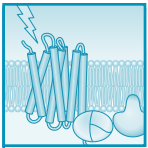


OXIDATIVE STRESS: AN ESSENTIAL FACTOR IN THE PATHOGENESIS OF GASTROINTESTINAL MUCOSAL DISEASES

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Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative Stress: An Essential Factor in the Pathogenesis of Gastrointestinal Mucosal Diseases. *Physiol Rev* 94: 329–354, 2014; doi:10.1152/physrev.00040.2012.—Reactive oxygen species (ROS) are generated as by-products of normal cellular metabolic activities. Super-oxide dismutase, glutathione peroxidase, and catalase are the enzymes involved in protecting cells from the damaging effects of ROS. ROS are produced in response to ultraviolet radiation, cigarette smoking, alcohol, nonsteroidal anti-inflammatory drugs, ischemia-reperfusion injury, chronic infections, and inflammatory disorders. Disruption of normal cellular homeostasis by redox signaling may result in cardiovascular, neurodegenerative diseases and cancer. ROS are produced within the gastrointestinal (GI) tract, but their roles in pathophysiology and disease pathogenesis have not been well studied. Despite the protective barrier provided by the mucosa, ingested materials and microbial pathogens can induce oxidative injury and GI inflammatory responses involving the epithelium and immune/inflammatory cells. The pathogenesis of various GI diseases including peptic ulcers, gastrointestinal cancers, and inflammatory bowel disease is in part due to oxidative stress. Unraveling the signaling events initiated at the cellular level by oxidative free radicals as well as the physiological responses to such stress is important to better understand disease pathogenesis and to develop new therapies to manage a variety of conditions for which current therapies are not always sufficient.

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I. INTRODUCTION

Reactive oxygen species (ROS), also referred to as reactive oxygen intermediates (ROI), are byproducts of normal cellular metabolism. Low and moderate amounts of ROS have beneficial effects on several physiological processes including killing of invading pathogens, wound healing, and tissue repair processes. As discussed in section IV, ROS act as essential signaling molecules. Cancer treatment by chemotherapeutic agents and radiotherapies depend largely on ROS generation to destroy malignant cells by inducing apoptosis. However, disproportionate generation of ROS poses a serious problem to bodily homeostasis and causes oxidative tissue damage. While natural antioxidant pathways can limit the adverse effects of ROS, their levels can be stimulated by many oxidative stressors and maintained

such that they contribute to tissue damage. ROS are produced in response to ultraviolet (UV) radiation, cigarette smoking, alcohol consumption, ingestion of nonsteroidal anti-inflammatory drugs (NSAIDs), and many other exogenous agents. Infections, ischemia-reperfusion (I/R) injury, and various inflammatory processes also result in elevated levels of ROS. Disruption of normal cellular homeostasis by redox signaling contributes to disease in virtually every organ including the development of cancer (FIGURE 1).

The gastrointestinal (GI) tract is a key source of ROS. Despite the protective barrier provided by the epithelial layer, ingested materials and pathogens can cause inflammation by activating the epithelium, polymorphonuclear neutrophils (PMNs), and macrophages to produce inflammatory cytokines and other mediators that contribute further to oxidative stress. Various GI pathological conditions including gastroduodenal ulcers, GI malignancies, and inflammatory bowel disease (IBD) arise in part from oxidative stress. Understanding the signaling events initiated by free radicals as well as the physiological response to such processes is key to furthering our understanding of ROS-mediated GI diseases with the potential to develop novel therapeutic interventions.

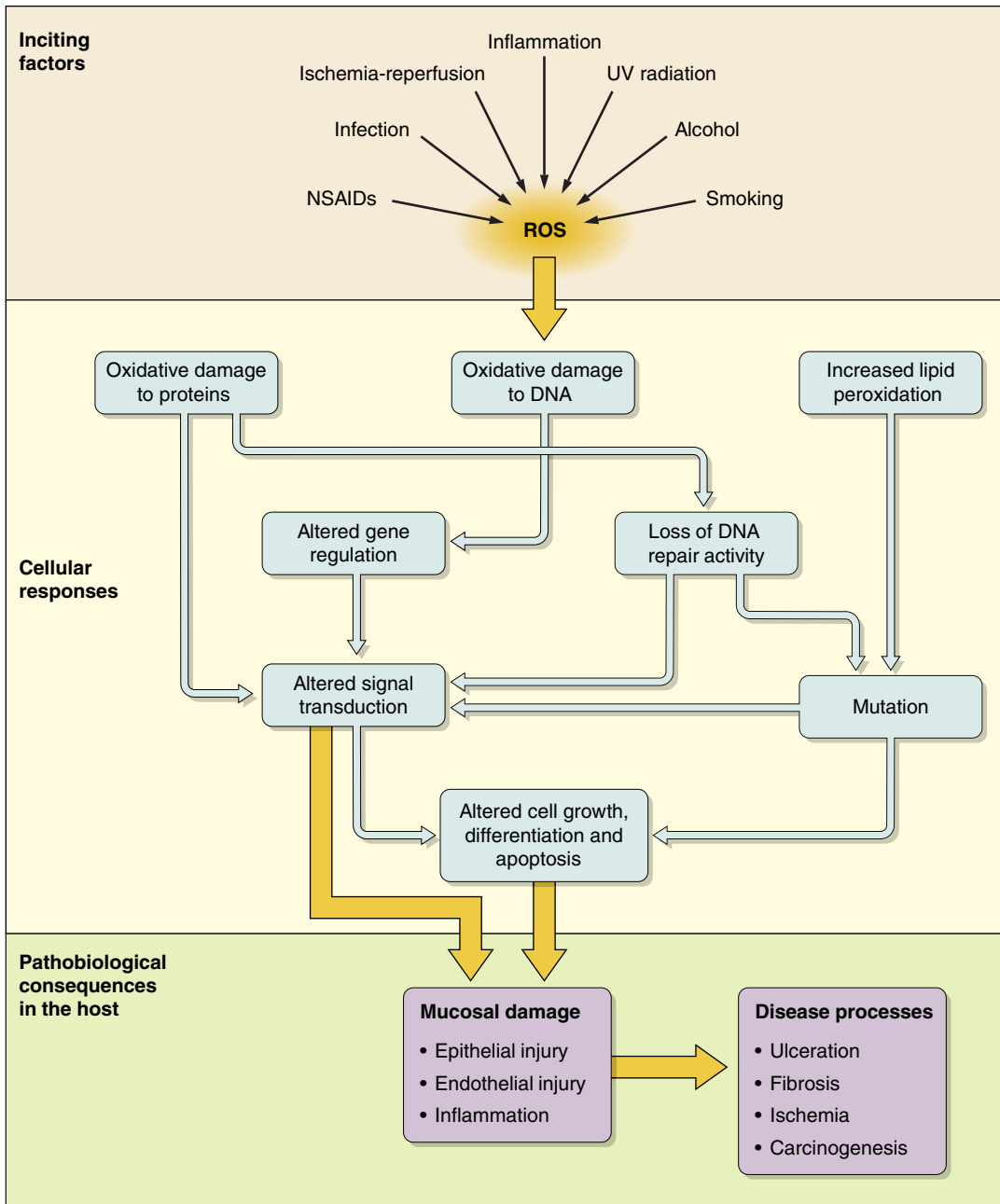


FIGURE 1. Schematic diagram showing the induction of oxidative stress and its pathophysiological effects. Oxidative stress damages internal organs by causing mucosal injury.

II. REACTIVE SPECIES AND THEIR FORMATION

A. ROS and Reactive Nitrogen Species

Molecular oxygen (O_2) is not only essential for the survival of aerobic organisms, its reduction to H_2O via mitochondrial respiration complexes provides ATP, but paradoxically contributes to cell death (164). Partially reduced O_2 , collectively named ROS, are highly reactive and continuously produced as by-products of cellular respiration. ROS are also generated during enzymatic reactions. ROS include radical compounds such as superoxide ($O_2^{\cdot-}$), hydroxyl

radicals (HO^{\cdot}), lipid hydroperoxides, and reactive non-radical compounds including singlet oxygen (1O_2), hydrogen peroxide (H_2O_2), hypochlorous acid ($HOCl$), chloramines ($RNHCl$), and ozone (O_3) (22). These oxygen-centered small molecules containing unpaired valence-shell electrons are unstable and highly reactive with proteins, lipids, carbohydrates, and nucleic acids inside the cells. These interactions can irreversibly inactivate target molecules. The redox state of major cellular antioxidants such as glutathione and thioredoxin are affected by the level of intracellular ROS accumulation. Alterations of the balance between ROS production and the capacity to rapidly detoxify reactive intermediates lead to oxidative stress.

Reactive radical compounds such as nitric oxide ($\cdot\text{NO}$), nitrogen dioxide (NO_2), and nonradical compounds, e.g., peroxyxynitrite (ONOO^-) and dinitrogen trioxide (N_2O_3), are collectively called reactive nitrogen species (RNS). These free radicals are unstable because of the presence of unpaired electrons in their outer electron orbit. RNS is often linked to ROS, e.g., in the formation of peroxyxynitrite causing nitrosative stress. Oxidative and nitrosative stress have been etiologically implicated in a wide variety of disease processes and states: aging, I/R injury, hypertension, atherosclerosis, diabetic neuropathies, renal diseases, neurological diseases including Alzheimer's disease and other forms of dementia, as well as cancers (20, 44, 92, 93, 110, 227). Oxidative stress also contributes to various GI diseases including gastroduodenal ulcers (226), inflammatory bowel disease (105, 223), and GI malignancies such as gastric (146) and colorectal cancer (130).

B. Mechanisms of ROS Generation

1. Endogenous sources

Intracellular compartments including mitochondria, the endoplasmic reticulum, peroxisomes, nuclei, the cytosol, plasma membranes, and even extracellular spaces are capable of ROS generation (13, 238). The mitochondrial electron transport chain is the major site of ROS production in most mammalian cells (237). Enzymes that catalyze ROS-generating chemical reactions are peroxidases, NADPH oxidase, NADPH oxidase isoforms (NOX), xanthine oxidase (XO), lipoxygenases (LOXs), glucose oxidase, myeloperoxidase (MPO), nitric oxide synthase, and cyclooxygenases (COXs) (164, 276).

A) MITOCHONDRIAL RESPIRATORY CHAIN. $\text{O}_2^{\cdot-}$ is the most crucial ROS as it can give rise to several other forms of reactive oxygen intermediates. The inner mitochondrial membrane (IM) contains a series of enzyme complexes referred to as the mitochondrial respiratory chain (MRC). These include complexes I-IV (NADH-ubiquinone oxidoreductase, succinate dehydrogenase, ubiquinol-cytochrome *c* oxidoreductase, and cytochrome *c* oxidase) along with coenzyme Q (CoQ) and a peripheral protein on the outer surface of the inner mitochondrial membrane, cytochrome *c*, which constitute the MRC. Electron leakage from MRC complexes I and III results in reduction of molecular oxygen, thus forming $\text{O}_2^{\cdot-}$ (157). Cytochrome *c* oxidase (complex IV) is the last enzyme component of the MRC which reduces O_2 to two molecules of H_2O via a four-electron reduction (59). Complex IV is not considered to be a biologically relevant source of ROS (17). Rather, studies indicate that cytochrome *c* may act as a mitochondrial antioxidant, oxidizing $\text{O}_2^{\cdot-}$ to O_2 (268). At high cellular O_2 concentration, cytochrome *c* oxidase is in an oxidized state and consumes $\cdot\text{NO}$. However, at low oxygen concentration, $\cdot\text{NO}$ is not used by cytochrome *c* oxidase, leading to $\cdot\text{NO}$ accumulation in the cell (280).

B) RESPIRATORY BURST AND NADPH OXIDASE. Respiratory burst is the process by which phagocytic cells consume large amounts of oxygen during phagocytosis, mainly via activation of NADPH oxidase and release $\text{O}_2^{\cdot-}$ into the extracellular space or phagosomes. NADPH oxidase is a multicomponent enzyme present in the plasma membrane and phagosomes of phagocytes such as monocytes, macrophages, neutrophils, and eosinophils (FIGURE 2, Eq. 1) (12). Phagocytic NADPH oxidase consists of six subunits: membrane-attached gp91^{PHOX} and p22^{PHOX} (PHOX = phagocytic oxidase), cytosolic p67^{PHOX}, p47^{PHOX} and p40^{PHOX}, and Rho GTPases, Rac1 or 2 (258). Activation of NADPH oxidase is caused by relocation of the cytosolic components to the cell membrane. The complex is normally latent in phagocytes but is activated and assembled in the membrane before respiratory burst. p47^{PHOX}, p67^{PHOX}, and either Rac1 or Rac2 can activate the membrane-bound, catalytic core of NADPH oxidase, flavocytochrome *b* (a heterodimer of gp91^{PHOX} and p22^{PHOX}) (140). p40^{PHOX} also regulates NADPH oxidase activity (66).

Six homologs of NADPH oxidase, namely, NOX1, NOX3–5, and DUOX1 and 2 (22, 101) have been identified with diverse intracellular localization. Phagocytic NADPH oxidase (NOX2/gp91^{phox}) and its homologs are collectively called the NOX family of NADPH oxidases. NOX1 and DUOX2 have important roles in GI pathology, especially in *Helicobacter pylori*-induced gastric inflammation, IBD, and tumor development.

C) XANTHINE OXIDASE. Xanthine oxidase (XO), found on the outer surface of the plasma membrane and also in the cytoplasm, is mainly expressed in the liver and small intestinal mucosa within the GI tract (298). It catalyzes oxidation of hypoxanthine (HX) to xanthine and then, to uric acid during purine catabolism (FIGURE 2, Eqs. 2a and 2b) (114). XDH can be converted to XO by utilizing NAD^+ . $\text{O}_2^{\cdot-}$ is generated during oxidation of hypoxanthine to xanthine as well as xanthine to uric acid. Both of these reactions are catalyzed by XO. $\text{O}_2^{\cdot-}$ is not a highly reactive free radical due to its short half-life and is eventually reduced to H_2O_2 . The charged moiety makes it impermeable to lipid membranes which keeps it restricted to its site of origin.

During ischemia, the production of xanthine and XO is greatly enhanced along with the loss of antioxidant enzymes. O_2 is an electron acceptor and cofactor for XO, thus generating $\text{O}_2^{\cdot-}$ and H_2O_2 . The intestinal mucosa has a tremendous capacity to oxidize hypoxanthine by XO (98). Therefore, it is not unexpected that I/R in the gut produces $\text{O}_2^{\cdot-}$ and H_2O_2 , the major ROS contributing to GI injury (256).

D) LIPOXYGENASES. Lipoxygenases (LOX) are nonheme iron enzymes catalyzing dioxygenation of polyenoic fatty acids yielding hydroperoxyl derivatives including hydroper-

	Enzymes/ Reaction name	Reaction catalyzed
Eq. 1	NADPH oxidase	$\text{NADPH} + 2\text{O}_2 \longrightarrow 2\text{O}_2^{\cdot-} + \text{NADP}^+ + \text{H}^+$
Eq. 2a	Xanthine oxidase	$\text{Hypoxanthine} + 2\text{O}_2 + \text{NAD(P)H} \longrightarrow \text{Xanthine} + 2\text{O}_2^{\cdot-} + \text{NAD(P)}^+ + \text{H}^+$
Eq. 2b		$\text{Xanthine} + 2\text{O}_2 + \text{NAD(P)H} \longrightarrow \text{Uric acid} + 2\text{O}_2^{\cdot-} + \text{NAD(P)}^+ + \text{H}^+$
Eq. 3	Lipoxygenases	<p>Diagram illustrating the lipoxygenase cycle: LOX_(ox) converts an unsaturated lipid to a lipid peroxyl radical, which then oxidizes another lipid to a lipid hydroperoxide, regenerating LOX_(red). The cycle is coupled with the reduction of NAD to NAD⁺.</p>
Eq. 4	Myeloperoxidase	$\text{H}_2\text{O}_2 + \text{Cl}^- + \text{H}^+ \longrightarrow \text{HOCl} + \text{H}_2\text{O}$
Eq. 5		<p>Diagram illustrating the myeloperoxidase reaction: HOCl is converted to ¹O₂ and H⁺ + Cl⁻ by the enzyme, while H₂O₂ is converted to H₂O.</p>
Eq. 6	Nitric oxide synthase	$\text{L-arginine} + \text{O}_2 \xrightarrow{2\text{NADPH} + 2\text{H}^+} \text{NO} + \text{citrulline} + 2\text{H}_2\text{O}$
Eq. 7		<p>Diagram illustrating the nitric oxide synthase pathway: L-arginine is converted to NO and citrulline. NO is further oxidized to NO₂⁻, NO₂, and NO₃⁻. NO₂⁻ reacts with RSH to form RSOH and NO₂⁻. NO₂⁻ also reacts with RSH to form RSNO and H⁺. NO₂⁻ is further oxidized to OONO⁻, OONOH, and CO₃⁻ radical, which then forms NO₂.</p>
Eq. 8	Cyclo-oxygenase	$\text{Arachidonic acid} + 2\text{O}_2 \longrightarrow \text{PGG}_2 \longrightarrow \text{PGH}_2$
Eq. 9a	Haber-Weiss Reaction	$\text{Fe}^{3+} + \text{O}_2^{\cdot-} \longrightarrow \text{Fe}^{2+} + \text{O}_2$
Eq. 9b		and
Eq. 9c		$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \longrightarrow \text{Fe}^{3+} + \text{OH}^- + \text{HO}^{\cdot}$
Eq. 10a	Superoxide dismutase	Net Haber-Weiss reaction-
Eq. 10b		$\text{O}_2^{\cdot-} + \text{H}_2\text{O}_2 \longrightarrow \text{HO}^{\cdot} + \text{HO}^{\cdot} + \text{O}_2$
Eq. 11	Glutathione peroxidase	$2\text{GSH} + \text{H}_2\text{O}_2 \longrightarrow \text{GSSG} + 2\text{H}_2\text{O}$
Eq. 12		or
Eq. 12	Glutathione reductase	$2\text{GSH} + \text{ROOH} \longrightarrow \text{GSSG} + \text{ROH} + \text{H}_2\text{O}$
Eq. 13		$\text{GSSG} + \text{NADPH} + \text{H}^+ \longrightarrow 2\text{GSH} + \text{NADP}^+$
Eq. 13	Catalase	$2\text{H}_2\text{O}_2 \longrightarrow 2\text{H}_2\text{O} + \text{O}_2$

FIGURE 2. Major endogenous oxidative enzymatic reactions.

oxyeicosatetraenoic acids (HPETEs) (255). Corresponding hydroxyl derivatives hydroxyeicosatetraenoic acid (HETE), leukotrienes (LT), and lipoxins are produced from HPETEs upon reduction (262). ROS can be generated by oxidation of arachidonic acid (AA) by LOX (87, 208) (**FIGURE 2, Eq. 3**).

AA is the substrate for LOX in animals while linoleic or linolenic acids serve as substrates in plants (259). Five LOX enzymes have been identified in humans that catalyze four different reactions producing fatty acid hydroperoxides (36) and are named based on position of oxygenated residues in arachidonic acid (277). 5-LOX produce proinflammatory leukotrienes (98, 255) in human monocytes and macrophages (323). The fact that LOX contribute to atherosclerosis (271) illustrates the potential importance of these reactions. Their location is cytosolic in neutrophils and nuclear in macrophages in the resting state, but neutrophilic LOXs also move to the nucleus upon stimulation (322). 12/15-LOX are also expressed in macrophages and are involved in atherosclerosis (162, 332). LTs and HETE can directly activate NADPH oxidase leading to ROS production by translocating the $p47^{PHOX}$ subunit to the plasma membrane (264).

Eicosanoids are produced in various cells of the GI tract including leukocytes, epithelial cells, and other mucosal cells (309). 15-LOX-1/-2 is downregulated in human colorectal tumors (118), and administration of 15-LOX-1 has shown anticarcinogenic effects (28). *H. pylori* induce 5-LOX-derived LT production in human gastric epithelial cells (GEC) contributing to the neutrophil infiltration characteristic of the inflammation associated with infection (104). 5-LOX-derived LTs contribute in *H. pylori*-mediated gastric carcinogenesis.

E) MYELOPEROXIDASE. Myeloperoxidase (MPO) is a heme-enzyme localized in lysosomes of neutrophils, macrophages, and monocytes. This enzyme chlorinates H_2O_2 to highly reactive HOCl (**FIGURE 2, Eq. 4**). It also catalyzes oxidation of thiocyanate (SCN^-) to generate another ROS, hypothiocyanite ($OSCN^-$) via a similar reaction (326). MPO normally exists in the ferric (Fe III) form, although it undergoes different stages of activation depending on the ligands, O_2^- or H_2O_2 (235). Lactoperoxidase present in the airway and digestive tract epithelia is also capable of generating $OSCN^-$ (88). HOCl reacts with H_2O_2 to generate singlet oxygen (1O_2) and chloride ion (Cl^-) (**FIGURE 2, Eq. 5**). 1O_2 is not a free radical, but has properties similar to ROS due to its electronic structure.

MPO activity is increased in *H. pylori*-infected subjects (257) and plays a role in the development of *H. pylori*-induced atrophic gastritis, a potential precursor of gastric cancer (250, 338). Increased MPO activity is also found in inflamed mucosa in ulcerative colitis, and this may contrib-

ute to the progression to malignancy associated with this disease (293).

F) NITRIC OXIDE SYNTHASE. Nitric oxide synthase (NOS) is a heme-containing monooxygenase that generates NO. Three different isozymes of NOS have been identified (274), constitutively expressed neuronal NOS (nNOS or NOS I) as well as endothelial NOS (eNOS or NOS III), and endotoxin or cytotoxin-inducible NOS (iNOS or NOS II) (224). All types of NOS catalyze the oxidation of L-arginine to an intermediate, N-hydroxy-L-arginine, followed by generation of L-citrulline and NO (**FIGURE 2, Eq. 6**) (37). At low L-arginine concentrations, L-arginine-uncoupled NOS can react with O_2^- to generate H_2O_2 .

NO is a weak oxidant, but when it combines with O_2^- to generate $OONO^-$, it becomes a potent ROS (155). NO and $OONO^-$ generate very stable nitrite (NO_2^-) and nitrate (NO_3^-) ions which accumulate in cells, leading to the formation of highly reactive intermediates, such as NO_2 , N_2O_3 , or NO (**FIGURE 2, Eq. 7**). These intermediates cause nitration and nitrosation of important biological macromolecules such as DNA, RNA, proteins, and lipids, thereby disrupting their function. 8-Nitroguanine, a nitration product of DNA and RNA, is a potent mutagen and pro-oxidant formed within cells (141). Nitrated lipids are capable of eliciting varied physiological responses and can also produce diffusible NO.

NOS are expressed in the GI tract. NO is involved in GI mucosal defense as well as injury. NO maintains normal functions of the GI mucosa and has a cytoprotective role. It maintains GI mucosal integrity by regulating gastric mucosal blood flow, epithelial secretion, and barrier function (16). However, NO can have deleterious effects, and increased iNOS expression is found in chronic ulcerative colitis and peptic ulcer patients (239). As such, RNS generated by iNOS have immense effects on the normal gut as well as pathophysiological conditions of the GI tract.

G) CYCLOOXYGENASE. Cyclooxygenase (COX) is a bifunctional enzyme (having both COX and peroxidase activities) that releases arachidonic acid (AA) from membrane phospholipids and catalyzes conversion of AA to prostanoids (**FIGURE 2, Eq. 8**). COX has two isoforms: COX-1 and COX-2. A splice variant of COX-1, COX-3 (also called COX-1b or COX-1 variant), has been reported. Initially it was thought to have no physiological role in humans, but recent reports indicate that this enzyme possibly has cytoprotective functions and is induced in human colon cancer cells (205) and gastric cancer cells during high osmotic stress (172). COX adds two O_2 molecules to AA by its bioxygenase activity to generate an unstable cyclic hydroperoxide, PGG_2 . Next, it reduces PGG_2 by its peroxidase activity to an endoperoxide, PGH_2 (266). PGH_2 is converted to biologically active and stable prostanoids such as PGE_2 , prostacyclins, and

thromboxane A_2 by various synthases. The peroxidase activity of COX generates $NAD^{\cdot-}$ and $NADP^{\cdot-}$ radicals. These radicals can eventually generate $O_2^{\cdot-}$ (163).

COX-1 and COX-2 are expressed in normal human gastric mucosa with increased levels at the edge of ulcers (133). *H. pylori* can upregulate both COX-1 and COX-2. COX-2 has been associated with precancerous changes in the GI mucosa including Barrett's esophagus, *H. pylori*-induced gastritis, as well as inflamed colonic mucosa (154) and is implicated in the development of cancers associated with these diseases (207). COX-1 has constitutive expression while COX-2 is upregulated by inflammation and tumorigenesis (179). Accordingly, selective COX-2 inhibitors (coxibs) have been developed as anti-inflammatory and antitumor drugs.

H) TRANSITION METALS. Transition metal ions such as iron (Fe^{2+}) and copper (Cu) carry out the Fenton reaction that generates HO^{\cdot} and OH^{\cdot} from H_2O_2 while being oxidized to Fe^{3+} and Cu^{2+} , respectively. These are reduced back by a reducing agent (FIGURE 2, Eq. 9b). The net Haber-Weiss reaction is shown in FIGURE 2 (FIGURE 2, Eq. 9c). The generation of HO^{\cdot} through this pathway accelerates lipid peroxidation (40). Oxidation of certain biological molecules during exercise generates superoxide anion radicals,

and this is mediated by trace amounts of transition metals (63). For example, ferrous ion (Fe^{2+}) can lose its electron to oxygen to produce $O_2^{\cdot-}$ and Fe^{3+} (97). Molecules that undergo such autooxidation are hemoglobin, myoglobin, catecholamines, reduced cytochrome *c*, and thiols.

2. Exogenous or environmental sources

There are multiple external triggers that induce oxidative stress that have direct or indirect effects on responses in the GI tract. Air pollutants, tobacco smoke, ionizing and non-ionizing radiations, foods and drugs, as well as xenobiotics can all contribute to oxidative stress. Chemical agents like quinones (33); heavy metals such as lead, arsenic, mercury, chromium, and cadmium; organic solvents; and pesticides are common exogenous sources of ROS (331). Various exogenous and endogenous sources of ROS are included in FIGURE 3, but this section focuses on those with the most relevance to the GI tract.

A) RADIATION AND CHEMOTHERAPY. Ionizing radiation, such as x-rays, neutrons, as well as α , β , and γ rays, can all cause oxidative stress. α Particles have weak penetrative power, but the rest are very penetrating through the human body. Ionizing radiation can produce HO^{\cdot} by radiolysis of water or ROS via secondary reactions (249). High levels of ioniz-

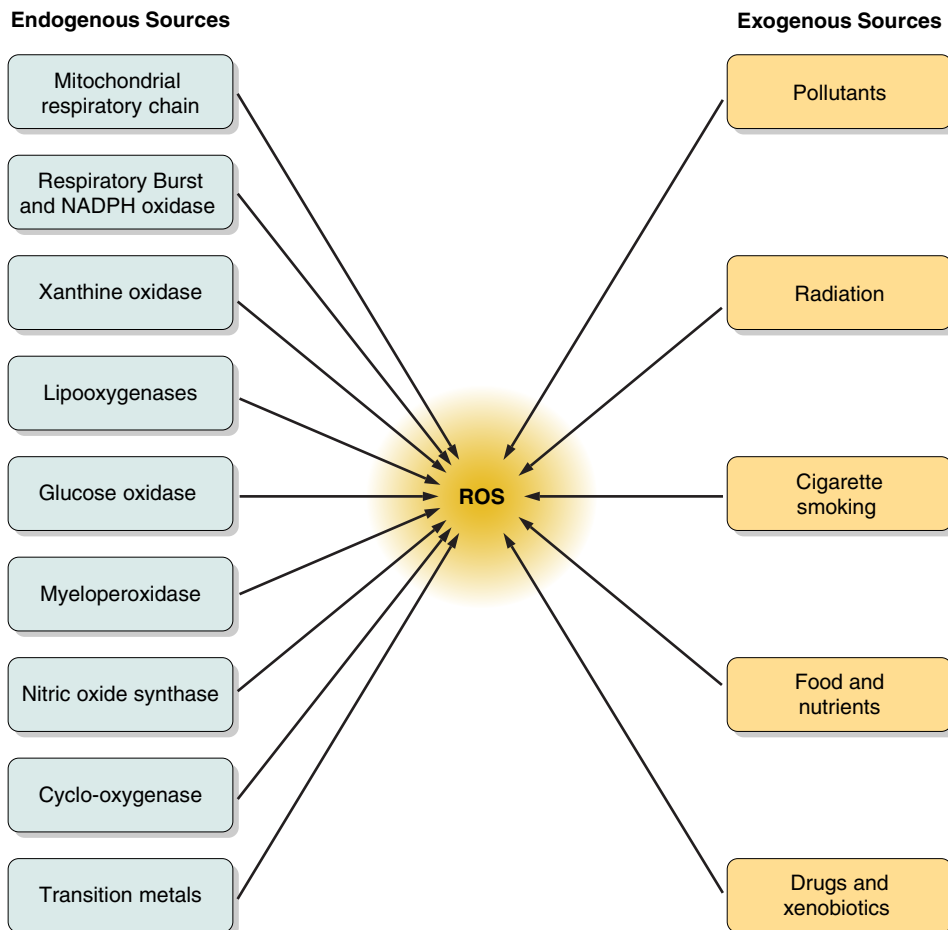


FIGURE 3. Endogenous and exogenous factors leading to reactive oxygen species (ROS) generation. Mitochondrial respiratory chain and various intracellular enzymes are the main generators of endogenous ROS. Environmental pollution, radiation, cigarette smoking, certain foods, and drugs are the major exogenous sources of ROS.

ing radiation cause injury to the cerebrovascular, GI, and hematopoietic systems. In the prodromal phase following damage to all of these tissues, GI symptoms appear including anorexia, nausea, vomiting, and diarrhea. When mice receive doses of 6–30 gray (Gy), GI injury syndromes appear, caused in part by p53-mediated death of GI epithelial cells (149). Radiation-induced cell death can be mitigated or even prevented in mice with the antioxidant *N*-acetylcysteine (NAC) (136) which establishes ROS as critical factors in development of radiation-induced GI syndromes.

Cancer chemotherapy is often accompanied by toxic side effects, and ROS generation by chemotherapeutic agents is the primary event leading to induced toxicity. This is evident by increased lipid peroxidation, and reduced antioxidant and tissue GSH levels during chemotherapy. Agents that produce high levels of ROS include anthracyclines (doxorubicin, daunorubicin), alkylating agents, platinum coordination complexes (e.g., cisplatin), epipodophyllotoxins (e.g., etoposide and teniposide), and the camptothecins (60). Methotrexate (MTX) is a widely used chemotherapeutic agent that causes gastrointestinal toxicity leading to diarrhea, nausea, and decreased nutrient absorption. The XO system is involved in MTX-mediated ROS production in an animal model (60).

Both radiation and chemotherapy induce systemic oxidative stress and reduce levels of vitamin E and beta-carotene in patients (57). Antioxidant vitamins have been used to treat these complications (192). Topical application of vitamin E enhances the rate of healing at sites of ulceration. Oral beta-carotene supplementation during the course of radiation and chemotherapy helps in the treatment of oral mucositis (192). Thus understanding the role of ROS in response to these antioxidant vitamins has helped in planning strategies to deal with some ROS-mediated tissue damages.

B) CIGARETTE SMOKE. Cigarette smoke is another significant generator of ROS (109) and has been shown to modulate GI disease. It is comprised of more than 7,000 chemical compounds and oxidative agents, and tobacco smoke contains 10^{14} – 10^{16} free radicals per puff (329). The active chemicals include aldehydes, quinones, benzo(a)pyrene, epoxides, and peroxides (55). Cigarette smoke has a gas phase which contains NO, peroxy radicals, and carbon-centred radicals as well as a tar phase containing relatively stable polycyclic aromatic hydrocarbons and nitrosamines (319). In the presence of iron, tar semiquinone can generate hydroxyl radicals (HO \cdot) and hydrogen peroxide (H $_2$ O $_2$).

Tobacco use is associated with various GI diseases including peptic ulcers, Crohn's disease (296), gastroesophageal reflux disease (GERD) (272), Barrett's esophagus (62), as well as carcinoma in the esophagus, gastric cardia (302),

and distal intestine (4). Interestingly, tobacco smoking has a protective effect in ulcerative colitis, which highlights the pathogenic differences between Crohn's disease and ulcerative colitis and reflects the complex mixture of compounds found in tobacco smoke (113, 169). Understanding the mechanisms underlying this difference may provide valuable information for developing new treatments for these two major forms of IBD.

C) FOODS AND ALCOHOL. Ingested food can generate O $_2^{\cdot-}$ and H $_2$ O $_2$ in the GI tract (67). Humans ingest macronutrients (carbohydrates, proteins, and fats), micronutrients (minerals and vitamins), food preservatives, as well as microorganisms. Dietary iron and also copper generate ROS by the Fenton reaction. Increased intake of Fe $^{2+}$ generates ROS and RNS, lipid peroxidation, and oxidative stress, and its accumulation in tissues increases the risk of cancer and inflammation (96). Trans fatty acids in processed foods also generate ROS (334). This may in part be attributable to the presence of acrylamide, which can be found in snack foods, breakfast cereals, and crackers. Acrylamide is absorbed mainly via ingestion and reacts with hemoglobin (26). Chronic acrylamide exposure gives rise to oxidative stress in humans by the increased production of ROS.

Lipids from vegetable and animal origin, when heated in microwave ovens, generate free radicals. In addition, foods from plants containing phenols supply oxidants to the body (3) while ethanol at high concentrations can directly damage the mucosal layer of the GI tract. Alcoholic liver disease (324) and alcoholic pancreatitis (217) occur in part due to ROS generated from ethanol. Furthermore, cancers of the oropharynx, larynx, esophagus, and liver are also associated with increased alcohol intake (225). Although these associations may be explained in part by the disruption of the intestinal barrier function due to alcohol-induced NO synthesis (279) and increased production of NF- κ B and tumor necrosis factor (TNF)- α (225), further studies are required to better understand the mechanisms of alcohol-induced GI injury.

D) DRUGS AND XENOBIOTICS. Many drugs and xenobiotics contribute to the formation of free radicals in the body. Anti-cancer drugs such as anthracyclines and analogs, mitoxantrone and other quinones, actinomycin D, enediynes such as bleomycin, chartreusins, elasmin A and related compounds can cause oxidative stress (76). The resultant oxidative stress facilitates their ability to kill tumor cells. Glucocorticoid therapy can lead to O $_2^{\cdot-}$ production (131), but its effects on inducing apoptosis in certain leukocyte populations confer a net decrease on oxidative stress. Volatile anesthetics may generate free radicals and change antioxidant levels in patients undergoing surgery (292). However, how these agents impact luminal GI pathophysiology is not well known.

Aspirin and antipyretic, analgesic NSAIDs such as ibuprofen and naproxen, also generate ROS. NSAIDs actions include nonselective inhibition of COX, thereby blocking formation of PGE₂ (241). Two main target organs of adverse reactions associated with NSAIDs are the GI tract and the renal system. NSAID-induced gastric injuries, including ulceration (320), occur in part due to induced aggregation of neutrophils in the gastric vascular endothelium (307). This can lead to ROS production and mucosal injury associated with NSAID treatment in rats (294). Acidic NSAID molecules irritate the gastric mucosa directly, but reduction of prostaglandin synthesis in rat gastric mucosa is more significant because this increases gastric acid secretion, and reduces bicarbonate secretion and mucosal blood flow, thereby increasing the risk of gastric ulceration and damage to the small intestine (315). A PGE₁ derivative, misoprostol, and agents that inhibit gastric acid secretion such as proton pump inhibitors and histamine receptor blockers are used for the treatment and prevention of NSAID-mediated gastroduodenal injury (229). It is believed that by removing bacterial populations of the stomach and the small intestine, NSAID-induced mucosal damage can be reduced (183). As mitochondrial oxidative phosphorylation mediates NSAID-induced mucosal injury of both of these organs, agents that prevent uncoupling of oxidative phosphorylation may be useful in treating NSAID-mediated GI injuries.

III. ANTIOXIDANT DEFENSE SYSTEMS

Oxidation reactions are crucial for aerobic life, but uncontrolled ROS generation is damaging. Although free radicals are continuously generated, the body is equipped to defend against the harmful effects of ROS with the help of antioxidants, collectively called the antioxidant defense system which comprises both enzymatic and nonenzymatic mechanisms. Antioxidants remove free radicals from the system and inhibit oxidation by being oxidized themselves. Dietary intake is another very important source of antioxidants and points to the potential effects of malnutrition or malabsorption of nutrients on the regulation of these mediators.

A. Endogenous Enzymatic Antioxidants

The major enzymatic antioxidants are superoxide dismutases, glutathione peroxidase, glutathione-reductase, catalase, and superoxide reductases. Superoxide reductase is an oxidoreductase present only in the anaerobic and facultative microorganisms (234). SOD and catalase provide major antioxidant defenses against ROS.

1. Superoxide dismutases

Superoxide dismutases (SOD) are metal ion cofactor-requiring enzymes that catalyze dismutation of O₂^{•-} into O₂

and H₂O₂ (FIGURE 2, Eqs. 10a and 10b). Three isoforms of SOD exist in humans (204): cytosolic copper and zinc-containing enzyme (Cu-Zn-SOD), manganese-requiring mitochondrial enzyme (Mn-SOD), and an extracellular Cu-Zn containing SOD (EC-SOD). Iron-containing SOD (Fe-SOD) is present in bacteria and plants but not in vertebrates and yeast, while nickel-containing SOD (Ni-SOD) is present only in prokaryotes (297). Mn-SOD is essential for survival as Mn-SOD null mice die soon after birth (188).

O₂^{•-} formed in the mitochondria is dismutated to H₂O₂ by Cu-Zn-SOD present in the mitochondrial intermembranous space and Mn-SOD present in the mitochondrial matrix (213). GPX present in the mitochondrial matrix can scavenge H₂O₂. Uncharged H₂O₂ crosses the mitochondrial membranes and in the cytosol can be scavenged by either cytosolic Cu-Zn-SOD or catalase (236). Gastrointestinal mucosal injury can be prevented by SOD in the gastrointestinal mucosa (150, 152). Intestinal tissues from IBD patients have increased levels of all three SOD isoforms, particularly in the epithelium (161).

Reduced SOD activity in the gut causes gastric ulcer, and increased SOD activity has been associated with ulcer healing in patients (200). These responses illustrate both the detrimental effects of ROS on tissue damage and the importance of antioxidant activity in promoting health. Gastric adenocarcinoma and squamous cell esophageal carcinoma tissues exhibit increased expression of Mn-SOD relative to the normal mucosa (134). Colorectal cancer is also associated with enhanced Mn-SOD expression. In contrast, Cu-Zn-SOD is slightly lower in cancer tissues than in normal tissues. Whether these changes are pathogenic or they simply reflect altered homeostasis has yet not been established.

2. Glutathione peroxidase

Glutathione peroxidase (GPX) converts glutathione (GSH), a tripeptide consisting of glutamate, cysteine, and glycine, into oxidized glutathione (also called glutathione disulfide, GSSG) and, during this process, reduces H₂O₂ to H₂O and lipid hydroperoxides (ROOH) to corresponding stable alcohols (FIGURE 2, Eq. 11). The GPX reaction is coupled to glutathione reductase (GSSG-R), which maintains reduced glutathione (GSH) levels (FIGURE 2, Eq. 12) (34). Neurons are most vulnerable to free radical damage as they have very low levels of GSH. GPX serves an important role in protecting cells from the harmful effects of peroxide decomposition.

Isozymes of GPX are found in the cytoplasmic, mitochondrial, and extracellular compartments (288). Humans have eight isotypes of GPX, most of which contain selenocysteine residues at their active site (74). GPX1 is ubiquitous, but GPX2 has epithelium-specific expression. GPX2 (originally named GPX-GI) was discovered in the gastrointestinal tract (52) which protects the gut against the absorption of dietary

hydroperoxides (318). GPX2 expression is detected in various parts of the GI tract and is induced in gastric cancer cells (153). GPX2 provides a first line of defense against ROS derived from inflammation associated with both pathogenic and nonpathogenic commensal bacteria in the gut (54). GPX1 and GPX2 double-knockout mice suffer from IBD-like symptoms as a result of induced oxidative stress and inflammatory responses (90). Understanding the mechanisms by which GPXs cause IBD and developing GPX-mimetics for future therapeutic approaches could enhance the management of human IBD.

3. Catalase

Catalase dismutates H_2O_2 to H_2O and O_2 (FIGURE 2, Eq. 13) and is found mainly in peroxisomes (261). Catalases are heme enzymes, but a manganese catalase is found in prokaryotes (333). In humans, catalase is found largely in liver, kidney, and erythrocytes, although all organs express this enzyme. Catalase-expressing pathogens such as *Campylobacter jejuni* (10), *H. pylori* (198), *Helicobacter hepaticus* (95), and enterobacteriaceae family bacteria (281) including *Escherichia coli*, *Shigella*, and *Salmonella* synthesize catalase to deactivate H_2O_2 to evade host response and survive within the host. Less catalase activity is noted in colorectal cancer (47), gastric adenocarcinoma, and *H. pylori*-infected stomach (196). Crohn's disease patients show permanent suppression of catalase activity in their mononuclear cells (126). Genetically modified Lactobacilli capable of producing catalase have been shown to reduce tumor in colon (75) and colitis in mice (166). Scientists even claim that catalase function is not to detoxify H_2O_2 , but to protect cells from apoptosis (203). Further support for this view comes from a study involving IBD patients (156). However, dissecting out the antiapoptotic and antioxidant roles of catalase in various GI diseases could help in developing more effective treatment strategies for inflammatory GI diseases.

4. Glutathione reductase

Glutathione reductase (GR or GSR) reduces oxidized glutathione disulfide (GSSG) to GSH (FIGURE 2, Eq. 12). GR is ubiquitously expressed except for *Drosophila*, Trypanosomes, and gram-negative bacteria (143). This homodimeric enzyme is a flavoprotein disulfide oxidoreductase. Each subunit contains four domains: FAD-binding and NADPH-binding domains, a central domain, and an interface domain. The active site is formed by dimerized interface domains, and only the dimer has catalytic activity (19). GR protects red blood cells, hemoglobin, and cell membranes from oxidative stress by generating GSH (48). Riboflavin deficiency leads to reduced GR activity (100). Increased level of GSH is often associated with drug resistance of various cancers including colon cancer (25, 246). Clinical trials of GR inhibitors and a better understanding of the

GST detoxification pathway will further help in developing chemotherapeutic regimens to treat colon cancer.

5. Heme oxygenase

Heme oxygenase (HO) catalyzes degradation of heme and generates CO, biliverdin, and iron (282). Two distinct HO isoforms, HO-1 and HO-2, have been reported (253). HO-2 is constitutively expressed, and HO-1 is inducible. There is a low expression of HO-1 at baseline in nearly all cells, but it is strongly induced by its substrate heme, heat shock, UV radiation, I/R injury, lipopolysaccharide (LPS), cytokines, and oxidative stress (312). Although HO-1 does not have a direct antioxidant enzymatic function, HO-1 and its product CO are believed to have indirect cytoprotective responses against oxidative stress (214, 304). HO-1 overexpression leads to resistance of hyperoxia-induced lung cell death, protein oxidation, and lipid peroxidation injury (228), whereas CO prevents oxidant-induced lung injury (215). HO-1 also has cytoprotective function in GI tumor cells. In an experimental colitis model, HO-1 was significantly upregulated in inflamed colon (310) as was also found in patients with IBD (222). Nrf2-deficient mice, which lack transcriptional regulation of Nrf2 on HO-1 gene, are more susceptible to dextran sodium sulfate, a chemical inducer of colitis, when compared with wild-type mice (147). HO-1 is crucial in modulating cell cycle, apoptosis, as well as oxidative stress in colon cancer cells (210). However, studies to understand HO-1's potential in treating free radical-induced GI diseases are still in their infancy.

B. Endogenous Nonenzymatic Antioxidants

1. Glutathione

Glutathione is found in all eukaryotic cells and is one of the key non-enzyme antioxidants in the body. It is generally present in its reduced form, GSH. This is ubiquitously expressed, and together with three enzymes, glutathione reductase GPX, and glutathione S-transferases (GST) (187), form the glutathione system. In the gut mucosa, the GSH system serves as an antioxidative barrier. High intake of fruits and vegetables stimulate GSH-dependent enzymes (120) which may account for at least some of the reported antioxidative benefit of these food groups.

GSH concentrations are much higher in the glandular gastric tissue, perhaps conferring some additional protection from the effects of gastric acid. While *H. pylori* infection-induced inflammation causes damage that in part is attributable to the production of ROS, this infection overwhelms the ability of mucosal cells and local glutathione to entirely prevent ROS-mediated damage. Therapeutic regulation of glutathione availability prevents the damage caused by *H. pylori* infection (184), which illustrates the impact of altering the relative balance of pro- and antioxidants in disease.

A very high correlation exists between high GST expression in the GI tract and tumor occurrence. Two isoenzymes of GPX, GPX-1 and GPX-2 or GPX-GI, catalyze reduction of hydroperoxides in the intestinal epithelia. GPX^{-/-} mice are susceptible to infection-induced inflammation and cancer (53). In humans, low GST activity is associated with high tumor incidence, and vice versa. Glutathione/GST causes neoplastic changes in *H. pylori*-infected gastric mucosa (301). GST activity is reduced in colon cancer. Again, dietary intake of fruits and vegetables reduces the risk of colorectal cancer (107), which may in part be attributable to their ability to favor an antioxidant environment.

2. Thioredoxin

The thioredoxin system is comprised of thioredoxin (Trx) and thioredoxin reductases (TrxR). Trx is disulfide-containing oxidoreductase that modulates activity of redox-sensitive transcription factors. Trx is present in the cytoplasm, membranes, mitochondria, and the extracellular space (151). Its active site contains a conserved sequence Cys-Gly-Pro-Cys. Oxidized Trx (Trx-S-S) is reduced by a flavoenzyme TrxR and NADPH (122) to its active dithiol form which scavenges ROS and helps maintain proteins in their reduced state (8). Several clinical conditions have been shown to involve Trx (21). Trx shields ocular lens from free radical damage (245) and inhibits reperfusion-induced arrhythmias in a rat cardiac tissue (7), indicating a protective effect during acute ischemic heart disease. TRX-1 shows cytoprotective action in various inflammatory conditions. For example, TRX-1 reduces DNA damage and neutrophil aggregation in the *Helicobacter felis*-infected stomach, suggesting a protective role in murine gastritis (144).

Thioredoxin binding protein-2 (TBP-2) is a negative regulator of Trx and has multiple regulatory functions in cellular redox regulation, growth, apoptosis, and aging. TBP-2^{-/-} mice die from GI bleeding under fasting conditions, indicating a protective role of TBP-2 in gut pathophysiology (212). Anti-ulcer drugs like geranylgeranylacetone can induce Trx production in rat hepatocytes. This drug also promotes secretion of Trx in rat gastric mucosa, suggesting that it has a protective role in at least experimental gastric ulceration (77). Bile acids upregulate TrxR mRNA expression in GI cancers via induced production of ROS (167).

3. Melatonin

Melatonin is a hormone synthesized from serotonin primarily in the mammalian pineal gland but is also found in the retina, lymphocytes, GI tract, and bone marrow (284). It is ubiquitous and can be found in dietary sources such as oats, yeast, and other plants. It is effective in both aqueous and lipid phases in neutralizing HO· and peroxy radicals, CO₃⁻, NO₂, O₂⁻, and HOCl (247) and can readily cross the blood-brain barrier. Melatonin as an antioxidant is ir-

reversibly oxidized and cannot be reduced. Thus it is referred to as a suicidal or terminal antioxidant (278). During the oxidative reaction, it is converted to several antioxidant intermediate metabolites, 6-hydroxymelatonin being the primary metabolite found in the nuclei and mitochondria. Mitochondria generate most free radicals generated within cells (112) and are particularly prone to oxidative damage as they lack protective histone proteins and have fewer DNA-repair enzymes. As melatonin can directly cross the mitochondrial membranes, it plays a very significant role in protecting mitochondria from oxidative damage. In this manner it protects vital organs including the liver from alcoholic damage (177). Other antioxidants can be converted to free radicals, but melatonin can never become a free radical as its oxidative role involves donation of two electrons. Melatonin's anti-inflammatory effects in animal studies and limited human studies suggest that supplemental melatonin may have a beneficial effect in colitis (284). Further studies are required to fully evaluate its anti-inflammatory and antioxidant functions.

C. Exogenous Antioxidants

1. Vitamin C

Vitamin C or ascorbic acid is the primary antioxidant in plasma and cells (185). It is synthesized from glucose in the liver of most mammalian species, but not by humans and therefore must be ingested to avoid scurvy, a potentially lethal condition (216). Vitamin C can be obtained from fresh fruits and vegetables. Vitamin C donates electrons to other compounds and prevents their oxidation. The many relevant species reduced by vitamin C include various ROS, RNS, sulfur radicals, O₃, nitrosating compounds, and HOCl. Vitamin C reduces heavy metal ions (Fe, Cu) that can generate free radicals via the Fenton reaction, and thus it can have pro-oxidant activity (273) although its main function is as an antioxidant.

2. Vitamin E

Vitamin E (the most biologically active form is α -tocopherol) is an important and abundant antioxidant that protects cell membranes from lipid peroxidation (LPO) (289). α -Tocopherol terminates the activity of LPO by scavenging lipid peroxy radicals (LOO·) but itself is converted into a reactive radical during this reaction (295). α -Tocopherol can also reduce Fe or Cu, as a pro-oxidant (327). The ability of α -tocopherol to act as a pro- or antioxidant depends on the amount of α -tocopherol available to scavenge ROS (327). However, according to some reports, α -tocopherol has no significant role in antioxidant metabolism (11). In one in vitro model, in the presence of Cu²⁺, α -tocopherol showed an oxidative DNA-damaging effect (328). Epidemiological studies indicate that food rich in fruits and vegetables lowers cancer rates, but supplementation of

exogenous vitamin E and other antioxidants have not been shown to prevent gastrointestinal cancers (32). This underscores the complexity of understanding the beneficial effects of foods beyond their individual antioxidant components.

3. Carotenoids including vitamin A

Vitamin A, which is found in food, is referred to as carotenoids or provitamin A. Yellow and orange fruits as well as green leafy vegetables provide most of the carotenoids in our diet. Alpha- and beta-carotene, lycopene, and cryptoxanthin are the main carotenoids in food as well as in the body (103). Beta-carotene and other carotenoids exhibit antioxidant properties depending on the *in vitro* experimental system used. Antioxidant properties of biological carotenoids depend on retinol-binding proteins and other endogenous antioxidants *in vivo* (243). Beta-carotene has been shown to suppress lipid peroxidation in mouse models (132). Antioxidant properties can be reversed to pro-oxidant behavior depending on O₂ tension or carotenoid concentration (336).

4. Minerals

Zinc (Zn), copper (Cu), manganese (Mn), iron (Fe), and selenium (Se) are key components of enzymes with antioxidant functions and are designated as antioxidant micronutrients. Zn, Mn, and Cu are cofactors of superoxide dismutase (Cu/Zn-SOD) (115). Fe is a component of catalase. Se is a major antioxidant in the form of selenoproteins that mitigates the cytotoxic effects of ROS. Cereals contain selenomethionine, a naturally occurring amino acid that is the most important nutritional source of Se. When Se-GPX is inhibited under physiological conditions, such as during Se deficiency, it leads to toxicity through increased O₂^{•-}, NO, and lipid peroxidation (189). Thus again, proper nutrition and absorption of these micronutrients is essential to maintain redox homeostasis.

5. Polyphenols including flavonoids

Plant polyphenols are important antioxidants, and dietary intake of these compounds can be up to 50–800 mg/day (232). Polyphenols comprise flavonoids, phenols, phenolic acids, lignins, and tannins. Flavonoid sources include fruits, vegetables, nuts, red wine, beer, tea, seeds, grains, spices, and medicinal plants. Flavonoids prevent superoxide anion production by inhibiting XO (111). In addition, they inhibit COX, LOX, GST, microsomal monooxygenases, and NADH oxidase (39). Many flavonoids chelate free Fe and Cu that could otherwise increase ROS generation, and also reduce ROS such as O₂^{•-}, and HO[•] (41).

IV. ROS AS SIGNALING MOLECULES AND ROS-MEDIATED REGULATION OF CELLULAR SIGNALING

Although often thought of as harmful molecules, ROS act as essential signaling molecules. Despite this function being widely reported, it has remained controversial or perhaps under investigated. **FIGURE 4** illustrates key ROS signaling events within cells. ROS modulate a number of redox-sensitive signaling pathways. Well-characterized targets are the catalytic Cys residues of tyrosine kinases and mitogen-activated protein (MAP) kinase phosphatases (protein tyrosine phosphatases). Oxidation of their Cys residues reversibly abolishes enzymatic activity (287). ROS also cause integration of cellular functions by regulating growth factor-mediated signaling pathways. Specificity of these signaling events is conferred by subcellular compartmentalization of H₂O₂ (305) and by local modulation of H₂O₂ concentration by scavengers (321).

In mammals, thiol-based peroxidases, peroxiredoxins (PRXs, a family of peroxidases), and GPX play important roles in ROS signaling. PRXs modulate H₂O₂ signaling downstream of growth factor tyrosine kinases and cytokine receptors (142). PRX1 is induced in macrophages by oxidized low-density lipoprotein and functions not only as an antioxidant and a reducer of ROS, but activates p38 MAPK, and enhances cell survival (61). PRX2 blocks H₂O₂ and TNF- α -induced upregulation of NF κ B (61). Overexpression of PRX4 also inhibits the above-mentioned event (138). Control of inflammatory responses by ROS is of course very relevant to GI diseases and is discussed in more detail in section V.

Another level of regulation of mammalian ROS is achieved by modulating mammalian antioxidant systems in a process involving several factors. p53 can be activated by NO, which decreases H₂O₂ accumulation by upregulating GPX1 (254). S-nitrosylation of the p53 inactivator Hdm2, a ubiquitin ligase (260), inhibits its interaction with p53, thereby blocking p53 ubiquitination and proteolysis. NO downregulates Mdm2 (mouse equivalent of Hdm2), similarly decreasing p53 ubiquitination (311). c-Myc can induce GSH formation, which potentiates its oncogenic functions (24). The class O forkhead box (FOXO) family of transcription factors is activated by H₂O₂ and imparts tolerance to oxidative stress by enhancing SOD1 expression (43). NRF2 transcription factor modulates the expression of defensive genes coding detoxifying enzymes and antioxidant proteins. KEAP1, the inhibitor of NRF2, helps in its retention in the cytosol. The NRF2-KEAP1 pathway is committed to xenobiotic and oxidant elimination. In response to attack by electrophiles or ROS, NRF2 is switched on and off via distinct mechanisms. Oxidative modification of KEAP1 at Cys residues and NRF2 phosphorylation results in release of NRF2 from KEAP1

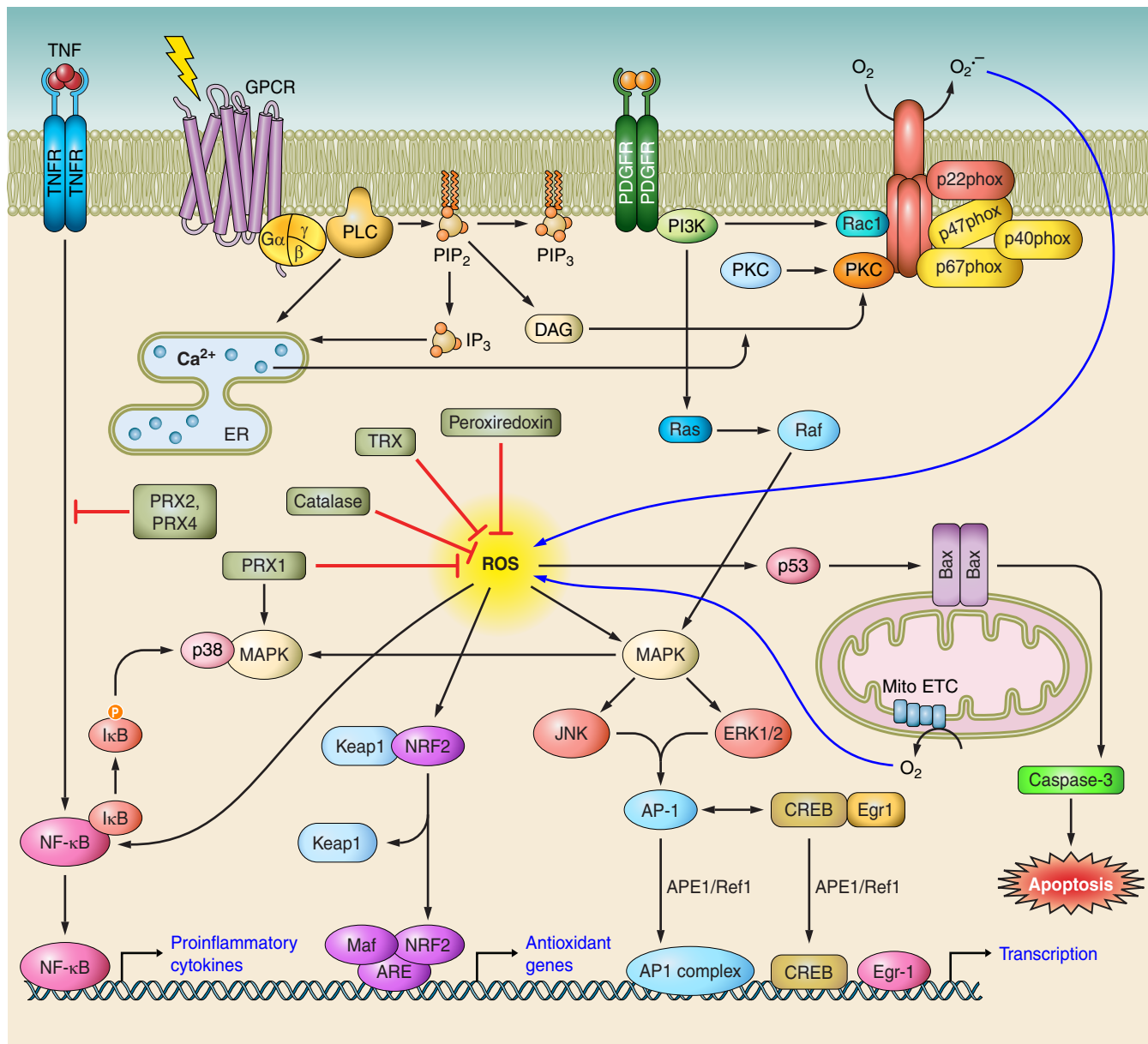


FIGURE 4. Schematic depiction of multiple signaling pathways that generate ROS and the intracellular events activated by ROS accumulation. Upon activation, G protein-coupled receptors (GPCRs) activate phospholipase C (PLC) leading to the activation of protein kinase C (PKC) molecules. Platelet-derived growth factor receptors (PDGFRs) activate phosphoinositide 3-kinase leading to activation of ras-related C3 botulinum toxin substrate 1 (RAC1). Both RAC1 and PKC activate membrane-bound receptors leading to membrane relocation and assembly of various components of phagocytic NADPH oxidases. Mitochondrial electron transport chain (Mito ETC) is another robust source of intracellular ROS generation. ROS in turn lead to enhanced production of (APE1/Ref1) and activation of several signaling events including p53-mediated apoptotic events, mitogen-activated protein kinase (MAPK) pathways, NF-E2-related factor (NRF2)-mediated activation of genes containing antioxidant response element (ARE), and nuclear factor- κ B (NF- κ B). Transcription factors including AP1, NF- κ B, cAMP response element-binding (CREB), and early growth response (EGR) protein, induced by these signaling events are kept in the active and reduced form by APE1/Ref1. Thus ROS signaling events play a central role in regulation of proinflammatory events, cell cycle, proliferation, and cell death. Antioxidant defense enzymes such as catalase, thioredoxins (TRX), peroxidases, and peroxiredoxins (PRX) contribute to preventing excessive levels of ROS from accumulating at the cellular and tissue level.

(335). Stabilized NRF2 translocates to the nucleus, interacts with various proteins, and binds with antioxidant response elements involved in activation of gene expression, thereby protecting cells from free radical damage.

Eukaryotic ROS sensing transcription factors, AP-1, and NF- κ B act as potent redox sensors due to the presence of single Cys in their DNA-binding domains (1). Oxidation of these Cys residues blocks their binding to the respective

consensus DNA sequences. Apurinic/aprimidinic (AP) endonuclease 1 (APE1), also known as Redox effector factor 1 (Ref1), functions as a reducing agent for various transcription factors (91). This ubiquitous multifunctional protein is induced by ROS (242) and is involved in base excision repair (78). The DNA-binding activity of transcription factors are restored by Trx (180). Although reducing condition is favorable for DNA binding, both AP-1 and NF- κ B can be activated by oxidative stress via induction of APE1/Ref1. A Zn-finger DNA-binding protein, early growth response gene-1 (Egr-1), is activated by ROS, and a positive feedback loop between APE1/Ref1 and Egr-1 regulates their early transcriptional activation after oxidative stress (233). Egr-1 also induces SOD1 and thus reduces free radical-induced damage (194). The functions of these signaling molecules will be discussed further in the next section.

V. PATHOPHYSIOLOGY OF OXIDATIVE STRESS IN THE GI MUCOSAL DISEASES

The GI tract is prone to ROS attack as it is accessed by the outside environment with resident immune cells and intestinal flora as well as dietary factors, all potential sources of ROS. Two main enzymatic reactions generate ROS in the GI tract—the HX/XO system and the NADPH oxidase system. In fact, the GI tract has the highest concentration of XO in the body, which along with numerous phagocytic cells (and a large number of catalase-negative bacteria in the colon), combine to generate large amounts of $O_2^{\cdot-}$ (181). ROS have been linked with various inflammatory GI disorders such as gastroduodenal inflammation, ulceration, and gastric cancer (230). Excessive levels of ROS damage cellular proteins (244) including cytoskeletal proteins (15) and, ultimately, disrupt GI tract barrier to increase gut permeability which contributes to inflammation in a variety of GI diseases. Furthermore, excess ROS induce inflammation by stimulating PMNs, thereby causing further damage to the tissue. As many GI diseases are initiated and promoted by oxidative stress, the mechanisms underlying the development of these pathophysiological conditions need to be elaborated.

A. Esophageal Diseases

1. Reflux esophagitis, Barrett's esophagus, and esophageal adenocarcinoma

GERD occurs due to prolonged contact of the esophageal mucosa with refluxed acidic gastric contents and is a very common disorder especially in developed nations. Acid, bile salts, and esophagitis (inflammation of the esophagus) that are associated with GERD lead to increased ROS, reduce the amount of antioxidants (for example, GSH and vitamin C), and increase expression of ROS-inducible genes. Reflux

esophagitis can lead to erosions or ulceration of the esophagus and also Barrett's esophagus (BE). In BE, specialized intestinal-type columnar epithelium replaces the normal squamous epithelial lining of the esophagus possibly due to the chronic exposure to gastroesophageal refluxate (316).

Bile acids, one of the major constituents of duodenogastroesophageal refluxate, are also believed to promote BE and esophageal adenocarcinoma (EA). Injection of SOD or buthionine sulfoximine reduces esophagitis in rat duodenogastroesophageal reflux model, suggesting that EA development is mediated by inflammation and oxidative stress (314). EA starts with metaplasia, followed by dysplasia, which ultimately develop into carcinoma (5). Individuals with BE are prone to develop EA, although more recent studies show a lower association between the two (121, 125). Prolonged contact with acid and bile of the gastroesophageal refluxate damages esophageal epithelium and induces inflammation. The amount of bile salts and acid in the esophageal refluxate is proportional to the degree of esophageal mucosal injury (202). Unconjugated bile acids are robust COX-2 inducers and activate PI3K/AKT and ERK1/2 pathways in BE and EA cells through ROS induction (270).

Comparison of mucosal biopsies from erosive gastritis and BE revealed that $O_2^{\cdot-}$ is the main oxidant responsible for reflux esophagitis (137). $O_2^{\cdot-}$, along with H_2O_2 , HO^{\cdot} , $ONOO^-$ produced by mitochondrial complexes, NADPH oxidase and NOS in inflammatory cells and the epithelium are linked with reflux esophagitis, BE, and EA (128). ROS activate varied signal transduction pathways such as AP1, NF- κ B, insulin receptor kinase, MAPKs, and Src kinases which regulate proliferation, differentiation, and apoptosis of epithelial cells (46, 102).

Various processes of reflux-induced free radical generation in the esophageal epithelium contribute towards BE pathogenesis. For example, lipid peroxidation is enhanced in BE (313), and along with reactive lipid-derivatives, NO and HOCl are also enhanced in BE and EA (317). Other changes include an increase in iNOS that is associated with inflammation and cell proliferation in BE and EA (56). COX-2 is overexpressed in human EA and is induced in biopsies of acid or bile acid-treated columnar-lined esophagus (252).

An inverse correlation exists between antioxidant supplementation and EA development (168). ROS scavengers can reduce esophageal mucosal damage. For example, SOD prevents the development to BE and EA in rats (231). Supplementation of α -tocopherol decreases EA progression in rats (50). Aspirin and NSAIDs reduce the risk of BE and EA, and the protective effect of NSAIDs targets an early stage of the metaplasia-dysplasia-carcinoma sequence (5). Aspirin may provide protection against BE and EA by inhibiting COX-2, while NSAIDs might serve as chemopreventive

agents by reducing neoplastic progression in BE (299), but further studies are needed to prove the purported benefits.

2. Esophageal squamous cell cancer

A second major type of esophageal cancer is squamous cell carcinoma (ESCC). ESCC is associated with a poor prognosis due to its typically late stage at the time of diagnosis and propensity for metastasis. It is a relatively aggressive form of squamous cell cancer compared with other tissues such as skin, head/neck, lung, and urogenital tract. Oxidative stress markers such as 8-hydroxydeoxyguanosine (8-OhdG) and thiobarbituric acid-reactive substances are elevated in tissues from ESCC (79). Alcoholism, cigarette smoking, as well as mineral and antioxidant vitamin deficiencies seem to promote ESCC (121), while consumption of antioxidants like vitamin C, β -carotene, and α -tocopherol are associated with a reduced risk of ESCC (283). Thus, by association, oxidative stress appears to be a major player in development of ESCC. Diakowska et al. (80) found that 8-OhdG level is high in advanced ESCC patients and total antioxidant levels are decreased (80). Thus the authors proposed that estimation of serum 8-OhdG and total antioxidant status can be used as diagnostic markers of ESCC progression. In vitro experiments showed that oxidative stress and radiation cause nuclear accumulation of FOXO3a, and its down-regulation reduced the radiosensitivity of esophageal cancers (49). The same study also reported that patient survival proportionately increases with nuclear FOXO3a accumulation and thus FOXO3a can act as a therapeutic marker for ESCC. Further studies in animals and other models are required to identify ROS-induced ESCC-associated proteins that could be successfully used as diagnostic and therapeutic markers of ESCC.

B. Gastroduodenal Diseases

1. Peptic ulcer disease and gastritis

Gastritis is defined as inflammation of the stomach mucosal lining and occurs in several conditions including *H. pylori* infection, NSAID use, alcohol consumption, and stress. Peptic ulcer disease (PUD) occurs in the proximal GI tract and is often associated with chronic gastritis. Gastric and duodenal ulcers represent the most common and chronic PUDs. On the basis of pathophysiology, PUD can be broadly classified into the following etiologic groups: 1) excessive acid secretion type (e.g., Zollinger-Ellison syndrome), 2) associated with infections, and 3) NSAID induced (174, 275). Gastritis and peptic ulcer are caused by multiple factors, both endogenous and exogenous, and free radicals are closely linked to both conditions.

There are several factors contributing to the accumulation of ROS in the stomach. Reduced antioxidant enzyme SOD levels (200) and antioxidant vitamin intake (206) contrib-

ute to the accumulation of ROS associated with gastroduodenal inflammatory diseases. Ethanol-induced gastric inflammation is associated with increased $O_2^{\cdot-}$ generation (117). Phagocytic leukocytes are the main source of ROS in chronic inflammation such as one observes in *H. pylori*-induced gastritis and IBD. Significant numbers of neutrophils and/or macrophages infiltrate the gastric mucosa during inflammation, generating large amounts of ROS.

Another cause of gastritis is ischemic injury, which is known to involve free radicals such as $O_2^{\cdot-}$, H_2O_2 , and HO^{\cdot} (191). Exogenous factors are also important. Smoking increases MPO activity in neutrophils (38) and in the extracellular spaces, thus enhancing gastric damage. XO activity is also higher in cigarette smoke-exposed rats (51). All these lead to neutrophil aggregation and vascular damage. Apoptosis, oxidative damage by ROS, and reduction of angiogenesis in the gastric mucosa lead to arrest of cell proliferation, and these events in turn induce ulceration (173). ROS-mediated increased lipid peroxidation, lowered GSH level, and antioxidant systems are involved in the pathogenesis of almost all forms of gastric ulcer (71, 108).

The role of *H. pylori* in development of gastritis and PUD deserves special mention as it is a major contributor to peptic ulcer and gastritis. When first determined to be a cause of peptic ulcer, this organism was found in ~95% of cases of duodenal ulcers and up to 70% of cases of gastric ulcers (94). Rates of infection in both types of ulcers in developed countries have decreased but still remain high in many developing nations. *H. pylori* strains isolated from duodenal ulcer patients induce higher neutrophil activity relative to gastritis strains (70). In the acute stage of *H. pylori* infection, neutrophil infiltration is observed, but unlike other bacterial infections, active inflammation persists throughout this lifelong infection that characterizes the majority of infected subjects worldwide. Chronic inflammatory cells including macrophages/monocytes, lymphocytes, and plasma cells are also present in the gastric mucosa of chronically infected humans, and thus these stromal cells contribute significantly to the development of gastritis and PUD. Gastric epithelial pit cells also produce ROS by activating nonphagocytic NADPH oxidase in response to *H. pylori* (286). Davies et al. (72) reported that mucosal samples from patients with duodenal ulcer and severe duodenitis generated significantly higher ROS levels than those from control subjects. This pathogen also enhances gastric antral ROS production which is correlated with bacterial load (73). The fact that infected individuals have significant reduction in vitamin E and C levels likely also contribute in the oxidative stress during infection (83), and low antioxidant levels are also linked to gastric ulcer disease (206). Therefore, the balance of factors that enhance or attenuate the local concentration of oxidants regulates disease progression.

Host and environmental factors such as genetics, diet, stress, tobacco, and levels of hygiene contribute significantly to the accumulation of ROS and the pathogenesis of *H. pylori* infection (173, 211). *H. pylori* possibly reduces GEC's ability to protect from ROS-mediated damage; however, the mechanisms that account for this are still elusive (269). High levels of lipid peroxidation and decreased mucosal GSH levels are noted in patients with *H. pylori* infection or uninfected peptic ulcer and gastritis (139). A chemotactic peptide from *H. pylori*, *N*-formyl-methionyl-leucyl-phenylalanine (fMLP) contributes to neutrophil accumulation and activation (197). The severity of *H. pylori*-induced GI diseases is often associated with the *cag* (cytotoxin associated gene) pathogenicity island (PAI), a 40-kb stretch of DNA encoding several components of a type IV secretion system (263). The *cag*(+) strains exhibit enhanced oxidative burst in PMNs (337). Intracellular GSH levels are lowered by *H. pylori* infection (23) and impairs GSH metabolism in the gastric epithelium (148). Kimura et al. reported that *H. pylori* vacuolating cytotoxin A (VacA) decreases GSH efflux and suppresses intracellular GSH turnover rate (148). Catalase and SOD released by *H. pylori* are insufficient to remove excess extracellular ROS but play important roles in the elimination of ROS generated by bacteria (198). NH₃ derived from *H. pylori* reacts with HOCl to generate monochloramine (NH₂Cl). NH₂Cl can penetrate cell membranes and damages intracellular components (106). Lipid peroxidation is increased in *H. pylori*-infected patients and is significantly lessened in the mucosa of patients after successful eradication of infection. (84).

H. pylori and ROS collaborate to activate the transcription factors NF- κ B and AP-1 in the gastric epithelium which upregulate the expression of chemokines including CXCL8 (IL-8) (69, 145). IL-8 also enhances neutrophil migration into the gastric epithelial layer and their subsequent activation (65, 145), thereby further contributing to inflammation. We have reported that the expression of APE1/Ref1, which reductively activates transcription factors, is induced in *H. pylori*-infected human gastric epithelia (82). We have also shown that APE1/Ref1 expressed in gastric epithelial cells enhances transcriptional activity of AP1 and NF- κ B and induces *H. pylori*-mediated IL-8 expression (209). As discussed, APE1/Ref1 reductively activates transcription factors to enhance their ability to bind to DNA. Thus these observations point to one potential mechanism whereby the oxidative stress signals are transduced to regulate the inflammatory response to *H. pylori*.

H. pylori colonization of the gastric pits is a major risk factor that predicts the severity of pathogenesis. Host cell death and survival depends on ROS produced in the infected stomach. *H. pylori* activate the intrinsic pathway of apoptosis (45). Induced expression of pro-apoptotic factors Bax and Bid and reduced expression of the anti-apoptotic factor bcl-2 have been reported in *H. pylori* infection (9).

Suppression of pit cell death by *H. pylori* is also reported and is believed to favor persistent bacterial colonization in the stomach as the rapid self-renewal of progenitor cells and apoptosis of gastric pit cells limit bacterial colonization. Infection with *cagA*(+) *H. pylori* but not with a mutant increases the survival factors phospho-ERK and antiapoptotic protein Mcl1 expression in the gastric pits (193). As APE1/Ref1 activates the tumor suppressor apoptotic protein p53, but forced overexpression of APE1/Ref1 prevents apoptosis (6), we examined this "paradoxical role" of APE1/Ref1 in *H. pylori*-mediated GEC apoptosis. We observed that acetylation of APE1/Ref1 as a result of *H. pylori* infection suppresses Bax expression, thereby preventing p53-mediated apoptosis in infected gastric epithelium (29). ROS are known to activate and stabilize hypoxia-inducible factor 1 α (HIF1 α) (248). Gastric epithelial ROS enhance normoxic stabilization of HIF1 α (218). We showed that APE1/Ref1 increases HIF1 α level in *H. pylori*-infected gastric mucosa, and in conjunction with transcriptional co-activator p300 induces transcriptional activity of HIF1 α (30). Further studies are needed to determine the role of HIF1 α in regulating oxidative stress in mucosal inflammation due to *H. pylori* infection and other factors.

RNS generation, in contrast, has been found to be helpful for maintaining gastric mucosal health. NO is a free radical that may inhibit pathogenic mechanisms of gastric ulcer by slowing disease progression (171). NO stimulates mucus secretion by gastric mucosal cells (170) and inhibits expression of adhesion molecule in the epithelium such as P-selectin, thereby reducing the ability of neutrophil to adhere in the gastric mucosa (306) and preventing neutrophil-released ROS-mediated ulceration. NO inhibits mast cell degranulation, thus providing another level of gastroprotection (306). Thus ROS or RNS species are important to identify as different entities can either enhance or attenuate gastric tissue damage.

2. Gastric adenocarcinoma and gastric cancer

Currently, gastric cancer is the fourth most common cancer in the world and second most common cause of mortality from cancer. Gastric cancer involves a number of genetic alterations of tumor-regulatory genes as well as epigenetic factors. *H. pylori* infection is the major cause of gastric cancer and a useful biomarker for this disease. So far, *H. pylori* gastritis is the only universal precursor condition for the diffuse form of gastric cancer. Correa (64) postulated that hyperproliferation found in *H. pylori* gastritis initiates a sequence of events leading to gastric cancer. This hyperproliferation possibly favors mutations which transform normal gastric mucosa to gastric carcinoma. Histopathologically, the sequence starts with superficial gastritis followed by multifocal atrophy, intestinal metaplasia and, lastly, dysplasia or cancer (64). *H. pylori* colonize the stomachs of ~50% of humans, and those who are infected have at least a twofold increased risk of gastric cancer relative to

the uninfected population (123). The *cag*⁺ *H. pylori* strains are more highly associated with gastric carcinogenesis than strains that do not have *cag* (221). Significantly high ROS or RNS production occur in *H. pylori*-infected gastric mucosa, vascular endothelium, as well as in neutrophils accumulated in the inflamed mucosa (199). Phagocytes accumulated in gastric mucosa after *H. pylori* infection produce O₂⁻, HO[•], and HOCl. O₂⁻ is not a very reactive molecule. The exact role of HO[•] is still not well understood, but OCl⁻ produced by phagocytic neutrophils reacts with NH₃ generated by the urease activity of *H. pylori* in the stomach lining and generates highly reactive molecule NH₂Cl. NH₂Cl has been reported to induce apoptosis in rat gastric mucosal cells (201). Epstein-Barr virus (EBV) is one of the major etiological agents of gastric cancer and represents 7% of gastric cancer cases (129). NH₂Cl derived from infiltrating neutrophils in *H. pylori*-infected stomach is able to convert latent EBV into lytic EBV which can further contribute in gastric carcinogenesis (195). Although the role of ROS produced in infected GECs is not clearly understood, those are believed to initiate signaling events in GECs which determine the course of *H. pylori* pathogenesis. In addition to stimulating host responses that contribute to ROS, *H. pylori* infection induces oxidative stress in GECs directly through the generation of ROS and regulates proinflammatory cytokine production, inflammation, and cell death (29, 81, 209). Persistent ROS causes proto-oncogene activation, oncogene/tumor suppressor gene mutations, and chromosomal aberrations, as a result of oxidative genome damage including oxidation of guanine to generate 8-OHdG and 8-oxo,7,8-dihydroguanosine (8-OHG) in DNA and RNA, respectively (68, 85).

Gastric adenoma and gastric cancer tissues (*H. pylori*-infected or uninfected) have increased mucosal expression of ROS and APE1/Ref1 compared with normal mucosa (99). Infection with *H. pylori* is associated with reduced amount of ascorbic acid in the gastric lumen and lowers its amount in the gastric juice. This antioxidant impairs effects of carcinogens, as it can reduce mutagenic agents such as nitrosamines and ROS. As the conventional therapeutic approach to kill cancer cells is via generation of ROS, depletion of cellular antioxidants increases the efficiency of ROS in killing cancer cells. Studies to inhibit various antioxidant mechanisms during neoadjuvant therapies will help us in controlling the disease.

C. Intestinal Diseases

1. IBDs (ulcerative colitis, Crohn's disease)

IBDs, both Crohn's disease and ulcerative colitis, involve chronic inflammation of the GI tract. In ulcerative colitis, only the colon is affected, whereas Crohn's disease may occur anywhere in the GI tract. Ulcerative colitis generally begins in the rectum, advancing proximally as the disease

progresses with continuous inflammation that affects only the mucosal layer of the gut wall. In contrast, Crohn's disease inflammation may occur in a segmental fashion and is transmural, involving all layers of the GI tract wall. The exact causes of IBD are not completely understood but are believed to result from inappropriate inflammatory response to commensal gut microbiota, which may be genetically regulated. Altered mesenteric circulation, intestinal microcirculation, and intestinal ischemia are potential etiologic factors in IBD, although their involvement could be secondary (127). The association of ROS with IBD is evident from the observation that increased ROS and decreased antioxidant levels contribute toward major pathogenic mechanisms in IBD (58, 126). ROS also potentiate immune reactions in IBD by inflammatory leukocytes, mainly PMNs, further augmenting tissue damage. O₂⁻, H₂O₂, and HO[•] secreted by phagocytes accumulate at the site of inflammation resulting in lipid peroxidation. No wonder that McCord described the GI tract as a "free radical time bomb" (186) and "in IBD, the fuse appears to be lit" (42).

Defects in mucosal antioxidant defenses are a contributing factor in ulcerative colitis as the redox status of mucosal glutathione is associated with inflammation and disease progression. The severity of ulcerative colitis in mice is related to SOD (159). Dextran sodium sulfate (DSS) is used to induce inflammatory responses in mouse models of ulcerative colitis. This results from activation of IκBα and NF-κB pathways via ROS generation. DSS increases sulfate load of cells which induces ROS, leading to activation of inflammatory response. Likewise, diets rich in sulfur in human ulcerative colitis induce ROS-mediated inflammation (31). Iron, which induces ROS production via the Fenton reaction, contributes to induction of colorectal tumor in a murine ulcerative colitis model (265). The antioxidant resveratrol significantly reduces inflammatory responses of ulcerative colitis in mice (330), further establishing the importance of ROS in ulcerative colitis.

Understanding the role of NO in intestinal inflammation is less straightforward. While some reports suggest NO's role in inflammation, other studies indicate that it has a protective role in the intestine (308). NO reacts with O₂⁻, produced by activated neutrophils, to form another potent oxidant, peroxynitrite (ONOO⁻). ONOO⁻ administration to the colon results in tissue injury (240). Thus iNOS might contribute in tissue injury via generation of ONOO⁻, and iNOS inhibitors have been shown to reduce colonic damage and inflammation (190). Oral administration of pre- and probiotics in DSS-induced acute murine colitis decreases NO levels in peritoneal macrophages and thus is reported to reduce colonic lesion (2). Further experiments to identify anti-inflammatory properties of probiotics that have been used to treat patients with IBD could determine underlying mechanisms of these potentially beneficial agents.

While the two forms of IBD share similar pathophysiology, HO[•] and O₂^{•-} are found to be responsible for Crohn's disease, while H₂O₂ and HOCl are more associated with ulcerative colitis (160). Increased XO and Mn-SOD activity are reported in inflamed mucosa of Crohn's disease patients (160). Crohn's disease patients exhibit elevated TNF- α in the colonic mucosa, and inflamed mucosa shows induction of iNOS, similar to ulcerative colitis (267). Enhanced oxidative stress and inflammation in conjunction with decreased antioxidant levels have been reported in patients with active Crohn's disease. However, with improvement of the patient's condition, these parameters of oxidative stress go back to normal levels (178). As NF- κ B is a major inducer of inflammatory cytokines, the effect of a potent NF- κ B inhibitor, vanillin, has been studied in mouse model of colitis (325). Vanillin was shown to suppress the production of Th1 cytokines as well as scavenges ¹O₂, underscoring its potential as a future treatment for IBD.

Lower plasma levels of vitamins A and E as well as beta-carotene and decreased antioxidant enzymes in the intestinal mucosa of IBD patients may determine the severity of IBD (116). Further studies are required to establish whether these vitamins affect the course of the disease. Antioxidants, to some extent, are effective in treating experimental colitis. SOD has had some success when used in the treatment of murine ulcerative colitis (89) and lecithinized SOD (PC-SOD), which overcomes the clinical limitations of SOD, treats ulcerative colitis more effectively. Further evidence that ROS contribute to the pathogenesis of IBD comes from the discovery that sulfasalazine, a drug with antioxidant properties, has some beneficial effects in the treatment of IBD. Despite these advancements, none could be claimed as a cure of IBD even with combinations of such agents. Disruption of intestinal homeostasis is now believed to be the major event in the development of IBD (176). Specifically, the role of gut microbiota in this process is developing as our knowledge in this field advances. Treating UC patients with fecal bacteriotherapy (infusion of fecal microbiota from healthy donors) is an emerging field of research and has been found to improve UC (35). Substantial research in this field is required to understand how fecal transplantation benefits patients with IBD and potentially other gastroenteric diseases.

2. Enteric infections

Intestinal epithelia produce ROS in response to microbial signals. The human large intestine contains $\sim 10^{14}$ prokaryotes from over 500 species (86). Epithelia contacted by enteric commensal bacteria rapidly generate ROS (165). Commensal bacteria-induced ROS generated in the intestinal epithelium modulate the protein degradation machinery of various signaling molecules and thus regulate diverse physiological events within the host cells (165). The early response to *S. enterica* serovar *Typhimurium* infection is ROS generation by NADPH oxidase with potent bacteri-

cidal effects (300). During the later stages of *Salmonella* infection, RNS are also generated (182). The antioxidant *N*-acetylcysteine (NAC) and NADPH oxidase inhibitor diphenyliodonium (DPI) have been reported to attenuate disease, implicating ROS generation as an important host response to gastrointestinal infection (165). A key issue to ascertain is whether therapeutic inhibition of ROS will protect the host from enteric infection-induced tissue damage or if any benefit would be offset by the impairment of antimicrobial host responses.

3. Ischemic intestinal injury

Intestinal I/R occurs in surgical and trauma patients and arises when blood flow to the intestine is interrupted due to various circumstances (291). I/R activates Toll-like receptors (TLRs) leading to acute intestinal and lung injury and inflammation observed during gut trauma (303). The tissue damage due to reperfusion is primarily caused by reentry of oxygen, rather than by ischemia itself. Ischemia followed by reperfusion is more damaging than ischemia without reperfusion (220). One explanation for this damage is that ATP becomes metabolized to HX during ischemia, while during reperfusion, oxygen reacts with HX to form xanthine and O₂^{•-}. Reperfusion enhances the damaging effects of ischemic injury due to accumulation of activated neutrophils and generation of ROS (175). These events are collectively called reperfusion injury. I/R-mediated GI injury is significantly reversed by both SOD and allopurinol. Observations that SOD prevents I/R-induced GI injury and oxidized glutathione is found in I/R-exposed GI mucosa establish the crucial role of ROS in the pathogenesis of I/R-mediated GI injury.

Blockage of blood supply to the colon leads to ischemic colitis. Ischemic colitis is of two types: occlusive and non-occlusive. Occlusive ischemic colitis occurs when a blood clot diminishes blood flow to the colon; nonocclusive ischemia develops because of narrowing of blood vessels or low systemic blood pressure (119). In its milder form, ischemic colitis leads to mild necrosis or ulceration, whereas severe ischemic colitis is characterized by sepsis, ulceration, and in some cases gangrene. Ischemic colitis has some pathological features in common with IBD. Enhanced lipid peroxidation is evident in ischemic colitis (135). Treatment of feline experimental I/R GI injury with antioxidants like SOD and drugs that attenuate the effect of inflammatory mediators have shown promising effects (219). While the morbidity and mortality of intestinal ischemia is best mitigated by preventing the ischemic insult, once injury occurs better strategies to reduce anoxia and oxidative stress are helpful, and further research in this field is needed.

4. Colorectal cancer

The development of colorectal cancer (CRC) is dependent on several mechanisms. Genes that affect the control of cell

growth are frequently mutated in colon cancer. In CRC, free radicals produced by the colonic bacteria *Enterococcus faecalis* may directly cause mutations in colonic DNA resulting in colon cancer (14). Free radicals convert dietary procarcinogens to carcinogens that may contribute to CRC (124). Lipid peroxidation of polyunsaturated fatty acids results in production of reactive metabolites that have also been implicated in the pathogenesis of CRC (18).

Chronic inflammation in IBD results in persistent oxidative stress and inflammation that contribute to dysplasia (251). A population-based study and one meta-analysis have shown that Crohn's disease and ulcerative colitis patients have an increased risk of developing small bowel carcinoma in Crohn's disease and colon cancer in both forms of IBD, compared with a non-IBD population (27). The risk of CRC increases with the duration of clinical disease, the severity of the inflammatory response, and the extent of IBD (290). CRC in IBD patients generally begins with low-grade dysplasia, progressing to indefinite dysplasia, then to high-grade dysplasia and eventually, invasive adenocarcinoma. DNA damage caused by ROS is a major contributor to CRC development in ulcerative colitis patients. Thus oxidative stress-induced cellular damage may provide a mechanistic basis for colon cancer by causing genetic instability, specific gene alterations, and aberrant methylation. Inflammation-induced ROS interact with cancer-regulating genes, transcription factors, as well as DNA mismatch repair genes (158). Cytokines such as IL-6, IL-8, TGF- β 1, and mediators such as COX-2 are pivotal factors in the development of CRC (158). Excess iron intake may also lead to CRC by inducing ROS and inflammation-mediated epithelial changes (265a). There is emerging information that chronic inflammation and host microbes may play key roles in not only IBD-related carcinogenesis but also in sporadic and other forms of CRC (285).

VI. CONCLUSIONS

Many cell types within the mucosa of the GI tract produce ROS as part of normal physiology, yet the gut mucosa also is a target of various oxidants that can lead to pathological conditions. Oxidative stress makes a substantial contribution to the pathogenesis of many GI mucosal diseases, and despite recent progress, mechanisms of ROS-mediated GI diseases are not well established. Understanding the cellular and molecular mechanisms including altered signaling caused by ROS are critical to developing future therapies for GI diseases mediated by oxidative stress. As discussed, major GI diseases in which ROS play a major role (e.g., gastritis, gastric cancer, IBD, colonic inflammation, and cancer) are substantially linked to the microbiota as well as disruption of the antioxidant balance in the mucosa. Future treatment strategies for these important mucosal GI diseases lie in a better understanding of free radical biology of the GI mucosa.

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DISCLOSURES

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