ANTIOXIDANTS AND ITS EFFECTS

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ABSTRACT

BACKGROUND

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecule. An antioxidant has been defined by Halliwell and Gutteridge as any substance that when present in low cone compared to the level of an oxidisable substrate significantly delays or prevents oxidation of that substrate. The term oxidisable substrate include proteins, lipids, carbohydrates, enzymes, nucleic acids, etc.

KEYWORDS

Oxidation, Oxidative Stress, Free Radicals, Scavengers, Antioxidants.

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Oxidation Reduction Reaction and their Importance in Biological Systems

Oxidation is a chemical reaction that transfers electrons from a substance to an oxidising agent.

The electron acceptor which gains the electrons is reduced and the process is called reduction. Thus, oxidation is always accompanied by reduction of an electron acceptor.

The principle of O-R applies equally to biochemical system and is an important concept underlying the nature of biologic oxidation. In all higher animals, biologic oxidation involutes using oxygen to liberate energy during aerobic metabolism. This energy phosphate bonds as in ATP, which acts as an important source of energy utilisation by the body.

Nature of Oxidants and the Concept of Free Radicals

Oxidants besides being derived from an aerobic metabolism are also products of the inflammatory response. They are mostly of the nature of free radicals.

A free radical is an atom or a molecule with one or more unpaired electrons.

A stable chemical molecule has no unpaired electrons in its electrons orbit. In a free radical, the presence of the unpaired electron makes it energetically unstable and so it will pair with an electron in the surrounding molecules, such as inorganic or organic chemicals, particularly with the key molecules in membranes and nucleic acid. Moreover, it initiates autocatalytic reaction.

These FRs Contribute to Varied Processes as

- Chemical injury.
- Radiation injury.
- Oxygen and other gaseous toxicities.
- Cellular ageing.
- Microbial killing by phagocytic cells.
- Inflammatory damage.
- Tumour destruction by macrophages.

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Oxidative stress and free radical; induced cellular damage: the ageing process.

In normal cells, the damage caused by FR and pro-oxidant process is kept in cells by protective mechanism. But in condition wherein the pro-oxidant process are dominant and the protective defences are lowered, there is a formation of a state called "Oxidative Stress." This state can result in serious cell damage if the stress is massive or prolonged.

Pro-oxidant process involute the formation of "Reactive Oxygen Species" [ROS] which is a collective term for free radical of oxygen.

- S/a - Superoxide (O2).
- Hydroxyl radical (OH).
- Peroxyl radical (ROO).
- Alkoxy radical (RO).
- Nitric oxide (NO).
- Carbon tetrachloride radical (CCl3).

ROS also includes substances, which are either oxidising agent and/or are converted to FRs. They are,

- Hypochlorous acid (HOCl).
- Ozone (O3).
- Peroxynitrite (ONOO).
- Singlet oxygen (O2).
- Hydrogen peroxide (H2O2).

Salvemini has described oxygen as a double-edged sword. It is vital to life, but leading to formation of by-products that are toxic such as formation of superoxide radical.

Now, O2 is more prone to produce O2. This is because molecular O2 is paramagnetic and contains 2 unpaired electrons with parallel spins. These unpaired electrons reside in separate orbitals unless their spins are opposed.

Reduction of O2 by direct insertion of a pair of electrons, e- into its partially filled orbitals is not possible. Hence, electrons are added to molecules O2 as single electron successively.

When O2 molecule takes up 1 electron by univalent reduction, it becomes superoxide anion.

$O2 + E\text{-} O_2 \longrightarrow$ (highly reactive and toxic to cell membrane)

O2 anion can capture further electrons to form hydrogen peroxide, H2O2 which is toxic and injurious.

H2O2 can further react with CO2 anion, in presence of Fe++ (ferrous) to form Hydrogen radical and Singlet oxygen.

This is known as Haber's reaction. Here, ceruloplasmin act as an antioxidant and converts Fe2+ to Fe3+, thus preventing further formation of highly reactive OH-FR.

O_2 anion can accept H+ and form "hydroperoxy" radical

O_2 HOO $H_2O_2+O_2$

Nitric oxide produced in the body from Arginine reacts with O_2 to produce "peroxynitrite" (ONOO), which decompose to form OH.

O2 anion can also be formed in metabolic pathway.

1. Cytosolic oxidation by enzyme.
2. During univalent oxidation with molecules in the respiratory chain.
3. During methemoglobin formation.
4. Cytosolic hydroxylation of steroids and drugs.
5. In tissues when exposed to ionising radiation.
6. During phagocytosis; by NADPH oxidising system during respiratory burst.

Scavengers of Free Radicals

1. Superoxide Dismutase: This enzyme is present in both cytosol and mitochondria and it can destroy the O_2anion.
   $2O_2$ Superoxide Dismutase $H_2O_2+O_2$
   $2H^+$
2. Catalase: This enzyme can destroy the H2O2 formed in the tissues to O_2
   H_2O_{2+} H_2O_2 Catalase $2\ H_2O_+O_2$
3. Glutathione Peroxidase: This is a Se-containing enzyme present in cytosol and mitochondria and it can destroy H_2O_2 with the help of reduced glutathione (G-SH).
   H_2O_2 + G-SH G. Peroxidase G-S-S-G $+2H_2O$
4. Ferricytochrome: $O_2 + Fe^{3t}$ $Fe^{2t} + O_2$
   Cyt C Cyt C

Harmful Effect of Free Radicals

FRs are highly reactive and are capable of damaging almost all types of biomolecules like lipids, proteins, polysaccharides and nucleic acids.

Lipid Peroxidation of Membranes

Free radicals in the presence of oxygen may cause peroxidation of lipids within plasma and membranes. Lipid peroxidation causes accumulation of lipid peroxides in biological membrane and disrupts its integrity and function. In addition, these lipid peroxides can break down to highly cytotoxic end products. LP is responsible for development of atherosclerosis and the vascular complication of DM.

Oxidative Modification of Proteins

FRs promote oxidation of amino acid residue side chains, formation of protein-protein cross linkages and oxidation of the protein backbone leading to protein fragmentation. This results in the degradation of critical enzymes by multicatalytic proteosome complex causing great damage throughout the cell.

Lesions of DNA

Reacting with thymine in nuclear and mitochondrial DNA produce single stranded breaks in the nucleic acid. The DNA damage has been implicated in ageing and in malignant transformation of cells.

Oxidative Damage to Carbohydrates

Oxidative FRs have been found to cause damage to hyaluronic acid which is responsible for the viscosity of synovial fluid in the joints of the body. Thus, the carbohydrate backbone of the joints are destabilised leading to weakness of such joints.

Role of FRs in Pathogenesis of Diseases with Multiorgan Involvement

1. Antoimmune Diseases.
2. Rheumatoid Arthritis.
3. Paracetamol and Alcohol toxicity.
4. Radiation injury.
5. Carcinogenesis.
6. Amyloidosis.
7. Ageing.

Disease having Specific Involvement

- CNS – Parkinsonism, Alzheimer's disease, Huntington's disease, Multiple scleroses.
- CVS – MI, Atherosclerosis.
- Endocrine – DM.
- GIT – PU, Cirrhosis, Pancreatitis.
- Renal – Nephrotoxicity due to aminoglycosides heavy metal poisoning.
- Respiratory system – Toxicity due to cigarette smoking.
- Eyes – Cataract and Retinopathy.

Role of Antioxidants

The term antioxidant originally was used to refer specially to a chemical that prevented the consumption of oxygen.

In the late 19th and early 20th century, extensive study was devoted to the uses of antioxidants in imp industrial processes sic as the prevention of metal corrosion, the vulcanisation of rubber and the polymerisation of fuels in the fouling of internal combustion engines. However, it was the identification of vitamins A, C and E as antioxidants that revolutionised the field and led to the realisation of the importance of antioxidants in the biochemistry of living organism.

Antioxidants are classified broadly into 2 divisions depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water soluble antioxidants reacts with oxidants in the cell cytoplasm and the blood plasma, while lipid soluble antioxidants protect cell membranes from lipid peroxidation. These compounds are either synthesised in the body or obtained from diet. The different antioxidants are present at a wide range of concentrations in body fluids and tissues. Some such as glutathione or ubiquinone are mostly present within cell, while other such as uric acid are more evenly distributed.

The action of one antioxidant may depend on the proper function of other members of the antioxidant system. The amount of protection provided by any one antioxidant will also depend on its concentration, its reactivity towards the particular ROS being considered, and the status of the antioxidants with which it interacts.

Classification

- Antioxidants in relation to lipid peroxidation.[1,2,3,4,5]
  1. Preventive antioxidant that will block the initial production of FR. Eg. Catalase and glutathione peroxidase.

2. Chain breaking antioxidants that inhibit the propagative phase of lipid peroxidation. Eg. Superoxide dismutase, vitamin E and uric acid.

- Antioxidants according to their location.
  1. Enzymatic antioxidants. Eg. Superoxide dismutase, catalase, glutathione peroxidase, glu reductase.
  2. Non-enzymatic antioxidants.
     a. Nutrient – Carotenoids, x-tocopherol, ascorbic acid selenium
     b. Metabolic – Glutathione, ceruloplasmin, albumin, bilirubin, ferritin and uric acid.

The Antioxidant Enzyme System
i.e. (Scavengers of free radicals)

Nutrient Antioxidants

Antioxidants	Dietary Source
1. Vitamin E (Tocopherol)	Unprocessed veg. oils (cotton seed, peanut and sunflower) whole grains, leafy veg, legumes.
2. Vitamin C (Ascorbic acid)	Citrus fruits (orange and grapes), gooseberry (amla), Guava, green veg(cabbage, spinach), cauliflower
3. B-carotene (Provitamin A)	Carrots, green fruits and veg, spinach, turnip, apricot
4. Lycopene	Tomatoes, papaya, pink guava and watermelon
5. Selenium	Sea foods, meats organ, whole grain
6. Coenzyme Q10	Organ meat, beef, green tea
7. Proanthrocyanidin	Grape seeds
8. Catechin	Green tea
9. Curcuminoids	Turmeric
10. Quercetin	Onions, Red Wine, Green Tea
11. X-lipoic acid	Red meat, guava, Yeast
12. Ellagic acid	Berries, Walnuts, Pomegranate
13. Hesperidin	Citrus fruits, lemon

Tocopherols (Vit E)
It is fat soluble and among the 8 tocopherol derivatives alpha-tocopherol is biologically the most active. It is an antioxidant present in all cellular membranes and protect against lipid peroxidation alpha-tocopherols can directly act on oxyradicals (O_2, OH, singlet oxygen).

It has the potential for protective effect in atherosclerosis, carcinogenesis, cataract formation and AMD.

However, the major biological role of Vit E is to protect Polyunsaturated Fatty Acid (PUFA) and other components of cell membranes from oxidation by FRs. It readily denotes the hydrogen from the hydroxyl group on its ring structure to the free radicals, rendering them unreactive and hence stable.

Lipid peroxidation is implicated in enhancement of platelet aggregation and thrombosis. Hence, vit E is considered beneficial in prevention and treatment of diabetic patients with hyperlipidaemia. In fact, vit E supplementation is reported to the atherosclerotic plaques in the coronary artery operations, the incidence of non-fatal MI and may play a role in 20 prevention of MI. By virtue of the same, it may have some role in pre-eclampsia.

Tocopherol, carotene and selenium has been shown to have anti-cancer activity in CA stomach.

It is also used in the treatment of nocturnal muscle cramps and intermittent claudication. Precisely not known, but it prevents oxidation of certain radicals and ensues better utilisation of O2 in muscle tissue, thereby improving muscle metabolism.

At very high dose, vit E has been reported to delay the progression of Alzheimer's disease.

RDA: 15 IU/day.

Ascorbic Acid (Vit C)
Vit C is a monosaccharide antioxidant found in both animals and plants. It acts as a first defence against FRs in the body, especially in the blood.

Vit C scavenges reactive nitric oxide species, nitrosamines in smoked fish, meat and pickles (which has been implicated in causation of CA stomach, etc.).

Diets low in ascorbic acid may be associated with increased risk of DU and SCC of the upper airways.

It is also believed to delay the onset of cataract and the risk of AMD of the eye.

Vit C further has the ability to regenerate Vit E

RDA: 60 mg/day

200 mg/day for better results.

Vitamin A, the Carotenoids and the Retinoids
Vit A is available as preformed vit, retinol or as carotenoids. In support of its antioxidant action, current RDA for retinol is 800-1000 ug/d.

Ingestion of retinol in dose of >10,000 IU/d, i.e. more than twice the RDA, modestly reduce the age adjusted risk of breast cancer. Further, low serum levels of serum retinoid have been associated with increased incidence of cervical dysplasia in women.[6,7]

Dietary precursors of vit A are called carotenoids. These are plant derivatives which usually have vit A actively. They act as preventive agent against certain cancers such as those of lung and spleen and especially the GIT. Betacarotene supplement has been reported to result in a substantial risk of Ca stomach. Foods, rich in p-carotene also lower the risk of heart disease, cataract and Age=Related Macular Degenerate of the eye (AMD).

Retinoids are substances having a structure similar to vit A, but they may or may not have vit A activity. They are modulators of epithelial cell differentiation, Eg. Tretinoin, Isotretinoin, Etretinate, Acitretin, etc.

Isotretinoin (cis-retinoic acid) has been shown in low doses to suppress leukoplakia of the aerodigestive tract.

High doses prevent the development of second primary tumours in patients with early SCC of the head and neck.

Etretinate (a derivative of tretinoin) and Acitretin (a metabolite of etretinate) have been used in the prevention and treatment of skin tumours, mycosis fungoides, superficial tumours of urinary bladder.

Minerals

Calcium and other trace elements like copper, zinc, manganese and selenium also serve antioxidant function. Most of them exert their actions by forming the prosthetic groups of intracellular enzyme and similar other substances.

Calcium supplementation over a four-year period has been shown to moderate the incidence of colorectal adenoma.

Copper prevents the development of generalised weakness and helps in maintaining healthy skin, thus preventing premature ageing. It also forms the prosthetic group of the antioxidant enzyme, SOD.

Zinc prevents the development of abnormal DNA and signs of non-physiological ageing like alopecia, stretch marks on skin, fatigue, depressed immunity, etc. It increases the risk of AMD and its loss of vision. Zinc is also a cofactor of Sodium.

Manganese is another trace element that acts as a cofactor of SOD.

Selenium is a component of glutathione, which acts as an antioxidant in the body. It has been reported to the risk of cancers of the breast, lung, stomach, colon and prostate. Selenium also has cardio-protective activity.

Coenzyme Q10 or Ubiquinone

It is a lipid soluble endogenous provitamin, present in the mitochondrial membrane. It is important components of the mitochondrial electron transport chain, which is involved in biologic oxidation. It possesses free radical condition properties.

Several medical condition like CCF, RF, male infertility, etc. are associated with CoQ 10 deficiency.

Another study has suggested that CoQ 10 slows down the functional decline in Parkinson's disease as evidenced by improvement in activities of daily living in patients receiving CoQ10 supplements.

Other Antioxidants Obtained from Plant and Animal Products Milk Thistle

It is a plant which contains the active principle, silymarin in its seeds and fruits. It has both cytoprotective and hepatoprotective activity and the chief mode of such action is provided by its antioxidant property.

Silymarin is a free radical scavenger and neutralise the hepatotoxicity of several agents including Amanita Phalloides, alcohol, paracetamol, halothane and CCl4.

Gingko Biloba

Its dried green leaves are the source of the popular herbal medicine known as Gingko. Its antioxidant property is useful in mild-to-moderate dementia (both Alzheimer's and multi-infarct dementia).

Camellia Sinensis (Tea)

The active principle of catechin possess antioxidant property. Green tea has more antioxidant than black tea.

Uses: Stomach, Pancreas, Lungs, Urinary bladder carcinoma.

Garlic, Grape fruits, Soya bean, Lycopene (Tomato), Lignan (oatmeal and barley), Lutein (Dark green veg):

One other herbal antioxidants used in Atherosclerosis, MI and Cancers.

Spirulina

It is a blue green algae from shallow pond water and is a good source of SOD, p-carotene and b-complex.

Curcuma Longa (Turmeric)

Contains a phenolic compound curcuminoids and acts as antioxidant anti-inflammatory antimicrobial and anti-carcinogenic.

Uses: Oral cancer, rheumatoid arthritis, against gram -ve bacterial infection, entamoeba histolytica.

Whole Grain

It has been reported that antioxidant identical to the body's own naturally occurring antioxidants like SOD, catalase, glutathione, etc. can be extracted from whole grain.

Fish and Fish Oil

They are rich sources of a special class of PUFA known as Omega-3 fatty acids. It inhibits VLDL, LDL and Apoprotein B synthesis. It is necessary for normal vision and neural development during first 6 months of life and so it should be supplemented in the diet of non-breast feeding infants.

Metabolic Antioxidants

1. Uric acid: is a powerful scavenge of singlet oxygen and OH radical.
2. Ceruloplasmin: inhibits iron and Cu dependant lipid peroxides.
3. Transferrin: binds to iron and prevents catabolism of free radicals.
4. Albumin: can scavenge free radical formation on its surface.
5. Bilirubin: protect the albumin bound FFA from peroxides.
6. Haptoglobin: binds to free Hb and prevents the ace of lipid peroxides.

Synthetic Antioxidants

1. Xanthine oxide inhibitors like allopurine is used in reperfusion injury, MI.

   Reperfusion injury: under certain conditions, when blood flow is restored to cell that have been previously rendered ischaemic, injury is often paradoxically exacerbated and proceed at a faster rate. As a consequence, tissues are subjected to loss of cell in addition to those that are irreversibly damaged due to ischaemia; this is seen in myocardial and cerebral infarction. Possible mechanism of such injury may involve free radicals, which may be generated by parenchymal and endothelial cell and infiltrating leucocytes as part of the inflammatory response. Further, cellular antioxidant reserves may be compromised. ROS also promote mitochondrial dysfunction ultimately leading to death.

2. Probucol: It is a phenolic chain breaking antioxidant has anti-inflammatory 5 anti-atherosclerotic effect. It inhibits lipid peroxidation, interfere the synthesis of eicosanoids.
3. Mercaptopropionyl glycine other thiols: They have been found to protect against reperfusion injury.
4. Desferrioxamine: It is an iron chelator. It can inhibit iron-dependant lipid peroxide and generate oxidative free radicals. It has been found to be useful in Alzheimer's disease reperfusion injury.

Glutathione

Reduced glutathiones play a key role in the biological antioxidant enzyme system. GSH $ H2O2 are the twin substrates for glut peroxidase. The reduced glutathione gets regenerated from the oxidised glutathione.

1. Vit A: Acute intoxication may result in anorexia, vomiting, abdominal pain, headache, diplopia, convulsions and coma. Chronic intoxication can lead to anorexia, hyperkeratosis (dry itchy skin) alopecia, oedema, bleeding tendency, fissures, non-healing ulcers, anaemia, liver damage, etc. B-carotene, in one particular large scale study was found to be risky in the development of lung carcinoma in smokers.
2. Vit C: Abdominal bloating and diarrhoea, depletion of VitB12 levels, rebound scurvy. People with a history of oxalate stones in their kidney should take large doses.
3. Vit E: Can worsen the status of vit 4-deficient condition (as in patients receiving treatment with warfarin).
4. Ubiquinone: Safe, occasionally causes GI upset.
5. Silymarin: Relatively safe.
6. Gingko: Serious intracerebral bleeding, especially if the patient is on concurrent therapy with anticoagulants may be seen.
7. Fish oils: are relatively safe. They may cause GI discomfort.
8. Probucol: is known to cause unfavourable effect on lipid profile on long-term use, in that HDL levels are lowered. It also increases QT interval and can result in arrhythmias in susceptible patients.

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