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Review Article

Reactive oxygen species and antioxidant defense in human gastrointestinal diseases

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ABSTRACT

Crohn's disease and ulcerative colitis, known together as inflammatory bowel diseases (IBDs), and celiac disease are the most common disorders affecting not only adults but also children. Both IBDs and celiac disease are associated with oxidative stress, which may play a significant role in their etiologies. Reactive oxygen species (ROS) such as superoxide radicals ($O_2^{\bullet -}$), hydroxyl radicals ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are responsible for cell death via oxidation of DNA, proteins, lipids, and almost any other cellular constituent. To protect biological systems from free radical toxicity, several cellular antioxidant defense mechanisms exist to regulate the production of ROS, including enzymatic and nonenzymatic pathways. Superoxide dismutase catalyzes the dismutation of $O_2^{\bullet -}$ to H_2O_2 and oxygen. The glutathione redox cycle involves two enzymes: glutathione peroxidase, which uses glutathione to reduce organic peroxides and H_2O_2; and glutathione reductase, which reduces the oxidized form of glutathione with concomitant oxidation of nicotinamide adenine dinucleotide phosphate. In addition to this cycle, GSH can react directly with free radicals. Studies into the effects of free radicals and antioxidant status in patients with IBDs and celiac disease are scarce, especially in pediatric patients. It is therefore very necessary to conduct additional research studies to confirm previous data about ROS status and antioxidant activities in patients with IBDs and celiac disease, especially in children.

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1. Reactive oxygen species and the antioxidant defense system

Reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet-}$), hydroxyl radicals ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), and singlet oxygen ($^1O_2$) are generated as by-products of normal metabolism in biological systems.[1,2] Low levels of ROS are essential for several physiological processes, including protein phosphorylation, transcription factor activation, cell differentiation, apoptosis, cell immunity, and as secondary messengers in the regulation of cardiac and vascular cell functioning.[3] Excessive ROS may have detrimental effects on target cellular components such as DNA, proteins, and lipids.[4] Accumulative evidence indicates that oxidative stress plays a major role in the initiation and progression of a number of human diseases such as cancer, hyperlipidemia, diabetes mellitus, metabolic disorders, atherosclerosis, cardiovascular diseases (hypertension, ischemic heart disease, chronic heart failure), and neurodegenerative diseases.[5]

Most cell types are capable of generating ROS under certain conditions. However, the major sources of these reactive molecules are phagocytic cells, especially macrophages, Kupffer cells, polymorphonuclear neutrophils (PMNs), endothelial cells, and various epithelial cell types, including enterocytes, hepatocytes, alveolar epithelial cells, and renal tubular epithelial cells.[6]

Mitochondria are the main organelles responsible for the production of ROS during physiological and pathological states. These organelles have their own ROS scavenging mechanisms required for cell survival.[7] Despite this, it has been shown that mitochondria generate ROS at an amount higher than their scavenging capacity.[8] $O_2^{\bullet-}$ is initially formed via a large number of pathways, including normal cellular respiration, the metabolism of arachidonic acid by lipoxygenases and cyclo-oxygenases and from inflammatory and endothelial cells.[9] The main sources of $O_2^{\bullet-}$ are respiratory complexes I (NADH dehydrogenase) and III (ubisemiquinone) located at the inner mitochondrial membrane, which generate a small amount of $O_2^{\bullet-}$ as a side product of electron transport during oxidative phosphorylation.[10] $O_2^{\bullet-}$ is released into the matrix by complex I, whereas it is released into both the matrix and the intermembranous space by complex III. Complex III forms $O_2^{\bullet-}$ during cycling of the electron acceptor ubiquinone, which can donate electrons to molecular oxygen on both the internal and the external face of the mitochondrial inner membrane.[11]

A variety of enzymatic and nonenzymatic processes can generate ROS. Among the most important sources are the reactions catalyzed by the enzymes nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and xanthine oxidase (XO).[6] NADPH oxidase (NOX family of enzymes) is an enzyme complex that is assembled after the activation of phagocytes by microbes or microbial products, such as lipopolysaccharide or various proinflammatory mediators. In resting cells, the components of NADPH oxidase are present in the cytosol and the membranes of various intracellular organelles. Upon cell activation, the components are assembled on a membrane-bound vesicle, which then fuses with the plasma membrane, resulting in the release $O_2^{\bullet-}$ outward into the extracellular milieu and inward into the phagocytic vesicle.[12] The reaction catalyzed by NADPH oxidase is critical for the formation of ROS in macrophages and PMNs. NADPH oxidase, however, is also present in other cell types, including vascular smooth muscle cells and endothelial cells.[13] The liver and the gut are rich sources of xanthine oxidoreductase (XOR), which catalyzes the production of uric acid. XOR exists in two interconvertible forms: XO and xanthine dehydrogenase (XDH).[14] Human XOR exists *in vivo* in the dehydrogenase form but is easily converted to XO by oxidation of the sulfhydryl residues or through proteolysis.[15] Differences are evident between the substrate affinities for the XO and XDH subforms. Additionally, XDH preferentially reduces NAD^+, whereas XO cannot reduce NAD^+, preferring molecular oxygen. Reduction of molecular oxygen by either form of the enzyme yields $O_2^{\bullet-}$ and H_2O_2.[14,15] Under certain circumstances, nitric oxide synthase (NOS) can generate $O_2^{\bullet-}$ in addition to nitric oxide (NO). If the concentration of L-arginine or BH4 is low, or if BH4 is oxidized, NOS becomes uncoupled and generates significant amounts of $O_2^{\bullet-}$.[16] This also occurs when NADPH oxidase activation leads to the oxidation of BH4.[17] In a separate reaction, myeloperoxidase (MPO), which is abundant in phagocytes, catalyzes H_2O_2 to produce HOCl and other oxidizing species.[18] It also utilizes NO to generate ROS, thereby reducing NO bioactivity and increasing oxidative stress.[19]

Peroxynitrite (ONO_2^-) is generated in a diffusion-controlled reaction of $O_2^{\bullet-}$ and NO, potentially causing oxidative damage via the nitration of tissues.[20] ONO_2^- is a key element in resolving the contrasting roles of NO in physiology and pathology.[21] Under proinflammatory conditions, simultaneous production of $O_2^{\bullet-}$ and NO can be strongly activated to increase production 1000-fold, which will increase the formation of peroxinitrite by a factor of 1 million. Without $O_2^{\bullet-}$, the formation of nitrogen dioxide by the reaction of NO with oxygen is miniscule by comparison. There is no requirement for NO and $O_2^{\bullet-}$ to be produced within the same cell to form peroxinitrite, as NO can readily move through membranes and between cells.[9,21]

The enzymatic–nonenzymatic antioxidant cellular defense system plays a key role in protecting biological systems from ROS by regulating the production of free radicals and their metabolites.[22] The primary antioxidant enzymes against superoxide radicals include superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). These enzymes act together in the metabolic pathway of ROS, and altered activity of one enzyme without compensatory changes in others may lead to lipid peroxidation.[9,22,23]

1.1. *Mechanism of $O_2^{\bullet-}$ scavenging*

SODs are enzymes based around a metal cofactor that functions to catalytically convert $O_2^{\bullet-}$ to oxygen (O_2) and H_2O_2.[24] These enzymes can be classified into four groups: iron SOD (Fe-SOD) is found in the chloroplasts of eukaryotic cells; manganese SOD (Mn-SOD) is typically found in mitochondria and can also be found in peroxisomes; copper–zinc SOD (Cu/Zn-SOD), which is usually the most abundant SOD located in the chloroplast, in the cytosol, and in the extracellular space; and nickel SOD (Ni-SOD), which has been isolated from a number of *Streptomyces* bacteria and from cyanobacteria.[25]