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Free Radicals: Generation, Defenses and Implications in Various Diseases

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ABSTRACT

Free radicals can be generated as a fall out of normal in situ cell metabolism or from various external sources like radiation, medication, etc. The balance between the free radicals and antioxidants is crucial for good health. Accumulation of free radicals in the body is a harbinger of various disorders due to damage to cellular structures, lipids, proteins, DNA and RNA. Free radicals have been implicated in various acute and chronic diseases like rheumatoid arthritis, artherosclerosis, liver disorder, lungs, kidney damage, diabetes, aging, cataract, cancer, cardiovascular, psoriasis, eye disorder, neurodegenerative diseases, etc. This review provides an overview of the mechanism of generation of free radical and implication of free radicals in human health. The role of various antioxidants as a defense against these diseases has also been dealt with.

Keywords: Free radicals, Superoxide dismutase (SOD), reactive oxygen species (ROS), catalase, antioxidants

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INTRODUCTION

In recent years it has become increasingly evident that free radicals play a major role in a variety of normal regulatory systems in man, the deregulation of which may lead to multifarious disturbances. They are cardinal to many biological processes, e.g., in prostaglandin synthesis, as an intermediate in enzyme-catalysed reactions, in tissue response to invading microorganisms and yet can prove disastrous if they go beyond the body's natural defense mechanism. Despite the fact that they have been accepted as important biochemical intermediates, the nature of free radicals remains too elusive and though other reactive biochemical entities exist in the body, but none is as imperceptible as free radicals. Their extremely short life-time (often measured in microseconds) and the difficulty to detect and measure per se, not least in the clinical conditions further aggravates the situation.

The fact that whether these free radical reactions are a cause of tissue damage or an accompaniment to or a consequence of such injury is yet to be established. However, it is apparent that disease or damaged tissues undergo radical reactions more readily than normal tissues [1], thus exacerbating the primary lesion. Free radicals have been reported to be involved in a number of clinical diseased states [2] as presented in table1.

Table 1: Representative diseased states in which oxygen radicals have been implicated [2]

Category	Disease(s)
Inflammatory-immune injury	Autoimmune disease, rheumatoid arthritis
Ischaemia-reflow states	Organ transplantation, stroke/ myocardial infarction
Erythrocyte disorders	Sickle cell anaemia, malaria, Fanconi's anaemia
Iron overload	Idiopathic haemochromatosis, thalassaemia, Bantu iron overload
Aging	Disorders of premature aging
Lungs	Emphysema, adult respiratory distress syndrome
Eye	Ocular hemorrhage, cataractogenesis
Heart and cardiovascular system	Artherosclerosis, doxorubicin toxicity
Brain	Parkinson's disease, Vitamin E deficiency
Skin	Solar radiation, porphyria

Free radicals can be defined as chemical species (molecules or molecular fragments) possessing one or more unpaired electron. Certain stable inorganic molecules like NO and NO_2 and also individual atoms like Na and Cl come under the purview of this definition. The free radicals have extremely short life in solutions but can be kept for longer periods frozen within the crystal lattices of other molecules. Due to the presence of one or more unpaired electrons, there is a net magnetic moment and the species thereby has the characteristic property of paramagnetism. The chemical reactivity of free radicals is usually high. The presence of unpaired electron is conventionally represented by a superscript bold dot ($R^{\bullet}$).

FREE RADICAL PROCESS

There are primarily two steps involved in a free radical process *viz*. the formation of free radicals and the destruction of the same. Formation of free radicals can take place either by hemolytic cleavage or by electron transfer reactions. In hemolytic cleavage covalent bonds of normal molecules cleave with each fragment retaining one of the paired electrons as in

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$$X : Y \longrightarrow X^{\bullet} + Y^{\bullet}$$

Depending upon the nature of bond this step may occur spontaneously or by light/ heat induction. Spontaneous or heat induced free radicals can be formed from peroxide, peracids and azo compounds. Chlorine, bromine and various ketones can generate free radicals by photochemical cleavage.

An alternate pathway for formation of free radical is via electron transfer reactions. These reactions usually involve inorganic ions or electrochemical process. The reaction can proceed either by loss of single electron from a normal molecule:

$$A - e^{-} \longrightarrow A^{+\bullet}$$

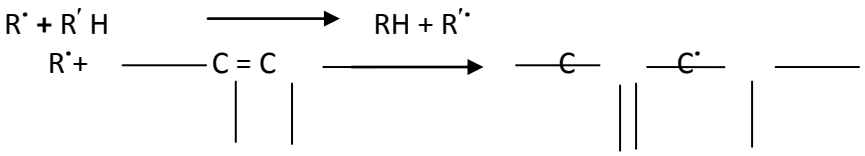
or by addition of single electron to a normal electron:

$$A^{+} - e^{-} \longrightarrow A^{\bullet}$$

Destruction of free radicals occurs by a process that is reverse to the first one, i.e. by combination of two like/unlike radical radicals leading to the formation of a new bond and thereby leading to the termination of reaction.

$$A^{\bullet} + B^{\bullet} \longrightarrow A—B$$

Alternatively if the free radical reacts with other molecule and not another radical then a new radical is generated.



The radical so produced can react with another molecule generating another new radical. This type of reaction is referred to as propagation. This process can go on until two radicals combine and terminate the sequence. Propagation reactions can also proceed without the involvement of a molecule *viz.*, by rearrangement of a radical to another by actual migration, in which migratory group moves with one electron or by cleavage of a radical to a molecule and a new radical.

$$R—A—B^{\bullet} \longrightarrow {}^{\bullet}A—B—R$$

$$Ph—C—O^{\bullet} \longrightarrow Ph^{\bullet} + CO_2$$

Propagation reactions can proceed by any of the following pathways:

(i) Abstraction of another atom/group

$$R^{\bullet} + R'H \longrightarrow R—H + R'^{\bullet}$$

(ii) Addition to a multiple bond

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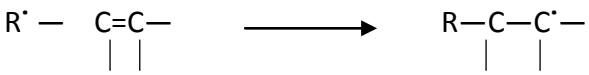
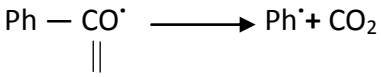
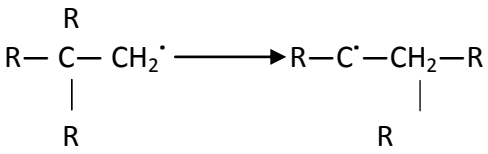


(iii) Decomposition



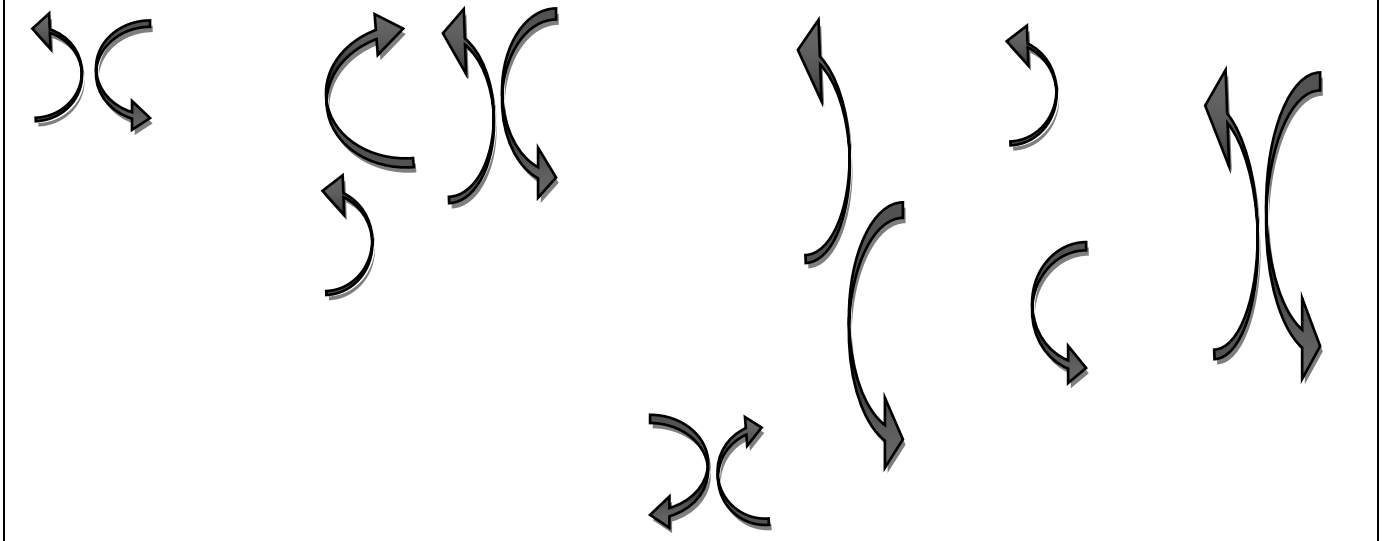
(iv) Rearrangement



FREE RADICALS IN BIOLOGICAL ENVIRONS

The major radicals that have unleashed a distress on the body's defense mechanism are free oxygen radicals and reactive oxygen species (ROS). Oxygen is one of the major intermediates of damaging radical reactions. Oxygen as such has low reactivity but it has the capacity to undergo a series of one-electron reductions producing a multitude of potentially damaging intermediates (Table 2) and this renders it toxic.

Table 2: Some redox reactions of biological relevance involving oxygen radicals

Reaction type	$1e^-$ oxidation (e.g., xanthine oxidase)	$1e^-$ dismutation (e.g., superoxidase dismutase)	$2e^-$ H_2O_2 forming oxidation (e.g., uricase)	$2e^-$ H_2O forming oxidation (e.g., peroxidase)	$2e^-$ dismutation (e.g., catalase)	$3e^-$ oxidation dismutation (metal catalysed Haber Weiss reaction)	$4e^-$ oxidation (e.g., cytochrome oxidase)
O_2 **$1e^-$** HO_2 **$2e^-$** H_2O_2 **$3e^-$** $OH^{\bullet}+OH^-$ **$4e^-$** $2H_2O$							
E.g. of localization in animal cells	Granulocyte membrane	Cytoplasm mitochondrion	Peroxisome	Cytoplasm granules of granulocyte	Peroxisome	Granulocyte	Mitochondrion

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Superoxide anion (O_2^-)

The univalent reduction of dioxygen to scavenger O_2^- occurs in both abiotic and biological systems and owing to the spin restriction this is obviously the most expedient pathway. Some of the sources of O_2^- include autooxidizable small molecule (like catecholamines, leucoflavins, tetrahydropterins), autoxidizable proteins (like reduced ferredoxins and hemoproteins), subcellular organelles (like mitochondria, chloroplasts, nuclei and microsomes), oxidative enzyme (like xanthine oxidase) and whole cells (like monocytes, neutrophils and macrophages) [3-15].

In respiratory cells, most of the dioxygen consumed is reduced to water without the release of intermediates such as O_2^- by the cytochrome oxidase and the "blue" copper oxidases [16].

O_2^- is an agent of oxygen toxicity and superoxide dismutase (SOD) provides an essential defense and plays a protective role by catalyzing the following reactions [17].

$$O_2^- + O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

Some specialized cells like phagocytes, on activation generate O_2^- as the major reduction product of dioxygen. Phagocytes during the respiratory burst produce O_2^- as a tool to kill microorganisms [14].

Superoxide radical ($O_2^{-\bullet}$)

$O_2^{-\bullet}$ is reported to be a major factor in oxygen toxicity and SOD constitutes an important defense against it. The deleterious effect of systems generating $O_2^{-\bullet}$ radicals are listed in Table 3. Some of the major sources of superoxide radicals are activated phagocytes, electron leakage from electron transport chains such as those in mitochondria and in endoplasmic reticulum to molecular oxygen, enzyme-flavin oxidase, autooxidation of ascorbic acid, thiols, adrenaline and flavin coenzyme and reductive activation of quinines, anthracyclines or aminoquinone anticancer drugs by microsomal reductases is reported to generate superoxide radicals [18].

Superoxide radicals are known to play a significant role in host defenses against microbial attack and in phagocytic bactericidal activity. Polymorphonuclear leucocytes (PMNL) and other phagocytic cells ingest opsonized particles by engulfing them into phagocytic vesicles formed from the plasma membrane. This is followed by a rapid cyanide-insensitive respiratory burst leading to the generation of $O_2^{-\bullet}$, H_2O_2 and $OH^\bullet$ [14]. A reduced pyridine mucleotide oxidase located in plasma membrane and thereby in phagocytic vesicles is reported to be responsible for the entire episode [19]. This system is however absent from PMNLs and its vesicles, which are unable to generate adequate concentrations of activated oxygen species to kill bacteria [14]. The PMN oxidase is reported to increase superoxide formation by intact leucocytes. Digitonin, tumor promoter agents and small synthetic N- formylmethionyl peptides are reported to activate the above system [20-22]. Autooxidation of reduced CoQ in antimycin A-blocked mitochondrial membranes generate superoxide and this could be the possible reason for formation of H_2O_2 in mitochondria in the presence of some substrates [23, 24].

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Table 3: Systems generating the superoxide radicals (O_2^-) and their lethal effects

Source of O_2^-	System studied	Damage	Protective mechanism
Autoxidation of dialuric acid	*Escherichia coli*	Viability lost	Protection by SOD
Autoxidation of dihydroxy fumarate	Rat thymocytes	Na^+- dependent amino acid uptake inhibited	SOD protects
Acetaldehyde + xanthine oxidase	Erythrocyte membranes	Lysis	SOD protects
	Arachidonic acid	Oxidation	Protection by SOD and catalase
	Staphylococcus aureus	Viability lost	Protection by SOD and catalase; for killing traces of iron chelates required
Heart muscle submitochondrial particles	Activity of NADH CoQ reductase complex	Activity lost	SOD protects
Hypoxanthine + xanthine oxidase	Rat brain membrane Na^+, K^+ - ATPase	Inactivation	Partial Protection by SOD
	DNA	Degradation, single-strand breaks attack on sugar moiety	Protection by SOD and catalase
	Cheek pouch of living hamster	Blood vessel permeability increased; leakage of contents	SOD protects
Xanthine +xanthine oxidase	Rat lungs *in vivo*	Acute lungs injury, edema	SOD protects
	Human synovial fluid	Loss of viscosity and lubricating ability	Protection by SOD and catalase
	Bacteriophage R17	Inactivation	Partial protection by SOD
Illuninated flavin mononucleotide (FMN)	Bacteria Ribonuclease Calf myoblast cells	Viability lost Activity lost Growth abnormality, some cell death	SOD protects Partial protection by SOD Partial protection by SOD

Hydrogen peroxide

This is not a free radical but falls under the category of 'Reactive Oxygen Species' (ROS). In the absence of metal catalysts, it is easily removed and is virtually harmless. Hydrogen peroxide is generated via dismutation reaction, where two superoxide molecules react together to form hydrogen peroxide and oxygen.

$$2O_2^{-\cdot} + 2H^+ \longrightarrow H_2O_2 + O_2$$

The source of intracellular H_2O_2 formation in liver cell is reported to be mitochondria, peroxisomes and endoplasmic reticulum [25].

Oxidases are reported to induce H_2O_2 formation in perioxisomes. In endoplasmic reticulum H_2O_2 formation is probably by dissociation of the oxygenated ferrous complex of cytochrome P-450 and autooxidation of reduced NADPH:cytochrome P-450. An augmentation in production of O_2^- and H_2O_2 is reported to occur owing to the uncoupling of the microsomal cytochrome P-450 monooxygenase system by some drugs [26].

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Hydroxyl radical

It is an extremely reactive oxidizing radical and is capable of causing damage within a small radius of its site of production. The primary pathway for the formation of $OH^{\bullet}$ in biological systems is believed to be via following reaction sequence:

$$Fe^{3+} \text{ or } Cu^{2+} + \text{reducing agent (e.g. } O_2^{-\bullet}\text{, Vit C)} \longrightarrow Fe^{3+} \text{ or } Cu^{2+}$$

$$Fe^{3+} \text{ or } Cu^{2+} + H_2O_2 \longrightarrow Fe^{3+} \text{ or } Cu^{2+} + OH^{\bullet} + OH^{-}$$

When $O_2^{-\bullet}$ is the reducing agent, this sequence is a metal-catalyzed reaction of $O_2^{-\bullet}$ with H_2O_2 (Haber-Weiss reaction):

$$O_2^{-\bullet} + H_2O_2 \longrightarrow O_2 + OH^{\bullet} + OH^{-}$$

$OH^{\bullet}$ radical is known to play a vital role in the mechanism of decomposition of H_2O_2 by catalase [27]. It is also proposed to be an intermediate in the Fenton reaction [28]. Owing to its reactive disposition, $OH^{\bullet}$ undergoes numerous reactions like:

$$OH^{\bullet} + OH^{\bullet} \longrightarrow H_2O_2$$
$$OH^{\bullet} + O^{-\bullet} \longrightarrow HO_2^{-}$$
$$OH^{\bullet} + O_2^{-\bullet} \longrightarrow OH^{-} + O_2$$
$$OH^{\bullet} + R\text{–}H \longrightarrow R^{\bullet} + H_2O$$
$$RCH = CH_2 + OH^{\bullet} \longrightarrow RC^{\bullet}H - CH_2OH$$
$$OH^{\bullet} + S \longrightarrow OH^{-} + S^{+}$$

$OH^{\bullet}$ radical is known to be involved in the inactivation of the oxygen carrier function of the hemoglobin. Some metalloenzymes, particularly with iron-sulfur centres and lacking a site for the complete reduction of oxygen are very sensitive to exposure to air. This aerobic inactivation is started by the primary formation of $O_2^{-\bullet}$ on oxidation of the reduced metal centres by O_2, followed by accumulation of H_2O_2 by spontaneous dismutation of $O_2^{-\bullet}$ and final attack of the enzyme by $OH^{\cdot}$ produced by a Fenton reaction of the reduced metals with H_2O_2.

Phagocytosis is reported to involve the formation of $OH^{\bullet}$ radicals. Many biochemical reagents are contaminated with iron salts and this may be sufficient for some $OH^{\bullet}$ formation. Reports indicate that iron saturated forms of lactoferrin and transferrin may participate in $OH^{\bullet}$ formation [29, 30]. A small pool of intracellular iron salts is reported to exist, chelated to phosphate esters as ADP, ATP and GTP [31].

Singlet oxygen (O_2)

Singlet oxygen is another non-radical that can lead to and be generated by free radical reactions. It can be produced under the effect of both photosensitized reactions and nonphotochemical processes [32]. The nonphotochemical reactions shown to produce

singlet oxygen involve oxidizing species (e.g. triphenylphosphite ozonide, sodium hypochlorite) that could react directly with oxidizable species present in a biological system.

One of the major biological targets for singlet oxygen is membranes, which are peroxidized and thereby leading to fragility and easy lysis. Other important targets are nucleic acid viz., guanine and proteins like histidine, methionine, tryptophan, tyrosine and cysteine [33].

Miscellaneous

Beside the free radicals discussed in the preceding section, some other free radicals of notable significance are carbon-centered radicals ($R^{\bullet}$), peroxy radicals ($ROO^{\bullet}$), alkoxy radicals ($RO^{\bullet}$) and sulphur-centered radicals (thiyl radicals, $RS^{\bullet}$).

Production of Free Radicals in Cells

Free radical production in cells can either be fortuitous or deliberate. They are produced deliberately by animal cells in special circumstances as they can be useful entities if constrained and targeted. Leakage of electrons from the electron transport chain results in accidental production of superoxide-radical and hydrogen peroxide. Free radicals generation in cells can occur due to any of the following:

Radiations

Ionizing radiations produce mainly hydrogen atom ($H^{\bullet}$), hydroxyl radical ($OH^{\bullet}$) and hydrated electron (e_{aq}^{-}) when directed onto aqueous solutions and these primary free radicals and hydrated electron react readily with neighboring biomolecules. Nonionising radiations of sufficient intrinsic energy produces free radicals and even radiations of low intrinsic energy can generate free radicals if an appropriate photosensitizer is present [32]. Thermal energy can produce free radicals. A relatively low temperature of 30–50°C is required to break unstable bonds. Very often these are used as initiator of free radical reactions, e.g. azobisiosobutyronitrile.

Redox reactions

These reactions among others include nonenzymic electron transfer reactions, e.g.

$$\text{Reduced quinine} + O_2 \longrightarrow \text{semiquinone}^{\bullet} + O_2^{-\bullet} + H^{+}$$

Enzyme such as flavinoxidase produces superoxide and hydrogen peroxide radicals also induce free radical generation. Transition metals such as iron and copper are reported to catalyse redox reactions and generate hydroxyl radical [34-37].

Activated phagocytes

They deliberately generate superoxide as part of their bactericidal role [14]. Autooxidation of certain compounds like ascorbic acid, adtrenaline, flavin coenzymes and thiols (glutathione and cysteine) generates superoxide radicals.

ISSN: 0975-8585

Toxic compounds

Agents like (redox cycling drugs) CCl_4 gets metabolized to trimethyl free radical by the action of cytochrome P-450 in the liver [38-39] and causes oxidative destruction to cellular membranes.

Damaging Reactions Produced by Free Radicals

The primary cause of cell damage by radiation is attributed to the free radicals generated by radiolysis of water *viz.* $H^{\bullet}$, $OH^{\bullet}$ and $HO_2^{\bullet}$**.** Free radicals when produced *in vivo* in amounts sufficient to overcome the normal protective mechanisms, major metabolic and cellular disturbances occur as summarized in Table 4 [40].

Table 4: Damaging reactions produced by free radicals at various sites

Target site	Damage	Consequences
DNA	Scission of the ribose ring, single strand breaks, interstrand cross-links and attack on bases	Mutations, translation errors, inhibition of protein Synthesis
Nucleotide	Free radicals added to the nucleotides	Significant changes in their biological properties
Nucleotides coenzymes	Oxidising free radicals can alter the redox state of the NAD^+ / NADH Destruction of coenzymes	$NADP^+$ / NADPH couples producing NAD (P)$^{\bullet}$ that dimerizes Enzyme inhibition
Thiol group	Protein and nonprotein thiol groups are oxidized to thiyl groups which dimerize $YSH + X^{\bullet} \longrightarrow YS^{\bullet} + XH$ $R_1S^{\bullet} + R_2S^{\bullet} \longrightarrow R_1SSR_2$	Modified ion transport, increase calcium influx, modified enzyme activity
Covalent binding to cellular components	Reactive free radicals interact with cell components such as protein, lipid and nucleic acid to form a stable covalently bound adduct	Structure and function distortion
Cellular membrane	Covalent binding to membrane/ enzyme and / or receptors Covalent binding to membrane components Protein oxidation Lipid peroxidation	Modification of the activities of membrane components Alteration of structure, function and/or antigenic character Aggregation and cross-linking, or fragmentation and breakdown Formation of toxic breakdown products and decreased lipid fluidity

Cellular damage

Cellular damage by free radicals in mainly due to the oxidation stress. Oxidation stress in cells arises due to excessive phagocytic action, influence of redox-cycling drugs (e.g. doxorubicin) or due to increased oxygen concentration.

This leads to the production of superoxide radical, hydrogen peroxide or hydroxyl radical which are the major cause of destruction. Nutrient uptake is reported to be affected by superoxide anion radicals. The transport of [^{14}C] leucine in *E.coli* [41] and of 2-amino [^{14}C] isobutyric acid in thymocytes [42] is impaired by xanthine/ xanthine oxidase system generated superoxide anion radicals.

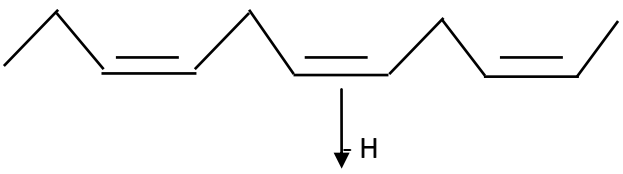
Lipid, proteins, carbohydrates and DNA are all vulnerable to such damage. The generation of free radical is catalysed by certain forms of iron and thus decompartmentalization and redistribution of iron from the blood is an extremely important consideration [43].

Damage to the lipids

Lipid is the most susceptible class to the attack by free radicals. Superoxide ($O_2^{-\bullet}$), hydroxyl ($OH^{\bullet}$) radicals and hydrogen peroxide are important mediators of cellular injury [40, 44, 45]. Cellular membranes are rich sources of polyunsaturated fatty acids (PUFAs), which are readily attacked by oxidizing radicals; the reaction is known as lipid peroxidation and is a continual biological process. This is a self perpetuating chain reaction and is damaging [46]. The lipid hydroperoxides so generated in the biological milieu are decomposed to many secondary products like alkanals, alkenals, hydroxyalkenals, ketones, etc. [47, 48]. The general process of lipid peroxidation is illustrated in Figure 1.

Lipid hydroperoxidase are fairly stable molecules at physiological temperatures but their decomposition is catalysed by transition metals and metal complexes especially iron (II) and iron (III) [49]. Alkoxy and proxy radicals formed further propagates chain reaction. Irradiation of cells, tissues or cell organelles may generate free radicals which can initiate lipid peroxidation and result in cell injury. Lipid peroxidation can also be facilitated by depletion of level of endogenous antioxidants or glutathione which play a crucial role in maintaining normal cellular protective mechanisms [50]. Incubation of the cells with drugs like benzphetamine, aminopyrine leads to depletion of intracellular glutathione and thereby lipid peroxidation. Cleavage of carbon bonds is depicted in Figure 2. Some of the consequences of attack on lipids are:

(i) Disorganisation of the structure and disturbance of membrane function [51].
(ii) Changes in the membrane permeability.
(iii) Various products formed like alkenals (e.g. malonaldehyde) crosslink the amino groups of lipids; and proteins [52] and alkenals (e.g. dihydroxy noneanal) [53] inhibit platelet aggregations.
(iv) Causes tissue injury and diseases as in CCl_4 hepatotoxicity [54] and in artherosclerosis [55].
(v) Decrease membrane fluidity [56].
(vi) Loss of functional integrity of membrane-bound enzymes.



Polyunsaturated fatty acid (PUFA)

Hydrogen atom abstraction

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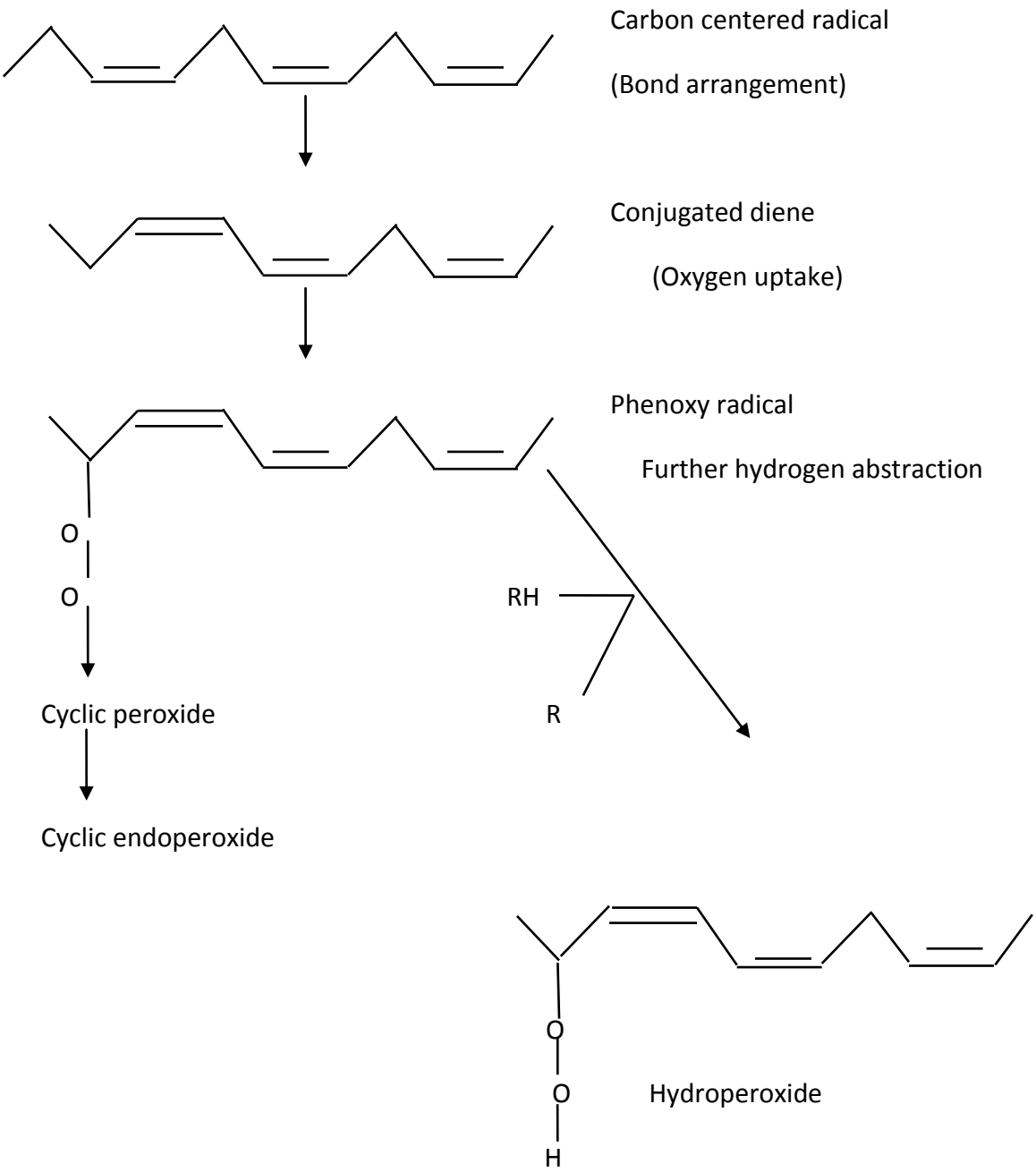

Fig. 1: Mechanism of peroxide degradation of polyunsaturated fatty acid

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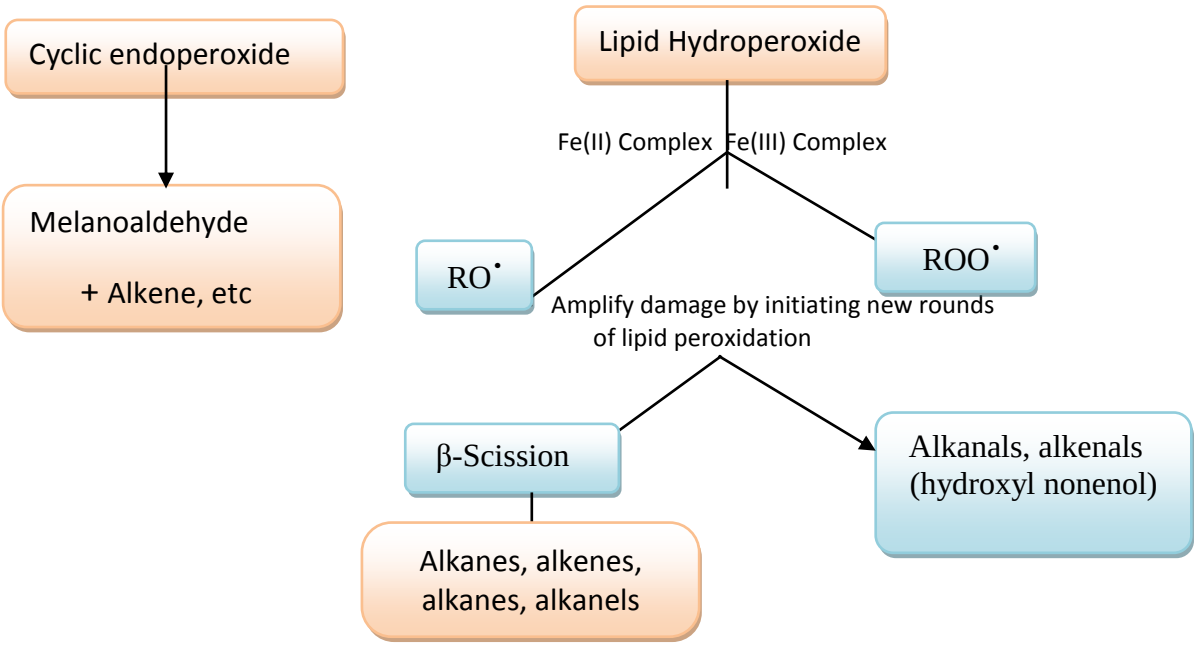

Fig. 2: Cleavage of carbon bonds during lipid peroxidation

Damage to proteins

Proteins are less susceptible to attack by free radicals and there is less possibility of destructive chain reactions being initiated. Several amino acids, crucial for protein function are particularly susceptible to radical damage (Figure 3). Some of the consequences of attack on protein are:

(i) Altered membrane and cellular function due to crosslinking of constituent proteins [57] and degradation and fragmentation of membrane proteins.
(ii) Altered enzyme activity.
(iii) Increased susceptibility of proteins to proteolytic attack [58].
These may lead to diseases like rheumatoid arthritis [59] and cataract [60] through oxidative damage to lens crystallins.

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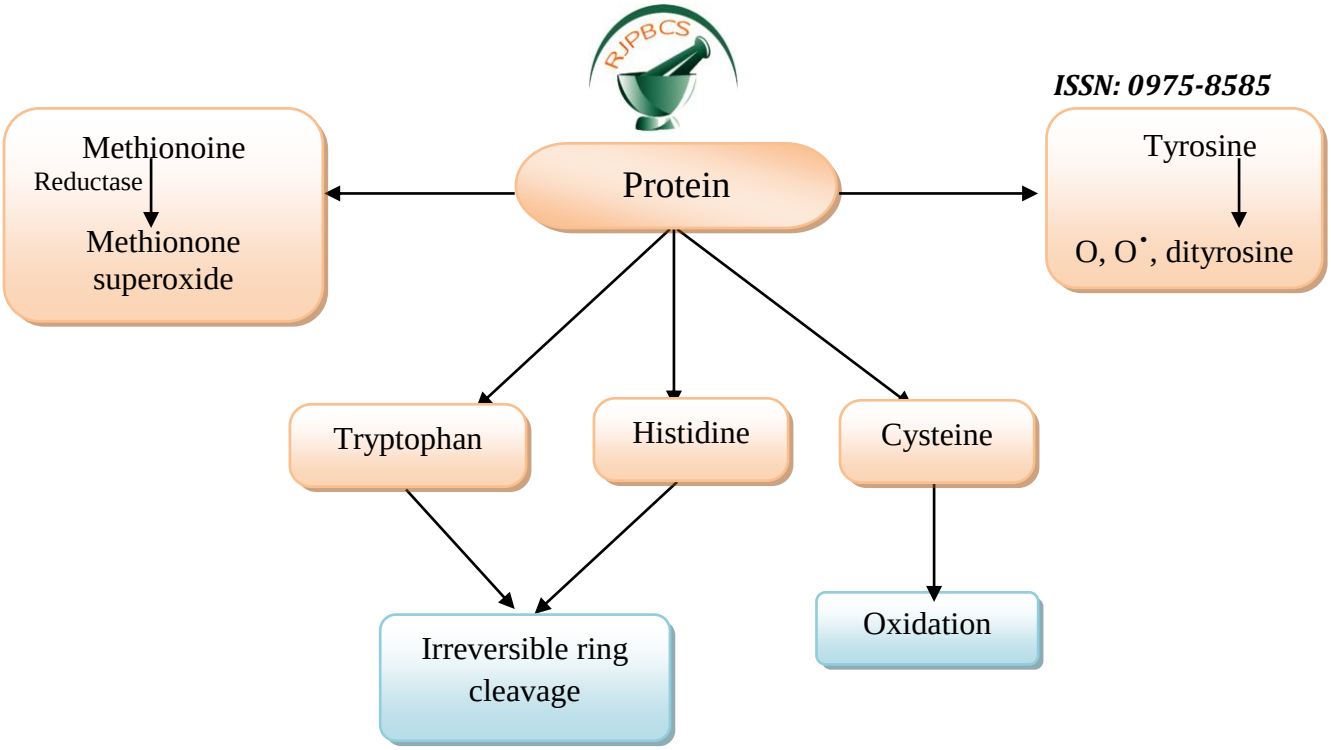

Fig. 3: Free radical attack on amino acid side chain of proteins

PROTECTIVE MECHANISM/ DEFENCES AGAINST FREE RADICALS

As the free radicals are damaging and their production in the cells is inevitable, defenses against the deleterious actions have evolved which are known as antioxidant defenses. Cells have antioxidant fortifications located intracellularly, extracellularly and membrane-bound. Some of the exogenous antioxidants for protection from free radicals are presented in Table 5.

Antioxidant defense can broadly be placed under two categories *viz.*, which prevent the generation of free radicals and which intercept any free radicals that are generated [61]. Various approaches available for defense against free radicals are:

Table 5: Exogenous antioxidants for protection from free radicals

Class of agent	Specific agent(s)	Mechanism of action
Xanthine oxidase inhibitor	Allupurinol, Oxypurinol, Folic acid, Pterin aldehyde, Tungsten	Inhibit superoxide generation by xanthine oxidase
Protease inhibitors	Soyabean trypsin inhibitor, Other serine protease inhibitor, Phenyl methyl sulfonyl fluoride	Block proteolytic activation of xanthine oxidase
Catalases	Native catalases, PEG-catalase, Liposome-encapsulated catalase	Catalyze $2H_2O_2 \longrightarrow 2H_2O_2 + O_2$
Free radical scavengers	Mannitol, Ethanol, Sodium formate Albumin Dimethyl sulphoxide Glutathione Water Caffeine	Scavenges $OH^{\bullet}$ Scavenges COOH, HOCL Scavenges $OH^{\bullet}$, Fe^{3+}, Fe^{2+}, O_2 Scavenges H_2O_2, $OH^{\bullet}$ Scavenges O_2, $OH^{\bullet}$ Scavenges $OH^{\bullet}$, e^{-} $^{\bullet}$eq
Inhibitors of iron redox cycling	Deformoximine, Apotransferrin, Ceruloplasmin	Binds free Fe^{3+}

Sequestration of transition metals

Iron is tightly bound to special proteins like transferrin and ferritin [44]. Transferrin sequesters iron (III) rendering it unavailable for catalyzing the Haber Weiss reaction, initiating lipid peroxidation or catalyzing the decomposition of lipid hydroperoxides. Desferrioxamine, diethylenetriamine pentaacetic acid and bathophenanthroline sulfonate can be employed to chelate iron salts [62-64]. Reports indicate that desferrioxamine also prevents reduction of Fe^{3+} salts by ascorbic acid, which is another source of $OH^{\bullet}$ radicals *in vivo* [65, 66].

Enzymic antioxidant defenses

The enzymic antioxidant defense include among other enzymes catalase, glutathione and SOD. Catalase acts on H_2O_2 and decomposes it as follows:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Glutathione acts on H_2O_2 and fatty acid peroxidase hydroperoxidase [67] and detoxify them.

$$RCHOOH + 2GSH \xrightarrow{\text{GSH Peroxidase}} ROH + GSSG$$

SOD is also an important antioxidant enzyme. Eukaryotic cells contain two SODs, the manganese containing enzyme (Mn-SOD) and the copper, zinc-containing enzyme (Cu, Zn-SOD). Mn-SOD is reported to be exclusively located in the soluble matrix of the mitochondria [68, 69] while Cu, Zn-SOD is accumulated primarily in the cytosol and are also suggested to be present in nuclei [70], chloroplasts [71, 72] and mitochondria [68, 69, 73]. Aerotolerant organisms possess detoxifying enzymes like SOD for detoxiction of $O_2^{\bullet-}$, and hydroperoxidases for detoxication of hydrogen peroxide. The above mentioned enzymes prevent the interaction between $O_2^{\bullet-}$ and H_2O_2 and thereby eliminating $OH^{\bullet}$ formation by the iron-catalysed Haber-Weiss reaction.

Nonenzymic defenses/ scavengers

α-Tocopherol (Vitamin E)

It is a chain breaking antioxidant as it functions to intercept lipid peroxy radicals $LOO^{\bullet}$ and hence terminates lipid peroxidation chain reaction.

$$COO^{\bullet} + \alpha\text{-tocopherol-OH} \longrightarrow COOH + \alpha\text{-tocopherol-}O^{\bullet}$$

α-tocopherol alone and in combination with glutathione peroxidase is reported to inhibit lipid peroxidation [74-76]. Specific physicochemical interaction between the phytyl side-chain of α-tocopherol and the fatty acyl chains of polyunsaturated phospholipids are known to augment this function of vitamin E, by presenting an anchor through which vitamin E can attach to membranes and thereby elicit its antioxidant behaviour [77]. Vitamin E due to its lipophilicity is primarily localized in subcellular membranes (like those of nuclei,

mitochondria and microsomes). At these sites it acts against peroxidative damage to maintain the integrity of the membrane [78].

Ubiquinol

Ubiqinol is a major antioxidant, whose physiological importance is not sufficiently characterized [19].

Ascorbic acid

Ascorbic acid is an important antioxidant which acts intracellularly and in plasma [80]. It is reported to be effective against γ-ray induced chromosomal aberrations. It also acts synergistically with tocopherol to combat damaging effects of free radicals [81, 82].

Uric acid

Another free-radical scavenger and biological antioxidant is uric acid [80]. It is also present in high concentration in biological fluids and also has high reactivity with singlet oxygen, superoxide anions and hydroxyl radicals. These attributes are reported to be major factors in employing urate in oxidant and radical induced aging and cancer [83]. Urate is reported to form complexes with iron and this leads to the inhibition of chelated as well as free iron dependent oxidation of ascorbic acid and the iron-dependent lipid peroxidation in the presence of hydroperoxides. Free radical formation and initiation of damaging reactions in membranes may be blocked by this inhibition without the apparent oxidation of urate [84]. Urate is also reported to regulate prostaglandin synthesis presumably by scavenging hydroxyl radicals [85].

Glutathione

Glutathione is located in cell cytosol and is reported to function as a major scavenger for free radicals [61].

Carotenoid pigments

Evidences indicate that they can function as effective quenchers of singlet oxygen [86, 87] and oxygen-centered radicals [88].

Repair process

Systems are known to exist that remove damaged biomolecules leading to altered cell metabolism and viability. These include specific enzymes that remove damaged nucleic acids and proteolytic systems that confiscate oxidized proteins and oxidized membrane lipids.

Antioxidant drugs

Metal chelating agents and free radical scavengers are reported to be employed as antioxidants. For e.g. probucol is used clinically as lipid lowering drug in artherosclerosis by low density lipoprotein [89]. Cysteine, 2-mercaptoethylamine, ethoxyquin and butylated hydroxyl toluene are reported to suppress lipid peroxidation of biological membranes *in vitro.*

ROLE OF FREE RADICALS IN HUMAN DISEASES

Inflammation

Inflammation is the response of host to injury or invasion by foreign material. Acute and chronic inflammations are fallout of the increased O_2^- generation particularly in the extracellular space. Inflammatory cells like PMNL, macrophages and monocytes are capable of releasing O_2^- [14, 90, 91]. Activated neutrophils generate superoxide radicals which damage the cells surrounding biological fluids further aggravating the inflammatory response [59]. As discussed earlier inflammatory cells undergo 'respiratory burst' leading to the generation of the free radicals, which otherwise is responsible for the destruction of bacteria [14], however on reversal of inflammatory change may damage cell membranes and biomolecules.

O_2^- is believed to play a vital role in the inflammatory process due to the conversion of a serum component into a powerful neutrophil chemoattractant [92]. Arachidonic acid bound to serum albumin may act as a chemoattractant. Activated neutrophils are also capable of generating a chemoattractant. Thereby the local congregation of neutrophils continues till the exhaustion of the activator takes place. At this stage the newly arrived neutrophils are not activated and lead to dissipation of the gradient of chemoattractant. The cases where the local activators are opsonized microorganisms, the above mentioned mechanism should work well. But, when the activator is generated endogenously, as in autoimmunity, it could get out of control. SOD act as an anti-inflammatory agent in such cases [93].

Thus O_2 acts as an intermediate of dioxygen reduction, an important cause of oxygen toxicity and of the oxygen-dependent toxicities of redox-active compounds. It also plays a key role in the micorbicidal action of neutrophils and in the inflammatory process.

The initiation of propagating free radical chain reactions in plasmalemmal membranes is reported to affect cellular integrity. The resultant lipid hydroperoxide products on being released in the extracellular space may augment microvascular permeability and alter leucocyte chemotaxis.

In the case of genetic disorder chronic granulomatous disease the ability to produce O_2^- and H_2O_2 is impaired due to loss of the microbicidal action of the phagocytes and thereby the susceptibility to infection is augmented.

Rheumatoid arthritis

The hazardous effects of oxygen free radicals, viz. the superoxide radical has been proposed to be involved in the aetiology and pathology of rheumatoid arthritis [94-97]. Excessive ROS formation by hyperactive neutrophils and macrophase may overcome antioxidant defense and this oxidation stress may lead to initiation of rheumatoid arthritis. SOD disrupts the sequence of biochemical inflammatory processes induced by free radicals, without a concomitant decrease in the immunological functions of phagocyte [95]. The activity of SOD is reported to be reduced in some inflammatory disorders.

Respiratory burst of neutrophils generates superoxide, hydroxyl radicals and hydrogen peroxide which along with hydrolases (produced by phagocytosis) cause a synergistic damage to cartilage, synovial membrane and hyaluronic acid which accounts for the decreased viscosity of synovial fluids [98]. PMNLs are reported to be involved in the pathogenesis of juvenile chronic arthritis [99]. It has been suggested that degradation of collagen by oxidant species affects progression of rheumatoid arthritis [100]. Studies have suggested that superoxide and oxygen radicals cause an alteration in immunoglobulin G and render it autoantigenic, resulting in the production of antibodies which thereby sustains the inflammatory response [59]. Direct injection of superoxide dismutase may relieve some of the symptoms.

Artherosclerosis

Lipid peroxidation is reported to be involved in the oxidative modification of low density lipoprotein (LDL) which ultimately results in the formation of artherosclerotic lesions [14]. Vitamin E, BHT and probucol drug can reduce the oxidation status of LDL and thus prevent artherosclerosis [101].

Cancer and cardiovascular diseases

Free radicals are involved in the aetiology of cancer [102] and cardiovascular diseases [103]. Poor plasma levels of antioxidant especially carotene and vitamin E increases the risk of cancer and ischemic heart disease respectively. Free radicals like superoxide, nitric oxide [104] generated by activated neutrophils, nitroxy and epoxy derivatives [105] produced by exposure to chemical carcinogens (polycyclic hydrocarbons and aromatic amines) and intake of transition metals (iron, chromium and nickel) lead to cancer development in humans as they are capable of modifying nucleic acid and DNA/RNA. Intake of antioxidants with a threshold values of 28-30 µmol/1 of vitamin E, 40-50 µmol/1 vitamin C, 0.4-0.5 µmol/1 carotene and 2.2-2.8 µmol/l of vitamin A is reported to reduce the effect of these diseases [106].

The importance of α-tocopherol as a biological antioxidant has been discussed earlier in this article. However, in many chemical systems, pro-oxidant properties of α-tocopherol have been noticed and it has been reported that it can induce tumor formation [107]. *In vitro* studies indicate that α-tocopherol can act as a potent DNA-damaging agent in the presence of Copper (II) ions by promoting the formation of copper-dependent ROS from molecular oxygen. This leads to DNA base oxidation and backbone cleavage.

In lung cancer patients and absence of catalase activity in plasma and lymphocytes is a marked feature of lymph [108].

Exposure to solar radiation is observed to cause cutaneous, malignant melanoma. Radiation absorbed by melanin in melanocytes generates free radicals that may initiate the carcinogenic process. Radicals generated in this manner in melanin in the epidermal upper layers are unable to diffuse as far as down to the melanocytes. This melanin may thus act as a protective, while the ones in the melanocytes may be a photocarcinogen [109].

Liver damage

This is induced by many prooxidant agents like chloroform [110], excess iron and ethanol. Certain drugs like paracetamol, halothane, paraquat, menadione, diquat, which start lipid peroxidation are reported to be involved in ischemic hepatitis.

Lungs and kidney damage

Reactive oxygen species (ROS) are implicated to be involved in a majority of lung and kidney diseases. ROS generated by infiltrating neutrophils or xanthine/xanthine oxidase systems cause inflammation to lungs, arachidonic acid induced by oxidation reactions results in both vaso- and bronchoconstriction [111, 112]. ROS are also reported to be causing damage to glomerular cells and thus implicated in the pathogenesis of ischemia, toxic and immunologically mediated renal injury [113, 114]. Chronic renal disease is reported to be associated with both increased extracellular SOD and increased infection rates [115].

Diabetes

Diabetes mellitus is a syndrome initially characterized by loss of glucose homeostasis. Abnormalities in the regulation of peroxide and transition metal metabolism are postulated in the establishment of disease [116- 118] as well as its long term complications like artherosclerosis, nerve damage, kidney damage and retinopathy. Free radical generation and oxidative stress are reported to play a role in the development of islet dysfunction in diabetes. These mechanisms affect membrane potential and cytosolic calcium in other cell type [119].

Senescence/ aging

Aging is the sum total of all the changes that occur in living organisms with passage of time leading to biochemical and physiological impairment, increased pathology and death [120]. Harman [121] proposed that free radicals are involved in the process of biological aging. Higher formation rates of free oxygen radicals in biological materials (especially mitochondria), accumulation of free radical damage and changes of antioxidant capacities further establish the role of free radicals in senescence. The cells are endowed with antioxidative defence but with the aging the generation of free radicals surpasses their disposal leading to age-related degenerative disease [122].

Free-radical attack on cell components leads to lipofuscin production. Lipofuscin granules (also referred to as age pigments) are autoflouroescent lipoprotein pigmented masses present within the cytoplasm of non-dividing cell such as neurons and cardiac myocytes. They may be debris after peroxidation of polyunsaturated lipids and their crosslinking with proteins in subcellular membranes. They are a prime indicator of the degree of cellular insufficiency in aging. However, reports do indicate that they do not interfere with normal cell processes. Lipofuscin granules are prone to progressive accumulation with advancing cellular age. The accumulation particularly takes place in postmitotoic cells like adrenals, brain, myocardium and skeletal muscle.

Psoriasis

It is a common skin disorder that is characterized by hyperproliceration and incomplete differentiation of epidermal keratinocytes. It has been hypothetised that the formation of clastogenic factors (CF) is similar to that in other diseases accompanied by oxidative stress and in particular chronic inflammatory diseases with autoimmune reactions like lupus erythematous, progressive systemic sclerosis and rheumatoid arthritis. Increment in superoxide production by phagocytes, formation of lipid peroxidation products and release of cytokines are reported to be responsible for the superoxide-stimulating and chromosome-damaging properties of patient's plasma. In diseases accompanied by CF formation the risk of cancer and leukemia is augmented [123].

Psychiatric disorders

Patients suffering from a variety of psychiatric disorders are reported to have increased Cu, Zn SOD, an enzymatic scavenger of O_2^-. It has been suggested that some of the mental aberrations observed in these patients can be related to the excess of SOD available for O_2^- scavenging. Neurodegeneration is characterized by a remarkable accumulation of iron in the affected brain regions [124]. New bioactive chelating agents including oxidative stress activable iron chelators are being developed that can flexibly respond to an increase of free radical formation in the cell.

CONCLUSION

This review has summarized the free radical generation in human cells and its involvement in the aetiology of various diseases. Hazardous effects on health result only when the free radical activities supersede the natural antioxidant defense mechanism. Endogenous antioxidants provide an important protection. However, in many pathophysiological conditions, local endogenous antioxidant capabilities can be exceeded and tissue injury results. In such cases exogenous antioxidants may be beneficial. If free radicals are aetiologically involved in major causes of death, optimization of antioxidants status should offer preventive prospects.

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