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 (O2•−), hydroxyl radicals (•OH), hydrogen peroxide (H2O2), and singlet oxygen (1O2) 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 O2•− to H2O2 and oxygen. The glutathione redox cycle involves two enzymes: glutathione peroxidase, which uses glutathione to reduce organic peroxides and H2O2; 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$_2^•$−), hydroxyl radicals (•OH), hydrogen peroxide (H$_2$O$_2$), and singlet oxygen (¹O$_2$) are generated as by-products of normal metabolism in biological systems. 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. Excessive ROS may have detrimental effects on target cellular components such as DNA, proteins, and lipids. 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.

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. 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. Despite this, it has been shown that mitochondria generate ROS at an amount higher than their scavenging capacity. O$_2^•$− 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. The main sources of O$_2^•$− are respiratory complexes I (NADH dehydrogenase) and III (ubisemiquinone) located at the inner mitochondrial membrane, which generate a small amount of O$_2^•$− as a side product of electron transport during oxidative phosphorylation. O$_2^•$− 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$_2^•$− 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.

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). 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$_2^•$− outward into the extracellular milieu and inward into the phagocytic vesicle. 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. 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). Human XOR exists in vivo in the dehydrogenase form but is easily converted to XO by oxidation of the sulfhydryl residues or through proteolysis. 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$_2^•$− and H$_2$O$_2$. Under certain circumstances, nitric oxide synthase (NOS) can generate O$_2^•$− 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$_2^•$−. This also occurs when NADPH oxidase activation leads to the oxidation of BH4. Peroxynitrite is generated in a diffusion-controlled reaction of O$_2^•$− and NO, potentially causing oxidative damage via the nitration of tissues. ONO$_2$− is a key element in resolving the contrasting roles of NO in physiology and pathology. Under proinflammatory conditions, simultaneous production of O$_2^•$− and NO can be strongly activated to increase production 1000-fold, which will increase the formation of peroxynitrite by a factor of 1 million. Without O$_2^•$−, the formation of nitric oxide by the reaction of NO with oxygen is miniscule by comparison. There is no requirement for NO and O$_2^•$− to be produced within the same cell to form peroxynitrite, as NO can readily move through membranes and between cells.

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. 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.

1.1. Mechanism of O$_2^•$− scavenging

SODs are enzymes based around a metal cofactor that functions to catalytically convert O$_2^•$− to oxygen (O$_2$) and H$_2$O$_2$. 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.
In eukaryotic cells, the intracellular level of $O_2^{•−}$ is regulated by the activities of the two fundamental scavenger enzymes, namely, Cu/Zn-SOD and Mn-SOD. These enzymes catalyze the dismutation of $O_2^{•−}$ to $H_2O_2$ and $O_2$. The Cu/Zn-SOD enzyme, located in cytosol, lysosomes, nucleus, and the mitochondrial intermembranous space, contains two protein subunits—each of which bears an active site containing one Cu and one Zn cation.26 Copper is the redox-active metal, changing between the 2+3+ oxidation states during catalysis, and zinc appears to play a role in overall enzyme stability and in facilitating function independent of pH.27 The proposed enzymatic mechanism for Cu/Zn-SOD follows two stages: the first is the reduction of the oxidized Cu(II) form of the enzyme by superoxide, releasing dioxygen; and in the second, the reduced Cu(I) is oxidized by another superoxide anion and two protons, generating $H_2O_2$. Mn-SOD in eukaryotic cells is expressed mainly in the mitochondria and contains manganese at its active sites. The MnSOD dismutation mechanism involves cycling between oxidized (Mn$^{3+}$) and reduced (Mn$^{2+}$) ions.25

$$O_2^{•−} + Cu(II)/Zn-SOD \rightarrow Cu(I)/Zn-SOD$$

$$O_2^{•−} + Cu(I)/Zn-SOD + 2H^+ \rightarrow H_2O_2 + Cu(II)/Zn-SOD$$

1.2. **Mechanism of $H_2O_2$ scavenging**

The dismutation of $O_2^{•−}$ generates $H_2O_2$, which is usually removed in cells by two types of enzymes, catalase and peroxidases.28

GPx, an enzyme dependent on the micronutrient selenium, plays a crucial role in the reduction of lipid and hydrogen peroxides. If GPx activity is decreased, more $H_2O_2$ is present, leading to direct tissue damage.29 There are four subspecies of GPx: GPx1 is ubiquitous and found in the cytosol of most cells, including red blood cells; GPx2 is also cytosolic but is confined to the gastrointestinal tract; GPx3 occurs in the plasma as a glycoprotein; and GPx4 is found in mitochondria, where it interacts with complex lipids, such as cholesterol and lipoproteins damaged by free radicals.30

CAT is a heme-containing peroxisomal enzyme important in the decomposition of intracellular $H_2O_2$.28 There are three types of CAT: typical or monofunctional catalases such as mammal-type catalases, bifunctional catalase-peroxidases, and pseudo catalases. Mammalian catalases are active as tetramers and show little peroxidase activity, and the target molecules are limited to small organic substrates. The second group, catalase-peroxidases, are bifunctional, acting both as catalase and peroxidase, and can use a variety of organic substances as hydrogen donors. In contrast to monofunctional catalases, catalase-peroxidases are active as dimers or tetramers. Pseudo catalases, also called non-heme manganese-containing catalases, are non-heme catalases containing only three characterized and sequenced enzymes from different bacterial species. Activity is derived from a manganese-rich reaction center rather than a heme group.31,32

Catalases from many species are known to be tetramers of subunits, each of which contains a ferric heme group (Fe-protoheme IX moiety) bound to its active sites.28 Each tetrameric molecule of mammalian catalases contains four molecules of tightly bound NADPH, which does not appear to be essential for the enzymic conversion of $H_2O_2$ to $H_2O$ and $O_2$, but protects catalase against inactivation by $H_2O_2$.23 The enzyme can function in two ways: catalytically, decomposing $H_2O_2$ into water and oxygen (α phase); or peroxidatively, by eliminating $H_2O_2$ by oxidizing alcohols, formate, or nitrate (β phase).34 In eukaryotes, catalase is localized in the peroxisomes. The catalytic mechanism is a two-step reaction: in the first, the heme Fe$^{3+}$ reduces $H_2O_2$ to $H_2O$ and generates a covalent Fe$^{4+}$ = O species with a porphin cation radical, referred to as compound I; in the second, compound I oxidizes a second peroxide molecule to $O_2$ and releases the ferryl oxygen species as $H_2O$. The catalase reaction is basically a dismutation reaction similar to SOD, without the production of free radicals.35

Catalase-Fe$^{3+}$ + $H_2O_2$ → Compound I + $H_2O$

$H_2O_2$ + Compound I → Catalase-Fe$^{3+}$ + $H_2O$ + $O_2$

GPx consists of four protein subunits, each of which contains one atom of selenium, comprising the integral structural component of the enzyme active site.30,36 This selenoprotein catalyzes the reduction of $H_2O_2$ using glutathione (GSH) as the reducing substrate.22 In short, the enzyme catalytic site includes a selenocysteine residue in which the selenium undergoes a redox cycle involving selenol (E-SeH) as the active form that reduces hydrogen peroxides and organic peroxides. The selenol is oxidized to selenenic acid (E-SeOH), which reacts with reduced GSH to form a selenenyl sulfide adduct (E-Se-SG). A second GSH then regenerates the active form of the enzyme by attacking E-Se-SG to form oxidized glutathione (GSSG). Thus, in the overall process, two equivalents of GSH are oxidized to disulfide and water, whereas the hydroperoxide is reduced to the corresponding alcohol.22,37

E-SeH + $H_2O_2$ → E-SeOH + $H_2O$

E-SeOH + GSH → E-Se-SG + $H_2O$

E-Se-SG + GSH → E-SeH + GSSG

The enzymatic activity of both CAT and GPx operates simultaneously in most cells. $H_2O_2$ synthesized by peroxisomal enzymes is mostly eliminated by CAT, whereas $H_2O_2$ arising from mitochondria or by the action of cytosolic Cu/Zn-SOD is eliminated by GPx.22,30

1.3. **Mechanism of *OH scavenging**

*OH is a very short-lived and highly reactive free radical formed by the successive monovalent reduction of $O_2$ in cell metabolism. It can potentially react with all biological molecules such as DNA, proteins, lipids, and almost any constituent of cells.38 Owing to the absence of any enzymatic mechanism for the elimination of this highly reactive ROS, excess production of *OH ultimately leads to cell death.39 It is generally assumed that *OH is synthesized in biological systems in the presence of $H_2O_2$ and iron ions, known as...
Haber–Weiss reaction. The first step involves the reduction of ferric into ferrous ion:

\[ \text{Fe}^{3+} + \text{O}_2^{••} \rightarrow \text{Fe}^{2+} + \text{O}_2 \]

The second step is the Fenton reaction:

\[ \text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \cdot\text{OH} \]

Recent studies have demonstrated that \( \cdot\text{OH} \) is formed not only in vivo under hypoxic conditions, but can be generated in vitro under reducing conditions in the presence of ascorbic acid and iron ions.\(^{40,41}\) An even more novel mechanism is a revelation of the generation of \( \cdot\text{OH} \) catalyzed by ferric ions without any additional redox agent, which can be considered a special case of the Fenton reaction:\(^{42}\)

\[ \text{Fe}^{3+} + \cdot\text{OH}^{•} \rightarrow \text{Fe}^{2+} + \cdot\text{OH} \]

It appears from this that the Fenton reaction is a convenient generator of \( \cdot\text{OH} \) with known implications in health and disease.\(^{43}\) There are many existing studies describing the effect of natural protein extracts and newly synthesized markers as \( \cdot\text{OH} \) scavengers. Nordihydroguaiaretic acid (NDGA), a plant phenolic lignan, scavenges \( \cdot\text{OH} \) efficiently.\(^{44}\) In addition, the reaction of NDGA and \( \cdot\text{OH} \) is predicted to be diffusion-controlled. The first step of this reaction is proposed to occur mainly by a sequential electron proton transfer from NDGA to \( \cdot\text{OH} \), generating a neutral radical of NDGA. After a second oxidation step, this gives a diradical that produces a cyclic compound after a cascade sequential complex reaction.\(^{45}\) The electrochemical studies performed in water support the formation of a cyclic compound (C2) as the main product of the reaction. It is concluded that NDGA can scavenge at least two \( \cdot\text{OH} \). Moreover, aminoantipyrines, pyrazolone derivates, were demonstrated to be highly efficient scavengers of \( \cdot\text{OH} \) owing the mechanism of hydroxylation of the aromatic ring.\(^{45,46}\)

### 1.4. Mechanism of \( \text{ONO}_2^{•−} \) scavenging

Inhibition of peroxynitrite-mediated oxidation reactions by excess NO or \( \text{O}_2^{•−} \) occurs via scavenging \( \cdot\text{OH} \) and nitrogen dioxide (\( \text{NO}_2 \)), which are formed from the decomposition of \( \text{ONO}_2^{•−} \).\(^{47}\) Upon protonation from \( \text{ONO}_2^{•−} \) to peroxynitrurous acid (\( \text{ONOOH} \), \( pK_a = 6.5–6.8 \)), the peroxynitrite anion rapidly decomposes at physiological pH to form nitrate (\( \text{NO}_3 \)), free \( \cdot\text{OH} \), and nitrogen dioxide (\( \text{NO}_2 \)). Recent studies indicate that the yield of \( \cdot\text{OH} \) and \( \text{NO}_2 \) formation reaches approximately 25–35% in the absence of competitive reactions.\(^{48}\) NADH reacts with \( \cdot\text{OH} \) and \( \text{NO}_2 \) formed during the self-decomposition of \( \text{ONO}_2^{•−} \), whereas GSH directly reacts with peroxynitrite at a neutral pH.\(^{49}\) Thus, NADH may only competitively inhibit those reactions that involve \( \cdot\text{OH} \) and \( \text{NO}_2 \) and not peroxynitrite itself.\(^{47}\)

A number of groups have investigated the reaction of uric acid with peroxynitrite. Kukuzawa et al\(^{50}\) have reported that the scavenging of peroxynitrite by uric acid is important in preventing the oxidation of BH4, which would lead to NOS uncoupling.

### 1.5. GSH redox cycle

Glutathione reductase (GR) is an equally important antioxidant enzyme, which plays a critical role in GSH metabolism, reducing glutathione disulfide (GSSG) to the sulfhydryl form, GSH, by the NADPH-dependent mechanism. The function of the enzyme is to keep the cellular concentration of reduced GSH high and that of its oxidized form, GSSG, low.\(^{51,52}\)

Regarding the nonenzymatic antioxidant defense system, GSH is mentioned in this review. GSH, the most abundant nonprotein thiol, is composed of the amino acids glutamine, cysteine, and glycine.\(^{53}\) This tripeptide can exist intracellularly in either an oxidized (GSSG) or reduced (GSH) state.\(^{53}\) Its function is regulation via several mechanisms: GSH nonenzymatically reacts with \( \text{O}_2^{•−} \) and \( \cdot\text{OH} \) is an essential cofactor for a number of enzymes (e.g., as an electron donor for the reduction of \( \text{H}_2\text{O}_2 \) or other peroxides catalyzed by GPx); modulates a variety of protein functions via S-glutathionylation; and has an important role to serve as a carrier/storage form for cysteine.\(^{52,55,56}\) Maintaining optimal GSH/GSSG ratios in the cell is critical to survival—that is, GSH is significantly advantageous over GSSG under healthy physiological conditions.\(^{55,56}\)

GR is a thermostable homodimeric flavoprotein in which each subunit contains four domains. The dimeric nature of the enzyme is critical for its function, because both subunits contribute essential residues to the constitution of the active site. It is noteworthy that different GR isoforms are found not only in the cytosol, but also in the mitochondrial matrix and chloroplasts.\(^{22,57}\) Functionally, GR is an NADPH:GSSG oxidoreductase.\(^{58}\) The enzyme essentially has three substrates, namely NADPH, \( \text{H}^+ \), and GSSG and two products, namely, GSH and \( \text{GSH} \).\(^{59}\) The catalytic cycle of GR has two phases: a reductive and oxidative half-reaction. During the reductive half-reaction, flavin adenine dinucleotide, a prosthetic group of GR, is reduced by NADPH and reducing equivalents are transferred to a reduct active disulfide. In the oxidative half-reaction, the resulting dithiol reacts with the GSSG, which is reduced to form two GSH at the active site of GR.\(^{58}\)

\[ \text{GSSG} + \text{NADPH} + \text{H}^+ \rightarrow 2\text{GSH} + \text{NADP}^+ \]

### 2. Evidence of ROS in human diseases

Currently published scientific studies have demonstrated that ROSs are involved in more than 60 health concerns.\(^{60}\) In rheumatoid arthritis, hydroxyl radicals cause degradation of proteoglycans and \( \text{H}_2\text{O}_2 \) easily inhibits cartilage proteoglycan synthesis by interfering with ATP synthesis. Peroxynitrite and hypochlorous acid may facilitate cartilage damage by inactivating tissue inhibitors of metalloproteinases and reacting with ascorbate. The resulting low levels of ascorbate in synovial fluid can be harmful as it is essential for normal cartilage function.\(^{61,62}\) In carcinogenesis, high reactive hydroxyl radicals cause oxidative DNA damage and peroxynitrite, which causes both oxidative damage and nitration of DNA bases.\(^{63}\) The majority of mutations induced by ROS appear to involve guanine modification, causing guanine (\( \text{G} \)) → thymin (\( \text{T} \)) transversions. If it relates to critical genes such as oncogenes
or tumor suppressor genes, initiation or progression of cancer can result. Various studies on diabetes, one of the most common chronic diseases worldwide, have shown an increased formation of free radicals and decrease in antioxidant potential. Free radicals, especially O₂•−, are formed excessively by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Sustained hyperglycemia may cause decreased radical scavenging by Mn-SOD and the GSH redox cycle.

ROSs are particularly active in neuronal tissue as the excitatory amino acids and neurotransmitters; nevertheless, they are involved in many neurodegenerative processes. In Alzheimer’s diseases, amyloid-β peptide and advanced glycation end products are potential sources of ROS. The toxicity of amyloid-β peptide is attributed to histidine residues. Binding of Cu²⁺ and Fe³⁺ produces toxic chemical reaction, altering the oxidation state of both metals, producing H₂O₂ catalytically in the presence of transition metals, finally producing toxic •OH. Parkinson’s disease is characterized by mutations of α-synuclein protein in substantia nigra. Evidence indicates that mutations in α-synuclein protein have a role in modulating the dopamine activity, which is responsible for ROS production. Dopamine is a very good metal chelator and electron donor with a high tendency to coordinate with Cu²⁺ and Fe³⁺. This binding leads to the reduction of the metals initiating Fenton’s chemistry to generate H₂O₂. Another identified mechanism of ROS synthesis is also examined in multiple sclerosis. Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine, thereby generating large quantities of ROS, including O₂•− and its dismutation product, H₂O₂.

The influence of oxidative stress was demonstrated in the pathogenesis of gastrointestinal diseases. It has been shown that the concentration of ROS is increased in patients with liver diseases such as alcoholic hepatitis and cirrhosis. Colon cancer, as well as acute and chronic pancreatitis, have also been associated with oxidative stress. It has been suggested that oxidative stress may have an important role in the pathogenesis of acquired megacolon. Necrotizing enterocolitis, a severe disorder found in infants, is another disease whose pathogenesis is attributed to oxidative stress as well as Helicobacter pylori infection, an important factor in the pathogenesis of gastric cancer. It is also followed by an increased production of ROS.

3. ROS and antioxidants in intestinal diseases

3.1. Free radicals in inflammatory bowel diseases

Inflammatory bowel diseases (IBDs), which include Crohn’s disease (CrD) and ulcerative colitis (UC), are caused by a chronic and uncontrolled inflammation of the intestinal mucosa, which can affect any part of the gastrointestinal tract. IBDs are most commonly diagnosed in adolescents, young adults, and also in children. The inflammation in UC is limited to the mucosa and submucosa of the colon. It begins in the rectum prior to spreading proximally in a continuous fashion and frequently involves the periappendiceal region. By contrast, CrD affects any part of the gastrointestinal tract, most commonly the terminal ileum or the perianal region.

The precise etiology of IBDs is still not fully clarified, so the number of patients has been increasing worldwide, especially in North America and Western Europe. Various effects such as environmental factors, disease susceptibility genes, and dysregulated immune reactions including ROS may play a considerable role in the pathogenesis of IBDs.

In the gastrointestinal tract, sources of ROS involve xanthine oxidase, amine oxidase, and aldehyde oxidase as well as the NADPH oxidase found in the macrophages of mucosal lamina propria. The mediator of toxicity seems to be •OH. However, it cannot be considered as the only strong oxidizing agent in the gut. Classically activated macrophages produce proinflammatory cytokines and NO through inducible NOS (iNOS). iNOS is a high-output source of NO as it is readily induced by proinflammatory cytokines and is unresponsive to changes in intracellular calcium. NO may mediate both antioxidant and pro-oxidant effects. The activation of guanylate cyclase to produce cGMP should also be mentioned in addition to other beneficial effects: its activation of cGMP-dependent kinases and modulation of calcium levels in the cells mediates and modulates many physiological functions and stress responses. Nitrosative stress acts in prevention of apoptosis by S-nitrosylation of caspases.

In chronic inflammation or relapsing immune activation within the gastrointestinal tract, the pro-oxidant phase begins to dominate, thus defeating the enteric smooth muscles, enteric nerves, and cells in close special contact, interstitial cells of Cajal (ICC), manifesting typical IBD symptoms. This suggests some previously established facts. In animal models of IBD, as well as CrD and UC, increased numbers of mast cells have been found. Functional activity of mast cells is mostly associated with degranulation and the release of heparin, histamine, serotonin, and proteases. Because the activity of proteases is in many ways beneficial to the inactivation of toxins or proinflammatory cytokines and influences activation of matrix metalloproteinase cascades even in nonmast cells, it also shows conflicting effects. In the inflamed mucosa of the intestine, there are a large number of polymorphonuclear leukocytes (PMNLs), monocytes, and lymphocytes. Activation of PMNLs and monocytes may induce enhanced formation of HOCl, which is more toxic than either O₂•− or H₂O₂ and nonspecifically reacts with sulphydryls, polyunsaturated fatty acids, DNA, pyridine, nucleotides, and aromatic amino acids. Intestinal macrophages isolated from CrD patients were shown to be more responsive to stimulation for O₂•− production than peripheral blood monocytes following increased NADPH-oxidase expression. The neutrophils from patients with UC were able to generate increased levels of O₂•− when compared with those of control individuals. Thus, increased O₂•− synthesis can be attributed to monocytes invading into the mucosa and increased vascular endothelium permeability.

It is important to note that in both predominant stimulations of iNOS activity, the formation of peroxynitrite is an inevitable process. It causes damage to enzymes and other structures more easily, either by strong oxidizing participates in radical reactions, inactivation of iron sulfur centers, inactivation by nitration, or by degranulating proteases. It is not surprising that recent studies showed that the mucosa
of patients with active CrD or UC had decreased levels of SOD. Recently, many studies have focused on direct activation of transient receptor potential (TRP) channels by $O_2^{**}$, $H_2O_2$, NO, downstream products of lipid peroxidation such as 4-hydroxynonenal, 4-oxononenal, 9-nitrooleate, dehydrated prostanooids, and by cysteine-modifying agents in a concentration-dependent manner. Since the task of the channels is to provide homeostasis of calcium and magnesium ions, pacemaker activity of the ICCs, and smooth muscle function, it is not surprising that the stress-induced activity of some channels (in particular TRPA1, TRPV1, TRPV4, TRPM4-8) is in the background of the pathophysiological signs of gastrointestinal diseases. For example, it was shown that degranulation of mast cells evoked just by psychological stress activates an “alarm program” in the enteric nervous system to produce symptoms such as diarrhea and abdominal distress. It is well documented that GPx, with respect to its isoforms, is transcriptionally upregulated by oxidative mechanisms and as a part of oxidative stress response. Understandably, in a study on UC patients either in active or remission stage, a significant increase in GPx activity was found in inflamed mucosa. Further studies also confirmed significantly higher plasma GPx levels in patients in the UC and CrD groups than those in the control group; this did not change based on whether the disease was in the active or remission phase.

However, the effects of oxidative stress on neurons differ. In an animal model of colitis, the changes in motility were proven to be due to selective loss of neuronal NOS immunoreactive neurons in myenteric plexus. Representing the opposite situation to that previously stated, via excessive production of NO by iNOS, De Giorgio et al demonstrated that neuronal loss was mediated through a caspase-3-dependent pathway. However, the loss of neurons was also associated with the appearance of eosinophilic and neutrophilic infiltrates into myenteric ganglia. Neuronal injury is also associated with remarkable loss of ICCs in myenteric plexus, which coordinate neuronal activity with gastrointestinal motility and smooth muscle function. Both immunologically activated mast cells and macrophages are found close to ICCs, providing cytoprotection for ICCs through the heme oxidase-1 (HO-1) pathway or mediating further dysfunction through fusion or transgranulation of granules. HO-1 is upregulated mainly in macrophages and has two main effects. First, it increases the expression of c-kit (tyrosine kinase acting as a receptor for stem cell factor) and neuronal NOS, and second, it mediates carbon monoxide cytoprotection.

### 3.2. Antioxidant defense in celiac disease

Celiac disease (CD) is an immune-mediated chronic inflammatory disorder of the upper small intestine induced by gluten and related prolamines in genetically susceptible individuals. As in other autoimmune conditions, environmental, genetic, and immunological factors may be involved in the pathogenesis of CD. In addition to this, oxidative stress is also implicated in the pathogenesis of CD. The results of various explorations showed that gliadin disturbs the pro-oxidant/antioxidant balance of affected individuals through overproduction of ROS. Several in vitro studies have also reported redox imbalance and increased levels of free radicals after the exposure of cells to gliadin. Earlier studies have noted that the activity of SOD markedly increases, whereas the activity of GPx decreases significantly. Selenium deficiency has already been reported in celiac patients with regard to low GPx activity. Stojiljkovic et al showed that the antioxidant capacity of celiac patients is weakened by a depletion of GSH and reduced activities of the GSH-dependent antioxidant enzymes GPx and GR. Conversely, the activity of CAT did not vary significantly in celiac patients with respect to the control group. CAT is most efficient against high $H_2O_2$ concentrations, and more effective protection is given by GPx when the $H_2O_2$ levels are lower.

### 3.3. Antioxidant status in intestinal diseases of children

IBDs and celiac diseases are the most common disorders diagnosed not only in adults but also in children, a currently expanding group affected by these diseases. Nevertheless, data concerning ROS status and antioxidant defense in children patients with UC, CrD, and CD are scarce. The study of Stojiljkovic et al showed increased SOD activity, whereas GPx and GR activities and GSH content were significantly reduced in children with celiac disease. CAT activity in celiac patients did not change in comparison to the control group. In this case, the antioxidant capacity of young celiac patients is significantly reduced, mostly through depletion of GSH. Activation of xanthine oxidase is one of the mechanisms of ROS overproduction in small intestinal mucosa of celiac patients. Earlier unpublished studies showed activation of xanthine oxidase in enterocytes in children with CD, which results in overproduction of ROS and further damage to the mucosa. Children with IBDs had increased GPx activity and GSH content compared with control children. These differences were found primarily in children with CrD.

Children are a very vulnerable group for research, and for this reason there is an important role for ethic codexes for research and consent of parents. Owing to the lack of relevant research data, it is necessary to carry out additional studies to build on the amount of previously conducted studies.

### 4. Conclusion

The role of oxidative stress in pathological changes in the gastrointestinal tract has mostly been studied in adults. It has been suggested that ROS plays an important role in the initiation as well as the progression of IBDs and celiac disease. In the pediatric age group, several epidemiological studies have been published with evidence suggesting that the incidence of IBD has increased recently. A similar trend is also observed in children with celiac disease. It is therefore necessary to continue research on these diseases to improve the quality of life of these patients, especially children.

### Conflicts of interest

The authors declare no potential conflict of interest.
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