

## Review Article

**Reactive oxygen species and antioxidant defense in human gastrointestinal diseases****Peter Patlevič<sup>a</sup>, Janka Vašková<sup>b,\*</sup>, Pavol Švorc Jr<sup>c</sup>, Ladislav Vaško<sup>b</sup>, Pavol Švorc<sup>d</sup>**<sup>a</sup> Department of Ecology, Faculty of Humanities and Natural Sciences, Prešov University in Prešov, Prešov, Slovak Republic<sup>b</sup> Department of Medical and Clinical Biochemistry, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic<sup>c</sup> Department of Physiology and Pathophysiology, Faculty of Medicine, University of Ostrava, Ostrava-Zábřeh, Czech Republic<sup>d</sup> Department of Medical Physiology, Faculty of Medicine, Pavol Jozef Šafárik University in Košice, Košice, Slovak Republic

## ARTICLE INFO

## Article history:

Received 18 May 2016

Received in revised form

11 July 2016

Accepted 25 July 2016

Available online 29 July 2016

## Keywords:

antioxidant enzymes

Crohn's disease

inflammatory bowel diseases

pediatric patients

radicals

## 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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<http://dx.doi.org/10.1016/j.imr.2016.07.004>

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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.<sup>1,2</sup> 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.<sup>3</sup> Excessive ROS may have detrimental effects on target cellular components such as DNA, proteins, and lipids.<sup>4</sup> 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.<sup>5</sup>

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

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.<sup>7</sup> Despite this, it has been shown that mitochondria generate ROS at an amount higher than their scavenging capacity.<sup>8</sup>  $O_2^{\bullet-}$  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.<sup>9</sup> 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.<sup>10</sup>  $O_2^{\bullet-}$  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.<sup>11</sup>

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).<sup>6</sup> 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.<sup>12</sup> 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.<sup>13</sup> 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).<sup>14</sup> Human XOR exists *in vivo* in the dehydrogenase form but is easily converted to XO by oxidation of the sulfhydryl residues or through proteolysis.<sup>15</sup> 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  $H_2O_2$ .<sup>14,15</sup> 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  $O_2^{\bullet-}$ .<sup>16</sup> This also occurs when NADPH oxidase activation leads to the oxidation of BH4.<sup>17</sup> In a separate reaction, myeloperoxidase (MPO), which is abundant in phagocytes, catalyzes  $H_2O_2$  to produce HOCl and other oxidizing species.<sup>18</sup> It also utilizes NO to generate ROS, thereby reducing NO bioactivity and increasing oxidative stress.<sup>19</sup>

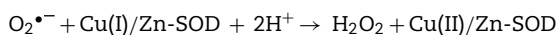
Peroxynitrite ( $ONO_2^-$ ) is generated in a diffusion-controlled reaction of  $O_2^{\bullet-}$  and NO, potentially causing oxidative damage via the nitration of tissues.<sup>20</sup>  $ONO_2^-$  is a key element in resolving the contrasting roles of NO in physiology and pathology.<sup>21</sup> Under 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 peroxynitrite 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 peroxynitrite, as NO can readily move through membranes and between cells.<sup>9,21</sup>

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.<sup>22</sup> 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.<sup>9,22,23</sup>

### 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  $H_2O_2$ .<sup>24</sup> 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.<sup>25</sup>

In eukaryotic cells, the intracellular level of  $O_2^{\bullet-}$  is regulated by the activities of the two fundamental scavenger enzymes, namely, Cu/Zn-SOD and Mn-SOD. These enzymes catalyze the dismutation of  $O_2^{\bullet-}$  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.<sup>26</sup> 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.<sup>27</sup> 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.<sup>25</sup>



### 1.2. Mechanism of $H_2O_2$ scavenging

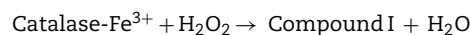
The dismutation of  $O_2^{\bullet-}$  generates  $H_2O_2$ , which is usually removed in cells by two types of enzymes, catalase and peroxidases.<sup>3,28</sup>

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.<sup>29</sup> 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.<sup>30</sup>

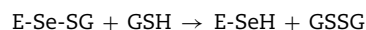
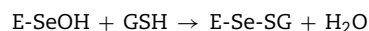
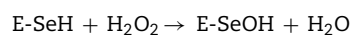
CAT is a heme-containing peroxisomal enzyme important in the decomposition of intracellular  $H_2O_2$ .<sup>28</sup> There are three types of CAT: typical or monofunctional catalases such as mammal-type catalases, bifunctional catalase-peroxidases, and pseudo catalases. Mammalian catalases are active 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.<sup>31,32</sup>

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.<sup>28</sup> 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  $H_2O_2$ .<sup>33</sup> The enzyme can function in two ways: catalytically, decomposing  $H_2O_2$  into water and oxygen ( $\alpha$  phase); or peroxidatively, by eliminating  $H_2O_2$  by oxidizing alcohols, formate, or nitrate ( $\beta$  phase).<sup>34</sup> 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 porphorin  $\pi$ -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.<sup>35</sup>



GPx consists of four protein subunits, each of which contains one atom of selenium, comprising the integral structural component of the enzyme active site.<sup>30,36</sup> This selenoprotein catalyzes the reduction of  $H_2O_2$  using glutathione (GSH) as the reducing substrate.<sup>22</sup> 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.<sup>22,37</sup>

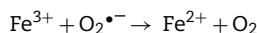


The enzymatic activity of both CAT and GPx operates simultaneously in most cells.  $H_2O_2$  synthesized by peroxisomal enzymes is mostly eliminated by CAT, whereas  $H_2O_2$  arising from mitochondria or by the action of cytosolic Cu/Zn-SOD is eliminated by GPx.<sup>22,30</sup>

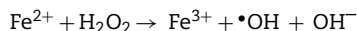
### 1.3. Mechanism of $\bullet OH$ scavenging

$\bullet OH$  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.<sup>38</sup> Owing to the absence of any enzymatic mechanism for the elimination of this highly reactive ROS, excess production of  $\bullet OH$  ultimately leads to cell death.<sup>39</sup> It is generally assumed that  $\bullet OH$  is synthesized in biological systems in the presence of  $H_2O_2$  and iron ions, known as

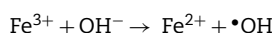
Haber–Weiss reaction. The first step involves the reduction of ferric into ferrous ion:



The second step is the Fenton reaction:



Recent studies have demonstrated that  $\bullet\text{OH}$  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.<sup>40,41</sup> An even more novel mechanism is a revelation of the generation of  $\bullet\text{OH}$  catalyzed by ferric ions without any additional redox agent, which can be considered a special case of the Fenton reaction<sup>42</sup>:



It appears from this that the Fenton reaction is a convenient generator of  $\bullet\text{OH}$  with known implications in health and disease.<sup>43</sup> There are many existing studies describing the effect of natural protein extracts and newly synthesized markers as  $\bullet\text{OH}$  scavengers. Nordihydroguaiaretic acid (NDGA), a plant phenolic lignan, scavenges  $\bullet\text{OH}$  efficiently.<sup>44</sup> In addition, the reaction of NDGA and  $\bullet\text{OH}$  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  $\bullet\text{OH}$ , 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.<sup>45</sup> 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  $\bullet\text{OH}$ . Moreover, aminoantipyridines, pyrazolone derivatives, were demonstrated to be highly efficient scavengers of  $\bullet\text{OH}$  owing the mechanism of hydroxylation of the aromatic ring.<sup>45,46</sup>

#### 1.4. Mechanism of $\text{ONO}_2^-$ scavenging

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

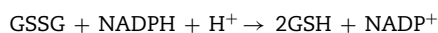
A number of groups have investigated the reaction of uric acid with peroxynitrite. Kuzkaya et al.<sup>50</sup> have reported that the scavenging of peroxynitrite by uric acid is important in preventing the oxidation of BH4, which would lead to NOS uncoupling.

#### 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.<sup>51,52</sup>

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.<sup>53</sup> This tripeptide can exist intracellularly in either an oxidized (GSSG) or reduced (GSH) state.<sup>53</sup> Its function is regulation via several mechanisms: GSH nonenzymatically reacts with  $\text{O}_2^{\bullet-}$  and  $\bullet\text{OH}$ ; 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.<sup>22,52,54</sup> Maintaining optimal GSH/GSSG ratios in the cell is critical to survival—that is, GSH is significantly advantageous over GSSG under healthy physiological conditions.<sup>55,56</sup>

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.<sup>22,57</sup> Functionally, GR is an NADPH:GSSG oxidoreductase.<sup>58</sup> The enzyme essentially has three substrates, namely NADPH,  $\text{H}^+$ , and GSSG and two products, namely, GSH and GSH.<sup>59</sup> 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 redox 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.<sup>58</sup>



## 2. Evidence of ROS in human diseases

Currently published scientific studies have demonstrated that ROSs are involved in more than 60 health concerns.<sup>60</sup> In rheumatoid arthritis, hydroxyl radicals cause degradations 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.<sup>61,62</sup> In carcinogenesis, high reactive hydroxyl radicals cause oxidative DNA damage and peroxynitrite, which causes both oxidative damage and nitration of DNA bases.<sup>63</sup> The majority of mutations induced by ROS appear to involve guanine modification, causing guanine (G)  $\rightarrow$  thymine (T) transversions. If it relates to critical genes such as oncogenes



or tumor suppressor genes, initiation or progression of cancer can result.<sup>64</sup> 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  $O_2^{\bullet-}$ , 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.<sup>65,66</sup>

ROSs are particularly active in neuronal tissue as the excitatory amino acids and neurotransmitters; nevertheless, they are involved in many neurodegenerative processes.<sup>67</sup> In Alzheimer's diseases, amyloid- $\beta$  peptide and advanced glycation end products are potential sources of ROS. The toxicity of amyloid- $\beta$  peptide is attributed to histidine residues. Binding of  $Cu^{2+}$  and  $Fe^{3+}$  produces toxic chemical reaction, altering the oxidation state of both metals, producing  $H_2O_2$  catalytically in the presence of transition metals, finally producing toxic  $\bullet OH$ .<sup>68</sup> Parkinson's disease is characterized by mutations of  $\alpha$ -synuclein protein in substantia nigra. Evidence indicates that mutations in  $\alpha$ -synuclein protein have a role in modulating the dopamine activity, which is responsible for ROS production.<sup>69</sup> Dopamine is a very good metal chelator and electron donor with a high tendency to coordinate with  $Cu^{2+}$  and  $Fe^{3+}$ . This binding leads to the reduction of the metals initiating Fenton's chemistry to generate  $H_2O_2$ .<sup>70</sup> Another identified mechanism of ROS synthesis is also examined in multiple sclerosis.<sup>71</sup> Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine, thereby generating large quantities of ROS, including  $O_2^{\bullet-}$  and its dismutation product,  $H_2O_2$ .<sup>72</sup>

The influence of oxidative stress was demonstrated in the pathogenesis of gastrointestinal diseases.<sup>73</sup> It has been shown that the concentration of ROS is increased in patients with liver diseases such as alcoholic hepatitis and cirrhosis.<sup>74</sup> Colon cancer, as well as acute and chronic pancreatitis, have also been associated with oxidative stress.<sup>75,76</sup> 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.<sup>77</sup>

### 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.<sup>78,79</sup> IBDs are most commonly diagnosed in adolescents, young adults, and also in children.<sup>80</sup> 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.<sup>81</sup> By contrast, CrD affects any part of the gastrointestinal tract,

most commonly the terminal ileum or the perianal region.<sup>82</sup> 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.<sup>83,84</sup>

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  $\bullet OH$ .<sup>85</sup> 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).<sup>86</sup> iNOS is a high-output source of NO as it is readily induced by proinflammatory cytokines and is unresponsive to changes in intracellular calcium.<sup>87</sup> 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.<sup>88</sup>

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.<sup>89–91</sup> Functional activity of mast cells is mostly associated with degranulation and the release of heparin, histamine, serotonin, and proteases.<sup>92</sup> 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.<sup>93</sup> In the inflamed mucosa of the intestine, there are a large number of polymorphonuclear leukocytes (PMNLs), monocytes, and lymphocytes.<sup>94</sup> Activation of PMNLs and monocytes may induce enhanced formation of HOCl, which is more toxic than either  $O_2^{\bullet-}$  or  $H_2O_2$  and nonspecifically reacts with sulfhydryls, polyunsaturated fatty acids, DNA, pyridine, nucleotides, and aromatic amino acids.<sup>95</sup> Intestinal macrophages isolated from CrD patients were shown to be more responsive to stimulation for  $O_2^{\bullet-}$  production than peripheral blood monocytes following increased NADPH-oxidase expression.<sup>96</sup> The neutrophils from patients with UC were able to generate increased levels of  $O_2^{\bullet-}$  when compared with those of control individuals. Thus, increased  $O_2^{\bullet-}$  synthesis can be attributed to monocytes invading into the mucosa and increased vascular endothelium permeability.<sup>97</sup>

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 particulates 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.<sup>83</sup> Recently, many studies have focused on direct activation of transient receptor potential (TRP) channels by  $O_2^{\bullet-}$ ,  $H_2O_2$ , NO, downstream products of lipid peroxidation such as 4-hydroxynonenal, 4-oxononenal, 9-nitrooleate, dehydrated prostanoids, and by cysteine-modifying agents in a concentration-dependent manner.<sup>98–100</sup> 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.<sup>100</sup> 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.<sup>101</sup> 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.<sup>102</sup> 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.<sup>103</sup>

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.<sup>104</sup> Representing the opposite situation to that previously stated, via excessive production of NO by iNOS, De Giorgio et al<sup>105</sup> 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.<sup>106</sup> Neuronal injury is also associated with remarkable loss of ICCs in myenteric plexus, which coordinate neuronal activity with gastrointestinal motility and smooth muscle function.<sup>91</sup> 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.<sup>91,107,108</sup> 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.<sup>107,109</sup>

### 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.<sup>91</sup> 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.<sup>110</sup> 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.<sup>111</sup> Selenium deficiency has already been reported in celiac patients with regard to low GPx activity.<sup>112</sup> Stojiljković 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.<sup>112</sup> 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.<sup>111,113</sup>

### 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.<sup>114</sup> Nevertheless, data concerning ROS status and antioxidant defense in children patients with UC, CrD, and CD are scarce.<sup>115</sup> The study of Stojiljković et al. showed increased SOD activity, whereas GPx and GR activities and GSH content were significantly reduced in children with celiac disease.<sup>116</sup> 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.<sup>111</sup> The results of Boda et al<sup>117</sup> 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.<sup>111,118,119</sup>

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.

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## 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.

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## Conflicts of interest

The authors declare no potential conflict of interest.

## Acknowledgments

This study was supported by Slovak Grant Agency for Science VEGA No. 1/0782/15.

## REFERENCES

- Sato H, Shibata H, Shimizu T, Shibata S, Toriumi H, Ebine T, et al. Differential cellular localization of antioxidant enzymes in the trigeminal ganglion. *Neuroscience* 2013;248:345–58.
- Navarro-Yepes J, Zavala-Flores L, Anandhan A, Wang F, Skotak M, Chandra N, et al. Antioxidant gene therapy against neuronal cell death. *Pharmacol Ther* 2014;142:206–30.
- Rajendran P, Nandakumar N, Rengarajan T, Palaniswami R, Gnanadhas EN, Lakshminarasaiah U, et al. Antioxidants and human diseases. *Clin Chim Acta* 2014;436:332–47.
- Wu JQ, Kosten TR, Zhang XY. Free radicals, antioxidant defense system, and schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 2013;46:200–6.
- Taniyama Y, Griendling KK. Reactive oxygen species in the vasculature. *Hypertension* 2003;42:1075–81.
- Fink MP. Reactive oxygen species as mediators of organ dysfunction caused by sepsis, acute respiratory distress syndrome, or hemorrhagic shock: potential benefits of resuscitation with Ringer's ethyl pyruvate solution. *Curr Clin Opin Nutr Metab Care* 2002;5:167–74.
- Hansen JM, Go YM, Jones DP. Nuclear and mitochondrial compartmentation of oxidative stress and redox signalling. *Annu Rev Pharmacol Toxicol* 2006:215–34.
- Glasauer A, Chandel NS. Targeting antioxidants for cancer therapy. *Biochem Pharmacol* 2014;92:90–101.
- Al-Gubory KH, Garrel C, Faure P, Sugino N. Roles of antioxidant enzymes in corpus luteum rescue from reactive oxygen species-induced oxidative stress. *Reprod Biomed Online* 2012;25:551–60.
- Halliwell B. Antioxidant defence system mechanisms: from the beginning to the end (of beginning). *Free Radic Res* 1999;31:261–72.
- Gill SS, Tuteja N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 2010;48:909–30.
- Ushio-Fukai M, Nakamura Y. Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy. *Cancer Lett* 2008;266:37–52.
- Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin I stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 1994;74:1141–8.
- George J, Struthers AD. Role of urate, xanthine oxidase and the effects of allopurinol in vascular oxidative stress. *Vasc Health Risk Manag* 2009;5:265–72.
- Pacher P, Nivorozhkin A, Szabo C. Therapeutic effects of xanthine oxidase inhibitors: renaissance half a century after the discovery of allopurinol. *Pharmacol Rev* 2006;58:87–114.
- Stuehr D, Pou S, Rosen GM. Oxygen reduction by nitric-oxide synthases. *J Biol Chem* 2001;276:14533–6.
- Landmesser U, Dikalov S, Price SR, McCann L, Fukai T, Holland SM, et al. Oxidation of tetrahydrobiopterin leads to uncoupling of endothelial cell nitric oxide synthase in hypertension. *J Clin Invest* 2003;111:1201–9.
- Winterbourn CC, Vissers MC, Kettle AJ. Myeloperoxidase. *Curr Opin Hematol* 2000;7:53–8.
- Eiserich JP, Baldus S, Brennan ML, Ma W, Zhang C, Tousson A, et al. Myeloperoxidase, a leukocyte-derived vascular NO oxidase. *Science* 2002;296:2391–4.
- Gersch C, Pali SP, Imaram W, Kim KM, Karumanchi A, Angerhofer A, et al. Reactions of peroxynitrite with uric acid. Formation of reactive intermediates, alkylated products and triuret, and in vivo production of triuret under conditions of oxidative stress. *Nucleoside Nucleotides Nucleic Acids* 2009;28:118–49.
- Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 2007;87:315–424.
- Deponte M. Glutathione catalysis and the reaction mechanism of glutathione-dependent enzymes. *Biochim Biophys Acta* 2013;1830:3217–66.
- Sindhi V, Gupta V, Sharma K, Bhatnagar S, Kumari R, Dhaka N. Potential applications of antioxidants — a review. *J Pharm Res* 2013;7:828–35.
- Glasauer A, Chandel NS. Targeting antioxidants for cancer therapy. *Biochem Pharmacol* 2014;2:90–101.
- Abreu IA, Cabelli DE. Superoxide dismutases — a review of the metal-associated mechanistic variations. *Biochim Biophys Acta* 2010;1804:263–74.
- Bonini MG, Gabel SA, Rangelova K, Stadler K, Derose EF, London RE, et al. Direct magnetic resonance evidence for peroxymonocarbonate involvement in the Cu, Zn-superoxide dismutase peroxidase catalytic cycle. *J Biol Chem* 2009;284:14618–27.
- Hough MA, Hasnain SS. Structure of fully reduced bovine copper zinc superoxide dismutase at 1.15 Å. *Structure* 2003;11:937–46.
- Aksoy Y, Balk M, Ogus H, Ozer N. The mechanism of inhibition of human erythrocyte catalase by azide. *Turk J Biol* 2004;28:65–70.
- Gamain B, Arnaud J, Favier A, Camus D, Dive D, Slomianny C. Increase in glutathione peroxidase activity in malaria parasite after selenium supplementation. *Free Radic Biol Med* 1996;21:559–65.
- Arthur JR. The glutathione peroxidases. *Cell Mol Life Sci* 2000;57:1825–35.
- Goyal MM, Basal A. Hydroxyl radical generation theory: a possible explanation of unexplained actions of mammalian catalase. *Int J Biochem Mol Biol* 2012;3:282–9.
- Chakravarti R, Gupta K, Majors A, Ruple L, Aronica M, Stuehr DJ. Novel insights in mammalian catalase heme maturation: effect of NO and thioredoxin-1. *Free Radic Biol Med* 2015;82:105–13.
- Kirkman HN, Rolfo M, Ferraris AM, Ferraris AM, Gaetani GF. Mechanisms of protection of catalase by NADPH. Kinetics and stoichiometry. *J Biol Chem* 1999;274:13908–14.
- Lardiniois OM, Rouxhet PG. Peroxidatic degradation of azide by catalase and irreversible enzyme activation. *Biochim Biophys Acta* 1996;1298:180–90.
- Putnam CHD, Arvai AS, Bourne Y, Tainer JA. Active and inhibited human catalase structures: ligand and NADPH binding and catalytic mechanism. *J Mol Biol* 2000;296:295–309.
- Hayes JD, McLellan LI. Glutathione and glutathione-dependent enzymes represent a co-ordinately regulated defence against oxidative stress. *Free Radic Res* 1999;31:273–300.
- Arner ES. Selenoproteins — what unique properties can arise with selenocysteine in place of cysteine? *Exp Cell Res* 2010;316:1296–303.
- Aprioku JS. Pharmacology of free radicals and the impact of reactive oxygen species on the testis. *J Reprod Infertil* 2013;14:158–72.
- Paiva CN, Bozza MT. Are reactive oxygen species always detrimental to pathogens? *Antioxid Redox Signal* 2014;20:1001–12.
- Michiels C. Physiological and pathological responses to hypoxia. *Am J Pathol* 2004;164:1875–82.



41. Reis KA, Guz G, Ozdemir H, Erten Y, Atalay V, Biczik Z, et al. Intravenous iron therapy as a possible risk factor for atherosclerosis in end-stage renal disease. *Int Heart J* 2005;46:255–64.
42. Bossmann SH, Oliveros E, Kantor M, Niebler S, Bonfill A, Shahin N, et al. New insights into the mechanisms of the thermal Fenton reactions occurring using different iron(II)-complexes. *Water Sci Technol* 2004;49:75–80.
43. Bochi GV, Torbitz VD, Cargnin LP, de Carvalho JA, Gomes P, Moresco RN. An alternative pathway through the Fenton reaction for the formation of advanced oxidation protein products, a new class of inflammatory mediators. *Inflammation* 2014;37:512–21.
44. Lü JM, Nurko J, Weakley SM, Jiang J, Kougius P, Lin PH, et al. Molecular mechanisms and clinical applications of nordihydroguaiaretic acid (NDGA) and its derivatives: an update. *Med Sci Monit* 2010;16:RA93–100.
45. Galano A, Macías-Ruvalcaba NA, Medina Campos ON, Pedraza-Chaverri J. Mechanism of the OH radical scavenging activity of nordihydroguaiaretic acid: a combined theoretical and experimental study. *J Phys Chem B* 2010;114:6625–35.
46. Hwu JR, Hsu CI, Hsu MH, Liang YC, Huang RC, Lee YC. Glycosylated nordihydroguaiaretic acids as anti-cancer agents. *Bioorg Med Chem Lett* 2011;21:380–2.
47. Jourd'heuil D, Jourd'heuil FL, Kutchukian PS, Musah RA, Wink DA, Grisham MB. Reaction of superoxide and nitric oxide with peroxynitrite. Implications for peroxynitrite-mediated oxidation reactions in vivo. *J Biol Chem* 2001;276:28799–805.
48. Coddington JW, Hurst JK, Lyman SV. Hydroxy radical formation during peroxynitrous acid decomposition. *J Am Chem Soc* 1999;121:2438–43.
49. Goldstein S, Czapski G. Reactivity of peroxynitrite versus simultaneous generation of (\*) NO and O(2) (\*) (-) toward NADH. *Chem Res Toxicol* 2000;13:736–41.
50. Kuzkaya N, Weissmann N, Harrison DG, Dikalo S. Interactions of peroxynitrite with uric acid in the presence of ascorbate and thiols: implications for uncoupling endothelial nitric oxide synthase. *Biochem Pharmacol* 2005;70:343–54.
51. Jozefczak M, Remans T, Vangronsveld J, Cuypers A. Glutathione is a key player in metal-induced oxidative stress defenses. *Int J Mol Sci* 2012;13:3145–75.
52. Ribeiro M, Rosenstock TR, Cunha-Oliveira T, Ferreira LI, Oliveira CR, Rego AC. Glutathione redox cycle dysregulation in Huntington's disease knock-in striatal cells. *Free Radic Biol Med* 2012;53:1857–67.
53. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. *Biomed Pharmacother* 2003;57:145–55.
54. Dickinson DA, Forman HJ. Cellular glutathione and thiols metabolism. *Biochem Pharmacol* 2002;64:1019–26.
55. Wu G, Fang YZ, Yang S, Luoton JR, Turner ND. Glutathione metabolism and its implications for health. *J Nutr* 2004;134:489–92.
56. Ballatori N, Krance SM, Notenboom S, Shi S, Tieu K, Hammond CHL. Glutathione dysregulation and the etiology and progression of human diseases. *Biol Chem* 2009;390:191–214.
57. Yan J, Ralston MM, Meng X, Bongiovanni KD, Jones AL, Benndorf R, et al. Glutathione reductase is essential for host defense against bacterial infection. *Free Radic Biol Med* 2013;61:320–32.
58. Berkholz DS, Faber HR, Savvides SN, Karplus PA. Catalytic cycle of humane glutathione reductase near 1 Å resolution. *J Mol Biol* 2008;382:371–84.
59. Outten CE, Cullota VC. Alternative start sites in *Sacharomycetes cerevisiae* GLR1 gene are responsible for mitochondrial and cytosolic isoforms of glutathione reductase. *J Biol Chem* 2004;279:7785–91.
60. Lesgards JF, Gauthier C, Iovanna J, Vidal N, Dolla A, Stocker P. Effect of reactive oxygen and carbonyl species on crucial cellular antioxidant enzymes. *Chem Biol Interact* 2011;190:28–34.
61. Hadjigogos K. The role of free radicals in the pathogenesis of rheumatoid arthritis. *Panminerva Med* 2003;45:7–13.
62. Rasheed Z. Hydroxyl radical damaged Immunoglobulin G in patients with rheumatoid arthritis: biochemical and immunological studies. *Clin Biochem* 2008;41:663–9.
63. Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. *Free Radic Biol Med* 1990;8:583–99.
64. Colis LC, Raychaudhury P, Basu AK. Mutational specificity of  $\gamma$ -radiation-induced guanine–thymine and thymine–guanine intrastrand cross-links in mammalian cells and translesion synthesis past the guanine–thymine lesion by human DNA polymerase  $\eta$ . *Biochemistry* 2008;47:8070–9.
65. Turko IV, Marcondes S, Murad F. Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA:3-oxoacid CoA-transferase. *Am J Physiol Heart Circul Physiol* 2001;281:H2289–94.
66. Matough FA, Budin SB, Hamid ZA, Alwahaibi N, Mohamed J. The role of oxidative stress and antioxidants in diabetic complications. *Sultan Qaboos Univ Med J* 2012;12:5–18.
67. Uttara B, Singh AV, Zamboni P, Mahajan RT. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol* 2009;7:65–74.
68. Murphy MP, LeVine H. Alzheimer's disease and the  $\beta$ -amyloid peptide. *J Alzheimers Dis* 2010;19:311.
69. Stefanis L.  $\alpha$ -Synuclein in Parkinson's disease. *Cold Spring Harb Perspect Med* 2012;2:a009399.
70. Yamato M, Kudo W, Shiba T, Yamada KI, Watanabe T, Utsumi H. Determination of reactive oxygen species associated with the degeneration of dopaminergic neurons during dopamine metabolism. *Free Radic Res* 2010;44:249–57.
71. van Horssen J, Witte ME, Schreiberlt G, de Vries HE. Radical changes in multiple sclerosis pathogenesis. *Biochim Biophys Acta* 2011;1812:141–50.
72. Cantu-Medellin N, Kelley EE. Xanthine oxidoreductase-catalyzed reactive species generation: a process in critical need of reevaluation. *Redox Biol* 2013;1:353–8.
73. Kim YJ, Kim EH, Hahm KB. Oxidative stress in inflammation-based gastrointestinal tract diseases: challenges and opportunities. *J Gastroenterol Hepatol* 2012;27:1004–10.
74. Muriel P. Role of free radicals in liver diseases. *Hepatol Int* 2009;3:526–36.
75. Leung PS, Chan YC. Role of oxidative stress in pancreatic inflammation. *Antioxid Redox Signal* 2009;11:135–65.
76. Sreevalsan S, Safe S. Reactive oxygen species and colorectal cancer. *Curr Colorectal Cancer Rep* 2013;9:350–7.
77. Otamiri T, Sjödaahl R. Oxygen radicals: their role in selected gastrointestinal disorders. *Dig Dis* 1991;9:133–41.
78. Kraus TA, Mayer L. Oral tolerance and inflammatory bowel disease. *Curr Opin Gastroenterol* 2005;21:692–6.
79. Huang H, Vangay P, McKinlay CHE, Knights D. Multi-omics analysis of inflammatory bowel disease. *Immunol Lett* 2014;162:62–8.
80. Sobczak M, Fabisiak A, Murawska N, Wesołowska E, Wierzbicka P, Wlazłowski M, et al. Current overview of extrinsic and intrinsic factors in etiology and progression



- of inflammatory bowel diseases. *Pharmacol Rep* 2014;66:766–75.
81. Jones-Hall YL, Grisham MB. Immunopathological characterization of selected mouse models of inflammatory bowel disease: comparison to human disease. *Pathophysiology* 2014;21:267–88.
  82. Bandzar S, Gupta S, Platt MO. Crohn's disease. A review of treatment options and current research. *Cell Immunol* 2013;286:45–52.
  83. Ishihara T, Tanaka K, Tasaka Y, Namba T, Suzuki J, Ishihara T, et al. Therapeutic effect of lecithinized superoxide dismutase against colitis. *J Pharmacol Exp Ther* 2009;328:152–64.
  84. Huang Y, Xiao S, Jiang Q. Role of Rho kinase signal pathway in inflammatory bowel disease. *Int J Clin Exp Med* 2015;8:3089–97.
  85. Grisham MB, Granger DN. Neutrophil-mediated mucosal injury. Role of reactive oxygen metabolites. *Dig Dis Sci* 1988;33:6S–15S.
  86. Martinez FO, Sica A, Mantovani A, Locati M. Macrophage activation and polarization. *Front Biosci* 2008;13:453–61.
  87. Nathan C, Xie QW. Regulation of biosynthesis of nitric oxide. *J Biol Chem* 1994;269:13725–8.
  88. Kim KM, Kim PKM, Kwon YG, Bai SK, Nam WD, Kim YM. Regulation of apoptosis by nitrosative stress. *J Biochem Mol Biol* 2002;35:127–33.
  89. Hori M, Nobe H, Horiguchi K, Ozaki H. MCP-1 targeting inhibits muscularis macrophage recruitment and intestinal smooth muscle dysfunction in colonic inflammation. *Am J Physiol Cell Physiol* 2008;294:C391–401.
  90. Rumessen JJ. Ultrastructure of interstitial cells of Cajal at the colonic submuscular border in patients with ulcerative colitis. *Gastroenterology* 1996;111:1447–55.
  91. Wang XY, Zarate N, Soderholm JD, Buorgeois JM, Liu LW, Huizinga JD. Ultrastructural injury to interstitial cells of Cajal and communication with mast cells in Crohn's disease. *Neurogastroenterol Motil* 2007;19:349–64.
  92. Mikkelsen HB. Interstitial cells of Cajal, Macrophages and mast cells in the gut musculature: morphology, distribution, spatial and possible functional interactions. *J Cell Mol Med* 2010;14:818–32.
  93. Caughey GH. Mast cell proteases as protective and inflammatory mediators. *Adv Exp Med Biol* 2011;716:212–34.
  94. Brazil J, Louis N, Parkos C. The role of polymorphonuclear leukocyte trafficking in the perpetuation of inflammation during inflammatory bowel disease. *Inflamm Bowel Dis* 2013;19:1556–65.
  95. Kawakami Y, Okada H, Murakami Y, Kawakami T, Ueda Y, Kunii D, et al. Dietary intake, neutrophil fatty acid profile, serum antioxidant vitamins and oxygen radical absorbance capacity in patients with ulcerative colitis. *J Nutr Sci Vitaminol* 2007;53:153–9.
  96. Alzoghbi MA. Concepts of oxidative stress and antioxidant defense in Crohn's disease. *World J Gastroenterol* 2013;19:6540–7.
  97. Naito Y, Takagi T, Yoshikawa T. Neutrophil-dependent oxidative stress in ulcerative colitis. *J Clin Biochem Nutr* 2007;41:18–26.
  98. Taylor-Clark TE. Role of reactive oxygen species and TRP channels in the cough reflex. *Cell Calcium* 2016, <http://dx.doi.org/10.1016/j.ceca.2016.03.007>.
  99. Ogawa N, Kurokawa T, Mori Y. Sensing of redox status by TRP channels. *Cell Calcium* 2016, <http://dx.doi.org/10.1016/j.ceca.2016.02.009>.
  100. Holzer P. TRP channels in the digestive system. *Curr Pharm Biotechnol* 2011;12:24–34.
  101. Lakhan SE, Kirchgessner A. Neuroinflammation in inflammatory bowel disease. *J Neuroinflammation* 2010;7:37.
  102. Lubos E, Loscalzo J, Handy D. Glutathione peroxidase-1 in health and disease: from molecular mechanisms to therapeutic opportunities. *Antioxid Redox Signal* 2011;15:1957–97.
  103. Piechota-Polanczyk A, Fichna J. Review article: the role of oxidative stress in pathogenesis and treatment of inflammatory bowel diseases. *Naunyn Schmiedebergs Arch Pharmacol* 2014;387:605–20.
  104. Auli M, Nasser Y, Ho W, Burgueno JF, Keenan CM, Romero C, et al. Neuromuscular changes in a rat model of colitis. *Auton Neurosci* 2008;141:10–21.
  105. De Giorgio R, Guerrini S, Barbara G, Staghellini V, de Ponti F, Corinaldesi R, et al. Inflammatory neuropathies of the enteric nervous system. *Gastroenterology* 2004;126:1872–83.
  106. Sanovic S, Lamb DP, Blennerhassett MG. Damage to the enteric nervous system in experimental colitis. *Am J Pathol* 1999;155:1051–7.
  107. Choi KM, Gibbons SJ, Nguyen TV, Stolz GI, Lurken MS, Ordög T, et al. Heme oxygenase-1 protects interstitial cells of Cajal from oxidative stress and reverses diabetic gastroparesis. *Gastroenterology* 2008;135:2055–64.
  108. Zarate N, Wang XY, Tougas G, Anvari M, Birch D, Mearin F, et al. Intramuscular interstitial cells of Cajal associated with mast cells survive nitrergic nerves in achalasia. *Neurogastroenterol Motil* 2006;18:556–68.
  109. Oliveira S, Queiroga CS, Vieira HL. Mitochondria and carbon monoxide: cytoprotection and control of cell metabolism – a role for Ca<sup>2+</sup>? *J Physiol* 2016;594:4131–8.
  110. Lauret E, Rodrigo L. Celiac disease and autoimmune-associated conditions. *Biomed Res Int* 2013;2013:127589.
  111. Ferretti G, Bacchetti T, Masciangelo S, Saturni L. Celiac disease, inflammation and oxidative damage: a nutrigenetic approach. *Nutrients* 2012;4:243–57.
  112. Stojiljković V, Todorović A, Pejić S, Kasapović J, Sačić ZS, Radlović N, et al. Antioxidant status and lipid peroxidation in small intestinal mucosa of children with celiac disease. *Clin Biochem* 2009;42:1431–7.
  113. Stazi AV, Trinti B. Selenium deficiency in celiac disease: risk of autoimmune thyroid diseases. *Minerva Med* 2008;99:643–53.
  114. Stojiljković V, Todorović A, Radlović N, Pejić S, Mladenović M, Kasapović J, et al. Antioxidant enzymes, glutathione and lipid peroxidation in peripheral blood of children affected by coeliac disease. *Ann Clin Biochem* 2007;44:537–43.
  115. Pascual V, Dieli-Crimi R, López-Palacios N, Bodas A, Medrano LM, Núñez C. Inflammatory bowel disease and celiac disease: overlaps and differences. *World J Gastroenterol* 2014;20:4846–56.
  116. Stojiljković V, Pejić S, Kasapović J, Gavrilović L, Stojiljković S, Nikolić D, et al. Glutathione redox cycle in small intestinal mucosa and peripheral blood of pediatric celiac disease patients. *An Acad Bras Cienc* 2012;84:75–84.
  117. Boda M, Németh I, Boda D. The caffeine metabolic ratio as an index of xanthine oxidase activity in clinically active and silent celiac patients. *J Pediatr Gastroenterol Nutr* 1999;29:546–50.
  118. Pavlick KP, Laroux FS, Fuseler J, Wolf RE, Gray L, Hoffman J, et al. Role of reactive metabolites of oxygen and nitrogen in inflammatory bowel disease. *Free Radic Biol Med* 2002;33:311–22.
  119. Hoffenberg EJ, Deutsch J, Smith S, Sokol RJ. Circulating antioxidant concentrations in children with inflammatory bowel disease. *Am J Clin Nutr* 1997;65:1482–8.