative stress responses and argue that treatment studies need indicator molecules to monitor oxidant and antioxidant balance. They suggest the Rlip protein levels provide an index of oxidative stress, and this protein needs to be studied in critically ill patients.[1]

Keywords: sepsis, oxidative stress, antioxidants, glutathione, neutrophils

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