Oxidative Stress and Antioxidant Defense

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Abstract: Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules and can damage cell structures such as carbohydrates, nucleic acids, lipids, and proteins and alter their functions. The shift in the balance between oxidants and antioxidants in favor of oxidants is termed "oxidative stress." Regulation of reducing and oxidizing (redox) state is critical for cell viability, activation, proliferation, and organ function. Aerobic organisms have integrated antioxidant systems, which include enzymatic and nonenzymatic antioxidants that are usually effective in blocking harmful effects of ROS. However, in pathological conditions, the antioxidant systems can be overwhelmed. Oxidative stress contributes to many pathological conditions and diseases, including cancer, neurological disorders, atherosclerosis, hypertension, ischemia/perfusion, diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease, and asthma. In this review, we summarize the cellular oxidant and antioxidant systems and discuss the cellular effects and mechanisms of the oxidative stress.

Key Words: antioxidant, oxidant, oxidative stress, reactive oxygen species, redox

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Reactive oxygen species (ROS) are produced by living organisms as a result of normal cellular metabolism. At low to moderate concentrations, they function in physiological cell processes, but at high concentrations, they produce adverse modifications to cell components, such as lipids, proteins, and DNA.[1–6] The shift in balance between oxidant/antioxidant in favor of oxidants is termed "oxidative stress." Oxidative stress contributes to many pathological conditions, including cancer, neurological disorders,[7–10] atherosclerosis, hypertension, ischemia/perfusion,[11–14] diabetes, acute respiratory distress syndrome, idiopathic pulmonary fibrosis, chronic obstructive pulmonary disease,[15] and asthma.[16–21] Aerobic organisms have integrated antioxidant systems, which include enzymatic and nonenzymatic antioxidants that are usually effective in blocking harmful effects of ROS. However, in pathological conditions, the antioxidant systems can be overwhelmed. In this review, we summarize the cellular oxidant and antioxidant systems and regulation of the reducing and oxidizing (redox) state in health and disease states.

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OXIDANTS

Endogenous Sources of ROS

ROS are produced from molecular oxygen as a result of normal cellular metabolism. ROS can be divided into 2 groups: free radicals and nonradicals. Molecules containing one or more unpaired electrons and thus giving reactivity to the molecule are called free radicals. When 2 free radicals share their unpaired electrons, nonradical forms are created. The 3 major ROS that are of physiological significance are superoxide anion ($O_2^{-}\cdot$), hydroxyl radical (•OH), and hydrogen peroxide (H_2O_2). ROS are summarized in Table 1.

Superoxide anion is formed by the addition of 1 electron to the molecular oxygen.[22] This process is mediated by nicotine adenine dinucleotide phosphate [NAD(P)H] oxidase or xanthine oxidase or by mitochondrial electron transport system. The major site for producing superoxide anion is the mitochondria, the machinery of the cell to produce adenosine triphosphate. Normally, electrons are transferred through mitochondrial electron transport chain for reduction of oxygen to water, but approximately 1 to 3% of all electrons leak from the system and produce superoxide. NAD(P)H oxidase is found in polymorphonuclear leukocytes, monocytes, and macrophages. Upon phagocytosis, these cells produce a burst of superoxide that lead to bactericidal activity. Superoxide is converted into hydrogen peroxide by the action of superoxide dismutases (SODs, EC 1.15.1.1). Hydrogen peroxide easily diffuses across the plasma membrane. Hydrogen peroxide is also produced by xanthine oxidase, amino acid oxidase, and NAD(P)H oxidase[23,24] and in peroxisomes by consumption of molecular oxygen in metabolic reactions. In a succession of reactions called Haber–Weiss and Fenton reactions, H_2O_2 can breakdown to OH^- in the presence of transmission metals like Fe^{2+} or Cu^{2+}.[25]

$$Fe^{3+} + \cdot O2 \rightarrow Fe^{2+} + O_2 \qquad \text{Haber–Weiss}$$
$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH \qquad \text{Fenton reaction}$$

O_2^- itself can also react with H_2O_2 and generate OH^-.[26,27] Hydroxyl radical is the most reactive of ROS

TABLE 1. Major Endogenous Oxidants

Oxidant	Formula	Reaction Equation
Superoxide anion	$O_2^{-\cdot}$	$NADPH + 2O_2 \leftrightarrow NADP^+ + 2O_2^{-\cdot} + H^+$ $2O_2^{-\cdot} + H^+ \rightarrow O_2 + H_2O_2$
Hydrogen peroxide	H_2O_2	$Hypoxanthine + H_2O + O_2 \rightleftharpoons xanthine + H_2O_2$ $Xanthine + H_2O + O_2 \rightleftharpoons uric\ acid + H_2O_2$
Hydroxyl radical	•OH	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$
Hypochlorous acid	HOCl	$H_2O_2 + Cl^- \rightarrow HOCl + H_2O$
Peroxyl radicals	ROO•	$R^\bullet + O_2 \rightarrow ROO^\bullet$
Hydroperoxyl radical	HOO·	$O_2^- + H_2O \rightleftharpoons HOO^\cdot + OH^-$

and can damage proteins, lipids, and carbohydrates and DNA. It can also start lipid peroxidation by taking an electron from polyunsaturated fatty acids.

Granulocytic enzymes further expand the reactivity of H_2O_2 via eosinophil peroxidase and myeloperoxidase (MPO). In activated neutrophils, H_2O_2 is consumed by MPO. In the presence of chloride ion, H_2O_2 is converted to hypochlorous acid (HOCl). HOCl is highly oxidative and plays an important role in killing of the pathogens in the airways.[28] However, HOCl can also react with DNA and induce DNA–protein interactions and produce pyrimidine oxidation products and add chloride to DNA bases.[29,30] Eosinophil peroxidase and MPO also contribute to the oxidative stress by modification of proteins by halogenations, nitration, and protein cross-links via tyrosyl radicals.[31–33]

Other oxygen-derived free radicals are the peroxyl radicals (ROO·•). Simplest form of these radicals is hydroperoxyl radical (HOO·•) and has a role in fatty acid peroxidation. Free radicals can trigger lipid peroxidation chain reactions by abstracting a hydrogen atom from a side-chain methylene carbon. The lipid radical then reacts with oxygen to produce peroxyl radical. Peroxyl radical initiates a chain reaction and transforms polyunsaturated fatty acids into lipid hydroperoxides. Lipid hydroperoxides are very unstable and easily decompose to secondary products, such as aldehydes (such as 4-hydroxy-2,3-nonenal) and malondialdehydes (MDAs). Isoprostanes are another group of lipid peroxidation products that are generated via the peroxidation of arachidonic acid and have also been found to be elevated in plasma and breath condensates of asthmatics.[34,35] Peroxidation of lipids disturbs the integrity of cell membranes and leads to rearrangement of membrane structure.

Hydrogen peroxide, superoxide radical, oxidized glutathione (GSSG), MDAs, isoprostanes, carbonyls, and nitrotyrosine can be easily measured from plasma, blood, or bronchoalveolar lavage samples as biomarkers of oxidation by standardized assays.

Exogenous Source of Oxidants

Cigarette Smoke

Cigarette smoke contains many oxidants and free radicals and organic compounds, such as superoxide and nitric oxide.[36] In addition, inhalation of cigarette smoke into the lung also activates some endogenous mechanisms, such as accumulation of neutrophils and macrophages, which further increase the oxidant injury.

Ozone Exposure

Ozone exposure can cause lipid peroxidation and induce influx of neutrophils into the airway epithelium. Short-term exposure to ozone also causes the release of inflammatory mediators, such as MPO, eosinophil cationic proteins and also lactate dehydrogenase and albumin.[37] Even in healthy subjects, ozone exposure causes a reduction in pulmonary functions.[38] Cho et al[39] have shown that particulate matter (mixture of solid particles and liquid droplets suspended in the air) catalyzes the reduction of oxygen.

Hyperoxia

Hyperoxia refers to conditions of higher oxygen levels than normal partial pressure of oxygen in the lungs or other body tissues. It leads to greater production of reactive oxygen and nitrogen species.[40,41]

Ionizing Radiation

Ionizing radiation, in the presence of O_2, converts hydroxyl radical, superoxide, and organic radicals to hydrogen peroxide and organic hydroperoxides. These hydroperoxide species react with redox active metal ions, such as Fe and Cu, via Fenton reactions and thus induce oxidative stress.[42,43] Narayanan et al[44] showed that fibroblasts that were exposed to alpha particles had significant increases in intracellular $O_2^{-\cdot}$ and H_2O_2 production via plasma membrane-bound NADPH oxidase.[44] Signal transduction molecules, such as extracellular signal-regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinase (JNK), and p38, and transcription factors, such as activator protein-1 (AP-1), nuclear factor-κB (NF-κB), and p53, are activated, which result in the expression of radiation response–related genes.[45–50] Ultraviolet A (UVA) photons trigger oxidative reactions by excitation of endogenous photosensitizers, such as porphyrins, NADPH oxidase, and riboflavins. 8-Oxo-7,8-dihydroguanine (8-oxoGua) is the main UVA-mediated DNA oxidation product formed by the oxidation of •OH radical, 1-electron oxidants, and singlet oxygen that mainly reacts with guanine.[51] The formation of guanine radical cation in isolated DNA has been shown to efficiently occur through the direct effect of ionizing radiation.[52,53] After exposure to ionizing radiation, intracellular level of glutathione (GSH) decreases for a short term but then increases again.[54]

Heavy Metal Ions

Heavy metal ions, such as iron, copper, cadmium, mercury, nickel, lead, and arsenic, can induce generation of reactive radicals and cause cellular damage via depletion of enzyme activities through lipid peroxidation and reaction with nuclear proteins and DNA.[55]

One of the most important mechanisms of metal-mediated free radical generation is via a Fenton-type reaction. Superoxide ion and hydrogen peroxide can interact with transition metals, such as iron and copper, via the metal catalyzed Haber–Weiss/Fenton reaction to form OH radicals.

$$Metal^{3+} + \cdot O_2 \rightarrow Metal^{2+} + O_2 \quad \text{Haber–Weiss}$$
$$Metal^{2+} + H_2O_2 \rightarrow Metal^{3+} + OH^- + \cdot OH \quad \text{Fenton reaction}$$

Besides the Fenton-type and Haber–Weiss-type mechanisms, certain metal ions can react directly with cellular molecules to generate free radicals, such as thiol radicals, or induce cell signaling pathways. These radicals may also react with other thiol molecules to generate $O_2^{-\cdot}$. $O_2^{-\cdot}$ is converted to H_2O_2, which causes additional oxygen radical generation. Some metals, such as arsenite, induce ROS formation indirectly by activation of radical producing systems in cells.[56]

Arsenic is a highly toxic element that produces a variety of ROS, including superoxide ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), peroxyl radical ($ROO^\bullet$), nitric oxide ($NO^\bullet$), hydrogen peroxide (H_2O_2), and dimethylarsinic peroxyl radicals [$(CH_3)_2AsOO^\bullet$].[57–59] Arsenic (III) compounds can inhibit antioxidant enzymes, especially the GSH-dependent enzymes, such as glutathione-*S*-transferases (GSTs), glutathione peroxidase (GSH-Px), and GSH reductase, via binding to their sulfhydryl (–SH) groups.[60,61]

Lead increases lipid peroxidation.[62] Significant decreases in the activity of tissue SOD and brain GPx have been reported after lead exposure.[63,64] Replacement of zinc, which serves as a cofactor for many enzymes by lead, leads to inactivation of such enzymes. Lead exposure may cause inhibition of GST by affecting tissue thiols.

ROS generated by metal-catalyzed reactions can modify DNA bases. Three base substitutions, G → C, G → T, and C → T, can occur as a result of oxidative damage by metal ions, such as Fe^{2+}, Cu^{2+}, and Ni^{2+}. Reid et al[65] showed that G → C was predominantly produced by Fe^{2+} while C → T substitution was by Cu^{2+} and Ni^{2+}.

ANTIOXIDANTS

The human body is equipped with a variety of antioxidants that serve to counterbalance the effect of oxidants. For all practical purposes, these can be divided into 2 categories: enzymatic (Table 2) and nonenzymatic (Table 3).

Enzymatic Antioxidants

The major enzymatic antioxidants of the lungs are SODs (EC 1.15.1.11), catalase (EC 1.11.1.6), and GSH-Px (EC 1.11.1.9). In addition to these major enzymes, other antioxidants, including heme oxygenase-1 (EC 1.14.99.3), and redox proteins, such as thioredoxins (TRXs, EC 1.8.4.10), peroxiredoxins (PRXs, EC 1.11.1.15), and glutaredoxins, have also been found to play crucial roles in the pulmonary antioxidant defenses.

Since superoxide is the primary ROS produced from a variety of sources, its dismutation by SOD is of primary importance for each cell. All 3 forms of SOD, that is, CuZn-SOD, Mn-SOD, and EC-SOD, are widely expressed in the human lung. Mn-SOD is localized in the mitochondria matrix. EC-SOD is primarily localized in the extracellular matrix, especially in areas containing high amounts of type I collagen fibers and around pulmonary and systemic vessels. It has also been detected in the bronchial epithelium, alveolar epithelium, and alveolar macrophages.[66,67] Overall, CuZn-SOD and Mn-SOD are generally thought to act as bulk scavengers of superoxide radicals. The relatively high EC-SOD level in the lung with its specific binding to the extracellular matrix components may represent a fundamental component of lung matrix protection.[68]

H_2O_2 that is produced by the action of SODs or the action of oxidases, such as xanthine oxidase, is reduced to

TABLE 2. Enzymatic Scavenger of Antioxidant Defense

Name of Scavenger	Acronym	Catalyzed Reaction
Superoxide dismutase	SOD	$M^{(n+1)+}$-SOD + O_2^- → M^{n+}-SOD + O_2
		M^{n+}-SOD + O_2^- + $2H^+$ → $M^{(n+1)+}$-SOD + H_2O_2
Catalase	CAT	2 H_2O_2 → O_2 + 2 H_2O
		H_2O_2 + Fe(III)-E → H_2O + O = Fe(IV)-E(.+)
		H_2O_2 + O = Fe(IV)-E(.+) → H_2O + Fe(III)-E + O_2
Glutathione peroxidase	GTPx	2GSH +H_2O_2 → GSSG +2H_2O
		2GSH+ ROOH → GSSG + ROH + H_2O
Thioredoxin	TRX	Adenosine monophosphate + sulfite + thioredoxin disulfide = 5'-adenylyl sulfate + thioredoxin
		Adenosine 3′,5′-bisphosphate + sulfite + thioredoxin disulfide = 3′-phosphoadenylyl sulfate + thioredoxin
Peroxiredoxin	PRX	2 R′-SH + ROOH = R′-S-S-R′ + H_2O + ROH
Glutathione transferase	GST	RX + GSH = HX + R-S-GSH

TABLE 3. Nonenzymatic Scavenger of Antioxidant Defenses

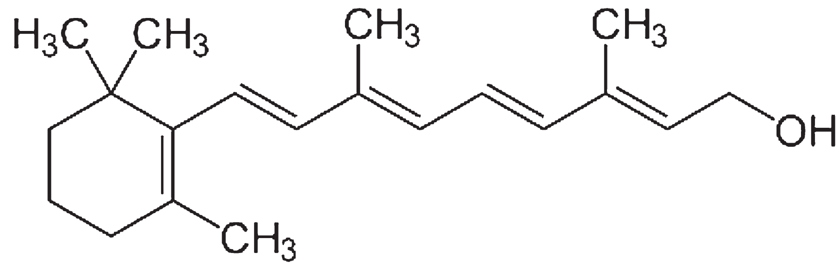
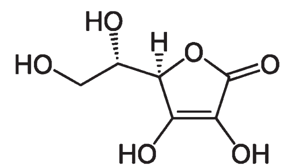
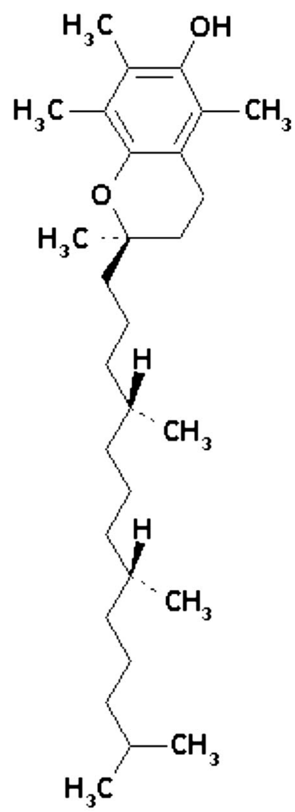
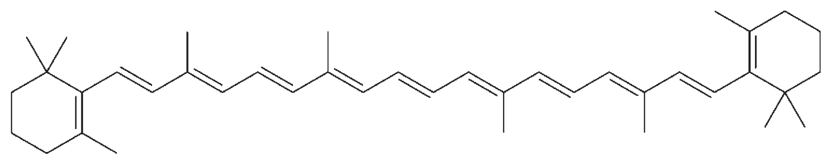
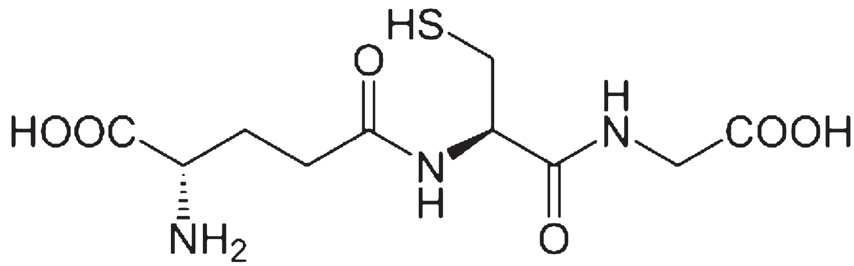
Chemical Name of Scavenger	Name of Scavenger	Structure
All trans retinol 2	Vitamin A	
Ascorbic acid	Vitamin C	
α-Tocopherol	Vitamin E	

TABLE 3. (*Continued*)

Chemical Name of Scavenger	Name of Scavenger	Structure
β-Carotene		
Glutathione		

water by catalase and the GSH-Px. Catalase exists as a tetramer composed of 4 identical monomers, each of which contains a heme group at the active site. Degradation of H_2O_2 is accomplished via the conversion between 2 conformations of catalase-ferricatalase (iron coordinated to water) and compound I (iron complexed with an oxygen atom). Catalase also binds NADPH as a reducing equivalent to prevent oxidative inactivation of the enzyme (formation of compound II) by H_2O_2 as it is reduced to water.[69]

Enzymes in the redox cycle responsible for the reduction of H_2O_2 and lipid hydroperoxides (generated as a result of membrane lipid peroxidation) include the GSH-Pxs.[70] The GSH-Pxs are a family of tetrameric enzymes that contain the unique amino acid selenocysteine within the active sites and use low-molecular-weight thiols, such as GSH, to reduce H_2O_2 and lipid peroxides to their corresponding alcohols. Four GSH-Pxs have been described, encoded by different genes: GSH-Px-1 (cellular GSH-Px) is ubiquitous and reduces H_2O_2 and fatty acid peroxides, but not esterified peroxyl lipids.[71] Esterified lipids are reduced by membrane-bound GSH-Px-4 (phospholipid hydroperoxide GSH-Px), which can use several different low-molecular-weight thiols as reducing equivalents. GSH-Px-2 (gastrointestinal GSH-Px) is localized in gastrointestinal epithelial cells where it serves to reduce dietary peroxides.[72] GSH-Px-3 (extracellular GSH-Px) is the only member of the GSH-Px family that resides in the extracellular compartment and is believed to be one of the most important extracellular antioxidant enzyme in mammals. Of these, extracellular GSH-Px is most widely investigated in the human lung.[73]

In addition, disposal of H_2O_2 is closely associated with several thiol-containing enzymes, namely, TRXs (TRX1 and TRX2), thioredoxin reductases (EC 1.8.1.9) (TRRs), PRXs (which are thioredoxin peroxidases), and glutaredoxins.[74] Two TRXs and TRRs have been characterized in human cells, existing in both cytosol and mitochondria. In the lung, TRX and TRR are expressed in bronchial and alveolar epithelium and macrophages. Six different PRXs have been found in human cells, differing in their ultrastructural compartmentalization. Experimental studies have revealed the importance of PRX VI in the protection of alveolar epithelium. Human lung expresses all PRXs in bronchial epithelium, alveolar epithelium, and macrophages.[75] PRX V has recently been found to function as a peroxynitrite reductase,[76] which means that it may function as a potential protective compound in the development of ROS-mediated lung injury.[77]

Common to these antioxidants is the requirement of NADPH as a reducing equivalent. NADPH maintains catalase in the active form and is used as a cofactor by TRX and GSH reductase (EC 1.6.4.2), which converts GSSG to GSH, a co-substrate for the GSH-Pxs. Intracellular NADPH, in turn, is generated by the reduction of $NADP^+$ by glucose-6-phosphate dehydrogenase, the first and rate-limiting enzyme of the pentose phosphate pathway, during the conversion of glucose-6-phosphate to 6-phosphogluconolactone. By generating NADPH, glucose-6-phosphate dehydrogenase is a critical determinant of cytosolic GSH buffering capacity (GSH/GSSG) and, therefore, can be considered an essential, regulatory antioxidant enzyme.[78,79]

GSTs (EC 2.5.1.18), another antioxidant enzyme family, inactivate secondary metabolites, such as unsaturated aldehydes, epoxides, and hydroperoxides. Three major families of GSTs have been described: cytosolic GST, mitochondrial GST,[80,81] and membrane-associated microsomal GST that has a role in eicosanoid and GSH metabolism.[82] Seven classes of cytosolic GST are identified in mammalian, designated Alpha, Mu, Pi, Sigma, Theta, Omega, and Zeta.[83–86] During nonstressed conditions, class

Mu and Pi GSTs interact with kinases Ask1 and JNK, respectively, and inhibit these kinases.[87–89] It has been shown that GSTP1 dissociates from JNK in response to oxidative stress.[89] GSTP1 also physically interacts with PRX VI and leads to recovery of PRX enzyme activity via glutathionylation of the oxidized protein.[90]

Nonenzymatic Antioxidants

Nonenzymatic antioxidants include low-molecular-weight compounds, such as vitamins (vitamins C and E), β-carotene, uric acid, and GSH, a tripeptide (L-γ-glutamyl-L-cysteinyl-L-glycine) that comprise a thiol (sulfhydryl) group.

Vitamin C (Ascorbic Acid)

Water-soluble vitamin C (ascorbic acid) provides intracellular and extracellular aqueous-phase antioxidant capacity primarily by scavenging oxygen free radicals. It converts vitamin E free radicals back to vitamin E. Its plasma levels have been shown to decrease with age.[91,92]

Vitamin E (α-Tocopherol)

Lipid-soluble vitamin E is concentrated in the hydrophobic interior site of cell membrane and is the principal defense against oxidant-induced membrane injury. Vitamin E donates electron to peroxyl radical, which is produced during lipid peroxidation. α-Tocopherol is the most active form of vitamin E and the major membrane-bound antioxidant in cell. Vitamin E triggers apoptosis of cancer cells and inhibits free radical formations.[93]

Glutathione

GSH is highly abundant in all cell compartments and is the major soluble antioxidant. GSH/GSSG ratio is a major determinant of oxidative stress. GSH shows its antioxidant effects in several ways.[94] It detoxifies hydrogen peroxide and lipid peroxides via action of GSH-Px. GSH donates its electron to H_2O_2 to reduce it into H_2O and O_2. GSSG is again reduced into GSH by GSH reductase that uses NAD(P)H as the electron donor. GSH-Pxs are also important for the protection of cell membrane from lipid peroxidation. Reduced glutathione donates protons to membrane lipids and protects them from oxidant attacks.[95]

GSH is a cofactor for several detoxifying enzymes, such as GSH-Px and transferase. It has a role in converting vitamin C and E back to their active forms. GSH protects cells against apoptosis by interacting with proapoptotic and antiapoptotic signaling pathways.[94] It also regulates and activates several transcription factors, such as AP-1, NF-κB, and Sp-1.

Carotenoids (β-Carotene)

Carotenoids are pigments found in plants. Primarily, β-carotene has been found to react with peroxyl (ROO•), hydroxyl (•OH), and superoxide ($O_2^{-\cdot}$) radicals.[96] Carotenoids show their antioxidant effects in low oxygen partial pressure but may have pro-oxidant effects at higher oxygen concentrations.[97] Both carotenoids and retinoic acids (RAs) are capable of regulating transcription factors.[98] β-Carotene inhibits the oxidant-induced NF-κB activation and interleukin (IL)-6 and tumor necrosis factor-α production. Carotenoids also affect apoptosis of cells. Antiproliferative effects of RA have been shown in several studies. This effect of RA is mediated mainly by retinoic acid receptors and vary among cell types. In mammary carcinoma cells, retinoic acid receptor was shown to trigger growth inhibition by inducing cell cycle arrest, apoptosis, or both.[99,100]

THE EFFECT OF OXIDATIVE STRESS: GENETIC, PHYSIOLOGICAL, AND BIOCHEMICAL MECHANISMS

Oxidative stress occurs when the balance between antioxidants and ROS are disrupted because of either depletion of antioxidants or accumulation of ROS. When oxidative stress occurs, cells attempt to counteract the oxidant effects and restore the redox balance by activation or silencing of genes encoding defensive enzymes, transcription factors, and structural proteins.[101,102] Ratio between oxidized and reduced glutathione (2GSH/GSSG) is one of the important determinants of oxidative stress in the body. Higher production of ROS in body may change DNA structure, result in modification of proteins and lipids, activation of several stress-induced transcription factors, and production of proinflammatory and anti-inflammatory cytokines.

Effects of Oxidative Stress on DNA

ROS can lead to DNA modifications in several ways, which involves degradation of bases, single- or double-stranded DNA breaks, purine, pyrimidine or sugar-bound modifications, mutations, deletions or translocations, and cross-linking with proteins. Most of these DNA modifications (Fig. 1) are highly relevant to carcinogenesis, aging, and neurodegenerative, cardiovascular, and autoimmune diseases. Tobacco smoke, redox metals, and nonredox metals, such as iron, cadmium, chrome, and arsenic, are also involved in carcinogenesis and aging by generating free radicals or binding with thiol groups. Formation of 8-OH-G is the best-known DNA damage occurring via oxidative stress and is a potential biomarker for carcinogenesis.

Promoter regions of genes contain consensus sequences for transcription factors. These transcription factor–binding sites contain GC-rich sequences that are susceptible for oxidant attacks. Formation of 8-OH-G DNA in transcription factor binding sites can modify binding of transcription factors and thus change the expression of related genes as has been shown for AP-1 and Sp-1 target sequences.[103] Besides 8-OH-G, 8,5′-cyclo-2′-deoxyadenosine (cyclo-dA) has also been shown to inhibit transcription from a reporter gene in a cell system if located in a TATA box.[104] The TATA-binding protein initiates transcription by changing the bending of DNA. The binding of TATA-binding protein may be impaired by the presence of cyclo-dA.

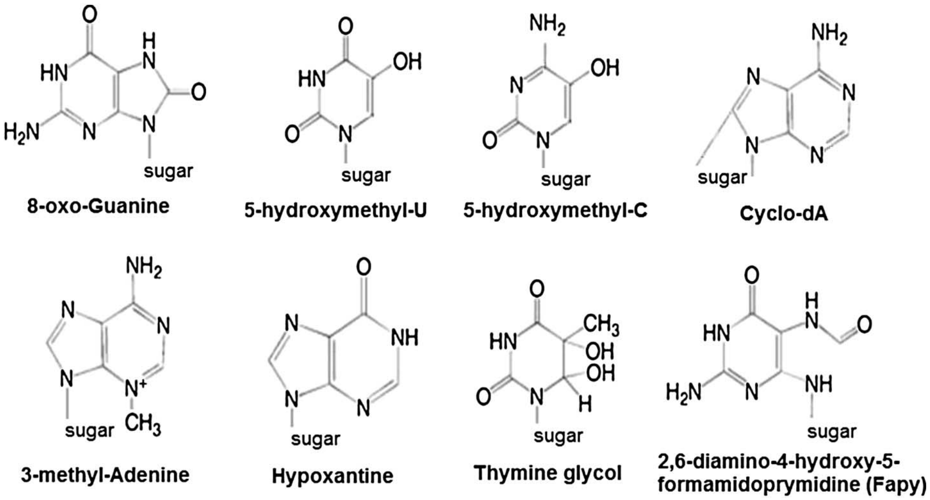


FIGURE 1. Base modifications introduced by reactive oxygen species.

Oxidative stress causes instability of microsatellite (short tandem repeats) regions. Redox active metal ions, hydroxyl radicals increase microsatellite instability.[105] Even though single-stranded DNA breaks caused by oxidant injury can easily be tolerated by cells, double-stranded DNA breaks induced by ionizing radiation can be a significant threat for the cell survival.[106]

Methylation at CpG islands in DNA is an important epigenetic mechanism that may result in gene silencing. Oxidation of 5-MeCyt to 5-hydroxymethyl uracil (5-OHMeUra) can occur via deamination/oxidation reactions of thymine or 5-hydroxymethyl cytosine intermediates.[107] In addition to the modulating gene expression, DNA methylation also seems to affect chromatin organization.[108] Aberrant DNA methylation patterns induced by oxidative attacks also affect DNA repair activity.

Effects of Oxidative Stress on Lipids

ROS can induce lipid peroxidation and disrupt the membrane lipid bilayer arrangement that may inactivate membrane-bound receptors and enzymes and increase tissue permeability.[109] Products of lipid peroxidation, such as MDA and unsaturated aldehydes, are capable of inactivating many cellular proteins by forming protein cross-linkages.[110–112] 4-Hydroxy-2-nonenal causes depletion of intracellular GSH and induces of peroxide production,[113,114] activates epidermal growth factor receptor,[115] and induces fibronectin production.[116] Lipid peroxidation products, such as isoprostanes and thiobarbituric acid reactive substances, have been used as indirect biomarkers of oxidative stress, and increased levels were shown in the exhaled breath condensate or bronchoalveolar lavage fluid or lung of chronic obstructive pulmonary disease patients or smokers.[117–119]

Effects of Oxidative Stress on Proteins

ROS can cause fragmentation of the peptide chain, alteration of electrical charge of proteins, cross-linking of proteins, and oxidation of specific amino acids and therefore lead to increased susceptibility to proteolysis by degradation by specific proteases.[120] Cysteine and methionine residues in proteins are particularly more susceptible to oxidation.[121] Oxidation of sulfhydryl groups or methionine residues of proteins cause conformational changes, protein unfolding, and degradation.[8,121–123] Enzymes that have metals on or close to their active sites are especially more sensitive to metal catalyzed oxidation. Oxidative modification of enzymes has been shown to inhibit their activities.[124,125]

In some cases, specific oxidation of proteins may take place. For example, methionine can be oxidized methionine sulfoxide[126] and phenylalanine to *o*-tyrosine[127]; sulfhydryl groups can be oxidized to form disulfide bonds;[128] and carbonyl groups may be introduced into the side chains of proteins. Gamma rays, metal-catalyzed oxidation, HOCl, and ozone can cause formation of carbonyl groups.[129]

Effects of Oxidative Stress on Signal Transduction

ROS can induce expression of several genes involved in signal transduction.[1,130] A high ratio for GSH/GSSG is important for the protection of the cell from oxidative damage. Disruption of this ratio causes activation of redox sensitive transcription factors, such as NF-κB, AP-1, nuclear factor of activated T cells and hypoxia-inducible factor 1 , that are involved in the inflammatory response. Activation of transcription factors via ROS is achieved by signal transduction cascades that transmit the information from outside to the inside of cell. Tyrosine kinase receptors, most of the growth factor receptors, such as epidermal growth factor receptor, vascular endothelial growth factor receptor, and receptor for platelet-derived growth factor, protein tyrosine phosphatases, and serine/threonine kinases are targets of ROS.[131–133] Extracellular signal-regulated kinases, JNK, and p38, which are the members of mitogen-activated protein kinase family and involved in several processes in cell including proliferation, differentiation, and apoptosis, also can be regulated by oxidants.

Under oxidative stress conditions, cysteine residues in the DNA-binding site of c-Jun, some AP-1 subunits, and inhibitory κ-B kinase undergo reversible S-glutathiolation. Glutaredoxin and TRX have been reported to play an important role in regulation of redox-sensitive signaling pathways, such as NF-κB and AP-1, p38 mitogen-activated protein kinase, and JNK.[134–137]

NF-κB can be activated in response to oxidative stress conditions, such as ROS, free radicals, and UV irradiation.[138] Phosphorylation of IκB frees NF-κB and allows it to enter the nucleus to activate gene transcription.[139] A number of kinases have been reported to phosphorylate IκBs at the serine residues. These kinases are the targets of oxidative signals for activation of NF-κB.[140] Reducing agents enhance NF-κB DNA binding, whereas oxidizing agents inhibit DNA binding of NF-κB. TRX may exert 2 opposite actions in regulation of NF-κB: in the cytoplasm, it blocks degradation of IκB and inhibits NF-κB activation but enhances NF-κB DNA binding in the nucleus.[141] Activation of NF-κB via oxidation-related degradation of IκB results in the activation of several antioxidant defense–related genes. NF-κB regulates the expression of several genes that participate in immune response, such as IL-1β, IL-6, tumor necrosis factor-α, IL-8, and several adhesion molecules.[142,143] NF-κB also regulates angiogenesis and proliferation and differentiation of cells.

AP-1 is also regulated by redox state. In the presence of H_2O_2, some metal ions can induce activation of AP-1. Increase in the ratio of GSH/GSSG enhances AP-1 binding while GSSG inhibits the DNA binding of AP-1.[144] DNA binding of the Fos/Jun heterodimer is increased by the reduction of a single conserved cysteine in the DNA-binding domain of each of the proteins,[145] while DNA binding of AP-1 can be inhibited by GSSG in many cell types, suggesting that disulphide bond formation by cysteine residues inhibits AP-1 DNA binding.[146,147] Signal transduction via oxidative stress is summarized in Figure 2.

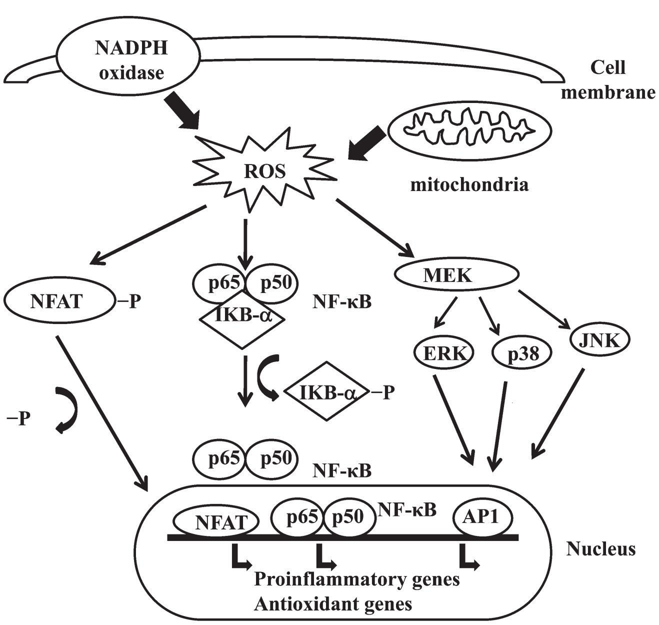


FIGURE 2. Effects of oxidative stress on signal transduction in the cell.

CONCLUSIONS

Oxidative stress can arise from overproduction of ROS by metabolic reactions that use oxygen and shift the balance between oxidant/antioxidant statuses in favor of the oxidants. ROS are produced by cellular metabolic activities and environmental factors, such as air pollutants or cigarette smoke. ROS are highly reactive molecules because of unpaired electrons in their structure and react with several biological macromolecules in cell, such as carbohydrates, nucleic acids, lipids, and proteins, and alter their functions. ROS also affects the expression of several genes by upregulation of redox-sensitive transcription factors and chromatin remodeling via alteration in histone acetylation/deacetylation. Regulation of redox state is critical for cell viability, activation, proliferation, and organ function.

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