REVIEW ARTICLE

Reactive oxygen species: Oxidative damage and pathogenesis

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Reactive oxygen species (ROS) such as O_2^-, H_2O_2 and $^{\cdot}OH$ are highly toxic to cells. Cellular antioxidant enzymes, and the free-radical scavengers normally protect a cell from toxic effects of the ROS. However, when generation of the ROS overtakes the antioxidant defense of the cells, oxidative damage of the cellular macromolecules (lipids, proteins, and nucleic acids) occurs, leading finally to various pathological conditions. ROS-mediated lipid peroxidation, oxidation of proteins, and DNA damage are well-known outcomes of oxygen-derived free radicals, leading to cellular pathology and ultimately to cell death. The mechanism of ROS-mediated oxidative damage of lipids, proteins, and DNA has been extensively studied. The site-specific oxidative damage of some of the susceptible amino acids of proteins is now regarded as the major cause of metabolic dysfunction during pathogenesis. ROS have also been implicated in the regulation of at least two well-defined transcription factors which play an important role in the expression of various genes encoding proteins that are responsible for tissue injury. One of the significant benefits of the studies on ROS will perhaps be in designing of a suitable antioxidant therapy to control the ROS-mediated oxidative damage, and the disease processes.

OXYGEN is vital for aerobic life processes. However, about 5% or more of the inhaled O_2 is converted to reactive oxygen species (ROS) such as O_2^-, H_2O_2, and $^{\cdot}OH$ by univalent reduction of O_2 (ref. 1). Thus cells under aerobic condition are always threatened with the insult of ROS, which however are efficiently taken care of by the highly powerful antioxidant systems of the cell without any untoward effect. When the balance between ROS production and antioxidant defenses is lost, 'oxidative stress' results which through a series of events deregulates the cellular functions leading to various pathological conditions including cardiovascular dysfunction, neurodegenerative diseases, gastroduodenal pathogenesis, metabolic dysfunction of almost all the vital organs, cancer, and premature aging[2]. The free-radical-mediated oxidative stress results in oxidation of membrane lipoproteins, glycoxidation, and oxidation of DNA: subsequently cell death results. Various necrotic factors, proteases, and ROS from damaged cells also attack the adjacent cells, resulting ultimately in tissue injury. Furthermore, tissue injury itself has been reported to cause severe oxidative stresses[3]. Injury caused by ischemia reperfusion, heat, trauma, freezing, severe exercise, toxins, radiation or infection; leads to the generation of ROS, and development of various disease processes[3].

The present review mainly deals with: (i) the mechanism of formation of ROS in respiring cells under aerobic conditions, (ii) the antioxidant systems involved in the scavenging process, and (iii) ROS-mediated lipid peroxidation, oxidation of proteins, and DNA damage, leading to cellular pathology and ultimately to cell death. The molecular mechanism of ROS-mediated diseases such as cardiac and cerebral ischemia, Alzheimer's disease and aging, rheumatoid arthritis, inflammatory bowel disease, and multistage carcinogenesis have been extensively studied[2]. A recent review by Thomas and Kalyanaraman[2] may be referred for current views on the ROS-mediated disease processes.

Reactive oxygen species: Their site of generation and their reactivity

Although O_2 can behave like a radical (a diradical) owing to the presence of two unpaired electrons of parallel spin, it does not exhibit extreme reactivity due to quantum-mechanical restrictions. Its electronic structure result in formation of water by reduction with four electrons, i.e:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O.$$

In the sequential univalent process by which O_2 undergoes reduction, several reactive intermediates are formed, such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and the extremely reactive hydroxyl radical ($^{\cdot}OH$): collectively termed as the reactive oxygen species (ROS). The process can be represented as:

$$O_2 \xrightarrow{e^-} O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} {}^{\cdot}OH \xrightarrow{e^-} H_2O.$$

For the production of O_2^-, normally the tendency of univalent reduction of O_2 in respiring cells is restricted by cytochrome oxidase of the mitochondrial electron transport chain, which reduces O_2 by four electrons to H_2O

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without releasing either O_2^- or H_2O_2. However, O_2^- is invariably produced in respiring cells[4]. This is due to the probable 'leak' of single electron at the specific site of the mitochondrial electron transport chain, resulting in inappropriate single electron reduction of oxygen to O_2^- (refs 5, 6). When the electron transport chain is highly reduced, and the respiratory rate is dependent on ADP availability; 'leakage' of electrons at the ubisemiquinone and ubiquinone sites[7] increases so as to result in production of O_2^- and H_2O_2 (refs 8, 9).

For the production of H_2O_2, peroxisomal oxidases and flavoproteins, as well as D-amino acid oxidase, L-hydroxy acid oxidase, and fatty-acyl oxidase participate[6,9]. Cytochrome P-450, P-450 reductase and cytochrome *b*-5 reductase in the endoplasmic reticulum under certain conditions generate O_2^- and H_2O_2 during their catalytic cycles[7]. Likewise, the catalytic cycle of xanthine oxidase has emerged as an important source of O_2^- and H_2O_2 in a number of different tissue injuries. Xanthine oxidase – produced by proteolytic cleavage of xanthine dehydrogenase during ischemia – upon reperfusion in presence of O_2, acts on xanthine or hypoxanthine to generate O_2^- and H_2O_2 (refs 10, 11) (Figure 1). The phagocytic cells, such as neutrophils, when activated during phagocytosis, generate O_2^- and H_2O_2 through activation of NADPH oxidase[12]. Neutrophil accumulation in inflammated tissue is one of the major reasons of oxidative damage due to generation of ROS. In addition, spontaneous dismutation of O_2^- at neutral pH or dismutation by superoxide dismutase, results in H_2O_2 production[4]. Various biological sources for the production of H_2O_2 have been reviewed[13].

Finally, for the production of $^{\bullet}OH$, except during abnormal exposure to ionizing radiation, generation of $^{\bullet}OH$ *in vivo* requires the presence of trace amount of transition metals like iron or copper. A simple mixture of H_2O_2 and Fe^{2+} salt forms $^{\bullet}OH$, as given by the following Fenton reaction:

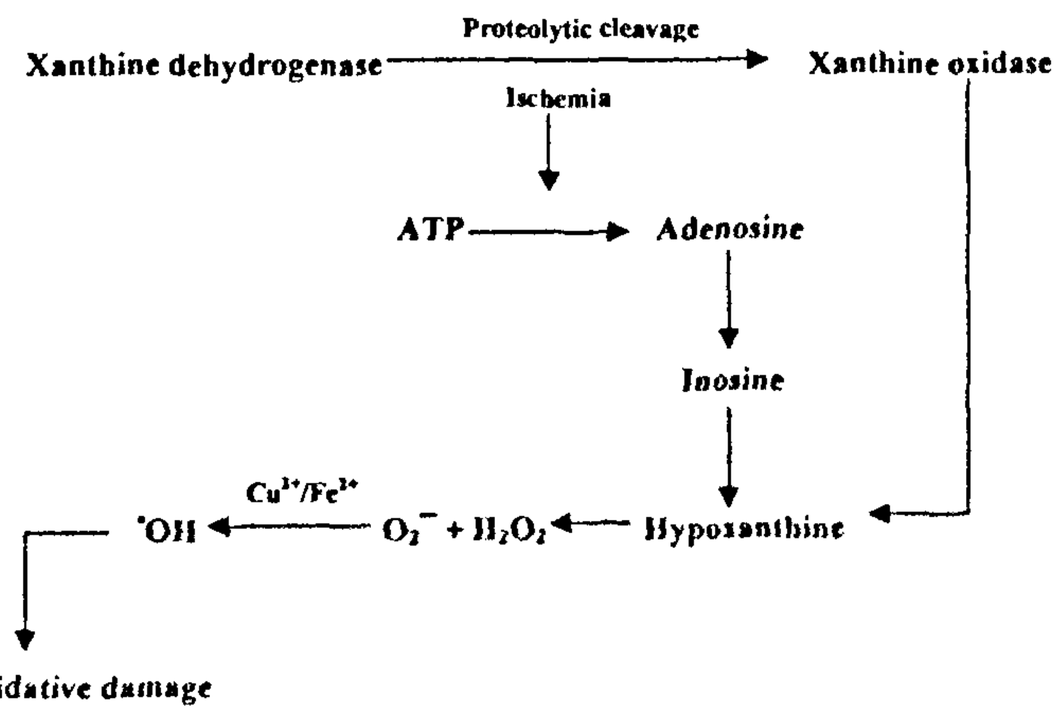

Figure 1. Plausible mechanism of ischemia-induced free radical generation and tissue damage.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-.$$

Traces of Fe^{3+} can react further with H_2O_2 to form the following products:

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + O_2^- + H^+.$$

Thus, a free-radical mechanism for the generation of $^{\bullet}OH$ may be deduced as follows:

$$O_2^- + H_2O_2 \rightarrow OH^- + {}^{\bullet}OH + O_2.$$

Unfortunately, the rate constant for the above reaction is very low but can be accounted for if the reaction is catalyzed by traces of transition metal ions – the metal-catalyzed Haber–Weiss reaction[14]. The various steps of this reaction are:

$$Fe^{3+} + O_2^- \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^-,$$

and the net result is:

$$O_2^- + H_2O_2 \xrightarrow{\text{iron salt}} O_2 + {}^{\bullet}OH + OH^-.$$

However, redox-active free iron or copper do not exist in biological systems, as these transition metal ions remain bound to proteins, membranes, nucleic acids or low-molecular weight chelating agents like citrate, histidine, or ATP (ref. 14). However during ischemic condition, and cellular acidosis, transition-metal ions may be released from some metalloproteins[15], resulting in generation of $^{\bullet}OH$, as shown in the above reaction. Therefore, use of chelation therapies, using desferrioxamine, controls (i) myocardial dysfunction following short-term ischemia, which is subsequently followed by reperfusion[16], and (ii) in stress-induced gastric ulcer[17]: indicating thereby the release of transition metal ion from ischemic organs[15]. In addition, Parkinson's disease is associated with high content of reactive iron in substantia nigra, and with an increased oxidative damage in nigral neuronal cells[18]. Similar changes were also observed in Huntington's disease and Alzheimer's disease. That 'catalytic' iron has a role in $^{\bullet}OH$-mediated damage of inflammatory joint disease as well, has also been suggested by Fairburn *et al.*[19]. Ferritin iron can be mobilized from the protein by O_2^-, ascorbate or low pH in the hypoxic tissue (acidosis), or from the microenvironment of phagocytes. The released iron can thereby generate $^{\bullet}OH$ (ref. 20). H_2O_2 also liberates redox-active iron from hemoglobin and myoglobin[11]. The release of these transition metal ions under some abnormal physiological conditions may further augment oxidative damage by $^{\bullet}OH$ (refs 11, 21).

O_2^- is toxic to a cell growing under aerobic conditions, and superoxide dismutase (SOD), which scavenges O_2^-,

offers cellular defense against this toxicity[22]. O_2^- shows high reactivity in hydrophobic environments, but poor reactivity in aqueous solutions. Due to its charged state, it cannot cross a biological membrane, with the exception of the erythrocyte membrane which has an 'anion channel' that helps in the crossing of O_2^-. An indirect deleterious action of O_2^- is mediated by its dismutation to H_2O_2, which is sensitive to catalase. In a transition-metal-free system, H_2O_2 shows limited toxicity. However, since it is long-lived, and membrane-permeable; it may diffuse considerable distance away from its site of generation[7]. However, in contrast, $^{\bullet}OH$ is extremely reactive having a very short half life, but with a very limited diffusion capacity. It can attack and damage almost every molecule in its vicinity at a 'diffusion-controlled rate'[23]. Thus, the extent of damage to the cells by O_2^- and H_2O_2 increases in presence of the transition metal ions due to the generation of more powerful $^{\bullet}OH$: The Haber–Weiss-catalysed reaction[14].

Primary defense against ROS: Catalytic removal of ROS by antioxidant enzymes

Superoxide dismutase (SOD), catalase, and peroxidases constitute a mutually supportive team of defense against ROS. While SOD lowers the steady-state level of O_2^-, catalase and peroxidases do the same for H_2O_2 (Figure 2).

Superoxide dismutase

The first enzyme involved in the antioxidant defense is the superoxide dismutase: a metalloprotein found in both prokaryotic and eukaryotic cells[4,22]. The iron-containing (Fe-SOD) and the manganese-containing (Mn-SOD) enzymes are characteristic of prokaryotes. In eukaryotic cells, the predominant forms are the copper-containing enzyme and the zinc-containing enzyme, located in the cytosol. The second type is the manganese-containing SOD found in the mitochondrial matrix[4]. The biosynthesis of SOD is mainly controlled by its substrate, the O_2^- (refs 4, 22). Induction of SOD by increased intracellular fluxes of O_2^- has been observed in numerous microorganisms[4], as well as in higher organisms[24,25].

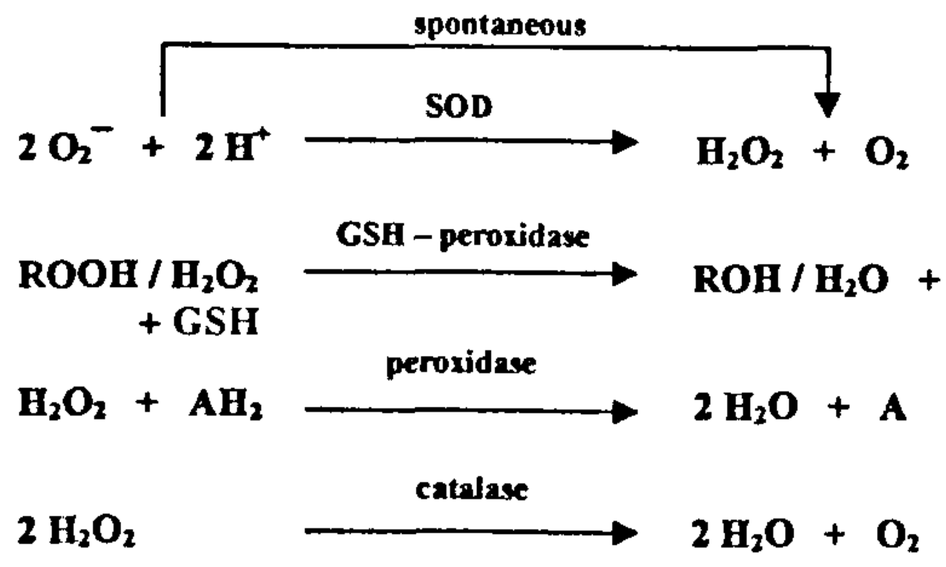

Figure 2. Catalytic removal of ROS by antioxidant enzymes. Superoxide dismutese (SOD), catalase, and peroxidases constitute a mutually supportive team of defense against ROS. SOD lowers the steady-state level of O_2^-. Catalase and peroxidases do the same for H_2O_2. ROOH refers to hydroperoxide and AH_2 acts as the electron donor.

Glutathione peroxidase

Glutathione peroxidase[26] catalyses the reaction of hydroperoxides with reduced glutathione (GSH) to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide[6] (Figure 2). This enzyme is specific for its hydrogen donor, GSH, and nonspecific for the hydroperoxides ranging from H_2O_2 to organic hydroperoxides. It is a seleno-enzyme; two-third of which (in liver) is present in the cytosol and one-third in the mitochondria[27].

Heme peroxidase

Heme peroxidases such as horseradish peroxidase, lactoperoxidase, and other mammalian peroxidases have been studied most extensively[28–31]. The enzyme catalyses the oxidation of a wide variety of electron donors with the help of H_2O_2 and thereby scavenges the endogenous H_2O_2 (refs 32, 33).

Catalase

Catalase present in almost all the mammalian cells is localized in the peroxisomes or the microperoxisomes[6]. It is a hemoprotein and catalyses the decomposition of H_2O_2 to water and oxygen and thus protects the cell from oxidative damage by H_2O_2 and $^{\bullet}OH$ (ref. 34).

Secondary defense against ROS: Free-radical scavengers

In addition to the primary defense against ROS by antioxidant enzymes, secondary defense against ROS is also offered by small molecules which react with radicals to produce another radical compound, the 'scavengers'. When these scavengers produce a lesser harmful radical species, they are called 'antioxidants'. For example, α-tocopherol, ascorbate, and reduced glutathione (GSH) may act in combination to act as cellular antioxidants (Figure 3). α-Tocopherol, present in the cell membrane and plasma lipoproteins, functions as a chain-breaking antioxidant[8]. Once the tocopherol radical is formed, it can migrate to the membrane surface and is reconverted to α-tocopherol by reaction with ascorbate or GSH. The resulting ascorbate radical can regenerate ascorbate by reduction with GSH, which can also directly

scavenge ROS, and the resulting GSSG can regenerate GSH through NADPH-glutathione reductase system (Figure 3).

However, ascorbate, in addition to its antioxidant capacity, at low concentrations and in the presence of catalytic metal ions (copper and iron) may generate $^{\cdot}OH$ by virtue of its metal-reducing capacity similar to O_2^- (refs 8, 23) together with generation of H_2O_2 (ref. 35).

$$\text{Ascorbate} + Cu^{2+} + O_2 + 2H^+ \rightarrow \text{Dehydroascorbate} + Cu^+ + H_2O_2$$

$$Cu^+ + H_2O_2 \rightarrow Cu^{2+} + {}^{\cdot}OH + OH^-$$

Ascorbate is the only cellular reducing agent to replace O_2^- in metal-catalysed Haber–Weiss reaction under physiological conditions[14]. Ascorbate up to a concentration of 0.2 mM potentiates catalytic amount of (10 μm) Fe^{2+}-induced lipid peroxidation. It is only when the concentration of ascorbate is above 0.2 mM (up to 2–4 mM), that it shows its antioxidant property[8]. The antioxidant and prooxidant role of ascorbate has recently been reviewed by Chatterjee[36].

Consequences of generation of free radicals *in vivo*

Reactive oxygen species can attack vital cell components like polyunsaturated fatty acids, proteins, and nucleic acids. To a lesser extent, carbohydrates are also the targets of ROS. These reactions can alter intrinsic membrane properties like fluidity, ion transport, loss of enzyme activity, protein cross-linking, inhibition of protein synthesis, DNA damage: ultimately resulting in cell death[11]. Some of the well-known consequences of generation of the free radicals *in vivo* are: DNA strand scission[37], nucleic acid base modification[38], protein oxidation[39,40], and lipid peroxidation[11].

A review of the current information available on these is discussed below.

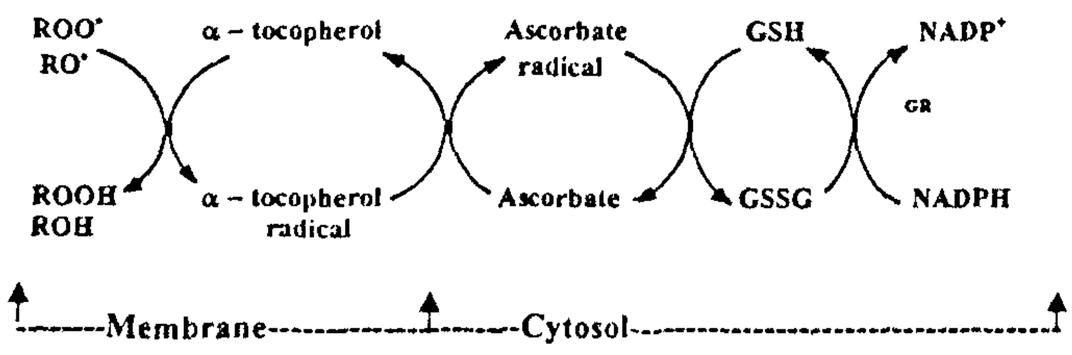

Figure 3. Mechanism of free-radical seavenging action of cellular low-molecular weight antioxidants—α-tocopherol, ascorbate, and reduced glutathione (GSH), through NADPH-glutathione reductase (GR) system.

Lipid peroxidation

Oxygen radicals catalyse the oxidative modification of lipids[41]. This peroxidation chain reaction is illustrated in Figure 4. The presence of double bond adjacent to a methylene group makes the methylene C–H bonds of polyunsaturated fatty acid (PUFA) weaker and therefore the hydrogen becomes more prone to abstraction. While lipid peroxidation is not initiated by O_2^- and H_2O_2, $^{\cdot}OH$, alkoxy radicals ($RO^{\cdot}$), and peroxy radicals ($ROO^{\cdot}$) result in initiating the lipid peroxidation[7]. This can lead to a self-perpetuating process since peroxy radicals are both reaction initiators as well as the products of lipid peroxidation. Lipid peroxy radicals react with other lipids, proteins, and nucleic acids; propagating thereby the transfer of electrons and bringing about the oxidation of substrates. Cell membranes, which are structurally made up of large amounts of PUFA, are highly susceptible to oxidative attack and, consequently, changes in membrane fluidity, permeability, and cellular metabolic functions result.

DNA damages

ROS can cause oxidative damages to DNA: both nuclear and mitochondrial. The nature of damages include mainly base modification, deoxyribose oxidation, strand breakage, and DNA–protein cross-links. Among the various ROS, $^{\cdot}OH$ generates various products from the DNA bases which mainly include C-8 hydroxylation of guanine to form 8-oxo-7,8 dehydro-2′-deoxyguanosine, a ring-opened product; 2,6-diamino-4-hydroxy-5-formamimodipyrimidine, 8-OH-adenine, 2-OH-adenine, thymine glycol, cytosine glycol, etc.[42]. ROS-induced DNA damages include various mutagenic alterations as well. For example, mutation arising from selective modification of G : C sites specially indicates oxidative attack on DNA by ROS. The action of 8-oxo-deoxy-guanosine as a promutagen, as well as in altering the binding of methylase to the oligomer so as to inhibit methylation of adjacent cytosine has been reported in cases of cancer development[43,44]. ROS have also been shown to activate

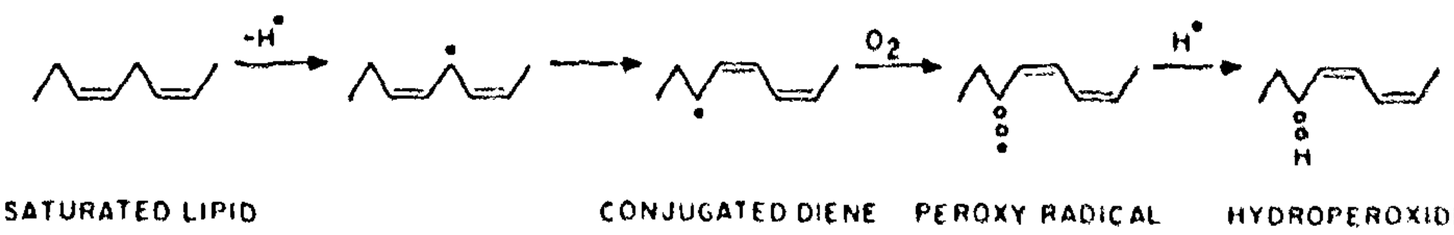

Figure 4. Mechanism of lipid peroxidation by ROS.

mutations in human *C-Ha-ras-1* protooncogene, and to induce mutation in the *p53* tumour-suppressor gene[45]. Besides, ROS may interfere with normal cell signalling, resulting thereby in alteration of the gene expression, and development of cancer by redox regulation of transcriptional factors/activator and/or by oxidatively modulating the protein kinase cascades. ROS also induce various early response or stress-response genes like *c-fos*, *c-jun*, *jun-B*, *jun-D*, *c-myc*, *erg-1*, and heme oxygenase-1. Activation of the early-response protooncogenes plays a vital role in signal transduction, leading to cell proliferation and transformation[46]. The oxidative damage of mitochondrial DNA also involves base modification and strand breaks, which leads to formation of abnormal components of the electron transport chain. This results in the generation of more ROS through increased leakage of electrons, and therefore further cell damage. Oxidative damage to mitochondrial DNA may promote cancer and aging, eventually[47].

Oxidative damage of proteins

During mitochondrial electron transport chain, free radicals are produced which can stimulate protein degradation. Oxidative protein damage may be brought about by metabolic processes which degrade a damaged protein to promote synthesis of a new protein. In the process of cataractogenesis, oxidative modification plays a significant role in cross-linking of crystalline lens protein, leading to high-molecular-weight aggregates, loss of solubility, and lens opacity[48]. Lipofuscin – an aggregate of peroxidized lipid and proteins – accumulates in lysosomes of aged cells, Alzheimer's disease brain cells, and iron-overloaded hepatocytes[49]. Within the rheumatoid joint, α-1-protease inhibitor is present in an oxidatively modified form[50]. On the basis of extensive studies on aging processes, it has been established that catalytically inactive or less active, more thermolabile forms of enzyme accumulate in cells during aging, and show a dramatic increase in the level of protein carbonyl content: an index of metal-catalysed oxidation of proteins[51–53]. In human erythrocytes, levels of glyceraldehyde-3-P-dehydrogenase, aspartate aminotransferase, and phosphoglycerate kinase decline with age together with an increase in protein carbonyl content[54]. The carbonyl content of protein in rat hepatocytes also increases with age along with decrease in the activities of glutamine synthetase and glucose-6-P-dehydrogenase, without any loss in the total enzyme protein[52]. An age-related oxidative modification of human ceruloplasmin, a copper-containing protein in human plasma, has also been reported[55]. An oxidative inactivation of glutamine synthetase occurs during ischemic-reperfusion injury of gerbil brain[56].

The mechanism of oxidative damage of proteins by ROS has been studied *in vitro* by generating these reactive species either in solution or 'site-specifically' within the protein. While the former damage is termed as non-specific (global), the latter damage is termed as site-specific (localized) damage[57]. Non-specific damage can be simulated by generating activated oxygen species *in situ*, using either a radiation source, ^{60}Co, or using pulse radiolysis techniques; which lead to aggregation and fragmentation of the protein and modification of almost all the amino acid residues[58,59]. In contrast, localized or 'site-specific' damage, which was extensively studied using glutamine synthetase as the model enzyme[57,60–62], can occur when the ROS, such as $^{\bullet}OH$, are formed at putative metal-binding sites in proteins. When these sites are occupied by iron or copper, they can – in the presence of suitable reductants, O_2^- or ascorbate – react with H_2O_2 to generate highly reactive $^{\bullet}OH$ which reacts preferably with specific amino acids present in the vicinity of the metal-binding site[39,53], inducing thereby specific damage (site-specific) that shows no gross structural modification. The concept of 'site-specificity' has been studied in detail[21,57,61]. It means: (i) catalytic metal ions – such as iron or copper – would be bound to the target molecule (protein, DNA, or cell membrane), and the $^{\bullet}OH$ produced by O_2^- (or ascorbate) and H_2O_2 produced at the iron- or copper-binding site would then react preferentially with the target moelcule; (ii) the damaging effect of $^{\bullet}OH$ is observed at a specific site where catalytic ions are bound, and (iii) the defensive action of the free-radical scavengers to remove $^{\bullet}OH$ from the specific site decreases dramatically, since they are unable to access the microenvironment. Although tryptophan, phenylalanine, and tyrosine residues of proteins are not the major sites of oxidation (as in the case of global damage) by site-specific oxidative system; arginine, lysine, histidine, cysteine and proline are particularly sensitive to this oxidation, resulting in the formation of carbonyl derivatives[60,63].

A series of detailed studies on site-specific, metal-catalysed oxidative damage of glutamine synthetase have been reported. This damage to glutamine synthetase is accompanied by the loss of a single histidyl and a single arginyl residue per subunit, both these subunits being situated in close proximity to one of the two divalent metal-binding sites of the enzyme[60,64]. The crystal structure of the modified glutamine synthetase has also confirmed these results[65]. Similarly, Cu^{2+}-superoxide dismutase and Zn^{2+}-superoxide dismutase, when exposed to H_2O_2, generate $^{\bullet}OH$, at or near the copper-binding site, which damages the adjacent histidine, releasing thereby the copper from the enzyme[66,67]. Other examples are serum acetylcholine esterase[68], muscle phosphoglucomutase[69], carbamoyl phosphate synthetase[70], alkaline phosphatase[71], and glucose-6-phosphatedehydrogenase[72]. Ceruloplasmin, albumin, and angiotensin are copper-containing proteins which undergo site-specific oxidative damage on being exposed to ascorbate[55,73]. Recently,

gastric peroxidase has also been shown to undergo site-specific oxidative damage with the loss of two lysine residues, upon the exposure of the enzyme to ascorbate–Cu^{2+}–H_2O_2 system, leading to generation of $^{\cdot}OH$ at the putative Cu^{2+}-binding site of the enzyme[74].

Reactive oxygen species and human diseases

Despite the existence of endogenous defense mechanisms against ROS, it has been observed that whenever either the level of the cellular antioxidant systems goes down or when the ROS reach abnormally high levels, oxidative damage to the cells occurs, finally leading to several pathological conditions[3]. About 100 disorders, like rheumatoid arthritis, hemorrhagic shock, cardiovascular diseases, cystic fibrosis, metabolic disorders, neurodegenerative disease, gastrointestinal ulcerogenesis, and AIDS, have been reported as the ROS-mediated disorders[3,11,17,42,75,76]. Some specific examples of the ROS-mediated diseases are Alzheimer's disease[77], Parkinson's disease[42,78], oxidative modification of low-density lipoprotein in atherosclerosis[79], cancer[80,81], Down's syndrome[82], and ischemic reperfusion injury in different tissues including heart, brain, kidney, liver, and gastrointestinal tract[10]. Among these, role of ROS in atherosclerosis[83], and ischemic injury in heart[84] and brain[85] have been studied extensively[11]. The major role played by ROS in stress-induced gastric ulcer and inflammatory bowel diseases has been recently established[17,86–88]. The involvement of ROS in aging has been documented as well[53,89,90]. The detailed mechanism of the oxygen-radical-mediated disease process has recently been reviewed[2]. Recently, Halliwell[76] has summarized the mechanism of formation of various oxygen-derived free radicals, and the role of antioxidant defense system in controlling development of pathological conditions.

Reactive oxygen species-mediated oxidative stress and apoptosis

ROS can also lead to cell death which can take place by two mechanisms: necrosis, and apoptosis. During necrosis the cell ruptures, spilling its contents that include copper and iron ions which act as prooxidants to generate ROS, specially $^{\cdot}OH$, which cause oxidative damage to the adjacent cells. Apoptosis or programmed cell death occurs as a result of activation of suicidal mechanism of the cell, and unlike necrosis, in apoptosis the cell does not normally rupture to affect the surrounding cells. ROS-mediated oxidative stress has been implicated in apoptic cell death that results in neurodegenerative diseases[75], progressive loss of T lymphocytes in human immunodeficiency virus infection (AIDS)[91,92], and in myocardial ischaemia-reperfusion injury[84,93]. That oxidative stress induces apoptosis in different cellular systems has been established by several groups[94–96]. Recently, Hasnain *et al.*[96] have demonstrated the critical role played by H_2O_2

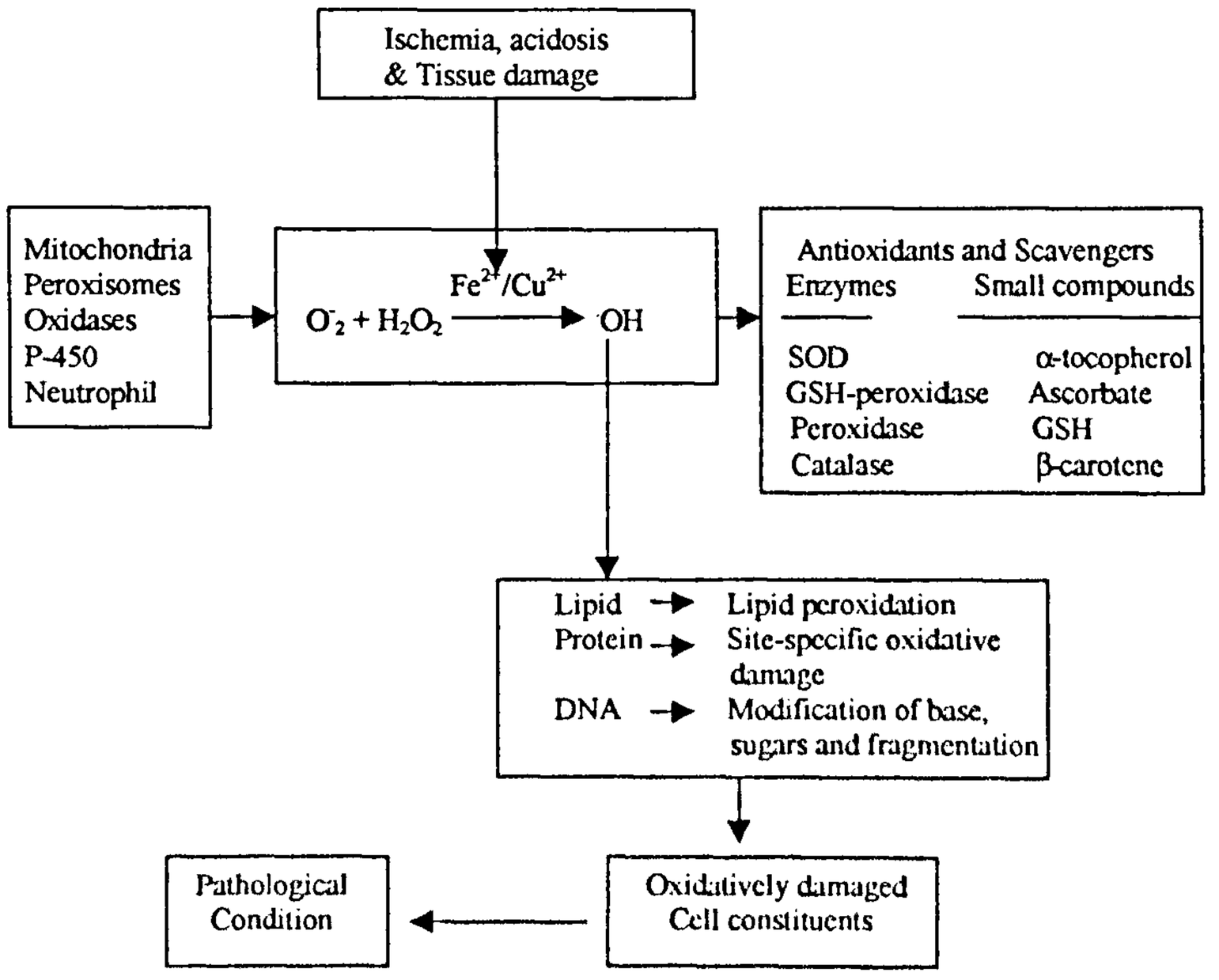

Figure 5. An overall picture of the metabolism of ROS and the mechanism of oxidative tissue damage leading to pathological conditions.

in bringing about apoptosis of *in-vitro*-cultured *Spodoptera frugiperda* insect cells. This may provide a suitable model to study the oxidative stress-induced apoptosis. High dose of UV-B radiation, which generates $^{\bullet}OH$, results in apoptosis without DNA repair[97]. Catalase sensitivity to this UV-B radiation-induced apoptosis clearly indicates the involvement of ROS in this process[98]. The mechanism of ROS-mediated apoptosis is not quite clear, except for the involvement of H_2O_2 which perhaps has a role in the signalling pathway that controls the activity of some transcription factors[99,100]. Caspases (cysteine aspartate protease) have been shown to be involved in the final stage of an apoptotic process[101,102]. Very recently Hasnain *et al.*[103] reported that an antiapoptotic *p35* gene prevents apoptosis, a result of the action of various apoptotic agents including oxidative stress. Through their elegant studies on Sf 9 insect cells, they established that *p35* gene product acts directly as an antioxidant, scavenging the free radicals, preventing thereby the ROS-mediated apoptosis.

Reactive oxygen species and aging

Eversince Harman[104] first proposed the free-radical theory of aging, as early as 1956, the molecular basis of aging and the role of ROS in this process has attracted considerable attention in recent years. It is now generally agreed that aging and age-related diseases result from ROS-mediated oxidative damage of lipid, protein, and nuclear and mitochondrial DNA molecules[105]. The concentration of oxidatively damaged proteins, lipids, and DNA has been reported to increase with age[106]. The hydroxyl and peroxy radicals cause extensive damage of proteins resulting in aging and age-related degenerative diseases[107]. Other than O_2^-- and H_2O_2-mediated oxidative damage, mutation in mitochondrial DNA also leads to the formation of defective respiratory enzymes[108] which not only result in decreased ATP synthesis but also generate more ROS to cause further oxidative damage. This vicious cycle is mainly responsible for aging and age-related disorders. Melatonin, a pineal hormone, has recently been implicated in oxidative damage and aging process[109,110]. Melatonin, having antioxidant property, declines significantly with the increase in age[110]. This decline in melatonin coincides with the increased oxidative damage and pathogenesis. The significance of melatonin in controlling oxidative stress, and age-related diseases has recently been reviewed[109–113]. Restricting the caloric intake has also been shown to delay aging through: (i) decreased production of mitochondrial O_2^- and H_2O_2, and (ii) increased production of antioxidant defenses, leading thereby to decreased production of oxidatively damaged proteins, lipids, and DNA[114,115]. Caloric restriction may thus decrease the oxidative stress and damage, and may prolong life in humans.

Current concepts to overcome oxidative tissue damage and associated diseases

Today, reactive oxygen species-mediated pathogenesis is of major concern. Figure 5 gives the overall picture of the sources, endogenous scavengers, and consequences of ROS. During abnormal physiological conditions such as stress, ischemia, acidosis and tissue damage; redox-active metal ions, like copper or iron, are liberated from the metalloproteins[11]. These released redox-active metal ions bind at specific sites of proteins, and to membranes and DNA, thereby increasing their susceptibility to site-specific oxidative damage by $^{\bullet}OH$ when there is increased accumulation of O_2^- and H_2O_2 due to derangement of the antioxidant enzymes. This results in abnormal metabolic functioning of the cell and, consequently, leads to various pathological conditions. Recently reports have been accumulating on (i) the role of endogenous H_2O_2 as a possible signal transduction messenger, and (ii) the role of intracellular redox state which is controlled by the ratio of oxidant to antioxidant, in regulating gene expression of the various proteins[99,100]. ROS have also been implicated in the regulation of at least two well-defined transcription factors, AP-1 and NF_kB (Figure 6)[99,100,116,117]. These transcription factors bind at the promoter regions of a

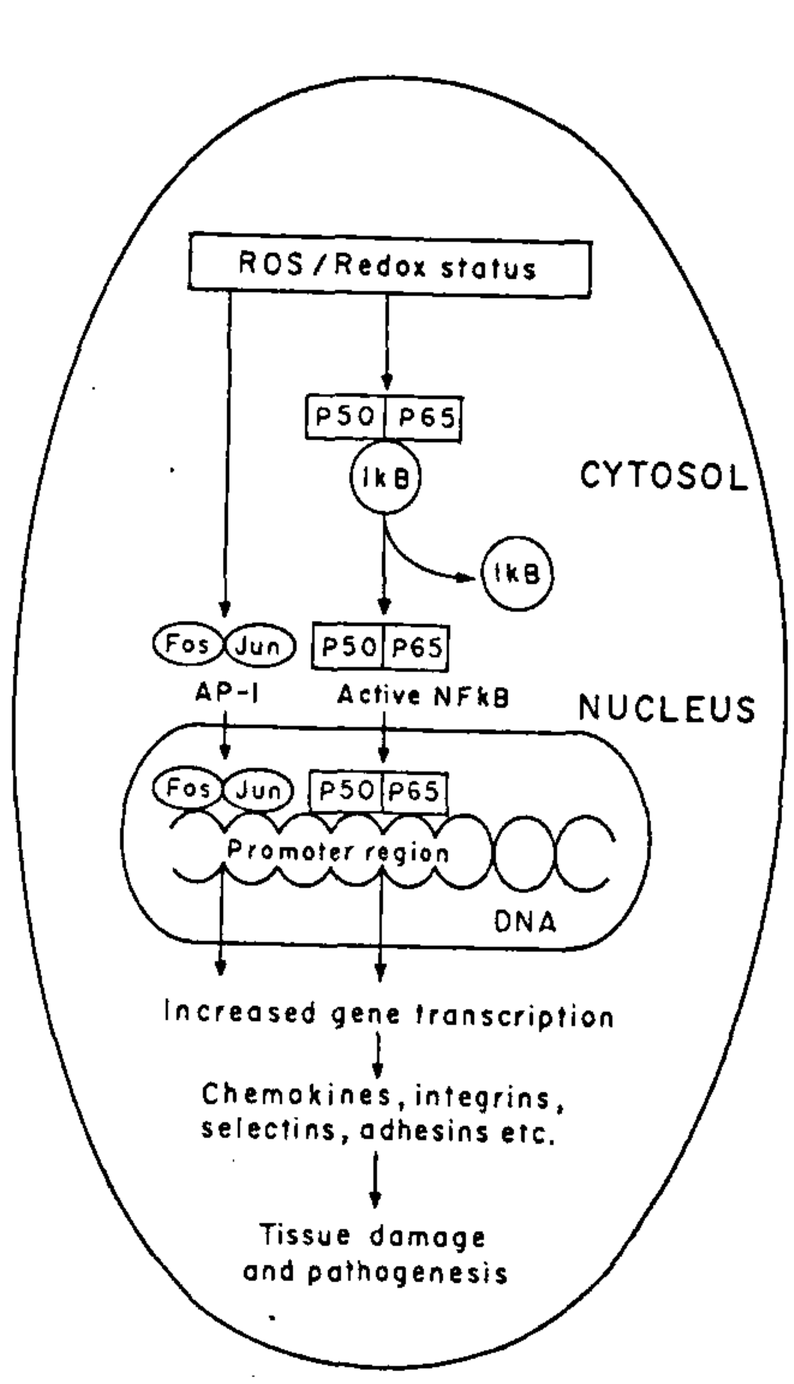

Figure 6. Plausible mechanism of ROS-induced activation of the two well-defined transcription factors, NF_kB and AP-1, leading to their binding at the promoter region of a large variety of genes, and their role in the expression of various proteins which are involved in inflammatory response and tissue injury.

large variety of genes and play a very significant role in the expression of various proteins such as TNFα, interleukin-1 and -2, collagenase, matrix metalloproteinase, etc. which are involved in inflammatory responses and tissue injury[78]. Blocking the expression of these genes by suitable antioxidants should be one of the approaches for controlling the ROS-mediated pathogenesis. Since ROS-mediated oxidative stress is now regarded as a major factor leading to aging and age-related neurodegenerative diseases[89,90,104], suitable antioxidant therapies to control these processes have already attracted world-wide attention in recent years. The pineal hormone, melatonin, having potent antioxidant activity, is a potentially promising candidate for the control of aging and other ROS-mediated pathogenesis. However, isolation of an antioxidant factor which is specific in its action, is nontoxic, and shows antistress property – from the natural sources, such as plants – and the therapeutic application of such an antioxidant factor would perhaps be one of the better approaches to control the ROS-mediated pathogenesis. We expect to make a breakthrough by the first decade of the next millennium in understanding the molecular mechanism of ROS-mediated pathogenesis, and its control by suitable antioxidant therapy/therapies.

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