Oxidative Stress Management in Living Cells

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Abstract:

Oxygen, being an indispensable element of aerobic life, plays an important role in different metabolic and physicochemical processes. However, in aerobic respiratory cells oxygen metabolism results in formation of highly reactive oxygen species that may be toxic to cell leading to a situation termed as oxygen paradox. In aerobic cells ROS are generated as a endogenous byproduct of the oxygen metabolism. Mitochondria are the major contributor of oxygen free radicals (ROS) generation in respiring cells. Total reduction potential (measure of reducing capacity) of all the redox couples found in biological cell is referred as redox state of the cell, which is highly maintained within narrow physiological limits. Prokaryotic as well as eukaryotic cells can be protected from oxidative stress by reestablishment of redox homeostasis. This process is achieved by ROS involving redox regulation. The persistent high-level production of ROS and RNS are responsible for redox imbalance. If cell fails to balance between the production and utilization or removal of free radicals, variety of chronic and degenerative diseases can be induced by oxidative stress.

Key word: Free radicals, ROS reductional potential, oxidative stress, lipid peroxidation, antioxidants.

Introduction:

Living system and Oxidative Environment :

Life was originated on this planet, earth in reduced environment that was characterized by presence of the gases like methane, ethane, hydrogen, Sulphur nitrogen etc. without oxygen. Evolution proceeds with the appearance of photoautotrophs, those are capable of making food by using sunlight and water. The process is photosynthesis, in which oxygen started to releases as a byproduct. Later on this oxygen became the most essential chemical entity of aerobic life. The presence of oxygen makes our planet earth unique for the existence of life. This overall process on geological time period, transformed initial reduced environment to the current oxidative environment.

Oxygen, is an indispensable element of aerobic life and plays an important role in different metabolic and physicochemical processes. In aerobically respiring cells oxygen metabolism results in formation of highly reactive oxygen species that may be toxic to cell. This dilemma in the role of oxygen in aerobic cell is considered as oxygen paradox. This **situation is tackled through** an adaptive strategy that has been evolved to use this oxygen as the terminal electron acceptor in mitochondrial respiration to form water. Thus oxygen formed from water during photosynthesis is again converted to water in aerobic metabolic cells and in this processes ATP is also synthesized from substrate biomolecules.

In addition to this, oxygen is utilized for synthesis of essential biomolecules including hormones, neurotransmitters etc. and in the detoxification of xenobiotics. This review emphasizes the endogenous and exogenous sources of free radicals such as reactive oxygen species –ROS and reactive nitrogen species – RNS, redox state of the living cells , its imbalance due to free radicals generating oxidative stress and the mechanisms in living cell to overcome this stress to obtain redox homeostasis.

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS):

Oxygen metabolism in all respiratory cells result in formation of highly reactive oxygen species (ROS) called oxygen free radicals. Free radical formation is thus an unavoidable physiological process in all such cells. ROS are generated in aerobic cells as a endogenous byproduct of the oxygen metabolism .

1.1.2: Endogenous sources of ROS:

In vivo production of oxygen free radical as a byproduct of enzymatic reaction was first hypothesized by Denham Harman. Endogenous sources of ROS can be enlisted as – mitochondria, peroxisomes , inflammatory cells and cytochrome P450 metabolism etc . Mitochondria is the major contributor of oxygen free radical (ROS) generation in respiring cells. Superoxide generation by mitochondria was first reported by Loschen et al. in 1971. (G.Loschen, B Flobe; , 1971)

In the process of electron transfer in mitochondrial electron transport chain molecular oxygen reduced to water through a generation of other intermediates like superoxide anion radical ($O_2^{\cdot -}$) , hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\cdot}$). Reaction is summarized as

$$\mathbf{O_2 \longrightarrow O_2^{\cdot -} \longrightarrow H_2O_2 \longrightarrow HO^{\cdot} \longrightarrow H_2O}$$

molecular oxygen **Superoxide anion** **Hydrogen peroxide** **hydroxyl radical** **water**

$O_2 \rightarrow O_2^{\cdot -}$

e- [Superoxide anion]

(Addition of an electron to molecular oxygen leads to formation of superoxide anion.)

$O_2 \xrightarrow{2e^-} H_2O_2$

[Hydrogen peroxide]

(Two electron reduction of molecular oxygen)

$\mathbf{O_2.^- \xrightarrow{e^-} H_2O_2}$

(One electron reduction of superoxide anion)

Therefore univalent reduction of molecular oxygen to hydrogen peroxide (H_2O_2) encompasses, superoxide anion ($O_2^{\cdot -}$), which by itself is not much reactive but when it react with hydrogen peroxide in presence of trace metal ions generates the hydroxyl radical (HO.), the highly reactive oxygen species. (K.I.Priyadarsini, ;2005:)

$$\mathbf{HO^{\cdot} + H_2O \rightarrow H_2O_2 + O_2.^-}$$

Hydroxyl radical **Hydrogen peroxide**

However superoxide anion $O_2.^-$ being short lived, can react with itself by the process of dismutation or disproportionation leads to formation of hydrogen peroxide ($\mathbf{H_2O_2}$) and molecular oxygen (O2).

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

Superoxide Anions hydrogen peroxide molecular oxygen

The process of protonation of superoxide anion (O2.-) in the vicinity of membrane leads to generation of an extremely reactive species i.e. perhydroxyl radical (HO_2.)

$O_2.^- + H^+ \rightarrow HO_2.$

Superoxide anion perhydroxy radical

Spin reversal of electron in outer orbit of molecular oxygen leads to formation of singlet oxygen (O2') considered as a highly potent oxidant. (L, 1996) Since H_2O_2 is not having free electron considered as a non radical . In spite of its little reactivity it can diffuse through the biological membranes as well as its hemolytic cleavage by transition metals yield the highly reactive hydroxyl radical (HO.) .

Thus the dismutation of superoxide radical (O2.-) yields H_2O_2 i.e. nonradical and hence decreases reactivity while the protonation reaction increases the reactivity by generating perhydroxyl radical (HO_2·) .

Superoxide radicals are generated as a byproduct through number of metabolic pathways , including enzymatic systems such as xanthine oxidase , NADH oxidase, NADPH-cytochrome P450 reductase etc. Xanthine oxidase (XO) has been widely present from bacteria to man . It is molybdenum iron- sulphur flavin hydroxylases that catalyses hydroxylation of purines .

Hypoxanthine → xanthine → Uric acid

In this reaction superoxide anion and hydrogen peroxides are generated. (M. Velko M. I., 266 ,(2004)

Autoxidation of Co-enzyme Q or ubisemiquinone leads to generation of superoxide radical . The superoxide radical ($O_2.^-$) generated can pass into cytosol through outer mitochondrial membrane by means of voltage dependant anion channel (VDAC).

Other important endogenous source involved in production of H_2O_2 is peroxisomes in which oxidation of fatty acids generates H_2O_2 . The enzymatic reactions catalyzed by D-amino acid oxidase, L-hydroxyl acid oxidase and fatty acid acyl co-A oxidase in peroxisomes are the important contributors of H_2O_2. Liver has been reported as major site of peroxisomal generation of H_2O_2 .(M. Velko M. I., Role of oxygen radicals in DNA damage and cancer incidence;, 266{2004), Microsomes are also producing near about 80% endogenous H_2O_2 at hyperoxia site. (M. Velko M. I., Role of oxygen radicals in DNA damage and cancer incidence; Metabolism of arachidonic acid and prostaglandin metabolism also result in production of a free radical source. (Dorge, 2002)

Some transition metals participating as a cofactors of different enzymes are also responsible for free radical formation, eg. iron has the property to gain or lose electron very easily ($Fe2^+$, Fe 3^+) and hence act as important catalyst of oxidation–reduction reaction. Iron is not allowed to be free entity. But excess superoxide result in release of free iron. Many pathophysiological conditions such as hemocromatosis, b-thalassemia , hemodialysis and free radical interactions with RBC membrane leads to RBC burst results in breakdown of hemoglobin and finally release of free iron. Thus the excess free iron act as pro-oxidant which is detrimental to cellular membranes and through a Fenton reaction produces free radicals. (F.Sibal Pala, 2007)

$Fe2^+ + H_2O_2 \rightarrow Fe3^+ + OH^{\cdot} + OH^-$

Fenton reaction

Beside from these internal sources wide range of external sources such as cigarate smoke (Dugan L.L. C. D., 1999)environmental pollutants, radiations, u.v.light certain drugs, pesticides ,anesthetics and other industrial solvents etc. are the major contributors of free radical sources.

Induction of oxidative stress in liver due to alcohol consumption had been reported, and results in sirrhosis and cancer.Different metal ions, such as Iron, Copper, Chromium, cobalt, vanadium, cadmium, arsenic, nickel etc. interact to cell result in free radical formation. Some may be the pro-oxidants at lower concentrations, but at high concentrations reacts with the cellular constituents and leads to formation of free radicals. (Dorge, Free Radical in the Physiological Control of Cell Function , 2002)

Endogenous sources RNS:

In higher organisms, the terminal guanido nitrogen of L-argenine is oxidized to form nitrogen free radical (NO H_2O_2),catalyzed by a flavohemoprotein ,the enzyme NOS,. NOS exists in different isoforms such as neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). These isoforms are present in different tissues in varying concentrations and their activity is depends on level of intracellular Ca ions. Various reactive nitrogen species (RNS) can be generated by NO. such as nitrosonium cation(NO^+), nitroxyl anion (NO^-) , peroxynitrite ($ONOO^-$). Snitroso-cysteine and S-nitroso- glutathione are the intermediate species that affects different physiological processes. (N.S, Nitrite in nitric oxide biology: cause or consequence? A system based review, 2006)

Activated phagocytic immune system cells such as macrophages and neutrophils, by engulfing and killing bacteria, also contributing in generation of ROS. Inducible NOS found to be highly expressed in macrophages in response to cytokines and other immune modulators. (Schreck R, A Role for oxygen radical as second messengers; , 1991;1)

1.1.3: Redox State and Oxidative stress:

Total reduction potential (reducing capacity) of all the redox couples such as GSSG/2GSH,NAD^+/NADH ASc.-/ACSH- etc. found in biological fluids, organelles , cells or tissues is referred as redox state of the cell (Kohen R, 2002; 30)

Under normal physiological conditions biological system shows more negative values of redox potential. Oxidative stress results in shifting of redox potential towards less negative values .The persistent high level production of ROS and RNS are responsible for redox imbalance may lead to changes in signal transduction that affects gene expression, results in different pathological symptoms as a result of redox dysregulation.

If cell fails to balance between the production and utilization or removal of free radicals, variety of chronic and degenerative diseases can be induced by oxidative stress such as aging, carcinogenesis, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, atherosclerosis, ischemic reperfusion injury, hypertension, muscle damage, liver injury, renal injury, respiratory diseases, asthma, etc.

1.1.4 : Redox homeostasis

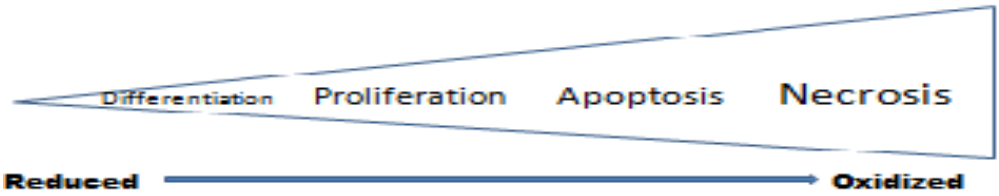
The cell evolved to adopt an unavoidable coexistence of unfriendly, dangerous free radical and started utilizing these entities for advantageous process. Redox state of the cell is highly maintained with in narrow physiological limits. Prokaryotic as well as eukaryotic cells can be protected from oxidative stress by reestablishment of redox homeostasis. The process is achieved through ROS involving redox regulatory mechanisms including reducing species in each cell such as GSH, NADH, FADH etc.

Redox signaling is based on the imbalance between ROS production and its neutralization by the mediators involved in different antioxidant systems. The temporary shift of the intracellular thiol / disulphide redox state towards oxidative conditions can be reestablished to the normal homeostasis by responding at different physiological, biochemical and metabolic levels that are beneficial to the cell. free radicals

Reduced → Oxidized

Inactive ***regulatory proteins*** — **Intracellular thiol / disulfide** — **Active** r***egulatory protein***

The cellular redox status depends on the steady state levels of H_2O_2 tat decides different cellular physical and metabolic processes from proliferation to apoptosis.



The magnitude, concentration and duration of ROS and RNS are important factors in deciding weather or not the interaction is beneficial to the system.

Secondary Reactive Oxygen Species that arises from the reaction of super oxide anion ($O2^{\cdot -}$) and $NO^{\cdot}$ serve as a mediators in different physiological regulatory processes. for eg. induction of protective enzymes such as Oxy R and Sox R by the redox sensitive bacteria.

The persistent high level production of ROS and RNS are responsible for redox imbalance may lead to changes in signal transduction that affects gene expression results in different pathological symptoms as a result of redox dysregulation.

Protection against oxidative stress is achieved through a large number of NAD(P)H oxidase. Isoforms of NOS are mediated by the production of number of secondary oxidative species that act as secondary messengers controlling different physiological responses . Production of superoxide ($O2^{\cdot -}$) and NO. by these enzymes is strictly regulated by hormones , cytokines and other inducing mechanisms .

The utilization of free radicals and its species in metabolic processes includes, the production of cGMP, as a secondary messenger due to activation of guanylate cyclase by superoxide anion ($O2^{\cdot -}$) and hydroxyl radical ($OH^{\cdot}$) was described by Mittal and Murad. Highly reactive nitric oxide ($NO^{\cdot}$) radical act as a major biological signaling molecule for different physiological processes such as a major biological signaling molecule for different physiological process such as neurotransmission , blood pressure regulation, defense mechanism, smooth muscle relaxation and immune regulation .

Nitric oxide radical ($NO^{\cdot}$) was declared as “molecule of the year” in 1992 (Science WD.) because of its different characteristics and importance in physiological processes.

The regulatory role of nitric oxide (NO) in controlling smooth muscle relaxation and in inhibition of platelet adhesion is important (Moncada et.al.). Roth and Droge explained the enhancing role of H2O2 at very low concentration in the production of IL-2, the T-cell growth factor . Some other reports explained the stimulatory role of H2O2 in the expression of heme oxygenase (HD-1) gene (Kerse and Tyrrell), activation of transcription factor NF-KB in mammalian cells (Baeuerie et al.) and induction of various genes in bacteria (Storz et. al.).

Microbial products such as bacterial lipopolysaccharides, lipoproteins or cytokines like interferon, interleukins act as a stimulator for phagocytic NADPH oxidase activation.

High amount of H_2O_2 is produced during the cellular immune response by activated macrophages . As a result accumulation of very high level of H_2O_2 in (10-100µM) in the surrounding inflammatory area leads to generation of reactive oxygen species. ROS in this area serves as a antimicrobial as well as tumoricidal role called “ Oxidative Burst” and considered as innate immune response . Phagocytic isoforms of NADPH oxidase catalyzes the

production of superoxides in these cells. A strong oxidants hypochlorous acid (HCIO) is produced in phygocytic cells catalysed by the enzyme myeloperoxidase . HCIO proved as a potent antimicrobial agent. Regulation of intracellular signaling in nonphagocytic cells such as smooth muscles cells, fibroblast, cardiac myocytes ,thyroid tissue is regulated by intracellular signaling triggered by NAD(P)H oxidases. ROS plays antitumorigenic role by inducing cellular senescence and apoptosis . Thus redox state of the cell decides the fate of the cell to induce either apoptosis or necrosis .

Secondary messengers are playing important role in regulation of respiratory ventilation, erythropoietin production and the enhancement of signaling cascades from various membrane receptors etc.

1.1.5: Interaction of ROS with

Bio molecules :

Denham Harman first time described the role of free radical in cellular damages, degenerative processes like aging, mutagenesis , cancer etc. ROS are responsible for stimulation of oncogene expression and induction of oncogenic phenotype in malignant cells. As well as ROS induces cellular senescence and apoptosis , hence have antitumorigenic activity.

Interaction with Nucleic Acids : Free radical interact with nucleic acids results in either strand breaks or base modification products. (J. F. R., 1990) Both purines and pyrimidines as well as deoxyribose sugar is sensitive to $OH^{\cdot}$ radical attack. Hydroxy radical ($OH^{\cdot}$) abstracts 'H'atom from methyl group of pyrimidine and from the sugar leads to allylic radical generation ,results in DNA strand break as well as it can add to DNA bases. Mitochondrial DNA is more susceptible than nuclear DNA as it is not protected by histones.

During oxidative stress ,8-hydroxyguanine (8-OH-G) , a oxidized DNA product is formed, that tautomerises to induce mutation. Along with ROS ,Reactive Nitrogen Species (RNS) like peroxynitrites and nitrogen oxides NO imparted in DNA damage. (G.C.Brown, 2001) 8-nitroguanine, a nitrogen adduct appears as result of interaction of peroxynitrites with guanine induces transversions(Purines to pyrimidines conversion & vice versa).

Interaction with Proteins:

Interaction with Lipids :Free radical interaction with lipids results in lipid peroxidation which is initiated by the interaction of hydroxyl radical ($HO^{\cdot}$) with fatty acids ($RH^{\cdot}$), called poly unsaturated fatty acids (PUFA), leads to generation of fatty alkyl free radical ($R^{\cdot}$) as in reaction 1.

Fatty alkyl free radical (R.)react with molecular oxygen to generate fatty peroxy radical ($ROO^{\cdot}$) having high oxidizing potential ,as in reaction 2.

ROO. Attacks unsaturated fatty acids (RH) in the membrane to form hydroperoxides and fatty alkyl radical (R.) as in reaction 3.

The reactions are summarized as follows;

$$RH + HO^{\cdot} \Longrightarrow R^{\cdot} + H_2O \quad \text{----(1)}$$

Fatty acid hydroxyl radical free fatty alkyl radical

$$R + O_2 \Longrightarrow ROO^{\cdot} \quad \text{----(2)}$$

$$ROO^{\cdot} + RH \Longrightarrow ROOH + R \quad \text{---(3)}$$

This autocatalytic cascade propagate until the free radical chain is terminated.

PUFA are extremely sensitive to oxidation. The maximum levels of lipid peroxidation are observed when the ratio of Fe II : Fe III is 1:1 (M. Velko M. I., Role of oxygen radicals in DNA damage and cancer incidence; , 2004) Several experimental models in vivo , of iron overload confirmed increased PUFA oxidation of hepatic mitochondria as well as lysosomal fragility. The mitochondrial PUFA are a preferential target for iron –driven peroxidation .Both Copper and Iron are pro-oxidants that catalyzes peroxidation of lipids.

The final aldehyde product of lipid peroxidation are lipid peroxidation are malondialdehyde (MDA) and 4-hydroxyl 2-nonenal (HNE). MDA is highly mutagenic and carcinogenic while HNE is toxic and affects the signal transduction of the cell. Interaction of MDA with DNA results in formation of nucleotide adducts which in turnsresposible for DNA-DNA interstrand crosslinks or DNA protein crosslinks.

Ethenoadducts formed when come in contact with peroxidising lipids such as etheno-dA, etheno-dC and eheno-dG are the exocyclic DNA adducts which are strongly genotoxic. Acrolein and crotonaldehyde generated by a lipid peroxidation are strong mutagen when interact with DNA a propanodeoxyguanosine (R.G.Nath, 1994)

Thus the lipid peroxidation generates reactive ,unstable, long lived toxic by products and thus interactions of these with cell membrane and other organelles is one of the reason of cell dysfunction may proceed to cell death.

1.2: Natural Defenses against damages induced by ROS :

The cell evolve to protect itself from free radical attack either by preventing the interaction of free radical with cellular moieties or by repairing the oxidative damage.

1.2.1: Antioxidants

1.1.2: Enzymatic Antioxidants :

Cell is deviced with a group of enzymes protecting form free radical attack. Major enzyme systems involved are , Catalases and Glutathione Peroxidases. These enzymes critically protect cellular components from interacting with ROS.

Superoxide dismutases(SODs) are metalloproteins containing Cu,Zn,Mn as a cofactors, eg; Cu-Mn Superoxide dismutase is present in cytosol, intermembrane space in mitochondria and extracellular spaces, Mn- Superoxide dismutases present in mitochondrial matrix etc. are involve in catalyzing dismutation of superoxide radicals.

Rapid conversion of superoxide radicals into H_2O_2 is highly accelerated (10000 folds) by the Superoxide dismutases in contrast to spontaneous dismutation.

$$O2.- + O2.- + 2H \xrightarrow[10^9 m^{-1}s^{-1}]{} H_2O_2 + O_2$$

The highest activity of these enzymes have been reported in liver.

Catalases and Glutathione peroxidase are the enzymes involved in removal of H_2O_2 formed by dismutation of superoxides as well as other spontaneous reactions. Even it is less reactive is a strong oxidant and also can be act as a precursor of hydroxyl radical (HO.) via fenton reaction.

Catalases also accelerate the interaction of H_2O_2 with few hydrogen donors results in fornation of water refered as peroxidatic activity of catalase.

Glutathione Peroxidase enzyme system consists of cofactors and enzymes as glutathione peroxidase, glutathione reductase , glutathione(GSH) & reduced nicotinamide adenosine dinucleotide phosphate (NADPH)

The co-factor GSH is a tripeptide containing three amino acids is an important component for the enzyme glutathione transperase engaged in removing of the free radicals, oxidized to glutathione disulfide (GSSG.)

$H_2O_2 + 2\ GSH \longrightarrow H_2O + GSSG$

Thus catalases & glutathione peroxidase are important enzymes those prevents participation of superoxide anion (O2.-)& H_2O_2 from the reactions results in generation of a hydroxyl radical (HO.), a more reactive oxidant.

1.2.3: Non enzymatic Antioxidants : Non enzymatic antioxidants are enlisted as ascorbic acid (Vitamin C),α-tocopherol (vitamin E),thiol antioxidants such as, glutathione (GSH), thioredoxin (TRX), lipoic acid, caretenoids , flavonoids, selenium etc.

The presence of sulphur atom remarks the antioxidant property of thiol compounds. Glutathione is a major soluble antioxidants considered as thiol –disulphide redox buffer of the cell (R.Masella, 2005)

Novel mechanisms of natural antioxidant compounds in biological systems: involvement of glutathione and glutathione-related enzymes, Glutathione (reduced) on interaction with free radical get oxidized to GSSG, a non radical form which in high concentration damages cellular enzymes and the ratio GSH/GSSG marks oxidative stress of the cell (C.Hwang, 1992) Glutathione plays versatile protective role because it act as a cofactor of several detoxifying enzymes against oxidative stress eg. glutathione peroxides (GPx,), glutathione transferase etc.GSH participates in amino acid transport through plasma membrane , Scavenges hydroxyl radical ,detoxifies hydrogen peroxide and lipid peroxide by using glutathione peroxidase as a catalyst. Vitamin C and vitamin E are regenerated back to their active forms by glutathione. (M. Velko M. I., Role of oxygen radicals in DNA damage and cancer incidence; , 2004) Ascorbic acid(Vitamin C) ,is a water soluble antioxidants in living cell. Along with Vitamin E it regenerates α-tocopherol from its radical in the membrane. α-tocopherol (Vitamin E) is a major fat soluble antioxidants in the cell that protects cell from lipid per oxidation. Carotenoids such as β- carotenoids pigments from various plants and microorganisms has remarkable antioxidant activity. Flavonoids and other phenolic compounds from dietary and medicinal plants have been proved to protect the cells from free radical attack.

1.2.4: Conclusion :

ROS and RNS generation is one of the natural process as a part of oxidative metabolism . Utilization of the free radicals and their interaction products with different cellular molecules may utilize by the cell as the signaling molecule. The imbalance between the production and utilization of these entities results in oxidative stress . Various cellular mechanisms are evolved to conquer these situations includes various enzymatic and non enzymatic antioxidant molecules. Wide range of antioxidant is available in nature to combat with free radicals and thus to prevent oxidation ,which is the major cause of various degenerative diseases and aging.

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