The Antioxidants-Scavengers of Free Radicals for Immunity Boosting and Human Health/ Overall Well Being

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ABSTRACT

The formation of free radicals is controlled by various beneficial compounds known as antioxidants. Antioxidants deficiency may lead to oxidative damage to DNA, proteins and other macro molecules and results in wide range of human diseases. Antioxidants are class of molecules which are considered as essential part of optimal health. The purpose of this article is to review the basic facts about antioxidants and their role in stabilizing or deactivating the free radicals. This review article has been prepared by doing a literature review from world-wide web and pubmed/medline.

Keywords: Antioxidants, free radicals, oxidization.

INTRODUCTION

An antioxidant inhibits oxidation reaction there by preventing cell damage and or death. Anything which acts against is called "anti" and "to oxidize" is to combine with oxygen hence antioxidants means to counteract oxidation.[1,2]

A dietary antioxidant is a substance in foods that significantly decreases the adverse effect of reactive oxygen species, reactive nitrogen species or both on normal physiological function in humans."[3] In 1992 Halliwell and Gutteridge defined antioxidants as those substances which when present at low concentrations, compared to those of an oxidizable substrate, will significantly delay or inhibit oxidation of that substrate.[4] Sics in 1996 defined antioxidants as the substances that neutralize free redicals or their actions.[5] Ternay and Sorokin in 1997 defined antioxidant as any substance that hinders a free radical reaction.[6] Azzi and Davies in 2004 defined antioxidant as the substances which counteract free radicals and prevent the damage caused by them. Antioxidants by virtue of scavenging the oxidants prevents chain reactions or activation of oxygen into highly reactive products before they affect the cells.[7]

"Free radicals" or highly reactive oxygen attack healthy cells leading to loss of their structure and function.[8]

Antioxidants are substances or agents that scavenge reactive oxygen metabolites, block their generation or enhance endogenous antioxidants capabilities. Role of antioxidants in metallurgy, rubber industries and petrochemicals was greatly explored and exploited in the 19th and early 20th century but recognition of Vitamins A,C and E as antioxidants transformed the field of human biochemistry.[9]

CLASSIFICATION OF ANTIOXIDANTS

The antioxidants defense systems of the human body are complex and various classification systems exist. Antioxidants can be categorized by several methods:

1. Their mode of function.
2. Their location of action.
3. Solubility.
4. Their structural dependents.
5. Their origin/source (Dietary or Non-dietary sources).[4]

1. According to mode of action

It can be classified as:

A. Intracellular: Superoxide dismutase enzyme 1 and 2, catalase, glutathione peroxidase, DNA repair enzymes e.g. poly (ADP-ribose) polymerase, others, reduce glutathione, ubiquinone (reduced form).

B. Extracellular: Superoxide dismutase enzyme3, selenium, glutathione peroxidase, lactoferrin, transferring, ascorbate, uric acid, carotenoids, ceruloplasmin.

2. According to location of action

A. Preventive antioxidants

Enzymes: Superoxide dismutase enzymes (1,2 and 3), catalase, glutathione peroxidase, DNA repair enzymes.

Metal ion sequestrators: Albumin, lactoferrin, transferrin, haptoglobin, ceruloplasmin, hemopexin, carotenoids, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, uric acid, polyphenolic flavenoids.

B. Scavenging antioxidants: Ascorbate, carotenoids, uric acid, α – tocopherol, polyphenols, bilirubin, albumin, ubiquinone, reduced glutathione and other thiols.

3. According to solubility

A. Lipid soluble: Haptoglobin, cerulopasmin, albumin, ascorbate, uric acid, polyphenolic flavenoids, reduce glutathione, and other thiols.

B. Water soluble: α- tocopherol, carotenoids, bilirubin, quinines.

4. According to structures they protect:

A. DNA protective antioxidants: Superoxide dismutase enzyme 1 and 2, glutathione peroxidase, DNA repair enzymes [poly (ADP) ribose polymerase], reduced glutathione, cysteine.

B. Protein protective antioxidants: Sequestration of transition metals by preventative antioxidants.

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Scavenging by competing substrates.
Antioxidant enzymes.

C. Lipid protective antioxidants- α- tocopherol, ascorbate, carotenoids, reduced glutathione, glutathione peroxidase, bilirubin.

5. According to their origin

A. Exogenous antioxidants: Carotenoids, ascorbic acid, tocopherols (a,b,c,d), polyphenols, folic acid cysteine.

B. Endogenous antioxidants: Catalase, superoxide dismutase, glutathione peroxidase, glutathione –S- transferase, reduce glutathione, ceruloplasmin, transferrin, ferritin, glycosylases.

C. Synthetic: N-acetylcysteine, penicillinamine, tetracylines.[4]

RH Liu in 2004 classified antioxidants in to two major groups:
A. Enzymatic antioxidants.
B. Non enzymatic antioxidants.(Figure:1)[10]

Antioxidants are grouped into two namely:
1. Primary or natural antioxidants.
2. Secondary or synthetic antioxidants.

Primary or natural antioxidants

Antioxidants minerals - Selenium, copper, iron, zinc and manganese.
Anti oxidants vitamins – They include- Vitamin C, Vitamin E, Vitamin B.
Phytochemicals – Flavonoids.

Secondary or synthetic antioxidants

Butylated hydroxyl anisole (BHA).
Butylated hydroxyrotoluene (BHT).
Propyl gallate (PG) and metal chelating agent (EDTA).
Tertiary butyl hydroquinone (TBHQ).
Nordihydro guaretic acid (NDGA).[11]

FREE RADICALS

Free radicals are highly reactive due to presence of unpaired electrons in the atomic or molecular orbitals. Dehnam Harman in 1956 proposed the concept of free radicals in the ageing process.[12-14] In 1969 McCord and Fridovich discovered the enzyme superoxide dismutase (SOD). Discovery of superoxide dismutase (SOD) provided a strong evidence about importance of free radicals.[15] Hydoxyl radical activates guanylate cyclase and genesis of "second messenger" cyclic guanosine monophospate (cGMP).[16]

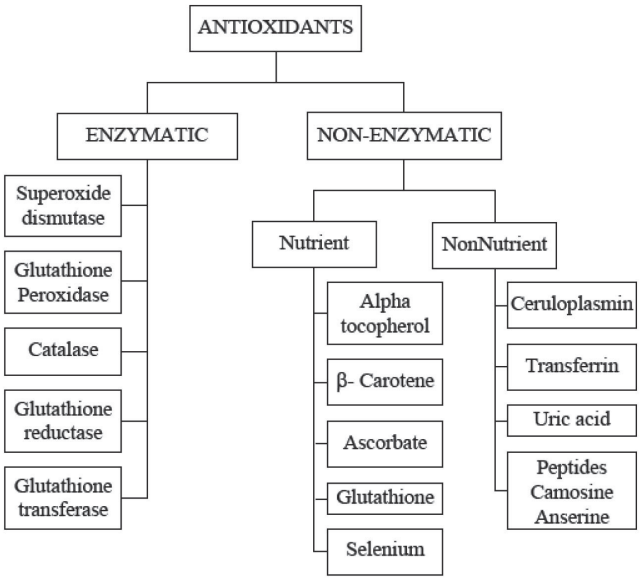

Figure-1: Antioxidants classified as enzymatic and non enzymatic

Normal cellular metabolism produced oxygen free radicals or, more generally, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS). Both ROS and RNS are like double-edged sword as they have both beneficial and deleterious effects on the living tissues.[17]

The term oxidative stress and nitrosative stress can be defined as the harmful effect of free radicals causing potential biological damage.

An overproduction of ROS/RNS and in the same time deficiency of antioxidants on the other, this process occurs. It can be said that, disturbance in the equilibrium status of antioxidants caused from the metabolic reactions that use oxygen results into oxidative stress.

The balance between beneficial and harmful effects of free radicals is achieved by mechanisms called "redox regulation". Redox regulation strikes the balance between beneficial and harmful effects through redox homeostatis hence protecting the living organisms from varied oxidative stresses.[18-20]

Reactive oxygen species (ROS)

Superoxide anion

The formation of superoxide takes place in the mitochondria of a cell. In mammalian cell mitochondrial electron transport chain is the main source of ATP in the mammalian cell. Few electrons leak during the energy transduction and form oxygen free radical superoxide which has proven to be deleterious for pathophysiology of living organisms leading to various diseases. Complexes I and III of the electron transport chain, produces superoxide and these become so strongly charged that they traverse out of the inner mitochondrial membrane.[21-23]

Hydroxyl Radical

Hydroxyl redical (•OH) is the neutral form of the hydroxide ion. The hydroxyl radical has a high reactivity, making it a very dangerous radical with a very short in vivo half-life of approx. 10^{-9}s. It has been demonstrated that superoxide release iron for [4Fe–4S] cluster containing enzymes of the dehydratase-lyase family. This iron Fe^{2+} can participate in the Fenton reaction, generating highly reactive hydroxyl radical as following:

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

Thus under stress conditions, $O2^{\bullet-}$ acts as an oxidant of [4Fe–4S] cluster-containing enzymes and H_2O_2 produces •OH which releases Fe2+ to be available for Fenton reaction.[24-26]

Peroxyl Radicals

The simplest peroxyl radical is (HOO•), which is the protonated form of superoxide ($O2^{\bullet-}$) and is usually termed either hydroperoxyl radical or perhydroxyl radical.

The protonated form of superoxide (O2•−) is the peroxyl radical (HOO•)or also termed as hydroperoxyl/perhydroxyl radical.[27,28]

Hydrogen Peroxide

H_2O_2 produced by oxygen consumption in peroxisome which oxidized variety of molecules. The organelle also contains catalase, which decomposes hydrogen peroxide and presumably prevents accumulation of this toxic compound.

In this way peroxisomes monitors the activities of these enzymes to ensue no net production of ROS.

Hydrogen peroxide plays a role as a second messenger in nuclear factor KB activation in some cells and where inflammation is present it may:

- Increase adhesion molecule expression.
- Cause cell proliferation.
- Induce apoptosis.
- Modulate platelet aggregation.[29]

Reactive nitrogen species (RNS)

NO• is a radical as it has one unpaired electron on the antibonding 2π* y orbital and specific nitric oxide synthases generate this radical in the biologic tissues, which metabolise arginine to citrulline with the formation of NO• via five electron oxidative reaction.[30]

Overproduction of reactive nitrogen species is called nitrosative stress. The oxidative burst triggered during the inflammatory processes initiates production of superoxide anion and nitric oxide by the cells of the immunity system. Superoxide anion and nitric oxide react and produce peroxynitrite anion (ONOO−) which causes DNA fragmentation and lipid oxidation.[31,32]

SOURCE OF FREE RADICALS

There are two major sources for generation of free radicals:
Normal biological process or cellular metabolism.
Environmental effect.

1. Normal biological process or cellular metabolism: Free radical generates due to:

a. Leakage of electrons from respiratory chain.
b. Production of H_2O_2 or O_2- by oxidase enzymes.
c. Chain reactions of membrane lipid peroxidation.
d. Peroxismal generarion of O_2 and H_2O_2.
e. During synthesis of prostaglandins
f. Production of nitric oxide by arginine.
g. During course of phagocytosis.
h. In oxidation of heme to bilepigments
i. Result of auto-oxidation.

Environmental effects

a Result of drug metabolism e.g paracetamol, halothane, cytochrome P_{450} related reactions.
b Damage caused by ionizing radiations on tissues.
c. Photolysis of O_2 by light.
d. Photoexcitation of organic molecules.
e. Cigarette smoke contains free radicals and trace elements that produce OH^-
f. Alchohol promoting lipid peroxidation.

Production of free radicals in cells

Free radicals can generate by two types of reactions:

1. Accidental generation
2. Deliberate synthesis

Accidental generation

This encompasses such mechanism as “leakage of electrons” in to oxygen from mitochondrial electron transport chains, microsomal cytochromes p450 and their electron donating enzymes and other systems.

Several compounds found in human body including O_2 to make $O2^-$ this explains why solutions of adrenaline deteriorate on standing; it reacts with O_2 to form O2- which then oxidizes more adrenaline in a chain reaction.

Deliberate synthesis

In 1991 Dupuy et al suggested deliberate synthesis of oxygen derived species for metabolic purposes. In the case of thyroid hormone synthesis, H_2O_2 generated by thyroid gland and used by peroxide enzyme to help attach iodine to thyroid hormrones. Babior and Weiss suggested the role of production of O_2 and H_2O_2 by phagocytic cells. NADPH oxidase in inflammatory cells produces superoxide anion by process of respiratory burst during phagocytosis. The superoxide is converted to hydrogen peroxide and then to hypochlorus acid with the help of superoxide dismutase and myeloperoxidase. Superoxide and hypochlorus ions are final effectors of bactericidal action. Thus generation of o2- and H_2O_2 by phagocytes is known to play an important role in killing of several bacterial strains.[33-35]

PROTECTIVE ROLES OF ANTIOXIDANTS IN CARCINOGENESIS

Antioxidants and their functions

The food factor was named “Vitamin A” in 1920. It comes in two different sources:

1. Retinol and retinoids
2. Carotenoids

The vitamin A is usually used to refer to both the metabolically active form (retinol) and other chemical forms that are converted into retinol within the body (carotinoids). Retinol is called preformed vitamin A and the carotinoids, of which beta carotene is most active are called provitamin A.[36]

Vitamin A (retinoids)

1. Inhibits keratinization and terminal differentiation of epidermal cells.
2. Enhancement of cellular immunity.
3. Arrest/reverse leukoplakia progression.
4. Induction of cytotoxic and cytostatic effects on cancer cells.
5. Influence DNA, RNA, and gene expression.
6. Interfere with carcinogenic stimulation and binding.

Beta-carotene

1. Precursor of Vitamin A.
2. Anti-oxidant and free radical scavenging.
3. It immunomodulate and stimulate production of T-helper and NK cells and IL-2 receptors.
4. Inhibition of mutagenesis
5. Inhibition of cancer cell growth

Vitamin E (A-tocopherol)

1. Scavenger of free radicals.
2. It maintains the integrity of membrane and immune function.
3. It inhibits the cancer cell growth and differentiation.
4. Has role in inhibition of mutagenicity and formation of nitrosamines.
5. In cancer cells it inhibits DNA, RNA and protein synthesis.
6. Cytotoxicity.

Vitamin C (ascorbic acid)

1. Has potent antioxidant activity
2. It inhibits vitamin E degradation.
3. It reduces the formation of nitrosamines.
4. Reduces oncogene expression.
5. It has also plays a important role in enhancement of

detoxification via cytochrome P450
6. Blocks formation of fecal mutagens.[37]

ANTIOXIDANT THERAPY IN ORAL LESIONS

Oral leukoplakia

Use of vitamin A therapy in treatment of cases of oral leukoplakia began in the early 1960s, but it was not widely accepted because of side effects.

Silverman et al (1963) showed that the administration of 300,000 to 900,000 units of vitamin A/day with 75, 000 IU vitamin A ester troches, which were dissolved orally and the saliva-solute swallowed, resulted in 7 (43.8%) of patient having partial or complete resolution of their oral leukoplakia.

Silverman et al (1965) revealed clinical efficacy with the use of vitamin A troches, but all six patient had side effects associated with increased serum levels of vitamin A.

Benner et al (1993) evaluated the administration of 800 IU of alpha tocopherol per day for 24 week on 43 patients at several different centers. A single-arm phase II study of alpha-tocopherol conducted in patients with oral leukoplakia involved 43 patients treated for 24 week. Twenty (46%) had clinical responses, and nine (21%)had histologic responses.

Zaridze et al (1993) found a singnificant reduction in oral leukoplakia prevalence in a study population that received B-carotene (40mg/d), retinol (100 000 IU/wk),and vitamin E 80 mg/wk) for 6 month.

Although the effect of the three test agents cannot be conclusively separated results of a secondary blood concentration analysis and the fact that the retinol and vitamin E were dosed weekly rather then daily suggest that β-carotene may be the primary agent responsible for the reduced frequency of lesions.[38]

Sankaranarayanan et al 1997 conducted a placebo controlled trial to evaluate chemopreventive potential of either vitamin A or beta carotene alone in keralan subjecs with oral leukoplakia. Their study was randomized to 160 fishermen and women with oral precancerous lesions who received vitamin A 300,000 IU/ week orally or beta carotene (360mg/week x12months, n=55) or placebo (n=55). The subjects were examined every 2 months to establish clinical response response of lesions and toxicity, if any. The complete regression rates were: 10% in placebo arm 52% with vitamin A and 33% with beta carotene the results of the study thus provided a strong evidence to justify long terms trials with vitamin A in subjects with high risk leukoplakia.[39]

Mohit pal et al in 2004 conducted a study to evaluate the efficacy of lycopene in the treatment of oral leukoplakia and compared two different doses with a placebo. He observed that patients receiving lycopene supplementatation in both 8 and 4 mg regimens showed highly significant difference in the response as compared to the placebo group and concluded that lycopene can be a safe and effective medication for the management of oral leukoplakia.[40]

ANTIOXIDANT THERAPY IN ORAL SUBMUCOUS FIBROSIS

Kerr RA in 2007 demonstrated the efficacy of lycopene in reducing both signs and symptoms of OSMF and as such it offers patients a noninvasive and safe alternative or adjunct to conventional therapies. The subjects with advanced OSMF (< 20 mm of opening) were poor responders.[41]

Kumar et al in 2007 evaluated the efficacy of lycopene therapy in patient with oral submucous fibrosis. 58 patients with oral submucous fibrosis found were randomly divided in to 3 groups, evaluated weekly over a 2- month period.

Group A and Group B were administered 16mg of lycopene and in Group B additionally intralesional steroid injection were administered biweekly. Group C subjects were given a placebo. Mouth opening values for the patients showed an average increase of 3.4 mm, 4.6 mm and 0.0mm for patients in group A,B and C repectively. The authors suggested that lycopene can and should be used as a first line of therapy in initial management of oral submucous fibrosis.

Lycopene has potent anti-carcinogenic and antioxidant properties. It exhibits the highest physical quenching rate constant with singlet oxygen. The precise reason for this efficacy may be because of two reasons:

Lycopene has been shown to inhibit hepatic fibrogenesis in LEC rats and may exert a similar inhibition on abnormal fibroblasts in oral submucous fibrosis. This would also be in concurrence with another study on oral submucous fibrosis performed by Haque et al, who reported a positive result in submucous fibrosis cases with a therapeutic modality used for liver fibrosis.

Lycopene also upregulates lymphocyte resistance to stress and suppresses the inflammatory response.[42]

Guruprasad R in 2014 estimated serum Vitamin C and Iron levels in Oral submucous fibrosis (OSMF). Out of 85 subjects the test group consisted of established 35 OSMF subjects and the control consisted of habit free 50 subjects. Phlebotomy was performed and two ml venous blood was withdrawn and serum vitamin C was estimated. He concluded iron and vitamin C deficiency in conjunction with usage of areca nut may lead to OSMF.[43]

Nallapu V et al in 2015 compared the intraoral Vitamin E supplement with the intralesional dexamethasone, hyaluronidase and local anaeathesia. Histopathologically confirmed cases of oral submucous fibrosis, twenty in number were selected and randomly divided into two groups. Group A was administered intralesional dexamethasone (2mg/ml), hyaluronidase (1500 IU), and lignocaine-0.2 cc (2%) every week. Group B was administered oral Vit E capsules, 400 IU OD for 8 weeks. He concluded that Vitamin E has a significant role in the improvement of oral submucous fibrosis.[44]

Anti oxidant therapy in Oral lichen planus

Retinoids have been proven of having antikeratinizing and immunomodulatory effects. Topical use of these agents is intended to enhance therapeutic effect on keratinocyte proliferation and differentiation, systemic use suggest the necessity for supplementation.

In 1992 Gorsky M et al evaluated the maximum permitted dose of etretinate (75 mg/day) as first mode of systemic treatment of patients with symptomatic oral lichen planus, 50% of the subjects became asymptomatic along with improved clinical signs and symptoms. Recurrence of few clinical signs were noted after discontinuation of treatment.[45]

Saawarn, *et al*. evaluated the efficacy of systemic lycopene in the management of OLP. In their study they administered lycopene 8mg/day and an identical placebo for two groups of 15 patients each for eight weeks. The patients in lycopene group marked reduction of 84% in burning sensation whereas

it was 67% reduction for the placebo group. He concluded that lycopene was very effective in the management of OLP, and oxidative stress may have a role in disease pathogenesis.[46]

Petruzzi M conducted randomized, placebo-controlled study addresses evaluation of the effects of topic tazarotene in the treatment of OLP. He randomly administered tazarotene gel 0.1% b.i.d. or with placebo for eight weeks to his subjects and concluded topical tazarotene may be a valuable therapeutic tool in the treatment of hyperkeratotic OLP.[47]

BURNING MOUTH SYNDROME

Femiano F et al examined the effect of ALA on the symptomatology of Burning mouth syndrome (BMS).

They divided 42 patients in to two groups (test and control) of 21 subjects each. The test group were administered with Tiobec (ALA) for 30 days orally, followed by 600mg orally for 20 days subsequently 200 mg per day orally for 10 days. About two-thirds of the patients were relieved of the symptoms of BMS who were administered alpha-lipoic acid (ALA), only 15% of patients were relieved of symptoms in the placebo group and remarkably another two-thirds of the patients in the placebo when switched to ALA protocol showed reduction in the BMS symptomatology.[48]

In 2008 Steele et al reviewed a series of patients with sore, burning mouth treated with alpha lipoic acid and subjectively evaluated improvement in symptoms and found that 35% reported benefit from taking alpha – lipoic acid.[49]

CONCLUSION

In the body the network of antioxidants is complex and composed of several components, they serve as electron donors which break the chain reaction of free radicals by sacrificing their own electrons to feed them without turning into free radicals themselves. Antioxidants are classical nature's way to provide adequate defense against reacting oxygen species attack. An individual with inadequate supply of antioxidants can be at the risk of oxidative stress which leads to accelerated tissue and organ damage.

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