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Review Article

Antioxidants Role in the Prevention of Free Radical Formation

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Abstract Oxygen and nitrogen species are very important in the biological reaction by these reaction toxic species are also produced some times these are involved in the prolongation of the reaction. These reactions cause many disorders and diseases. These reactions are controlled by using the antioxidants. In this article we mainly discuss about what are antioxidants, classification of anti-oxidants, the formation of ROS and NOS what is the importance in the free radicals formation, what is oxidative stress, antioxidants mechanisms, in vivo and *in vitro* determination methods and discuss about only some of the drugs used for the treatment, what is the reason are explained in this article. Controlling of the free radicals (FR) formation is the very important in the any living system. These FR are involved in the cell damage and cases the many disorders and many diseases.

Keywords: Superoxide Anion, Hydroxyl Radical, Atherogenesis, ROS, NOS, Peroxynitrite (ONOO-), Alzheimer 's disease.

Introduction

An antioxidant is a molecule capable of inhibiting the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons or hydrogen from a substance to an oxidizing agent. Oxidation reaction can produce free radicals. These free radicals can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death to the cell. Anti-oxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. These anti-oxidants are oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols[1].

Antioxidants are important additives in gasoline. These anti-oxidants are prevents the formation of gums that interfere with the operation of internal combustion engines, such as substituted phenols and phenylenediamine derivatives.

Oxidation reactions are crucial for life, they can also be damaging plants and animals maintain complex systems of multiple types of antioxidants. These are glutathione, vitamin C, Vitamin E, some enzymes like catalase, superoxide dismutase and various peroxidases. Low level of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress and may damage or kill cells.

Types of Anti-Oxidants:

Anti-oxidants are classified into several types based on the different aspects these are,

1. Source of anti-oxidants
2. Solubility nature of the anti-oxidants
3. Based on importance
4. Anti-oxidants mechanism

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Source of anti-oxidants:

Plant source: The numbers of plant species in India having the medicinal value in these some of the plants are having the anti-oxidative property. Some of the natural anti-oxidant plants are.

Aerva lanata, Amaranthus paniculatus, Aristolochia bracteolate, Cissapelos pareira, Cocinia indica wight, Coriandrum sativum, Coscinium fenestratum colebr, Cynodon dactylon, Cyperus roundus, Enicostemma littorale blume, Evolvulus alsinoides, Fagonia cretica, Gymnem montanum, Hygrophil auriculata, Phyllanthus spices like P. amarus, P. debils klein, P. mader aspatensis, P. niruri, P. urinaria, P. Virgatus, Plumbago zeylanica, Rubia cordifolia, Striga orobanchioides and *Trichopus zeylanicus.*

Synthetic anti-oxidants:

Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)

Based on solubility nature:

1. Watersoluble anti-oxidants
   E.g.: Ascorbic acid, Uric acid etc.
2. Lipid soluble anti-oxidants
   Eg: Carotens, Ubiquinolin etc.

Based on importance of anti-oxidants:

Primary anti-oxidants: Eg: glutathione & sod (these are produced by the body)

Secondary anti-oxidants: Eg: vitamins A, C, E and super fruit juices (rich in antioxidants, polyphenols alpha-lipoicacid, Punicalagins, resveratrol and phyto-nutrients).

Based on mechanism of action:

Destroys hydrogen peroxide in high concentration.
Eg: Catalase, glutathione peroxidase, super oxidase dismutase.

Inhibition of topoisomerase1&2
Eg: alkaloids

Enhancing activity of SOD and catalase
Eg: ctechins

Mainly act as physical quenchers of reactive oxygen
Eg: carotenoids

Scavenges lipid peroxyl radicals through hydrogen atom transfer
Eg: alpha tocopherol

Decreases the expression of proapototic genes
Eg:(-)-EGCG

Decrease lipid peroxidation and enhance the level of glutathione and antioxidant enzymes
Eg: ferulic acid

Maintains the redox state of critical protein sulphahydrals
Eg: glutathione

Inhibition effect on proapoptotic and cardio regulatory genes
Eg: proanthocyanidin

Inhibit the oxidation of lipids fats and proteins
Eg: phenolics

Suppresses COX-2 expression
Eg: quercetin, kaempferol, genistein, resveratrol

Nitric oxide synthesis enhancers
Eg: tannins

Mechanism of Anti-Oxidant:

Four possible mechanisms have been suggested by which antioxidants function to reduce the rate of oxidation of fats and oils.

These are:

1) Hydrogen donation by the antioxidant.
2) Electron donation by the antioxidant.
3) Addition of the lipid to the antioxidant.
4) Formation of a complex between the lipid and antioxidant.

However, it is thought that the first two mechanisms (hydrogen donation and electron donation) are the most probable modes of action of antioxidants. It should also be noted that some other compounds (citric acid, ascorbic acid, lecithin, etc.) have synergistic effects with antioxidants preventing the oxidation of fats and oils. Thus it is possible that a feed manufacturer may employ a mixture of antioxidants and synergistic compounds in order to reduce oxidation.

Oxygen Role in Formation of Free Radicals:

The vast of majority of complex life on earth requires oxygen for the life activates. Oxygen is a highly reactive molecule that damages living organisms by producing reactive oxygen species. The use of oxygen as part of the process for generating metallic energy produces reactive oxygen species. The maximum organisms are having complex network of antioxidant metabolites and enzymes these are together to prevent the formation of free radicals and protect the cellular components like DNA, proteins and lipids. The formations of free radicals do to the reactive oxygen species. These are produced in the cell by the hydrogen peroxidase (H_2O_2), hypochlorous acid (HClO) and free radicals such as the hydroxyl radical (.OH) and superoxide anion (O_2_). The hydroxyl radical is particularly unstable and will react rapidly non-specifically with most biological molecules. These are damages the DNA. Do to cases the mutations and possibly cancer in plants, algae and cyanobacteria, reactive oxygen species are also produced during photosynthesis process.

Nitrogen Radicals Formation:

Nitric oxide synthase (NOS) catalyses the synthesis of radical species like nitric oxide (NO) from the catalytic conversion of arginine to citruline[3]. NO itself is un reactive with most biological molecule but becomes toxic when converted to secondary species. The reactive nitrogen species (RNS)[4]are radical nitrogen based molecules that can act to facilitate nitrosylation reactions. Non radical product peroxynitrite (ONOO-)[5]. This powerful oxidizing and nitrating agent can case direct damage to proteins lipids and DNA. Three different isoforms of NOS are endothelial (e NOS) are neuronal (n NOS) and inducible NOs (i NOS). All NOS isoforms are up regulated after hypoxia –ischemia. n NOS and e NOS are activated early in the process of hypoxia–ischemia.

Oxidative Stress:

Oxidative stress is thought to contribute to the development of a wide range of diseases including Alzheimer's disease, Parkinson's disease, the pathologies caused by diabetes, rheumatoid arthritis and neuro-degeneration. In motor neuron diseases and many of these cases, it is unclear if oxidants trigger the disease. The low density lipoprotein (LDL) oxidation appears to trigger the process of atherogenesis.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of normal cellular metabolism. Each plays a dual role

as a deleterious and beneficial elements these both either harmful or beneficial to living systems[6, 7]. Number of physiological functions are controlled by redox responsive signaling path ways including regulation of vascular tone, defensive mechanism against microorganisms and malignant cells, monitoring of oxygen tension in the control of ventilation and erythropoietin production and signal transduction from membrane receptors in various physiological processes neurotransmission, reproduction, and normal growth and development[8] The harmful, potentially biologically damaging, effects of FR generation of oxidant and nitrosative stress[9,10]. Results from an imbalance between excessive generation of oxidant compounds and insufficient anti-oxidant defense mechanisms[11].OS can cause tissue damage can occur and has been implicated in a number of human diseases[12]. Birth is an oxidative challenge for the new born. The trantions from foetus to neonate exposes the new born to much more oxygen rich world than the intrauterine environment. This oxidative reactions and the production of the FR[13] in the new born can be regulated by anti-oxidants. Neonatal plasma has low levels of glutathione peroxidase, SOD beta carotene, riboflavin, riboproteinase, vitamin E, selenium, copper, zinc, ceruloplasmin, transferrin, and other plasma factors[14,15].

Anti-Oxidants in Defense Net Work in *In Vivo* and *In vitro* Against Oxidative Stress:

A number of methods and variations have been developed and applied for the measurement of antioxidant capacity and efficacy.

***In vivo* determination**:

From the view point of mechanistic action the anti-oxidants may be classified into preventing antioxidants, scavenging anti-oxidants and repair and de novo anti-oxidants[16]. The preventing antioxidants function as the first line defense by suppressing the formation of ROS & RNS. The scavenging anti-oxidants remove active species rapidly before the active species attack biologically essential molecules. Various antioxidant compounds and enzymes play their roles at respective site and constitute a total defense network system *in vivo*.

The free radicals scavenging antioxidants (IH) function by scavenging active free radicals (X.) before they act biologically essential molecules by donating hydrogen atom or electron followed by proton transfer to give a stable compound (XH) and antioxidant derived radical (I.) the rates of these reactions are determine primarily by the redox property such as I-H bond dissociation energy and ionization potential of the antioxidant.

$$X\bullet + IH \longrightarrow XH + I\bullet$$

$$X\bullet + IH \longrightarrow X. + IH+: \longrightarrow XH + I\bullet$$

Factors effecting on the antioxidant capacity and efficiency:

1. The chemical reactivity towards free radicals and stoichiometric number, that is rate of radicals scavenging and number of radical molecule scavenged.
2. Fate of antioxidant derived radical.
3. Inter reaction with other antioxidant.
4. Concentration and mobility at the environment.
5. Absorption distribution, retention and metabolism.

These factors[17,18] determine the antioxidant capacity and efficacy.

***In vitro* determination**:

The free radical scavenging capacity of antioxidant in *in vitro* evaluated through different methods under different conditions. It has been expressed by the terms such as ability, activity, capacity, efficacy, parameter, potential, power and reactivity. The capacity of anti-oxidants for scavenging free radicals has been assessed more often and widely by the two methods.

In this two methods are;

1. Reaction with stable reference radical
2. Competition methods using the conventional UV visible absorption spectrophotometer.

Reaction with stable free radicals: Galvinoxyl, DPPH, ABTS, and Nitroxide

The capacity of antioxidant compound for scavenging free radicals should be assessed by two factors those are rate of scavenging radicals and number of radicals each antioxidant molecule can scavenge which are determined inherently the chemical structure of the antioxidant compound and also free radicals these two parameter are measured by the following the reaction with stable reference free radical such as galvinoxyl[19] and DPPH[20].

$ABTS^{+}$ is also stable and used similarly[21,22]. ABTS cation radical is soluble in both water and organic solvent and can be applied similarly as galvinoxyl and DPPH[23,24].

Competition method:

The capacity of antioxidant and mixtures containing antioxidant compounds for scavenging free radicals has been assessed widely by competition method using a reference compound as a probe. The capacity of antioxidant for scavenging free radicals has been assessed by the extent of suppression of probe consumption, UV or visible absorption, florescence, chemilumiescence are EPR spectroscopy. Various methods have been developed and applied and of these, oxygen radical

absorbance capacity (ORAC), total radical antioxidant parameter (TRAP), Trolax equivalent antioxidant capacity (TEAC), and total oxiradical scavenging capacity (TOSC) have been used most frequently in this methods[25-32]. ORAC is one of the most widely methods the TRAP method was first applied for human plasma by measuring oxygen absorption with oxygen electrode[33, 34] and then by a decrease in fluoresces by R-phycoerithrin[35].

Why Antioxidants Are Not Much In Clinic:

Many antioxidants show strong positive effects in the laboratory but only a few of these drugs are used as anti-oxidants in humans. Because of;

ROS production occurs ubiquitously in aerobic cells. And chemical reactivity of ROS takes place in a nonspecific manner to find statistically significant differences in different cells or tissue is difficult.

Many synthetic compounds have been used in clinical trials, but some, especially enzyme inhibitors, showed side effects, such as liver function disturbance. As a result of this, it was necessary to withdraw some promising drug candidate from further development.

Human blood and tissues already contain an abundance of antioxidants. Moreover, many anti-oxidative enzymes are readily induced by many cytokines and stresses. For example, preconditioning phenomenon in ischemia-reperfusion could be due to the production of induced antioxidatives enzymes, such as Mn-SOD, while xanthine oxidase is induced which is a potential source of ROS (i.e. O2•−). Because of this, when anti-oxidative drugs are administered to humans, their clinical benefits are frequently difficult to verify.

Many natural substances are used as dietary supplements, in spite of the scientific evidence, and beneficial evidence is poorly validated in many cases. This leads to delays in initiating authentic clinical trials.

Some compounds produced from natural substances contain contaminants, especially endotoxins. Endotoxins are capable of inducing the production of a wide variety of enzymes and cytokines, thus making the results of such trials difficult.

By the above reasons some of the synthetic drugs are only used .the clinical trials also go on the some of the anti-oxidants. Some of the synthetic drugs and where they are using are mentioned given table.1.

Conclusion

Many of the disorders and diseases are mainly caused by the involvement of ROS and NOS. These reactive species are neutralized or inhibits the long chain reactions by using the antioxidants. Some of the antioxidants are pre pared by living systems and some of the antioxidants are taken by the diet. These antioxidants are control the formation of the ROS, NOS, free radicals and biochemical reactions. The capacity of antioxidants *in vitro* as assessed by competition method such as ORAC and TEAC methods may be useful for evaluation of radical scavenging. However, this is not suitable for the assessment of ant oxidation capacity such as efficiency of lipid peroxidation inhibition. The capacity of antioxidants should be evaluated by two factors separately, that is, rate and number of radical scavenging, which can be assessed by competition method with conventional UV/ visible absorption spectroscopy by using probes. The capacity of antioxidant compounds or products containing mixture of antioxidants *in vivo* may be assessed from their effect on the level of oxidative stress biomarkers after intake.

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