Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Suppl 4, 2011

Review Article

ANTIOXIDANTS AND THEIR PROTECTIVE ACTION AGAINST DNA DAMAGE

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Received: 11 May 2011, Revised and Accepted: 14 June 2011

ABSTRACT

In recent years many articles have been published on oxidative stress. Perhaps the knowledge about free radicals like reactive oxygen and nitrogen species metabolism; definition of markers for oxidative damage provide evidences linking chronic diseases and oxidative stress. Identification of flavonoids and other dietary polyphenol antioxidants present in plant foods and data supporting the idea that health benefits are associated with fruits, vegetables and red wine in the diet are probably linked to the polyphenol antioxidants they contain. Excessive reactive oxygen species may cause irreparable DNA damage, leading to mutagenesis and perhaps cancer. Investigation into the nature of DNA damage and repair have provided valuable insight into aging, human genetics and cancer .Now, there is deep interest in identifying free radical scavengers or antioxidants that inhibit oxidative DNA damage. In this review we discuss DNA damage due to oxidative stress, and the role of potent Antioxidants in its protection.

Keywords: Antioxidants, Prooxidants, Free Radicals, DNA Damage

INTRODUCTION

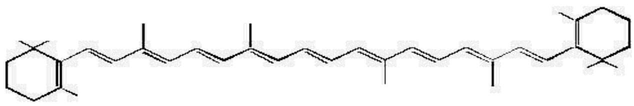
Chemical compounds that can generate free radicals are called Prooxidants. Free radicals are atoms or group of atoms with an unpaired electron. Once formed these highly reactive radicals can start a chain reaction. In 1954, the lethal effects of ionizing radiation were identified. Since then free radicals such as ROS and reactive nitrogen species (RNS) have gained notoriety.[1] Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane.[2] Cells may function poorly or die if this occurs. They are highly reactive that have short half-life and strong damaging activity towards macromolecules like DNA, Proteins lipids etc due to their oxidation properties. Free radicals have been implicated in the etiology of several human diseases as well as ageing [3,4,5] Cells are protected from adverse effects of Free radicals due to well-developed Antioxidant system. Antioxidants [6,7] are molecules, which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged.

Oxidative stress

In a normal cell there is a proper balance between pro-oxidants and antioxidants but when the level of pro-oxidants increases as compared to antioxidants, they lead to oxidative stress.[8] It's the major cause behind most of the diseases. It leads to aging and several degenerative diseases such as heart diseases and cancer.[9] It is caused by exogenous factors like exposure to ionizing radiation or heat or by desiccation or mechanical shearing or smoking as well as endogenous processes during normal cell metabolism. Excess oxidative stress can lead to oxidative damage of DNA causing significant base damage, strand breaks, altered gene expression, and ultimately mutagenesis. Continuous oxidative damage to DNA[10,11] is believed to be a significant contributor to the age-related development of many cancers, such as those of the breast, colon/rectum, and prostate.

Antioxidants and their defense system

Antioxidants' are substances that neutralize free radicals or their actions. Nature has endowed each cell with adequate protective mechanisms against any harmful effects of free radicals: superoxide dismutase (SOD),glutathione peroxidase, glutathione reductase, thioredoxin, thiols and disulfide bonding are buffering systems in every cell.Dietary antioxidants are potent solution to overcome the action of ROS.The most important dietary antioxidants are Plant polyphenols, carotenoids, xanthophylls, flavonoids etc.Many studies have suggested that they exhibit strong antioxidant activity as they can reduce free radical formation and scavenge free radicals.[12,13] Antioxidant defense system against oxidative stress is composed of several lines, and the antioxidants are classified into four categories based on function. First line of defense comprises preventive antioxidants, which suppress formation of free radical (enzymes: glutathione peroxidase, catalase; selenoprotein, transferrin, ferritin, lactoferrin, carotenoids etc.) Second line of defense is the radical scavenging antioxidants suppressing chain initiation and/or breaking chain propagation reactions: radical scavenging antioxidants.Third line of defence consist of repair and de novo antioxidant (some proteolysis enzymes, repair enzymes of DNA etc)A fourth line of defence is an adaptation where the signal for the production and reactions of free radicals induces formation and transport of the appropriate antioxidant to the right site.



Beta-carotene

DNA damage: Types and causes

DNA is under constant attack from many sources: Radiation, ultraviolet light, and contaminants in our food and in our environment can all wreak havoc on our genetic material, potentially leading to cancer and other diseases.[14] A convenient biomarker of oxidative stress is the extent of oxidation of bases in DNA (8-Oxo-7,8-dihydroguanine) either chromatographically (gas chromatography-mass spectrometry, HPLC with electrochemical detection, or HPLC-tandem mass spectrometry)[15] or enzymically, with the use of the enzyme formamidopyrimidine DNA glycosylase to convert 8-oxo-7,8-dihydroguanine to DNA breaks, which are detected with alkaline elution, alkaline unwinding, or the comet assay.[16] DNA damage induction is a fundamental unavoidable process and plays a key role in cancer development and the induction of heritable genetic defects[17] DNA damaging agents (genotoxins) are nearly always identified as carcinogens. Humans are constantly exposed to natural DNA damaging agents such as sunlight, dietary agents (such as cooked meat, acrylamide) and endogenously formed oxygen free radicals.[18]

DNA Damages are physical abnormalities in the DNA, such as single and double strand breaks, 8-hydroxydeoxyguanosine residues and polycyclic aromatic hydrocarbon adducts. Enzymes can recognize DNA damages, and thus they can be correctly repaired if information, such as the undamaged sequence in the complementary DNA strand or in a homologous chromosome, is available for copying. DNA damage is caused due to interaction of DNA with ROS or RNS. Free radicals such as •OH and H• react with DNA by addition to bases or abstractions of hydrogen atoms from the sugar moiety. The C4-C5 double bond of pyrimidine being sensitive to attack by •OH, generates a spectrum of oxidative pyrimidine damage products, including thymine glycol, uracil glycol, urea residue,5-hydroxydeoxyuridine, 5-hydroxydeoxycytidineetc. Similarly, interaction of •OH with purines generate 8-hydroxydeoxyguanosine (8-OHdG), 8-hydroxydeoxyadenosine, formamidopyrimidines and other purine oxidative products. Several repair pathways repair DNA damage. 8-OHdG has been implicated in carcinogenesis and is considered a reliable marker for oxidative DNA damage. DNA damage accumulates in brain, muscle, liver, kidney, and in long-lived stem cell. These accumulated DNA damages are the likely cause of the decline in gene expression and loss of functional capacity observed with increasing age. Methods like HPLC-ECD, LC-MS/MS, total antioxidant capacity (FRAP, ORAC) are used to determine antioxidant defenses and biomarkers19 of oxidative stress in vitro and in vivo including antioxidant nutrients (ascorbate and tocopherols) and phytochemicals carotenoids and flavonoids.

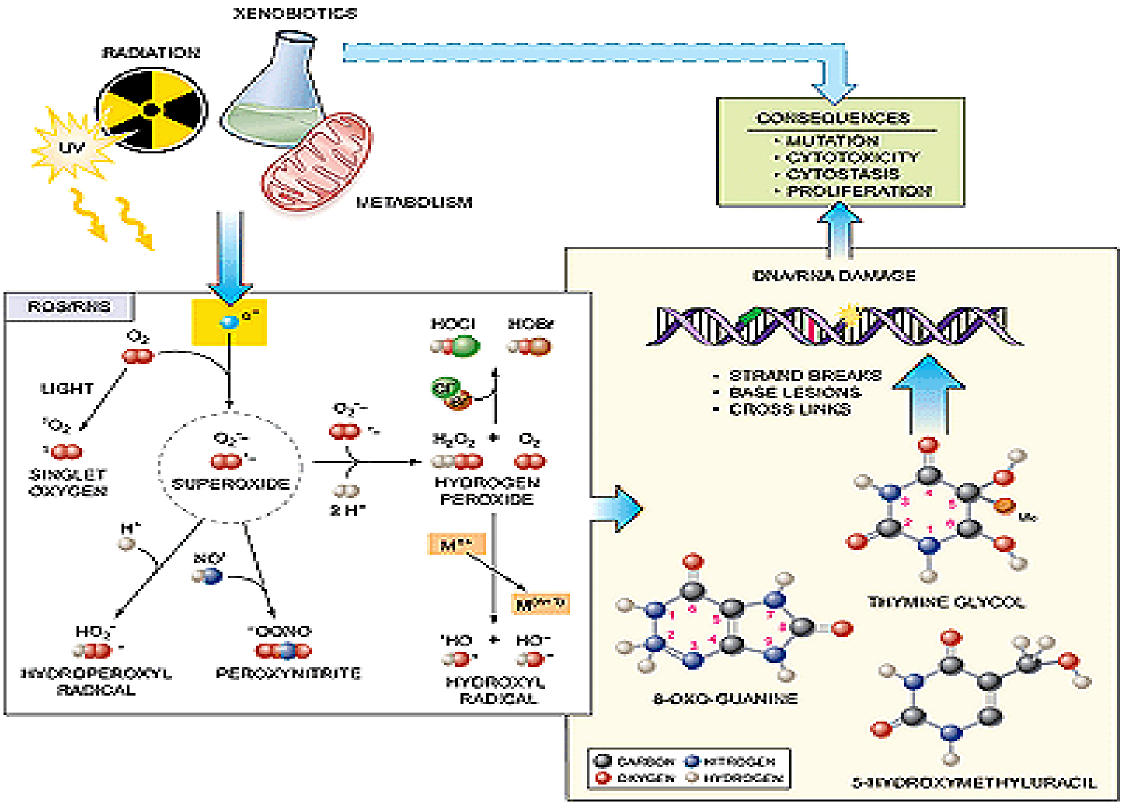


Fig. 1: Reactive oxygen species: causes and consequences [20]

Antioxidants Role to protect DNA damage

Natural compounds, especially derived from dietary sources provide a large number of antioxidants.[22] Some beverages such as tea and coffee are also rich sources of antioxidants.

Experimental as well as epidemiological data indicate that a variety of nutritional factors can act as antioxidants and inhibit the process of cancer development and reduce cancer

Risk. Some of these include vitamins A, C, E, beta-catotene, and micronutrients such as antioxidants and anticarcinogens.[23]Numerous epidemiological studies to date highlight the importance of consuming dietary products rich in antioxidants Recent studies in humans have shown that supplementation with antioxidant compounds such as vitamins E and C, lycopene and ß-carotene can help reduce levels of free-radical damage his lends support to the hypothesis that dietary products high in antioxidants potentially exert a protective effect against degenerative disorders, such as cancer, by a decrease in DNA damage .[24,25]

In a randomized crossover study of healthy nonsmoking males ages 27 to 40 years, Pool-Zobel et al. [26] found that supplementing the diet with tomato, carrot, or spinach products resulted in significantly decreased levels of endogenous strand breaks in lymphocyte DNA.[27,28,29]

Results from most observational studies provide support for a protective association of high dietary intakes and/or blood levels of antioxidant vitamins, especially β-carotene and vitamin C, on cancer risk and oxidative DNA damage. Several interventions with supplemental doses of antioxidants resulted in a significant decrease in endogenous DNA damage .[30,31]

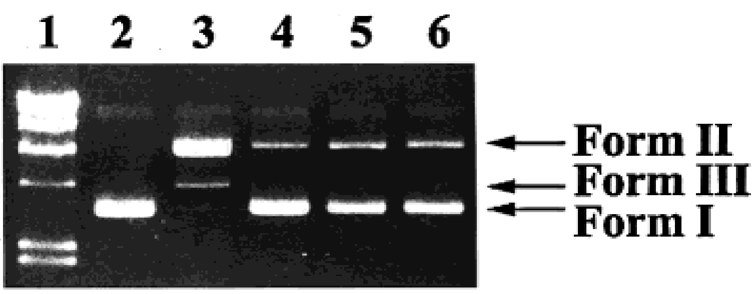


Table 1: Types of DNA damage[21]

Source of DNA	Potential damage	Comments	References
Ancient DNA	abasic sites, deaminated cytosine, oxidized bases, fragmentation, nicks	Cytosine deamination has been reported to be the most prevalent cause of sequencing artifacts in ancient DNA.	Gilbert, M.T. et al. (2007) Nuc. Acid Res., 35, 1–10. Hofreiter, M. et al. (2001) Nuc. Acid Res., 29, 4793.
Exposure to Ionizing Radiation	abasic sites, oxidized bases, fragmentation, nicks	Ionizing radiation is used to sterilize samples.	Sutherland, B.M. et al. (2000) Biochemistry, 39, 8026.
Exposure to Heat	fragmentation, nicks, abasic sites, oxidized bases, deaminated cytosine, cyclopurine lesions	Heating DNA accelerates the hydrolytic and oxidative reactions in aqueous solutions.	Bruskov, V.I. (2002) Nuc. Acids Res., 30, 1354.
Exposure to Light (UV)	thymine dimers, (cyclobutane pyrimidine dimers) pyrimidine (6–4) photo products	UV trans-illumination to visualize DNA causes thymine dimer formation.	Cadet, J. et al. (2005) Mutat. Res., 571, 3–17. Pfeifer, G.P. et al. (2005) Mutat. Res., 571, 19–31.
Mechanical Shearing	fragmentation, nicks	Normal DNA manipulations such as pipetting or mixing can shear or nick DNA.	
Storage in Aqueous Solution	abasic sites, oxidized bases, deaminated cytosine, nicks, fragmentation	Long term storage in aqueous solution causes the accumulation of DNA damage.	Lindahl, T. et al. (1972) Biochemistry, 11, 3610 and 3618.
Exposure to Formalin	DNA-DNA crosslinks, DNA-protein crosslinks	Formaldehyde solution that has not been properly buffered becomes acidic, increasing abasic site formation.	Workshop on recovering DNA from formalin preserved biological samples. (2006) The National Academies Press.

Inhibition of DNA damage by potent antioxidants

Initially a Fe^{3+}-dependent system is designed to test the scavenging activity of plant extracts on radicals generated by iron, because hydroxyl radicals are known to be the most reactive of all the reduced forms of dioxygen and are thought to initiate cell damage.[32,33] Next deoxyribose assay system is taken to confirm the antioxidant activity of potential extract. To the scavenging effect of plant extract on Fe^{3+}-dependent hydroxyl radicals, it was investigated whether the extract reduced Fe^{3+}-dependent DNA nicking. When pBR322 plasmid DNA was dissolved in the reaction mixture, a time-dependent increase in the formationof single-stranded nicked DNA (Form II) and of double-stranded nicked and linear DNA (Form III) was observed. However, the addition of 20 íg of OFS extract to the nicking reaction mixture increased Form I DNA formation Consequently, the treatment caused Fe3+-mediated Form III DNA formation to disappear and reduced Form II DNA formation, lane 4). This OFS ethanol extract-mediated antioxidant activity was similar to that of 2 U of superoxide dismutase (SOD) and 5 U of catalase, as shown in lanes 5 and 6, respectively. These results indicate that the OFS ethanol extract effectively mitigates the oxidative stresses on susceptible biomolecules, such as DNA.[34] Similarly extracts of Curcumin is a non-toxic, highly promising natural antioxidant compound having a wide spectrum of biological functions. Also Sunflower, Trapa, Onion, lotus extracts etc showed very good protection from DNA damage. [35] Lycopene probably acted by suppressing the oxidative stress caused due to high levels of iron that may be carcinogenic. A Brazilian study led by Matos[36] demonstrated that rats treated with a high level of iron (ferric nitrilotriacetate) significant increased DNA damage and malondialdehyde (indicator of lipid oxidation) level in the prostate. The pre-treatment of the rats with lycopene and beta-carotene reversed these effects and DNA damage was almost completely prevented.

Inhibitory effects of Opuntia ficus-indica (OFS) extract on DNA nicking caused by hydroxyl radicals. The DNA nicking reaction was initiated by adding 0.5 íg of pBR322 plasmid DNA to Fenton's reaction solution in the absence(lane 3) or presence (lane 4) of OFS extract for 30 min at 37 ^{0}C. Lanes1 and 2 show the ì/*Hin*dIII DNA marker and native plasmid DNA, respectively. Lanes 5 and 6 show the results for reaction mixtures containing 2 U of SOD and 5 U of catalase, respectively. *From J. Agric. Food Chem.* 2002, *50,* 6490–6496.[37]

Many research studies have identified potent plants that are effective against DNA damage.Pudina extract (*Mentha spicata* Linn.)[38] used as flavoring in culinary preparation throughout the plains of India was examined for its DNA damage protecting activity and antioxidant potential. . *n*-Butanol soluble fraction (PE) derived from methanol extract of *Mentha spicata* Linn. exhibited significant protecting activity against DNA strand scission by •OH on pBluescript II SK(−) DNA.Unani plants like *C. icosandra, R. damascena and C. scariosus* showed significant oxidative DNA damage preventive activity and antioxidant activity.70% methanol extract of the fruits of *Terminalia chebula, Terminalia belerica and Emblica officinalis*, imposes the fact that they might be useful as potent sources of natural antioxidant. In vitro and in vivo studies indicate that several botanicals such as *Gingko biloba, Centella asiatica, Hippophae rhamnoides, Ocimum sanctum, Panax ginseng, Podophyllum hexandrum, Amaranthus paniculatus, Emblica officinalis, Phyllanthus amarus, Piper longum, Tinospora cordifoila, Mentha arvensis, Mentha piperita, Syzygium cumini, Zingiber officinale, Ageratum conyzoides, Aegle marmelos and Aphanamixis polystachya ,Paeonia mffiticosa.* protect against radiation-induced lethality, lipid peroxidation and DNA damage.[39,40]

CONCLUSION

Free radicals can adversely affect many crucial biological molecules leading to loss of form and function leading to diseases. Antioxidants can protect against this damage. Dietary and other components of plants form major sources of antioxidants.Oxidants can react with DNA Bases or sugars.Guanine is the most sensitive base towards oxidative attack.Increasing understanding of the conditions under which each of these oxidants are produced and methods of inhibiting their formation will likely prove very important in preventing cancer and other diseases of previously unknown origin. Recent research centers on various strategies to protect crucial DNA from oxidative damage induced by free radicals [41,42] Many novel approaches are made and significant findings have come to light in the last few years. The traditional Indian diet, spices and medicinal plants are rich sources of natural antioxidants. Higher intake of foods high level of antioxidants in functional foods is one strategy that is gaining importance in advanced countries and is making its appearance in our country. Biomedical scientists, nutritionists and physicians together can make significant contribution in improving human health by their teamwork in the coming decades. High dietary intakes of antioxidant vitamins and phytochemicals are associated with better maintenance of physiologic function and a lower prevalence of many degenerative conditions in older adults. Antioxidants reduce oxidative stress and present opportunities for health promotion and alternative therapy as protection against DNA damage and ultimately cancer.[43]

Future prospects

Many randomized trial data are necessary to evaluate definitively the potential role of antioxidants in DNA damage.[44]Various in vivo experiments should be conducted to prove the antioxidant properties of plants against DNA damage. Not only this but genetic variation studies should also be performed in order to find if there is any difference in protective role of antioxidants in males and females. Recent research on the effect of antioxidants on DNA repair enzymes suggest that they remove oxidized purines, whereas mRNA levels of the relevant DNA repair genes appears to be unaffected by an antioxidant-rich diet. In the future intervention studies and considerations of genotypes of defence enzymes as well as DNA repair capacity should be kept in mind. Fruits and vegetables are rich in hundreds of phytochemicals that act as antioxidants but which component is responsible for the most beneficial effects is still unknown. Various factors are still not known so their protective role should be identified against DNA damage. Well-designed long-term research can determine whether any of these chemicals, taken in a pill, would be useful for preventing DNA damage leading to infertility and Cancer. There are many methods used to measure antioxidants and DNA Damage. A small alteration in methodology can vary the result by a great degree. So one should identify the four or five best methods and make the research result consistent. Dietary antioxidants are not very effective due to their poor solubility, inefficient permeability, and instability due to storage of food. Novel drug delivery systems (NDDS) like liposomes, microparticles, nanoparticles and gel-based would also help in delivery of these antioxidants by oral route, as this route is of prime importance when antioxidants are intended for prophylactic purpose against DNA damage. Some components of plants may act as prooxidant if taken at higher dose in presence of free transition metals. Future experiments should be aimed at purifying and characterizing the specific components that are responsible for its relatively high antioxidation activity.

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