ANTIOXIDANTS: BASIC CONCEPTS IN RELATION TO THE EYE

Pradeep Venkatesh, Garg Satpal, Lalit Verma, Tewari Hem Kumar and Sheena Garg

Dr. Rajendra Prasad Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi-29

ABSTRACT

Antioxidants are a small group of substances that protect living cells from the destructive consequences of powerful oxidizing intermediates that can be formed from oxygen. Situations in which pro-oxidant mechanisms within the body are more active than the antioxidant mechanisms (oxidative stress) predispose and contribute to the pathogenesis of several ailments in various organs of the body. In the eye, pro-oxidant factors have been blamed for the causation of diseases such as age related macular degeneration and senile cataract. The role of pro-oxidants in the genesis of certain diseases is well established however, the effectivity of antioxidants provided to the body by dietary supplementation is inconclusive. In this article we provide a review on the basic concepts of antioxidant-pro-oxidant interaction in relation to its effects on the eye.

KEY WORDS : Antioxidants, Pro-oxidants, Oxidative stress

Our body has several biomolecules such as proteins, lipids and nucleic acids, either as constituents of cell membranes or of DNA and RNA respectively. These biomolecules are very sensitive to degradation by various environmental toxins, of which, intermediates generated during the reactions of oxygen are an important source. Antioxidants are a small group of substances that protect living cells from the destructive consequences of powerful oxidizing intermediates that can be formed from oxygen.

It is widely believed that earth's atmosphere was formed nearly 4-5 billion years ago. At this period and for nearly 3 billion years thereafter, oxygen was yet to become a component of our atmosphere (1, 2). The biological evolution of blue-green algae, the first ever photosynthetic organisms, lead to a gradual accumulation of oxygen in our atmosphere and till date photosynthetic organisms remain the only source of atmospheric oxygen. Prior to the evolution of blue green algae and the accumulation of oxygen, the earth

was inhabited by only anaerobic living forms. These organisms also extract energy from glucose similar to aerobes but, the oxidation of glucose is incomplete and so correspondingly less energy is generated during anaerobic metabolism of glucose. In contrast, aerobic organisms by using oxygen as a terminal oxidizing agent are able to generate more energy from reduced food substrates such as glucose. This is because glucose can now be more completely oxidized to carbon dioxide. The accumulation of oxygen released by these photosynthetic organisms was an important precursor to the evolution of aerobic organisms about 1.5 billion years ago. The aerobes also began to thrive because of their capacity to extract more energy from food substrates by complete oxidation. It is also believed that the increasing oxygen concentration within the earth's atmosphere caused extinction of several anaerobic organisms. It is well known that obligate anaerobic forms cannot thrive in the presence of oxygen. The carbon-oxygen cycle shown below depicts the relationship between carbon-oxygen interaction.

Author for Correspondence :

Dr. Satpal Garg,
Additional Professor of Ophthalmology, at above address

To comprehend how, oxygen, the very essence of life, can itself become a toxin and endanger cellular components, including those of the eye, it is important

Photosynthesis and the carbon-oxygen cycle

During photosynthesis, solar energy is trapped by chlorophyll and this is followed by a catalytic conversion of carbondioxide and water to glucose. Oxygen is formed as a byproduct during this interaction.

$\text{C}_6\text{H}_{12}\text{O}_6$ (glucose) is a high energy reduced carbon and oxygen is a strong oxidizing agent. The products of photosynthetic organisms (glucose and oxygen), become the reactants of the non photosynthetic aerobic organisms. The products of this non-photosynthetic reaction are again the reactants of the photosynthetic organisms i.e. carbondioxide and water. This completes the carbon-oxygen cycle.

to know in brief the chemical nature and reactivity of oxygen and its intermediates as shown in table 1.

Oxidative stress is a term used to describe any challenge in which pro-oxidants predominate over antioxidants (3,4). Oxidative stress has been shown to be involved in the pathogenesis of several ailments afflicting various organs in the body including the eye. This is as shown in chart 1 below.

Chart 1: Effect of oxidative stress on various organs of the body

Table 1. Properties of Oxygen

- Molecular oxygen in its stable ground state is not toxic.
- Molecular oxygen has unpaired electrons in its outer orbital. However it is chemically not reactive because of spin restrictions involving these electrons. This spin restriction limits insertion of electron pairs into oxygen but not of single electrons.
- Oxygen is a strong oxidizing agent and is one of the primary oxidants for biological as well as non biological oxidation reactions.
- Oxygen can get reduced by photo-excitation or by accepting electrons involving univalent, divalent and tetravalent chemical interactions.
- It is now very clearly evident that the toxicity of oxygen is necessarily because of its intermediates formed.
- Toxic intermediates are generated only during photo-excitation and reduction by the univalent pathway. Divalent and tetravalent interactions of oxygen do not generate toxic intermediates.
- Toxic oxygen intermediates are formed from endogenous and several exogenous sources.
- The toxic metabolites liberated during oxygen interactions are :
 1. Superoxide anion radical (O_2^-) or the Superoxide radical.
 2. Hydroxyl radical ($\text{OH}^\cdot$), is the strongest oxidizing agent known.
 3. Hydrogen Peroxide (H_2O_2), by its ability to get oxidized into hydroxyl radical.
- These intermediates are highly reactive because they do not face spin restrictions. Being highly reactive, they have a strong tendency to oxidize biological molecules like lipids, proteins and the nucleic acids. Oxidation of these biomolecules degrades them and disrupts cellular activity.
- These toxic metabolites rapidly undergo catalytic conversion into water during the following chemical reactions :

In the body, a balance exists between the pro-oxidant factors that tend to increase oxidative stress and the antioxidant factors that enable the cellular milieu to overcome the risk of oxidative stress (Table 2). Pathological manifestations begin to occur whenever there is an increase in the activity of the pro-oxidant factors or when the antioxidant system becomes either malfunctional (e.g. Chidiak-Higachi Syndrome) or less efficient with passing age. In most cases the pathological changes result not due an episode of acute oxidative stress but due to cumulative stress occurring over several decades.

Mechanism of damage to biomolecules by pro-oxidants

Oxygen, as mentioned earlier, allows complete oxidation of reduced food substrates and so enables aerobes to derive more energy. However, oxygen itself can become toxic in the following conditions:

1. When the oxygen concentration increases above 21%, e.g. with hyperbaric oxygen.
2. When oxygen concentration is normal but the antioxidant defense mechanisms are immature, e.g. in premature babies.
3. When oxygen concentration is normal but the antioxidant defense mechanisms are "worn-out". The causes of this wearing out of antioxidant defenses are presently ill understood. Both genetic and environmental factors contribute but, how and why remain unraveled.

Often, this production of oxygen free radicals (OFR) in a controlled manner during any inflammatory process has however an important indirect protective effect also. This is distinctively demonstrated in *chronic granulomatous disease*. Children suffering from this disease develop life threatening and fatal bacterial infections because of a genetic defect that prevents OFR generation within their leukocytes during any "inflammatory" threat from bacterial invasion. Chart 2 shows in a simplified way the possible manner in which free radicals bring about tissue trauma.

Biological sources of free radicals and pro-oxidants

Oxygen free radicals (OFRs) and other free radicals are generated by normal metabolic processes such as the reduction of oxygen to water by the electron transport chain within the mitochondria. The concentration of molecular oxygen in various organs is highly variable and so is the amount of reduced oxygen species that is generated. The predominant mechanisms by which superoxide radical is generated in the cells is by catalytic reduction of oxygen by membrane bound enzymes (mitochondrial, microsomal and endoplasmic reticular) as well as by enzymes located in the cytosol; by the auto-oxidation of haemoglobin, catecholamines, flavins, glutathione, ascorbate, melanin etc.(5, 6). The maximum superoxide production occurs at the level of cellular membranes and this probably accounts for the increased propensity of these membranes to become affected early and more severely during any form of damage by oxidative stress.

Table 2.

Hydrogen peroxide is generated during the

Pro-oxidants	Antioxidants	
Ultraviolet light	Enzymes :	Superoxide dismutase
Photo-sensitizers :		
Riboflavin		Catalases
Kyneurine Derivatives		Glutathione peroxidase
Oxygen Intermediates :		
Singlet oxygen	Vitamins :	Ascorbic acid (Vit.C)
Hydroxyl radical		Tocopherol (Vit.E)
Hydrogen peroxide	Pigments :	Xanthophyll
		Melanin

Chart 2.

dismutation of the superoxide radical by the superoxide dismutase enzyme. Hydroxy radical is derived by the reaction of the superoxide radical with hydrogen peroxide. Nitric oxide has also been shown to have weak pro-oxidant and stronger anti-oxidant properties. Similar dual properties have been demonstrated with ascorbic acid.

Tissue inflammation, trauma or damage, particularly ischemia is a potential source of free radicals. This has been well established in myocardial infarction and the transplantation of organs. Leukocytes have been largely implicated as the source of OFRs and other free radicals during these processes.

Environmental factors are also an important source of reduced oxygen species. These factors include ultraviolet light, X-rays, metal ions (iron, copper), ultrasound and g-rays. Sunburn is an usually innocuous manifestation of UV damage. In the eye however it leads to visual impairment by a solar burn

of the fovea. Depletion in the ozone layer increases the risk of exposure to UV light.

Biological sources of anti-oxidants

There exists an array of intra-cellular and extracellular antioxidant defenses within the body. Chief among these are vitamin A, a cytosolic antioxidant; vitamin E, a membrane linked antioxidant and glutathione and glutathione dependent enzymes (mitochondrial, cytosolic and extracellular forms of these have been identified) (7). Important antioxidant enzyme systems include glutathione peroxidase (GPX), glutathione reductase, glutathione transferase, superoxide dismutase (SOD) and catalase. Superoxide dismutase is one of the most important enzymes that function as cellular antioxidants (8).

Antioxidants mentioned above are either themselves micronutrients (e.g. Vit.C, Vit.E) or are dependent on micronutrients (e.g. GPX and SOD are dependent on selenium and zinc respectively for their activity). Hence, it is possible that diet may have a

protective effect on the deleterious properties of pro-oxidants. Another source of antioxidants are the synthetic antioxidants such as butylated hydroxytoluene, added to processed foods, to prevent their oxidation during storage and hence to improve shelf life.

Does diet/supplementary antioxidants have a role in protection against oxidative stress?

As a consequence of the well established role of micronutrients in antioxidative mechanisms within the body, there remains a great deal of interest and debate on the possible prophylactic and therapeutic benefits of dietary supplementation of these micronutrients. Several studies, both experimental and clinical have indeed revealed an increased prevalence of various conditions such as cataract and age related macular degeneration in population groups exposed to oxidative stress (9-13). In contrast, results from studies aimed at determining any beneficial role of dietary supplementation of antioxidants in either preventing, delaying or reversing the effects of OFR and other free radical induced damage have been largely inconclusive (14-19). This is explainable by the following facts:

1. The absorption and transport of micro-nutrients within the body are regulated closely by homeostatic

processes. This implies that increased bioavailability of these micro-nutrients does not occur by dietary supplementation unless a state of deficiency exists (rarely encountered clinically).

2. Oxidative stress is a continuously ongoing process and the stress becomes enhanced by increasing environmental pro-oxidant exposure (e.g. UV light). This can be solved only by decreasing exposure to these harmful toxins and not by increasing intake of antioxidants.

3. Other associations may be involved in bringing about the effects of these vitamins and micronutrients rather than just their mere presence.

While experimental conditions can be tailored to suit the study conditions, the same is not true whenever a prospective, randomized clinical trial is undertaken. This is particularly so for studies involving antioxidants and free radical damage. Owing to this most efforts to identify the role of antioxidants have been limited to experimental studies only. Because of a lack of conclusive evidence about the prophylactic and therapeutic role of antioxidant supplementation in clinical trials and also because cumulative toxicity with some of these are known to be possible their prescription to patients seems unwarranted. However adequate intake of fruits rich in antioxidants should be encouraged.

REFERENCES

1. de Duve, C. (1995) The beginnings of life on earth. *Amer. Sci* 83, 428-437.
2. Lazearo, A. and Miller, S.L. (1996) The origin and early evolution of life: prebiotic chemistry, the pre-RNA world, and time. *Cell* 85, 793-798.
3. Jones, D.P. and DeLong, M.J. (2000) Detoxification and protective functions of nutrients. In : *Biochemical and physiological aspects of human nutrition*. Ed. Stipanuk, M.H. W.B Saunders Company, Philadelphia. p. 901-916.
4. Sies, H. (1985) Oxidative stress: Introductory remarks. In : *Oxidative stress*. Ed. Sies, H. Orlando, Florida: Academic Press, p. 1-10.
5. Halliwell, B., Murcia, M.A., Chirico, S. and Aruoma, O.I. (1995) Free radicals and antioxidants in food and in vivo: What they do and how they work. *Crit. Rev. Food Sci. Nutr.* 35, 7-20.
6. Halliwell, B. and Gutteridge, J.M.C. (1989) In : *Free radicals in Biology and Medicine*. 2nd Edition, Oxford University Press (Clarendon), Oxford, p. 422-438.
7. Sies, H. and Stahl, W. (1995) Vitamins E and C, betacarotene and other carotenoids as antioxidants. *Amer. J. Clin. Nutr.* 62, 1315S-1321S.

8. Fridovich, I. (1989) Superoxide dismutases J. Biol. Chem. 264, 7761-7764.
9. Leske, M.C., Chylack, L.T. and Suh-Yuh, W. (1991) The lens opacities case-control study: Risk factors for cataract. Arch. Ophthalmol. 109, 244-251.
10. The Italian-American study group (1991) Risk factors for age related cortical, nuclear and posterior subcapsular cataracts. Amer. J. Epidemiol. 133, 541-552.
11. The Italian-American study group (1994) Incidence and progression of cortical, nuclear and posterior subcapsular cataract. Amer. J. Ophthalmol. 118, 623-631.
12. Bochow, T.W., West, S.K. and Emmet, E.A. (1989) Ultraviolet light exposure and risk of posterior subcapsular cataracts. Arch. Ophthalmol. 107, 369-372.
13. Mohan, M., Sperduto, R.D. and Angra, S.K. (1989) India-US case-control study of age related cataracts. Arch. Ophthalmol. 107 : 670-676.
14. Jacques, P.F. and Chylack, L.T. (1991) Epidemiologic evidence of a role for the antioxidant vitamins and carotenoids in cataract prevention. Amer. J. Clin. Nutr. 53, 352S-355S.
15. Goldberg, J., Flowerdew, G. and Tso, M.O.M. (1988) Age related macular degeneration and cataract. Are dietary antioxidants protective? Amer. J. Epidemiol. 128, 904-905.
16. Hankinson, S.E., Stampfer, M.J. and Seddon, J.M. (1992) Nutrient intake and cataract extraction in women: A prospective study. Brit. Med. J. 305, 335-339.
17. Leske, M.C., Wu, S.Y. and Hyman, L. (1995) The lens opacities case-control study group. Arch. Ophthalmol. 113, 1113-1119.
18. Rouhiainen, P., Rouhianen, H. and Salonen, J.T. (1996) Association between low plasma vitamin E concentration and progression of early cortical lens opacities. Amer. J. Epidemiol. 144, 496.
19. Sperduto, R.D., Hu, T.S. and Milton, R.C. (1993) The Linxian cataract studies: Two nutrition intervention trials. Arch. Ophthalmol. 111, 1246.