FREE RADICALS IN HEALTH AND DISEASES – A MINI REVIEW

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Summary

Free radicals and related species have attracted a great deal of attention in recent years. They are mainly derived form oxygen (reactive oxygen species/ROS) and nitrogen (reactive nitrogen species/RNS), and are generated in our body by various endogenous systems, exposure to different physicochemical conditions or pathophysiological states. Free radicals can adversely alter lipids, proteins and DNA and have been implicated in aging and a number of human diseases. Lipids are highly prone to free radical damage resulting in lipid peroxidation that can lead to adverse alterations. Free radical damage to protein can result in loss of enzyme activity. Damage caused to DNA, can result in mutagenesis and carcinogenesis. In contrast, physiological effects of ROS/RNS (e.g. superoxide radical and nitric oxide) occur at low/moderate concentrations and involve in cellular responses to noxia, as for example in defence against infectious agents, in the function of a number of cellular signaling pathways and act as second messengers in intracellular signaling cascades. The harmful effect of ROS is neutralized by a broad class of protective agents termed antioxidants, which prevents oxidative damage by reacting with free radicals before any other molecules can become a target. Therefore, the best recommended action is to increase the intake of natural antioxidant by consuming cereals, pulses, nuts, fruits, vegetables, which seems to be a safe approach.

Key words: Free radicals, Lipid peroxidation, Antioxidant, Diseases,

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Introduction

In recent years there is an upsurge in the areas related to newer developments in prevention of disease especially the role of free radicals in health and disease. Free radicals are fundamental to any biochemical process and represent an essential part of aerobic life and our metabolism. They are continuously produced by the body's normal use of oxygen (1). One can have too much of a good thing including oxygen, which is necessary for life, but in the form of free radical, it can cause harm. Oxygen is a dangerous friend. The by-products of its metabolism called free radicals are unstable, violently reactive, potentially destructive and short lived. Free radicals are the new "buzz word" in pathophysiology today. They have a special affinity for lipids, proteins and nucleic acids (DNA). Most molecules have all their electrons in pairs and are therefore not free radicals. Molecules are held together by pairs of electrons forming stable bonds, but breaking a bond forms highly reactive free radicals (2).

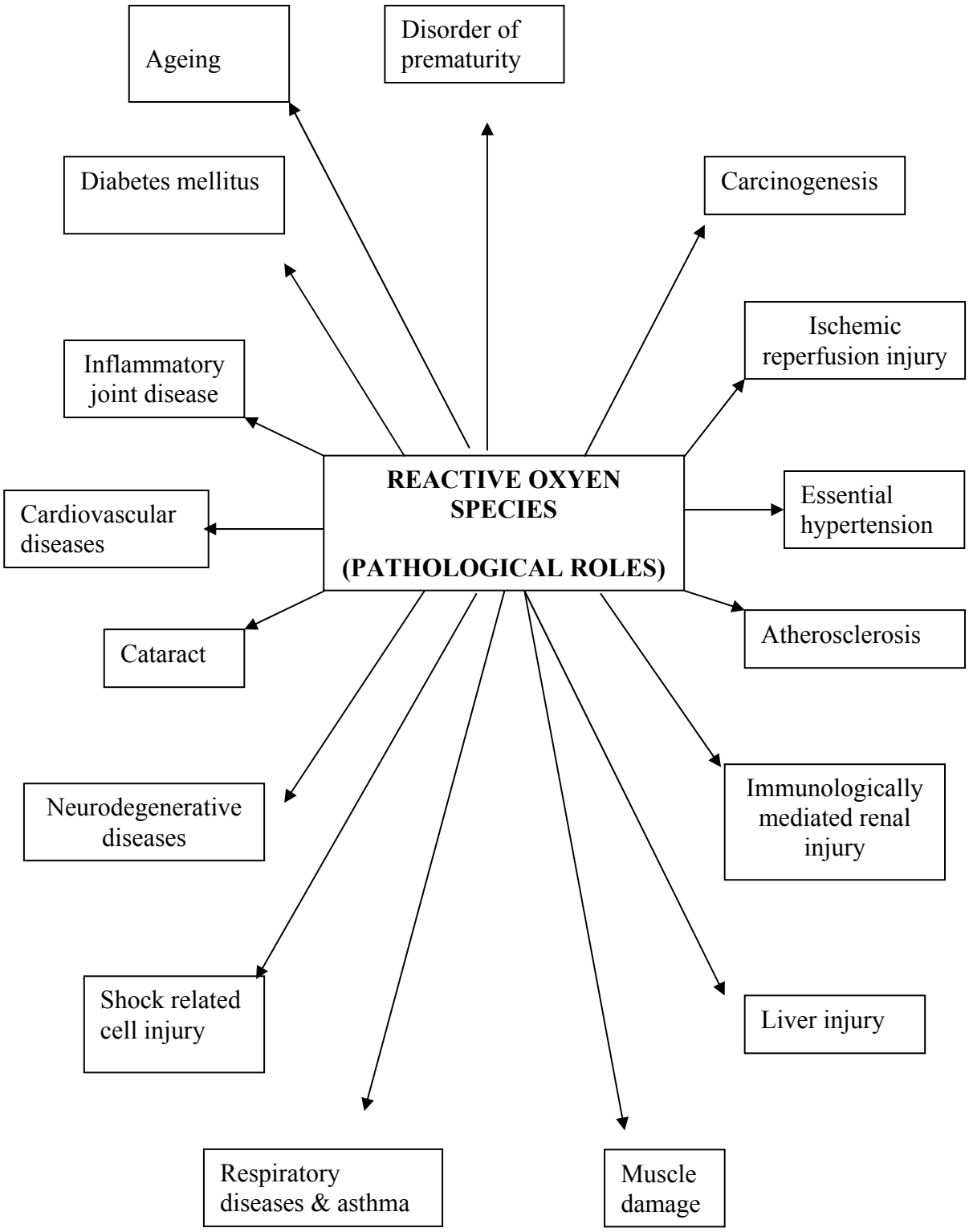
A free radical is an atom, ion or molecule, possessing an unpaired electron in an outer orbit. It is harmful because in search for a pairing electron, the free radical takes one electron from a stable molecule, in turn the stable one becomes a free radical and the resulting chain reaction can injure tissues and impair their functions. Most common radical derivatives of oxygen like superoxide free radical anion ($O_2^{\bullet}$), hydroxy free radical ($^{\bullet}OH$), lipid peroxyl ($LO^{\bullet}$), lipid alkoxyl ($LOO^{\bullet}$) and lipid peroxide (LOOH) as well as non-radical

derivatives such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2^{\bullet}$) are collectively known as reactive oxygen species (ROS) (3). The nitrogen-derived free radicals are nitric oxide ($NO^{\bullet}$) and peroxynitrite anion ($ONOO^{\bullet}$) (4). ROS have been implicated in over a hundreds of diseases states (Fig 1) which range from arthritis and connective tissue disorders to carcinogenesis, ageing, physical injury, infection and acquired immunodeficiency syndrome (5).

Production of free radicals in the human body

Most of the oxygen taken up by cells is converted to water by the action of cell enzymes. However, some of these enzymes "leak" electrons into the oxygen molecules, and lead to the formation of free radicals. They are formed during normal biochemical reaction involving oxygen. Metals containing proteins, as well as other sources of metals, are potent electron transferring agents. There are two important sources of reactive oxygen species generated in the biological system. One of the internal factors i.e. normal cellular metabolism like mitochodrial electron transport, endoplasmic reticulam oxidation, Enzymatic activity including NADPH oxidase, xanthine oxidase, monoamine oxidase, tyrosine hydroxylase, L-amino oxidase, Diamine oxidase,glycolate oxidase,alpha hydroxy acid oxidase and L-Gluconolactone oxidase, prostaglandin synthesis, auto-oxidatioon of adrealine, thiol, ascorbic

Fig 1 Pathological roles of free radicals (3)



acid, reduced riboflavin ($FMNH_2$ $FADH_{2)}$, nitric oxide synthase, stimulated neutrophils, activated phagocytic cells and cytochsrome P_{450} (3,6-9).

Other external factors i.e. environmental sources like radiation-constituents of ozone, oxidant of engine exhaust, carbon tetrachloride, paracetamol, pesticides, transition metals, alcohol, pollutants, cigarette smoke and inflammation are other sources of oxygen free radical; exposure to too high concentration of oxygen itself can be a cause of free radicals (3,8).

Superoxide ($O_2^{\bullet}$): The body makes another oxygen radical (i.e. unpaired electrons is located on oxygen), superoxide. Adding one electron to the oxygen molecule makes superoxide, which is generally a poor reactive radical. Some superoxide is made by "accidents of chemistry", in that many molecules in the body react directly with oxygen to make superoxide. Examples include the catecholamine and some constituents of mitochondria electron transport chains. Such superoxide generation is unavoidable. In addition, some superoxide is made deliberately For example activated phagocytes generate large amounts of superoxide as part of the mechanism by which foreign organisms are killed (3).

Hydrogen peroxide (H_2O_2) is the most stable reactive oxygen metabolites. H_2O_2 may be generated directly by divalent redacting of O_2 or indirectly by univalent reduction of $O_2^{\bullet}$. Hydrogen peroxide is the primary product of the reduction of O_2 by numerous oxidases. H_2O_2 is very sensitive to decomposition by redox-active metal complexes, of which catalase and peroxidase arre the most effective exponents. Metal ions have a strong effect on the chemistry of

O_2 and its reduction products. The well-known Fanton reaction is initiated when Fe^{2+} comes in contact with H_2O_2 to product $^{\bullet}OH$ (10).

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

H_2O_2 also reacts with $O_2^{\bullet}$ to initiate Haber-Weiss reaction producing $^{\bullet}OH$ in the presence of Fe^{2+} (11).

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

Hydroxyl radical ($^{\bullet}OH$): Radiation can split water in the body to generate the hydroxyl radical. This is a highly reactive radical, which once generated, attacks wherever it is next to. Its lifetime *in vivo* is very short because the hydroxyl radical reacts at its site of formation usually leaving behind a legacy in the form of a propagating free-radical chain reaction (12).

Singlet oxygen ($^1O_2$): It is also not a free radical and is formed as a result of spin reversal of electron in outer orbital of oxygen molecule. It is considered highly potent oxidant with short half-life causing tissue damage (13).

Nitric oxide ($NO^{\bullet}$): Another physiological free radical is nitric oxide, which is produced by the vascular endothelium as a relaxing factor and also by phagocytes and in the brain. Nitric oxide has many physiological functions, but excess nitric oxide can be toxic (14).

Molecular damage induced by free radicals

Most molecules in the body are non-radicals. Hence any reactive free radical generated is likely to react with a non-radical, a free radical chain reaction results and new radicals are formed. All the biological molecules

present in our body are at risk of being attacked by free radicals, which seem to be one of the final common pathways of cell damage by cross-linking of proteins and by critical alterations of lipids. Such damaged molecules can impair cell functions and lead to aging and cell death eventually resulting in diseased states. There are three important reactions on this type. They are lipid oxidation, protein oxidation and DNA damage (3,15).

Lipid peroxide (LOOH): when (LH) target polyunsaturated fatty acid (PUFA) is attacked by free radical R (initiating oxidizing radical). Oxidation of the PUFA generates fatty acid radical ($L^{\bullet}$**)** that rapidly adds oxygen to form fatty acid peroxy radical ($LOO^{\bullet}$**)**, which are the carrier of chain reaction, they can oxidize further PUFA molecules tto produce LOOH (lipid hydroperoxide) that can break down to yet more reactive radical species like llipid peroxyl, lipid alkoxyl and aldehydes like malondialdehyde (MDA). This phenomenon of oxidative destruction of PUFA's is called as lipid peroxidation. These metabolic byproducts can cause direct destruction of structure of membrane or indirectly can damage other structures of cell e.g., DNA, RNA, Proteins synthesis and enzymes mainly by aldehydes like (MDA). A measured level of MDA is used as a direct index of tissue damage associated with lipid peroxidation. It reacts with thiobarbituric acid and produce red-coloured product (16).

$$LH + R^{\bullet} \rightarrow L^{\bullet} + RH$$

$$L^{\bullet} + O_2 \rightarrow LOO^{\bullet}$$ (Fatty acid peroxy free radical)

$LOO^{\bullet}$ + L'H (New PUFA) $\rightarrow$ LOOH (Lipid hydroperoxide) + L

LOOH $\rightarrow$ $LOO^{\bullet}$ (Lipid peroxyl), $LO^{\bullet}$ (Lipid alkoxyl, Aldehydes (MDA)

Protein oxidation: Proteins are also targets for free radicals. Oxidative modification of proteins by reactive oxygen species (ROS) or reactive nitrogen species (RNS) is implicated in the pathogenesis of various diseases. Oxidative damage to a specific protein, especially at the active site, can induce a progressive loss of a particular biochemical function. Several types of ROS-induced protein modifications have been demonstrated (17), including the loss of sulfhyryl (SH) groups, formation of carbonyls, disulphide crosslink, methionine sulfoxide, dityrosine cross-links, nitro tyrosine, and glyoxidation and lipid peroxidation adducts, among others. Alterations of signal transduction mechanisms, transport systems, or enzyme activities have been shown (18). Protein oxidation may be at least in part responsible for atherosclerosis, many forms of cancer, ischemia-reperfusion injury and may also be associated with aging (19).

Carbohydrates: Free radicals such as OH react with carbohydrates by randomly abstracting a hydrogen atom from one of the carbon atoms, producing a carbon-centered radical. This leads tto chain breaks in important molecules like hyaluronic acid. In the synovial fluid surrounding joints, an accumulation and activation of neutrophils during inflammation produces

significant amounts of oxyradicals that is also being implicated in rheumatoid arthritis (15).

DNA oxidation: The oxidative damage to DNA may be the most dangerous for the cell because it affects the cell cycle and leads to mutations and cancer. The oxidation of guanine by the hydroxyl radical ($OH^{\bullet}$) to 8-hydroxy-2-deoxyguanosine (8-OHdG), which eventually leads to GC→ TA transversions during subsequent DNA replication (20). DNA alteration has been suggested to be responsible in part in the processes of aging (21), diabetes mellitus (22), inflammatory diseases, and liver disease (23).

Antioxidants

The imbalance between ROS production and antioxidant defense leads to 'oxidative stress'. Any compound, natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of this type of damage. The harmful effect of ROS is neutralized by a broad class of protective agents termed antioxidants, which prevents oxidative damage by reacting with free radicals before any other molecules can become a target. Antioxidants are probably now regarded as the new generation 'superheroes' to maintain the health (24). Every living organism has antioxidant defense to cope up with the ROS. The enzymatic antioxidant such as SOD, CAT, GPx etc., and non enzymatic antioxidants are vitamin C, vitamin E ceruloplasmin, albumin and reduced glutathione play an important role in the protection of cells against free radical mediated damage (14).

Antioxidants may exert their effects by different mechanisms, such as suppressing the formation of active species by reducing hydro peroxides ($ROO^{\bullet}$) and H_2O_2 and also by sequestering metal ions, scavenging active free radicals, repairing and/or clearing damage. Similarly, some antioxidants also induce the biosynthesis of other antioxidants or defence enzymes (1).

Research in the recent past has accumulated enormous evidences revealing that enrichment of body systems with natural antioxidants may correct the vitiated homeostasis and can prevent the onset as well as treat diseases caused and/or fostered due to free-radical mediated oxidative stress. These developments accelerated the search for antioxidant principles that lead to the identification of natural resources, isolation of active principles and further modification and refinement of active antioxidant molecules (5, 25).

Plant and plant products are being used as a source of medicine since long. Plant extracts increasingly used as phytotherapeutics and are still a large source of natural antioxidant. Natural antioxidants strengthen the endogenous antioxidant defense from ROS ravage and restored the optimal balance by neutralizing the reactive species (26). Particularly, flavonoids and phenolics are consider as potential therapeutic agents a wide range of ailments and are widely distributed in the plant kingdom and therefore an integral part of diet, with significant amount reported in vegetables, fruits and beverages (27).

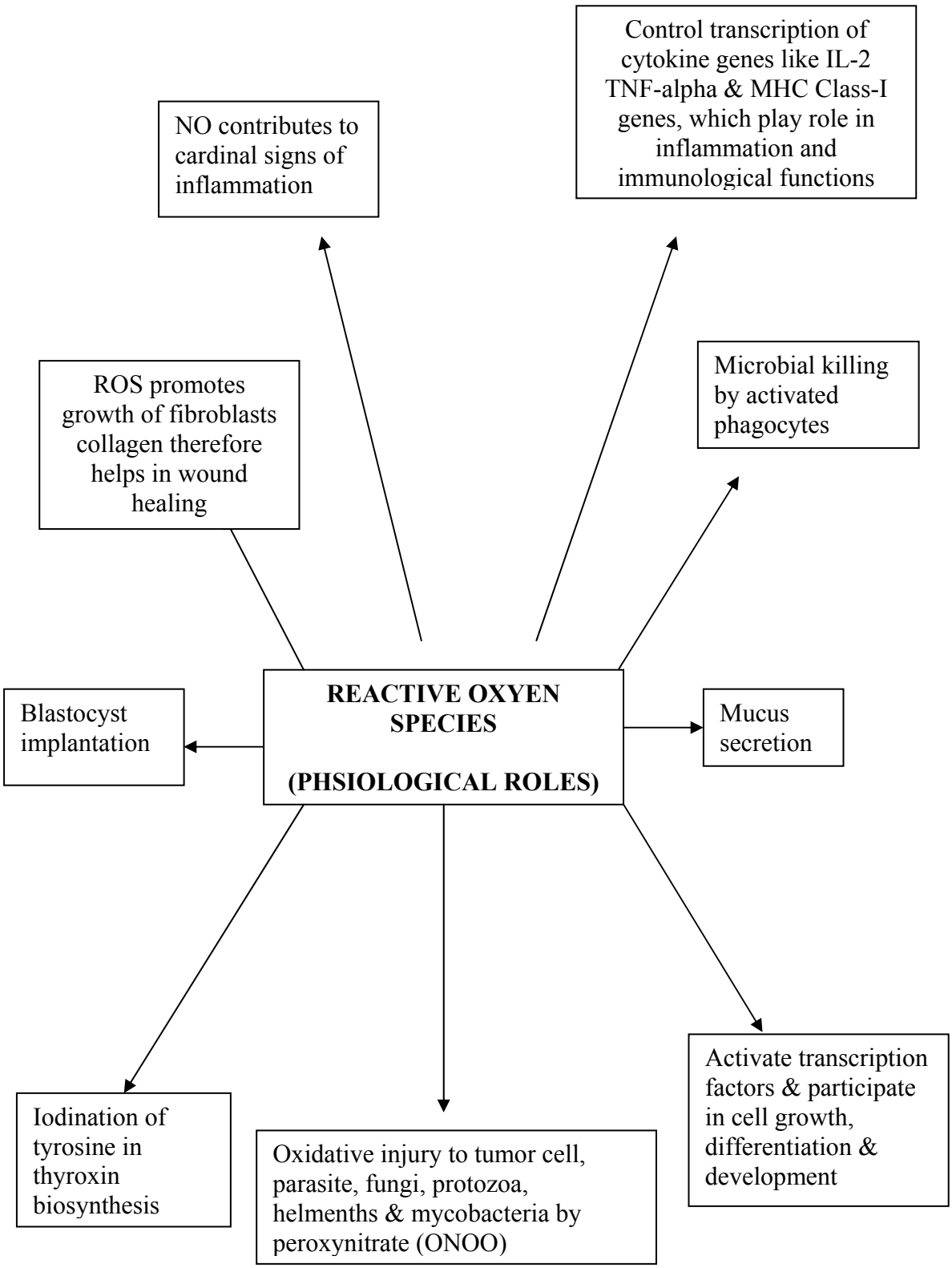
Physiological roles of free radical

Free radicals have been implicated in the etiology of several human diseases (28). But it has to be emphasized that ROS and RNS are both produced in a well-regulated manner to help maintain homeostasis at the cellular level in the normal healthy tissues and play an important role as signaling molecules. The physiological and pathological roles of reactive oxygen species are shown in fig I and fig II. Most cells can produce superoxide ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2) and nitric oxide ($NO^{\bullet}$). Hence, it is worth emphasizing the important physiological role of free radicals (Fig 2).

1. Generation of ATP (universal energy currency) from ADP in the mitochondria: oxidative phosphorylation
2. Detoxification of xenobiotics by Cytochrome P_{450} (oxidizing enzymes)
3. Apoptosis of effete or defective cells
4. Killing of micro-organisms and cancer cells by macrophages and cytotoxic lymphocytes.
5. Oxygenases (eg. COX: cyclo-oxygenases, LOX: lipoxygenase) for the generation of prostaglandins and leukotrienes, which have many regulatory functions.

In recent years, it has become increasingly clear the ROS such as $O_2^{\bullet}$ and H_2O_2 may act as second messengers. Observations made some twenty years age had suggested that ROS may play a role in modulating cellular function. Studies done then revealed that exogenous H_2O_2 could mimic the action of the

Fig 2 Physiological roles of free radicals (3)



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vasodialation and neurotransmission through activation of soluble guanylated cyclase further supported the concept that ROS and RNS can act as second messengers to modulate signaling pathways. This led to the renaissance of the field of redox signaling and with the accumulation of data in various systems, a clearer picture is emerging of the signaling pathways and specific targets affected by ROS/RNS (29).

In conclusion, free radical/reactive oxygen species is continuous process in the biological system and there are substantial evidences for there involvement in many pathophysiological states, where antioxidant can play very important role but there are number of problems at present associated with the practical application of antioxidant therapy like choice of nature of antioxidants, dose and duration of therapy to be employed, delivery at specific site, specificity of targeting free radicals and direct of assay of free radical/reactive oxygen species. Moreover, irrational and non judicial use of antioxidant can also increase the risk of potential toxicity, as many antioxidants can also act as pro-oxidants under a range of circumstances as well as it still difficult to ascertain that free radicals are cause or consequence of pathology and there are inadequate evidences to support therapeutic efficacy of various antioxidant.

Therefore, the best recommended action is to increase the intake of natural antioxidant by consuming cereals, pulses, nuts, fruits, vegetables, which seems to be a safe approach.

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