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Role of Free Radicals and Reactive Oxygen Species in Biological Systems-A Comprehensive Review

Vidya M. A[1*], Krishnanand P. S[2], Mohamed Nizam Al Deen Shah[3]

[1]Senior Lecturer, Department of Oral Pathology, Dayanand Sagar College of Dental Sciences, Bangalore, Karnataka, India.

[2]Professor and Head, Department of Oral Pathology, Dayanand Sagar College of Dental Sciences, Bangalore, Karnataka, India.

[3]Assistant Professor, Department of Physiology, Dayanand Sagar College of Dental Sciences, Bangalore, Karnataka, India.

Review Article

Address of Correspondence Author
Vidya M. A; Department of Oral Pathology, Dayanand Sagar College of Dental Sciences, Bangalore, Karnataka, India.
E-mail: vidyama@dscds.edu.in

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ABSTRACT

A free radical is an atom, molecule, or compound that is highly unstable because of its atomic or molecular structure (i.e., the distribution of electrons within the molecule). This instability makes free radicals very reactive, and they attempt to pair up with other molecules, atoms, or even individual electrons to create a stable compound. To achieve a more stable state, free radicals can "steal" a hydrogen atom from another molecule, bind to another molecule, or interact in various ways with other free radicals.

Free radicals play an important role in a cell's life and death. These are unstable/unpaired electrons in their outermost shell and may become highly reactive. Reactive oxygen species (ROS) are generated from molecular oxygen/nitrogen through Electron Transport Chain (ETC), cytochrome P450, and other cellular and sub-cellular functions. They affect beneficial metabolic and cellular processes and also play a key role in pathological conditions of the body. It is normally balanced by an endogenous antioxidant system. Imbalances in redox status may develop cellular oxidative stress. If the endogenous antioxidants fail to overcome the reactive metabolites production, then exogenous antioxidants would be necessary to balance redox status. Dietary sources, including plants, herbs, spices, vitamins, and herbal extracts, play an important role in this regard.

Excess of everything is harmful such as no oxygen supply will prevent aerobes to live and similarly 100% oxygen can also lead to the death of aerobes. Excessive ROS can cause chronic diseases including cancer but inflammation cannot be controlled without ROS. Also excess or decrease of ROS can affect fertility.

This review article summarizes the functional role of Reactive Oxygen Species (ROS), its origin, and pathological importance. Elevated rates of reactive oxygen species (ROS) have been detected in almost all cancers, where they promote many aspects of tumor development and progression.

Keywords: Reactive Oxygen Species (ROS), Free Radical (FR), Reactive Nitrogen Species (RNS).

Introduction

Reactive species refers to substances that take part in oxidation-reduction reactions which include free radical species that possess an unpaired electron in their outermost orbit. These include reactive oxygen or nitrogen species. Beneficial biological functions such as apoptosis, necrosis, and phagocytosis are mediated by reactive oxygen species.[1]

Our planet is unique in that it possesses life. This is due to the presence of elements like carbon, nitrogen, and oxygen. Though other planets (rocky planets) have oxygen, it is bound to their rocks it is lacking in their atmosphere. However as life took hold on earth the organisms eventually developed mechanisms to produce and utilize energy like photosynthesis, whilst other organisms like diatoms learned to consume sand particles composed of Silicon dioxide and liberate the oxygen into the atmosphere which is a major source of oxygen for life. This oxygen constitutes 20.89% of the earth's atmosphere.

However, this was not always the case as the levels of oxygen would change over the past 500 million years from 10% up to 35%.

The Organisms eventually adapted and developed new mechanisms that utilized the available levels of oxygen as part of their metabolism, especially a key role in generating energy. This is reflected by the significantly larger size of insect fossils which reflected the higher levels of oxygen in the atmosphere at that time when compared to their modern-day descendants.[2]

Oxygen is quite misunderstood because though it is vital to our survival excess quantities can be toxic to living beings this can be seen when rats that are subjected to pure oxygen at 2 times sea level atmospheric pressure developed convulsions in a matter of hours and died. This is why oxygen has been called a double-edged sword by salvemini.[3]

Oxygen has some interesting characteristics, it has an atomic number of 8 and contains 2 unpaired electrons in its outermost orbital; because of the spin constraints of the orbitals in oxygen it has 2 electrons to be unpaired, and it has the lowest energy configuration. So oxygen exists as a biradical making it a strange radical. Dioxygen is quite unreactive in the atmosphere due to its levels and atmospheric temperature and pressure. The excitability of oxygen can be altered from a low energy state (triplet state) to a high energy state (singlet state) by altering parameters like temperature, irradiation, etc. The cells make elevate oxygen to a more excitable state through reactions with other radicals with unpaired electrons of transition metals like copper, iron, zinc, manganese, and molybdenum; or by reducing oxygen. Apart from ROS we also have reactive nitrogen species:

Reactive nitrogen species are composed of nitrogen; the important one is nitric oxide NO form which other radicals can be derived such as peroxynitrite which causes cellular damage, Peroxynitrite in combination with the reduced oxygen superoxide $O2^{-4}$.

Origin of Free Radicals

Reactive species are created via enzymatic and non-enzymatic reactions in both physiologic and pathological conditions.

Oxygen-derived free radicals are generated in physiologic conditions like oxidative phosphorylation in the mitochondria dioxygen obtains 4 electrons yielding 2 molecules of water and an ATP. However, due to

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entropy, not all the oxygen molecules would obtain all 4 electrons that are necessary for the completion of this reaction which gives rise to free radicals. Oxygen obtains the 4 electrons in a sequential order yielding intermediate products before the production of water.

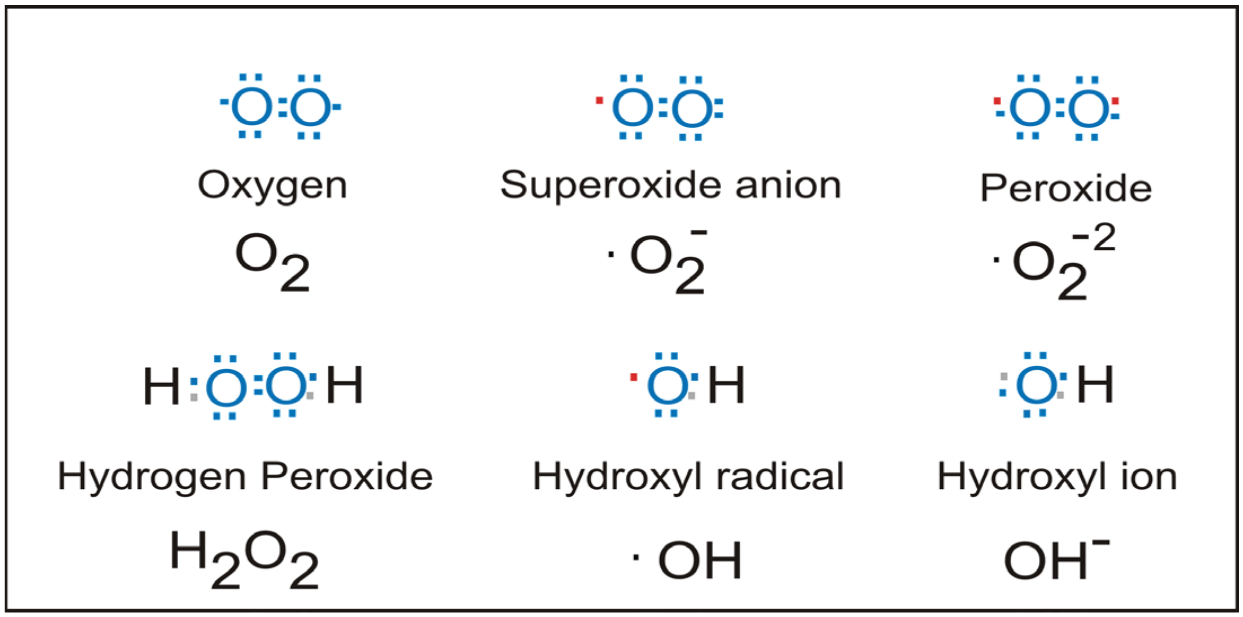
Production of Reactive Species in the Mitochondria

Most of the reactive oxygen species (abbreviated as ROS) were produced in the mitochondria in eukaryotic organisms. More than 90% of oxygen in the body is converted into water by cytochrome oxidase by reduction process in the electron transport chain (ETC) through the four-electron mechanism, but ROS were not released. The electron transport chain in eukaryotes was generally present at the interior membrane of mitochondria but in prokaryotes in the plasma membrane.[5]

On sequentially adding 1electron oxygen will yield superoxide anion O_2^- which further yields hydrogen peroxide, followed by Hydroxyl and finally water.

Most reactive oxygen species are generated as by-products during mitochondrial electron transport. In addition, ROS are formed as necessary intermediates of metal-catalyzed oxidation reactions. Atomic oxygen has two unpaired electrons in separate orbits in its outer electron shell. This electron structure makes oxygen susceptible to radical formation.

The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including superoxide; hydrogen peroxide; hydroxyl radical; hydroxyl ion; and nitric oxide.



Electron structures of common reactive oxygen species. Each structure is provided with its name and chemical formula. The •designates an unpaired electron.

Inflammatory processes that depend on oxygen such as the respiratory oxidative burst to combat infections utilizing NADPH to yield O_2^- which would further yield HOCl. This reaction is catalyzed by myeloperoxidase which is a heme-containing enzyme. The inability to generate ROS by leucocytes is seen in the chronic granulomatous disease.

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Production of endoplasmic reticulum: via cytochrome p450 and cytochrome B5 detoxifies lipid-soluble drugs and substances into hydrophilic byproducts and yields superoxide anions with the help of NADPH and NADH providing the electrons for the reaction.

Non Enzymatic Production of ROS

The production of Reactive species can take place pathologically by ionizing radiation, inflammation, metals, drugs, and chemicals.

The ionizing Radiation can occur in nature from cosmic rays or from technologies involved in nuclear energy production or even destruction. The g photons of the ionizing radiations possess the energy to split a molecule of water yielding OH^- and an electron.

Metals mainly when in the unbound state like copper and iron (and others like Mn-Mo and Zn) will also play a role in reactive species generation.

It is required for the Haber Weiss Fenton reaction which produces Hydroxyl radicals from peroxide.

H_20_2 can react non-enzymatically with Fe^{2+} and Cu^{1+} or chelates in Fenton-type Reactions, thereby being converted into reactive 'OH radicals.

$H_20_2 + Fe^2$ ·····'O1 H + $^-$OH + Fe^{3+}.....

The Fenton reaction can be augmented by the reduction of Fe^{3+}- by 0_2- regenerating Fe^{2+}.

The net result is the production of 'OH as in the iron catalysed Haber-Weiss type of reaction.

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

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

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

So in conditions of increased levels of iron in the unbound state like hemochromatosis where there is a genetic deficiency of transferrin a carrier molecule of iron, there is increased hydroxyl production in organs like the liver causing cirrhosis and liver failure. Also in Wilson's disease which is a condition of ceruloplasmin deficiency increased levels of unbound copper cause increased liver damage cirrhosis and even liver failure.

Cytochrome p450 is another enzymatic source of free radicals its main function in the liver is the metabolism of steroid hormones and lipids and bile acids.

Physiologically the P450 can generate free radicals through metabolic pathways involving arachidonic acid metabolism (Cox and Lipoxygenase pathways).

Certain drugs especially being metabolized in the liver by the **cytochrome P450** system will also produce free radicals that can damage the liver such as acetaminophen (Tylenol), Carbon tetrachloride CCl4 is used commonly in the dry cleaning industry to yield CCl3 which will cause damage to cell structures; in the early stages, the damage is reversible and can be identified as cellular swelling where the rough endoplasmic

reticulum swells causing the ribosomal detachment from the cisterns of the endoplasmic reticulum. Due to the disruption of the structure of the endoplasmic reticulum is unable to carry out packaging and folding of fats and proteins where they accumulate in the liver not being released into the blood.

Rotenone is a pesticide that also gets metabolized to yield reactive species.

Other substances from the environment like smoke from exhausts and industries and tobacco can also yield reactive species.[6]

In the oral cavity, cells are uniquely susceptible to free radical damage as the mucus membranes rapidly imbibe the substances across their surfaces. The increase in free radicals from oxidative stress contributes to the further breakdown of cell walls and oral tissue. In the oral cavity, oxidative stress is associated with gingivitis and periodontitis.[7]

Factors including alcohol consumption, exposure to nicotine, dental procedures, bleaching agents, dental cement, composite fillings, and metals used in dentistry also lead to oxidative stress.[8]

The oxidative killing mechanism of neutrophils and other phagocytes involves the formation of reactive oxygen species (ROS).[9]

Whilst most ROS have extremely short half-lives, they can cause substantial tissue damage by initiating free radical chain reactions. Therefore the body contains a number of protective Anti-Oxidant (AO) mechanisms, whose specific role is to remove harmful oxidants (ROS), as soon as they form, or to repair the damage caused by ROS in-vivo.[10]

ROS alteration on DNA causes mutations that lead to cell injury. The hydroperoxide of linoleic acid (1 3-hydroperoxy linoleic acid) was found to cause guanine-site-specific double-stranded DNA breakage. FR/ROS activate transcription and Encode transcription factors participating in the modulation of cell growth, differentiation, and development and can activate apoptosis, a "programmed" form of cell death. FR/ROS have been implicated in the regulation of mammalian transcription factors such as nuclear factor (NF)-κB and activator protein-1 (AP-1) and of so-called "heat shock" (or stress protein) transcription factors (HSTF).[11]

Free radicals and reactive oxygen species (ROS) are essential to many normal biologic processes. At low concentrations, these free radicals stimulate the growth of fibroblasts and epithelial cells in culture, but at higher concentrations, it may result in tissue injury.[12]

A variety of biological processes eg. antimicrobial defense, inflammation, carcinogenesis, radiation damage, photobiological effects, and aging, involve reactive oxygen species.[13]

Types of ROS, Source of Synthesis and the Damage Caused by the Production of ROS

Types of Reactive Species

The highly reactive molecules: reactive molecules •NO, ONOO– are the most widely studied species and play important roles in diabetic cardiovascular complications. Superoxide ((.O^{-}_{2}) is produced by one electron reduction of oxygen by different oxidases including dihydro nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, xanthine oxidases, cyclooxygenase as well as by the mitochondrial electron transport chain during the course of normal oxidative phosphorylation which is essential for generating ATP.[14]

Cellular Response of ROS

Oxidants are generated as a result of normal intracellular metabolism in mitochondria and peroxisomes, as well as from a variety of cytosolic enzyme systems. In addition, a number of external agents can trigger ROS production. A sophisticated enzymatic and non-enzymatic antioxidant defense system including catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) counteracts and regulates overall ROS levels to maintain physiological homeostasis.

Carbohydrates Oxidation by ROS

Free carbon and hydrogen of deoxy sugars are attributed to the oxidation of carbohydrates, e.g. mannitol and glucose. The free radicals bind with these carbohydrates and form carbon-centered radicals. These carbon-centered radicals interact with other carbohydrates, and thus series of autocatalytic chain reactions commence resulting in the destruction of the cells. Ketamine and ketoaldehydes are the most common oxidative products of carbohydrates.

Protein oxidation by ROS

Reactive oxygen species interact on protein molecules at the specific amino acid side chain and form the modification in protein structure results fragmentation of the peptide chain, and alteration in electrical charges; peroxynitrite nitrate protein is accumulated and thus, increases the proteolysis. Garrison has found that active oxygen has the potential to react with amino acid side groups and cleave the polypeptide chain, thus resulting in the formation of reactive carbonyl groups.[15]

Stedman and Oliver proposed the protein oxidation mechanism in which lysine residue is converted to α amino-adipic semialdehyde. He found that ferrous ions formed by reduction through superoxide anion from ferric ion bind to the cationic side of amino acid on a protein molecule, in which one amino acid is lysine. This bound metal reacts with hydrogen peroxide and forms hydroxyl radicals, which helps in the production of the carbonyl radical. This radical in turn cleaves the polypeptide chain. Oxidative markers are protein carbonyl groups.

Lipid Peroxidation by ROS

Oxidative stress causes damage to cellular macromolecules such as nucleic acids, proteins, and lipids. The lipids mostly affected are those possessing unsaturated bonds like polyunsaturated fatty acids. Cholesterol or unsaturated fats are not a common target of reactive species. It is usually the carbon atom that is in between 2 unsaturated carbon bonds that are affected. The hydroxyl ion captures an electron from a phospholipid molecule leaving it in an unstable state which will be able to do the same to a molecule in its vicinity triggering a chain reaction that will damage the cell membrane as a whole.

Among these targets, the peroxidation of lipids is particularly more damaging because the formation of lipid peroxidation products leads to a facile propagation of free radical reactions. Abstraction of a hydrogen atom from the Poly Unsaturated Fatty Acid (PUFA) moiety of membrane phospholipids initiates the process of lipid peroxidation.

Alkyl radicals are formed which are ultimately rearranged to form conjugated **dienes**, and stimulate the autocatalytic lipid peroxidation cascades.[16]

Reactive species damage to nucleic acids

Amongst the nucleic acids guanine is the most susceptible to oxidative damage by reactive species due to its high oxidation potential followed by cytosine and the others.

This is due to the fact that the pyrimidine has an imidazole ring component which gets oxidized to 8- Hydroxy guanine. The ROS can also react with sugars in the DNA and RNA causing strand breaks which may or may not be repaired by the cells.

Defenses against the reactive species:

1. Antioxidants are small molecules they can be divided into lipophilic or hydrophilic. Lipophilic antioxidants like Vitamins A and E present near the membranes. They work by donate electrons to the reactive species and stabilizes the molecule.
   Other known antioxidants: Acetyl-L-carnitine, Alpha lipoic acid, Coenzyme Q10 (also known as ubiquinone is the antioxidant of the mitochondria that gets reduced to Ubisemiquinine), Curcumin (from turmeric powder), N-Acetyl cysteine (NAC), Resveratrol, Selenium, Zinc, Vitamins A (limited role as an antioxidant) that limits the production of iNOs in endothelial cells, vascular muscles cells, cardiac myocytes, mesangial cells. Vitamin C (the main antioxidant of GIT and in the cytoplasm), and Vitamin E (major antioxidant of blood). Vitamins exhibit anti-atherogenic and anti-inflammatory roles in the neurons. Vitamin D3, K2, and niacin inhibit iNOS activity in brain cells (macrophages, microglia, and astrocytes).
   The mechanism of how small molecule antioxidants work is that the antioxidant will accept its electron from a radical becoming an antioxidant radical itself. The antioxidant radical is not reactive because the electron it accepted is delocalized making it less reactive. There is an interplay that occurs between the vitamins C and E where the reduced vitamin C can regenerate reduced vitamin E.
2. Metal carrier proteins like ceruloplasmin and transferrin bind to free metals preventing them from generating reactive species through reactions like the Haber Weiss Fenton reaction.
3. Enzymes such as superoxide dismutase, catalase, and glutathione peroxidase play a protective role against reactive species.

Superoxide dismutase is an enzyme that catalyses the conversion of 2 superoxide anions to peroxide. Superoxide exists in 2 forms based on which metal it is dependent on to function:

The first one is Manganese dependent which is found in the mitochondria of the cell. The other one is dependent on copper and zinc which are found in the cytoplasm. SOD takes 2 of the same molecule and causes the oxidation of one at the expense of the reduction of the other hence the name dismutase.

Superoxide dismutase works in 2 steps:

Step 1: the SOD (oxidized version) will react with single superoxide as an electron acceptor to give O_2 while getting reduced.

Step 2: The regeneration of dismutase.

Here the reduced dismutase will donate the electron obtained from the previous reaction to a superoxide giving one peroxide molecule.[19]

The peroxide molecule itself belongs to the reactive species but it will eventually be converted to water by the peroxidase enzymes like catalase enzyme.

Peroxidases like glutathione (GSH) are very important in the inactivation of reactive species GSH has incorporated selenium as selenocysteine. GSH works by: 2 molecules of GSH donate electrons to 1 peroxide molecule giving 2 molecules of water. The 2 GSH molecules combine to form one molecule of GSSG. The GSSG is reconverted back to GSH by the enzyme glutathione reductase with NADPH reduced to NADP.

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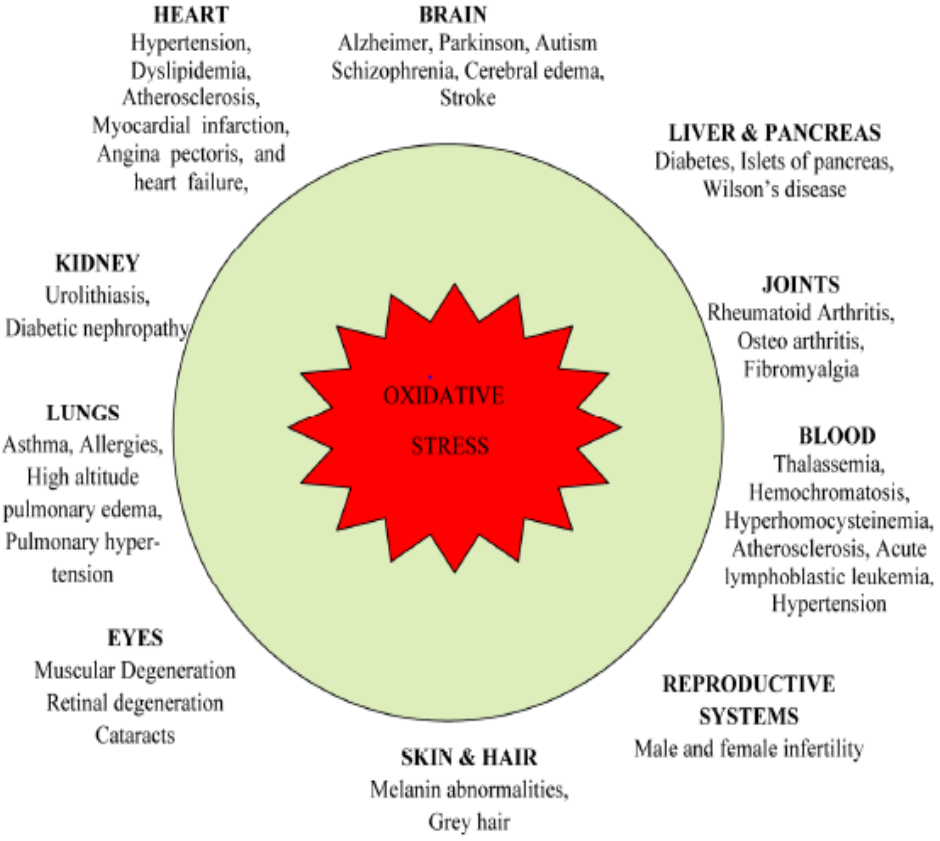
Catalase contains the Heme group and takes 2 peroxide molecules and Catalyses their conversion to 2 molecules of water. Exercise increases the levels of SOD and Catalase levels.[20]

Function of ROS in Cells

ROS performs beneficial functions in our body. Redox level should be maintained. It is the mediator of phagocytosis, apoptosis, detoxification reactions, executioner of precancerous cells and infections, etc. It is beneficially involved in signaling pathways to maintain cellular homeostasis in the body. The ROS regulates many metabolic and cellular processes including proliferation, migration, gene expression, immunity, and wound healing.[21]

Human disorders clinically linked to oxidative stress

Oxidative stress has been implicated in several diseases including cancer, atherosclerosis, malaria, chronic fatigue syndrome, rheumatoid arthritis, and neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease, and Huntington's disease.[22]



ROS Function as Redox Signaling

Oxidative stress has been linked to a myriad of pathologies. However, elevated ROS also act as signaling molecules in the maintenance of physiological functions — a process termed redox biology.

Redox biology involves a small increase in ROS levels that activates signaling pathways to initiate biological processes, while oxidative stress denotes high levels of ROS that result in damage to DNA, protein, or lipids. Thus, the response to ROS displays hormesis, given that the opposite effect is observed at low levels compared with that seen at high levels.[23]

ROS and cancer

It is currently believed that there are two major mechanisms by which normal cells can be converted into malignant cells. These include the conversion of normal proto-oncogenes into activated oncogenes and the inactivation of normal tumor suppressor genes.

It is hypothesized that oxidants might contribute to carcinogenesis by causing oncogene activation or Tumor Suppressor Gene inactivation.[24]

In cancer cells, the high rate of ROS production is counterbalanced by an equally high rate of antioxidant activity in order to maintain redox balance.

If cancer cells do not control their ROS levels then they are susceptible to oxidative-stress-induced cell death. Steady-state ROS levels in cancer cells are determined by both the rate of ROS production and also the rate of ROS scavenging.

Thus, at steady state, cancer cells can display either an increase or a decrease in ROS compared with normal cells. Additionally, the signaling pathways that are responsive to H_2O_2 are localized close to the sources of ROS generation, allowing activation of these pathways despite the high overall antioxidant activity in cancer cells that protects against oxidative-stress-induced cell death. The major mechanism by which cancer cells increase their antioxidant proteins is through activating the transcription factor nuclear factor erythroid 2-related factor 2 (NRF2).

Cancer cells 'hijack' normal cell machinery by constitutively activating growth factor pathways to sustain cellular growth and proliferation.

Warburg noticed that cancer cells have an increased expression of anaerobic glycolytic enzymes causing the cancer cells to sustain themselves in an anaerobic environment i.e in the relative deficiency of blood vessel proliferation to the cells. This allows cancer cells to take up abundant nutrients, survive stress, and continuously proliferate. Consequently, the 'hyper-metabolism' of cancer cells causes the abundant generation of ROS from mitochondria and the endoplasmic reticulum, as well as by the action of NADPH oxidases. The level of ROS production by the cancer cells is in the middle of the spectrum between low (that is conducive to life as in non-cancerous cells) and high (that is lethal as in apoptotic cells).[25]

The aim of cancer chemotherapy is to raise the levels of ROS within the cancer cells to a high enough level to induce apoptosis in them. And while there would also increase the levels of ROS in non-tumor cells it would not be sufficient to induce apoptosis in them as they had a low level to begin with. This is also the reason why antioxidants are not given to patients on the day of therapy and the day before and the day after it.[26]

Reactive Oxygen Species and Periodontics

The majority of periodontal tissue destruction is caused by an exaggerated host response to those organisms and their products.[27]

The neutrophils play a pivotal role in host defence and are the first line of defence against infectious periodontal disease.

Neutrophils have several selective mechanisms for controlling bacterial invasion; including both intracellular and extracellular oxidative and non-oxidative killing mechanisms.[28]

The oxidative killing mechanism of neutrophils and other phagocytes involves the formation of reactive oxygen species (ROS).[29]

Effects of ROS on Periodontal Tissues and Components:

The reactive oxygen species cause periodontal tissue damage by,

1. Ground substance degradation.
2. Collagenolysis either directly or indirectly or as a result of oxidation of proteases.
3. Stimulation of excessive pro-inflammatory cytokine release through NF-κB activation.
4. PG-E2 production via lipid peroxidation and superoxide release, both of which have been linked with bone resorption.
5. Since IL-1 & TNF-α positively regulate their own production, the additive effects of endotoxin-mediated cytokine production and that arising from a respiratory burst of PMNLs in response to the same organisms, lead to periodontal inflammation and subsequent attachment loss.[30]

Phagocytic Cells

The stimulated production of reactive oxygen species by phagocytic cells was originally called "the respiratory burst" due to the increased consumption of oxygen by these cells. This process is catalyzed by the action of NADPH oxidase, a multicomponent membrane-bound enzyme complex, and is necessary for the bactericidal action of phagocytes.[31]

While several enzymes are recognized as being able to produce ROS moieties, NADPH oxidase is the most significant.[32]

NADPH oxidase activity is controlled by a complex regulatory system that involves the G-protein Rac.[33]

Signal Transduction

Reactive oxygen species have a role in a number of cellular processes. High levels of ROS, which can lead to cellular damage, oxidative stress, and DNA damage, can elicit either cell survival or apoptosis mechanisms depending on the severity and duration of exposure. Nitric oxide (•NO) has been shown to serve as a cell-to-cell messenger, being responsible for such effects as decreasing blood pressure.

Intra-cellular, ROS species, in conjunction with antioxidant enzymes, are believed to play a role in turning enzymes on and off by redox signalling in a manner akin to that of the c AMP second messenger system.[34]

Summary

Reactive Oxygen Species (ROS) is a phrase used to describe a number of reactive molecules and free radicals derived from molecular oxygen. The production of oxygen based radicals is the bane to all aerobic species. These molecules, produced as by products during the mitochondrial electron transport of aerobic respiration or by oxidoreductase enzymes and metal catalyzed oxidation, have the potential to cause a number of deleterious events. It was originally thought that only phagocytic cells were responsible for ROS production as their part in host cell defence mechanisms. Recent work has demonstrated that ROS have a role in cell signalling, including; apoptosis; gene expression; and the activation of cell signalling cascades. It should be noted that ROS can serve as both intra- and intercellular messengers.

The interest in reactive oxygen species originally revolved around the pathology associated with the deleterious effects of aerobic respiration: the necessary evil caused by the leakage from the electron

transport chain in mitochondria. In this context, research involved the role that these agents played in aging, chronic diseases and cancer.

A new frontier was born with the discovery that the "oxidative burst" by phagocytic cells was actually the result of the intentional production of reactive oxygen species. This was thought to be a very specific application where specific cells produced what can only be described as toxic agents in order to kill invading microorganisms. Further recent work has shown that ROS are produced in all cell types and serve as important cellular messengers for both intra- and inter-cellular communications. It is now apparent that a very complex intra-cellular regulatory system involving ROS exists within cells. Cells respond to ROS moieties in different ways depending on the intensity, duration and context of the signalling.

In regards to intracellular signalling it appears that hydrogen peroxide (H_2O_2) is the most interesting candidate, while nitric oxide (•NO) is involved primarily with intercellular signalling.

To cope with the oxidative stress elicited by aerobic metabolism, animal and human cells have developed a ubiquitous antioxidant defense system, which consists of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase together with a number of low molecular-weight antioxidants such as ascorbate, α-tocopherol and glutathione, cysteine, thioredoxin, vitamins, etc. However, this antioxidant defense system may be overwhelmed by various pathological or environmental factors so that a fraction of ROS may escape destruction and form the far more reactive hydroxyl radicals.

The involvement of ROS and the antioxidant defense mechanisms in human saliva has been demonstrated in various processes of the oral cavity: healing periodontal disease, preventing oral carcinogenesis, reducing oral mucosa inflammatory reactions, and ameliorating metal-based restoration reactions.

Antioxidant enzymes such as SOD and catalase can directly counter balance the oxidant attack and may protect cells against DNA damage. SOD inhibits OH- production; therefore it acts as inhibitor at the initiation and promotion stages during carcinogenesis.

It is found that the generation of reactive metabolites plays a central role in a cell's life playing important biological functions in cells in a controlled environment. The impairment of antioxidant status, either by exogenous or endogenous sources, may disturb the cellular redox balance and the pathological conditions would be the main characteristics and forms oxidative stress in cells or tissues. In this scenario, exogenous antioxidants which are dietary sources could be beneficial to level the redox balance status. It is important to note that increased antioxidants may interrupt the normal biological oxidant processes, and thus stop the reactive metabolites to perform their normal biological functions, which prevents the cancerous cells to undergo apoptosis.

Studies over the past two decades in various organisms, tissues and cell types have led to a shift in our understanding of ROS: we no longer view them just as molecules that invoke damage (i.e. oxidative stress) but now also appreciate their role in regulating signaling pathways that impinge on normal physiological and biological responses (i.e. redox biology).

Despite a wide variety of studies on oxidative stress phenomenon and role of scavengers, there are still various gaps in the explanation of the exact mechanism of these scavengers function.

Conclusion

Studies over the past two decades in various organisms, tissues, and cell types have led to a shift in our understanding of ROS: we should no longer view them just as villains that wreak havoc on biological systems, or as molecules that invoke damage (i.e. oxidative stress) but rather as a double-edged sword that play key roles in immunity as is evident in the case of chronic granulomatous diseases, and also in signaling processes. The abrasive effects of Free radicals can be exploited in the treatment of cancers by inducing damage to tumor cells and should not be interfered with in such situations with the misuse of self-prescription due to widespread misbeliefs. There is much-needed research to be conducted to fill in the gaps of the exact mechanism of how scavengers of free radicals function and even more need for awareness of the importance of free radicals amongst the medical fraternity and the general populous.

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