Oxidants, antioxidants and carcinogenesis

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Free radical can be defined as chemical species possessing an unpaired electron, which is formed by homolytic cleavage of a covalent bond of a molecule, by the loss of a single electron from a normal molecule or by the addition of a single electron to a normal molecule. Most of the molecular oxygen consumed by aerobic cells during metabolism is reduced to water by using cytochrome oxidase in mitochondria. However, when oxygen is partially reduced it becomes 'activated' and reacts readily with a variety of biomolecules. This partial reduction occurs in one-electron steps, by addition of one, two, and four electrons to $O_2$, which leads to successive formation of reactive oxygen metabolites (ROMs). These are five possible species: $O_2^-$, hydroperoxy radical ($HO_2^\cdot$), peroxide ion ($HO_2^-$), hydrogen peroxide ($H_2O_2$) and hydroxyl radical ($OH^-$).

$$O_2 \rightarrow O_2^-$$

$$O_2^- \rightarrow HO_2^-$$

$$HO_2^- \rightarrow H_2O_2$$

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H$_2$O$_2$ $\rightarrow$ Fe$^{3+}$/Cu$^{2+}$ $\rightarrow$ OH + OH$^-$

The $O_2^-$ and $H_2O_2$ so formed, in presence of metal catalyst such as Cu$^{2+}$/Fe$^{3+}$, may lead to the formation of most reactive $OH^-$.$^3$ $O_2^-$ is reduced to $H_2O_2$ by the catalytic activity of superoxide dismutase (SOD). Another main enzymatic antioxidants, namely glutathione peroxidase (GPx) and catalase (CAT) again convert $H_2O_2$ to $H_2O$. The most potent enzyme is CAT. GPx and CAT are important in the inactivation of many environmental mutagens. CAT is also found to reduce the SCE levels and chromosomal aberrations. Antioxidative vitamins such as vitamin A, E, and C have a number of biological activities such as immune stimulation, inhibition of nitrosamine formation and an alteration of metabolic activations of carcinogens. They can prevent genetic changes by inhibiting DNA damage induced by the ROMs. Therefore, these antioxidants may be helpful in the treatment of human cancer. However, detailed studies are required to draw a definite conclusion.

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The most potent enzyme is CAT. $H_2O_2$ is further converted to $H_2O$ with the help of GPx and CAT. SOD inhibits $OH$ production. SOD also acts as anti proliferative agent, anticarcinogens, and inhibitor at initiation and promotion/ transformation stage in carcinogenesis. GPx is another antioxidative enzyme which catalyses to convert $H_2O_2$ to $H_2O$. The most potent enzyme is CAT. GPx and CAT are important in the inactivation of many environmental mutagens. CAT is also found to reduce the SCE levels and chromosomal aberrations. Antioxidative vitamins such as vitamin A, E, and C have a number of biological activities such as immune stimulation, inhibition of nitrosamine formation and an alteration of metabolic activations of carcinogens. They can prevent genetic changes by inhibiting DNA damage induced by the ROMs. Therefore, these antioxidants may be helpful in the treatment of human cancer. However, detailed studies are required to draw a definite conclusion.
is one of the most commonly occurring product of these DNA modifications. We discuss here the deleterious action of ROMs in relation to carcinogenesis, and various enzymatic and non-enzymatic antioxidants that prevent cellular and molecular damage caused by the ROMs.

1 Oxidants

**Superoxide anion (O$_2^-$, CAS# 11062-77-4)**

Superoxide anion is the first reduction product of O$_2$. It is a base with the equilibrium with its conjugate acid, the hydroperoxyl radical HO$_2^*$, whose pKa is 4.8. In aqueous solution, at neutral or slightly acid pH, O$_2^-$ is a relatively non-reactive species and dismutates to H$_2$O$_2$. This reaction either occurs spontaneously or is catalysed by intracellular enzyme SOD. It has been proposed that O$_2^-$ owing to its unreactivity can diffuse through a long way from its site. At low pH in the cell, it becomes protonated (HO$_2^*$) and, hence, reactive. The lifetime of O$_2^-$ in the water cellular environment is approximately 10$^8$s (ref. 13). O$_2^-$ can be produced either by the univalent reduction of O$_2$ or by the univalent oxidation of H$_2$O$_2$. The most important source of O$_2^-$ is oxidative enzymes, among which XO and NADPH/NADH oxidase are the most effective sources.

These enzymes possess flavin or transition metal such as Zn, Cu, Fe, which serve as electron donors. Several oxidative enzymes such as aldehyde oxidase and dihydroorotic dehydrogenase have been shown to produce substantial amounts of O$_2^-$ (ref. 16).

O$_2^-$ itself directly affects the activity of catalase and peroxidase. Experimental studies showed that O$_2^-$ directly affected some intracellular enzymes, changing their activities, such as epinephrine and creatine phosphokinase, lactate dehydrogenase-bound NADH, aconitase, 6-phosphogluconate dehydrogenase. Research demonstrated an increased production of O$_2^-$ during the proliferation of endothelial cells and involvement of this species in proliferation of B-lymphocytes. O$_2^-$ is able to cause the oxidation of epinephrine. It may also be capable of initiating the peroxidation of unsaturated lipids. It is also able to cause the oxidation of thiols. Thus, XO acting on xanthine in the presence of oxygen may cause the cooxidation of cysteine. Photochemical or enzymatic generation of O$_2^-$ resulted in an increase in chromosome breakage, rearrangement, and sister chromatid exchanges (SCEs). Thus O$_2^-$ may be one of the possible factors for increased risk of carcinogenesis. Our recent study found higher generation of O$_2^-$ in breast cancer patients.

**Hydrogen peroxide (H$_2$O$_2$; CAS# 7722-84-1)**

Hydrogen peroxide is the most stable ROMs. This is to say that it is the least reactive and the most readily detected. H$_2$O$_2$ may be generated directly by divalent reduction of O$_2$ or indirectly by univalent reduction of O$_2^*$. H$_2$O$_2$ is the primary product of the reduction of O$_2$ by numerous oxidases, such as XO, uricase, D-amino acid oxidase, and α-hydroxy acid oxidase localized in peroxisome. In any system producing O$_2^*$, substantial amount of H$_2$O$_2$ is formed. The H$_2$O$_2$ is decomposed, although not readily, to H$_2$O and O$_2$. H$_2$O$_2$ like most peroxides, is very sensitive to decomposition by the species that react with it. The reaction is catalysed by redox-active metal complexes, of which catalase and peroxidase are the most effective exponents. Metal ions have a strong effect on the chemistry of O$_2$ and its reduction products. Experiments with antioxidant enzymes show that H$_2$O$_2$, rather than O$_2^*$ is the more essential species to induce cell injury. Other researches also indicated H$_2$O$_2$ as the most effective species for cellular injury. It has been demonstrated that H$_2$O$_2$ stimulates proliferation of smooth muscle cells. Addition of exogenous H$_2$O$_2$ has been found to activate NF-$k$B. The well-known Fenton reaction is initiated when Fe$^{2+}$ comes in contact with H$_2$O$_2$. Ions of Cu, Co, and Ni can also participate in a similar reaction:

$\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \cdot\text{OH} + \text{OH}^-$

H$_2$O$_2$ also reacts with O$_2^-$ to initiate Haber-Weiss reaction producing $\cdot\text{OH}$ in presence of Fe$^{2+}$:

$\text{O}_2^- + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \cdot\text{OH} + \text{OH}^-$

Prise et al. demonstrated that DNA SSB was produced at relatively low concentrations in cells on ice without toxicity. However, 1-10 mmol H$_2$O$_2$ produced significant toxicity and caused DNA DSB in cells after 20 min. H$_2$O$_2$ has been found to be effective in activating DNA binding with NF-$k$B in vitro but not in vivo.

Certain estrogen metabolites such as catecholestrogens are involved in carcinogenesis, where H$_2$O$_2$ plays an important role. The most active catecholestrogens are the 4-hydroxy derivatives, which produce about 2.5 times more DNA DSB than the 2-hydroxy derivatives, while estradiol and 16α-hydroxyestrone are inactive. In addition, results show that 4-hydroxyestradiol (4-OHE$_2$) at physiological concentrations is capable of exhibiting DNA cleaving activity. The formation of these catecholestrogen-
induced DNA strand breaks have been associated with the utilization of O₂ and the generation of H₂O₂ (ref. 34). H₂O₂ exposed to cultured MCF-7 cells has been shown to inhibit binding of estrogen receptor to DNA.35

H₂O₂ has been associated with the induction of cancer in animals and has been found to induce molecular damage that leads to the formation of transformed cells in vitro.36 It has also been known to be mutagenic and carcinogenic.37 Studies also demonstrated that H₂O₂ stimulated the proliferation of smooth muscle cells.38 H₂O₂ is believed to be involved in initiation and promotion of carcinogenesis.39,40 Many reports suggested that H₂O₂ could induce DNA breaks in intact cell and purified DNA.41 H₂O₂ has been known to cause DNA damage in the form of SSBs and DSBs,42 chromosomal aberrations,43 and SCEs.44 The induction of chromosomal aberrations by H₂O₂ was also reported in a retrospective study.45 Significantly higher H₂O₂ concentration26 and SCEs41 have been reported in breast cancer patients. The studies found comparatively lower H₂O₂ concentration in stage IV than stage II, however the frequency of SCEs were found to be higher in stage IV. An experimental study suggested that H₂O₂ could induce higher frequency of SCEs when applied at low concentrations.46 Studies by Ray et al.47 also suggested that there might be an optimum concentration of H₂O₂ that could induce higher DNA damage and higher frequencies of SCEs in breast cancer.

**Hydroxyl radical (•OH)**

Hydroxyl radical is highly reactive. It can react with practically any molecule present in cells. For this reason it is short-lived. This insufficient stability does not allow it to diffuse through the cells. Therefore, it reacts with an organic substrate at the sites or near the sites of its formation. The life span of •OH at 37°C is 10⁻⁹ s. It does not survive for more than a few collisions after its formation. The reactions of •OH are thus site-specific. Due to such short life time, it is very difficult to investigate the •OH by conventional methods.48 This •OH is produced following the reaction of O₂⁻ and H₂O₂ in presence of metallic ions such as Fe²⁺/Cu²⁺. Lipid is very susceptible to •OH attack and initiate LPO.49 As a result of interaction of •OH with DNA, formation of many types of oxidized nucleoside have been reported. 8-OhdG is one of the most commonly occurring product of these DNA modifications.50 Formation of 8-OhdG is thought to be a promutagenic lesion since this induces G:C to T:A transversion unless repaired prior to replication.43 Cheng et al.51 further reported a negative correlation between the level of 8-OhdG and clinical stages as well as lymph node status of breast cancer. They also believed that because of the increased activities of repair enzymes for damaged DNA in advanced-staged breast cancer tissue, DNA adducts may be diluted by DNA replication during rapid cell turnover, which may be the reason for negative correlation between 8-OhdG levels and progression of breast cancer.44 •OH is the most potent among ROMs, reacting with a wide range of macromolecules at a high rate constant.45 •OH is known to induce conformational changes in DNA including strand breaks, base modifications, damage to tumour suppressor gene and enhanced expression of protooncogenes.46,47 •OH is responsible for DNA damage, high frequency of SCEs, and LPO.48

**Malondialdehyde (MDA; CAS #: 542-78-9)**

Malondialdehyde is the major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acid (PUFA)49. MDA, a secondary product of LPO, is used as an indicator of tissue damage by a series of chain reactions.50 MDA is also a by-product of prostaglandin biosynthesis.51 It reacts with thiobarbituric acid and produce red-coloured products. It has been proposed that the tumourigenic effect of high dietary fat, rich in PUFA takes place via increased synthesis of prostaglandin.52 It is said to be a product of normal metabolism, and is present in a variety of fat-containing foodstuffs.53 Furthermore, the direct interaction of DNA with lipid hydroperoxides produced via a chain reaction and/or other highly reactive compounds formed during LPO has also been proposed as plausible mechanism for the association between high dietary fat intake and carcinogenesis.54 It has also been proposed that the effect of dietary fat on cancer occurs through the activation of procarcinogens to ultimate carcinogens by fat oxidation products such as lipid hydroperoxides.55 MDA can modify xanthine oxidoreductase activity through interaction with XO and/or xanthine dehydrogenase (XDH).56 MDA is mutagenic and genotoxic agent that may contribute to the development of human cancer.57 Lipid hydroperoxides may directly induce DNA strand breaking,58 and lipid peroxyl and alkoxyl radicals may cause base oxidation in DNA.59 Figure 1 demonstrates the formation of lipid hydroperoxides and conjugate diene during lipid peroxidation of PUFA.
Peroxide and hydroperoxides have also demonstrated tumour-promoting activity in vivo. It can result in the formation cyclic DNA adduct, which contributes to the carcinogenicity and mutagenicity in mammalian cells. MDA induced mutations include frameshifts and base-pair substitutions. The frameshifts are G → T transversions and C → T and A → G transitions. Large body of evidence has suggested that MDA is a mutagen and a potential carcinogen.

There are reports that MDA can form DNA adducts which may be responsible for the development of breast cancer. Several reports have also suggested increased MDA levels in breast cancer patients. Higher MDA levels have also been reported in solid tumour and human cell lines. Significantly higher plasma MDA level was found in patients with gastric carcinoma. Higher plasma MDA level in breast cancer was also found by Ray et al. On the contrary, Gerber et al. demonstrated lower MDA concentration in breast cancer patients. They also demonstrated a negative correlation between MDA level and tumour size in the patients. There are number of observations that indicate an apparent negative correlation between levels of cellular LPO and rates of cell proliferation. A negative correlation was also established between plasma MDA level and progression of breast cancer.

**Nitric Oxide (NO; CAS# 10102-43-9)**

Nitric oxide is an inorganic free radical gas, containing odd number of electrons and can form a covalent link with other molecules by sharing a pair of electrons. It is synthesized by a family of isoenzymes called nitric oxide synthase (NOS; EC 1.14.13.39) located in various tissues, and plays an active role in free radical and tumour biology. There are three isoenzymes of NOS. Synthesis of NO from NOS-I and NOS-III is calcium-dependent, whereas NOS-II is calcium independent. The calcium-dependent NOSs (I and III) produce a small amount of NO (pmole), whereas much larger amounts of NO (nmole) are released by NOS-II in cells in response to cytokines, lipid lipopolysaccharide (LPS), immune complexes, endotoxins, some co-factors such as colmodulin, FAD, FMN, NADPH, and tetrahydrobiopterin. Figure 2 shows the formation of NO from NOS. NO plays a vital role as a cell signalling molecule in vascular, nervous and immune systems. It regulates numerous physiological processes, including neurotransmission, smooth muscle contractibility, platelet reactivity and the cytotoxic activities of immune cells. Prolonged exposure to NO inhibits the activity of number of enzymes such as aconitase, complexes I and II, and cytochrome c oxidase. On the other hand, excessive and upregulated NO synthesis has been implicated as causal or contributing to pathological conditions, including many lethal and debilitating diseases.

NO and its derivatives produced in inflamed tissues contribute to carcinogenesis. It is believed that NO plays a duel role in cancer. It is a cytostatic/cytotoxic agent, which causes tumour cell killing when generated at higher concentrations by cytokine-activated macrophages and endothelial cells, but comparatively at low concentrations, it promotes tumour growth and metastasis.

NO plays an active role in free radical and tumour biology. Moreover, it may have a role in carcinogenesis by inducing DNA strand breaks. It has been reported that NO can induce DSB by overlapping SSB in the chromosome. NO exerts direct damages including DNA base deamination, peroxynitrite-induced adducts formation and strand breaks in the DNA. NO is known to be a potential mutagen. It can bind nonheme iron of ribonucleotide reductase to
inhibit DNA synthesis. NO may have a role in carcinogenesis by impairing the tumour suppressor function of p53 (ref. 53). Ohue et al. hypothesised that NO may either generate or select for the high frequency of p53 mutations that arise at the transition from adenoma to carcinoma in situ. Exact mechanism by which NO affects the p53 functions is not clear. However, it is proposed that the p53 DNA-binding domain contains several cysteine residues, which play an important role in its DNA binding activity. As NO can modify cysteine residues leading to the formation of disulfide bonds, it can thus affect the biological function of p53. DNA damage triggers the accumulation of p53 protein. Forrester et al. reported that protein accumulates in human cells when it is exposed to NO. Deamination of DNA by NO may represent an important endogenous mechanism of genomic alteration. NO-mediated deamination of 5-methylcytosine produces thymine. Hence, the overrepresentation of point mutations in human disorders at methylated CpG sites and the high frequency of mutation at CpG sites in the p53 tumour suppressor gene in human cancers may reflect the etiological contributions of NO in human carcinogenesis.

ONO' produced during the reaction of NO and O2' is probably responsible for genetic damage. The reaction of O2' with NO, depending on the relative amounts present, can be 5 times faster than the decomposition of O2' by SOD. ONOO' is a potent mutagen that can induce transversion mutations (mainly G → T) at G-C pairs.

The effect of NO has been shown in Fig. 3. McRitchie et al. have investigated the role of estrogen in the NO pathway. Estrogen has been reported to upregulate eNOS gene expression. A correlation between eNOS expression and estrogen receptor (ER) expression has been reported in several human breast cancer cell lines. In ER-positive cells, it is thought that estradiol may enhance the production of NO, which then acts as a free radical to induce mutations leading to a more malignant phenotype.

Human endothelial cells may be activated/modulated by ROMs with the release of NO. It has been suggested that NO can stimulate O2'/H2O2/·OH-induced LPO. Human erythrocytes possess a NO synthase (NOS) that can be activated by oxidative stress and Ca2+ accumulation to produce NO, and this activation could be involved in the pathogenesis of toxic anaemia in breast cancer patients.

By causing oxidative stress in human erythrocytes with H2O2 or by increasing the intracellular calcium a gradual increase in both NO and ONOO- is observed. Furthermore, it has been shown that erythrocytes from breast cancer are subjected to higher oxidative stress by ONOO- (100 μmoles), with a consequential increase of membrane rigidity, than erythrocytes from healthy individuals. Such mechanical changes may result in shortening of the lifespan of erythrocytes, a feature of toxic anaemia in cancer patients. Research suggested that NO was directly involved in the increased frequency of SCEs. Reports, are also there which suggest that NO also plays important role in increasing frequencies of micronuclei, and chromosomal aberrations. In another report, NO was found to induce micronuclei. Increased levels of NOS expression and/or activity have also been reported in human gynaecological and breast tumours. In human gynaecological and breast cancer, the increased expression was inversely associated with the differentiation of tumour grade. Rajnakova et al. found that the expression of NOS was more abundant in early (T2) lesions than in advance (T4) ones in gastric cancer specimens. Studies using the well-characterized murine K-1735 melanoma system of clones, cell lines, and somatic cell hybrids (between non-metastatic and
metastatic cells) conclude that non-metastatic cells exhibit high levels of inducible NOS (iNOS), whereas metastatic cells do not. Ray et al. found significantly higher concentration of NO in breast cancer cases. Lower NO concentration in stage IV may be also because of L-arginine depleted cells which produce lower O$_2^-$ together with the production of NO. The O$_2^-$ and NO may result in the formation of OONO$^-$ which acts on molecular level and thereby resulting in higher SCE frequency in stage IV.

Experimental studies suggested that NO contributes to the destruction of circulating tumour cells. High NO concentrations are required to induce apoptosis in mammalian cells and low concentrations of NO protect from apoptotic cell death. High concentration of NO kills not only tumour cells but also the normal cells. However, experimental studies demonstrated that transfection of highly metastatic K-1735 murine melanoma cells (which express low iNOS) with an enzymatically active iNOS expression vector suppresses tumorigenicity and metastasis by inducing high levels of NO production. The systemic administration of MLV-31362 (with IFN-gamma) induces iNOS gene expression in M5076 hepatic metastases, which in turn resulted in their regression. To our knowledge, it is not clear whether metastatic breast tumour cell lines exhibit low NOS expression. If so, NOS gene therapy may be useful in breast cancer treatment.

Sources of oxygen free radicals

ROMs are generated by specialised phagocytic cells (neutrophils and macrophages) as cytotoxic agents to fight invading microorganisms, a process known as the respiratory or oxidative burst. For this purpose, phagocytes use the membrane-bound NADPH oxidase complex which catalyses the one-electron reduction O$_2$ to O$_2^-$, The NADPH oxidase of phagocytic leukocytes transfers electrons from NADPH at the cytosolic side of the membrane to molecular O$_2$ at the other side of the membrane. The NADPH oxidase contains plasma membrane cytochrome b$_{558}$ and at least four cytosolic factors. NADPH + 2O$_2$ → NADP$^+$ + 2O$_2^-$ + H$^+$ The ROMs are generated in biological systems, via several enzymatic and non-enzymatic pathways. A variety of mammalian cell types are able to produce ROMs after specific stimulation. The production of free radical from various sources has been illustrated in Fig. 4. In these cells there exists an enzyme that is similar to NADPH oxidase of phagocytes. The ROMs are also produced by electron leakage from the transport chain in mitochondria and endoplasmic

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Fig. 3—Mechanisms of NO genotoxicity. The genotoxicity of NO is due to its reaction either with oxygen or superoxide. The reaction lead to direct genotoxicity mediated by N$_2$O$_3$ and peroxynitrite, or to indirect genotoxicity due to activation of nitrosamines, apoptosis, inhibition of DNA repair enzymes, or LPO-induced DNA damage.
reticulum where molecular \( \text{O}_2 \) is sequentially reduced to \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \). A large amount of \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) are also produced by human tumour cells \(^{106}\). The major source of ROMs in tissue is probably hypoxanthine/xanthine oxidase system, and they are believed to be formed mainly during reperfusion following ischaemia.

Several enzymes, such as glucose oxidase, uric acid or amino acid oxidase in mammalian cells produce \( \text{H}_2\text{O}_2 \) directly \(^{109}\). The oxidative stress hypothesis of carcinogenesis asserts that many carcinogens can generate ROMs that damage cells, predisposing these cells to malignant conversions \(^{111}\). Recent evidence suggested that human eosinophil activated by tumour necrosis factor-alpha (TNF-\( \alpha \)) produces a significant amount of \( \text{H}_2\text{O}_2 \) (ref. 112). The cell free system containing the substrate hypoxanthine and xanthine oxidase may produce considerable amount of superoxide anions \(^{113}\).

**Xanthine oxidase (XO; EC: 1.1.3.22)**

Xanthine oxidase derives from xanthine dehydrogenase (XD), an initial translation product, by proteolysis \(^{114}\). Hypoxia has been shown to cause a significant increase in the XO-to-XD ratio, indicating a conversion XD to XO \(^{115}\). In diseased conditions, large amounts of XO and XD are believed to be released into circulation to produce significant amount of ROMs \(^{116}\). Studies reported that OONO\(_2\) was produced indirectly by co-incubation of NO with hypoxanthine/xanthine oxidase \(^{117}\). XO catalyses the oxidation of hypoxanthine to UA reducing \( \text{O}_2 \) by one or two electrons resulting in the formation of \( \text{O}_2^- \) or \( \text{H}_2\text{O}_2 \), respectively. Figure 5 indicates the formation of \( \text{O}_2^- \) from XO. It has also been demonstrated that XO produces \( \text{O}_2^- \) (ref. 118). Recent studies also described XO as a potential source of \( \text{O}_2^- \) (ref. 119).

XO is a well-characterized flavoenzymes containing transitional metals, such as molybdenum and iron, required for the catalysis. For XO, it is the \( \text{C}_8 \) position that undergoes oxidation in production of UA, which is then excreted. XO has been purified from buttermilk. The enzyme is a dimer of 260,000 mol wt. Each subunit has one molecule of FAD and one molecule of molybdenum. At the MoVI oxidation state there are also 4 atoms of iron and equivalent amount of inorganic sulphide releasable with UA. XO is reported to be cytotoxic. XO has been shown to reduce iron.

![Fig. 4 — Production of ROMs from various sources](image-url)
from ferritin\textsuperscript{120}. In the cell free system containing the substrate hypoxanthine, XO can produce considerable amount of \( \text{O}_2^- \), which may cause DNA-strand breaks\textsuperscript{113}. Recent studies suggested that XO is the principal mediator of remote tissue injury (lung, heart, liver)\textsuperscript{121}. XO generates ROMs as by-product while catalysing their reactions\textsuperscript{122}.

**Antioxidant**

Oxygen is essential for aerobic life process. However, cells under aerobic condition are threatened with the insult of ROMs that are efficiently taken care of by the powerful antioxidant system in human body. Aerobic life is characterized as continuous production of oxidants balanced by equivalent synthesis of antioxidants\textsuperscript{123}. A shift of the balance on the oxidant side may trigger a cascade of reaction leading to the formation of highly reactive cytotoxic compounds such as ROMs\textsuperscript{8}. The improper balance between ROMs production and antioxidant defences results in "oxidative stress", which deregulates the cellular functions leading to various pathological conditions including cancer\textsuperscript{124}. ROMs overproduction induced by different exogenous and endogenous mechanism may exhaust the antioxidant system of cells and contribute to a number of destructive processes and diseases, including cancer\textsuperscript{125}. Epidemiological studies have suggested that high endogenous level of oxidative adducts and deficiencies in antioxidant levels are likely to be important risk factors for cancer\textsuperscript{126}. In order to counteract the lethal effects of oxidative damage of DNA, normal living cells have developed multiple antioxidative defences and DNA repair systems\textsuperscript{127}.

**Enzymatic antioxidant**

The first lines of defence against \( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \) mediated injury are antioxidant enzymes: SOD, GPx, and CAT. The term antioxidant has been defined by Getteride and Halliwell\textsuperscript{3} as “any substance that delays or inhibits oxidative damage to a target molecule”. Antioxidant enzymes, together with the substances that are capable of either reducing ROMs or preventing their formation, form a powerful reducing buffer which affects the ability of the cell to counteract the action of oxygen metabolites. All reducing agents thereby form the protective mechanisms, which maintain the lowest possible levels of ROMs inside the cell\textsuperscript{128}.

**Superoxide dismutase (SOD; EC: 1.15.1.1)**

SODs are a family of metalloenzymes that convert \( \text{O}_2^- \) to \( \text{H}_2\text{O}_2 \) according to the following reaction:

\[
\text{O}_2^- + \text{O}_2^- \xrightarrow{\text{SOD}} \text{H}_2\text{O}_2 + \text{O}_2
\]

SOD is the most important enzyme because it is found virtually in all aerobic organisms. There are four families of SOD: Cu-SOD, Cu-Zn-SOD, Mn-SOD, and Fe-SOD. Human SOD is the Cu-Zn-SOD enzyme. The transition metal of the enzyme reacts with \( \text{O}_2^- \) taking its electron. \( \text{O}_2^- \) is the only known substrate for SOD. Each type of SOD has its own peculiarities; however, all types of the enzyme have similar properties\textsuperscript{129}.

Cu–Zn–SOD is found in the cytosol of most eukaryotic cell. A different form of Cu–Zn–SOD is found in extra cellular fluids, where it is called EC-SOD\textsuperscript{30}. Mn–SOD is located in the mitochondrial matrix and bacteria, while Fe–SOD is present in many aerobic bacteria. Cu–Zn–SOD is sensitive to cyanide but resistant to chloroform-ethanol treatment. In contrast Mn–SOD is resistant to cyanide, but is destroyed by the treatment with chloroform plus ethanol\textsuperscript{131}. Human encoding Cu,ZnSOD and MnSOD are found on chromosome 21q22.1 and 6q21, respectively\textsuperscript{132}. Moreover, SOD is considered to be a stress protein.
which is synthesized in response to oxidative stress\(^ {133}\). SOD has been detected in a large number of tissues and organisms, and is thought that it is present to protect the cell from damage caused by \(O_2^+\) (ref. 134). \(\cdot OH\) or \(Fe(II)O\) generated from the metal-catalysed interaction of \(O_2^+\) with \(H_2O_2\), the in vitro process is inhibited by SOD, or catalase, or by chelating agents. The Cu–Zn–SOD is reported to inhibit \(\cdot OH\) production. The possible mechanism is that \(H_2O_2\) rapidly reduces \(Cu\) (II) at the active site and then more slowly inactivated the reduced enzyme. The inactivation could be prevented by xanthine, urate and formate\(^ {135}\). SOD inhibits nuclear transcription factor AP-1 and NF-xB in human breast cancer cell\(^ {136}\). During oxidative stress, cell responds to ROMs with SOD\(^ {137}\). Accumulation of \(O_2^+\) and \(H_2O_2\) results in the production of \(\cdot OH\) by a metal-catalysed reaction. SOD is reported to inhibit \(\cdot OH\) production\(^ {133}\). SOD can act as anticarcinogens, and inhibitor at initiation and promotion/transformation stage in carcinogenesis. Mutation caused by potassium superoxide in mammalian cells can be blocked by SOD\(^ {138}\). From the results of experiments with ROMs and antioxidant enzymes, Simon et al.\(^ {139}\) however concluded that elevation of intracellular SOD increased the cell damage, allowing more \(H_2O_2\) to be generated.

Increased plasma levels of various antioxidants enzymes in cancer patients has been suggested\(^ {140}\). SOD activity increased significantly in Hodgkin's disease\(^ {141}\) and leukaemia\(^ {142}\). Significantly high SOD was also found in breast cancer\(^ {143}\). Breast cancer patients have been reported to possess increased levels of plasma copper and zinc\(^ {144}\), which further support increased Zn–Cu–SOD activity in breast cancer patients. Elevated SOD activity in various diseases including breast cancer has been reported\(^ {145}\). The exact cause of this elevation in SOD activity in these studies is not clear. Production of ROMs has been found to be higher in various pathological conditions. To counter the deleterious action of ROMs, antioxidant enzymes are also synthesized in response to the higher production of ROMs\(^ {133}\). SOD, a primary enzymatic antioxidant, mainly dismutates \(O_2^+\) to \(H_2O_2\). Thus, increased production of SOD in various genetic diseases may be in response of higher production ROMs in those diseases.

**Glutathione peroxidase (GPx; EC: 1.11.1.9)**

Glutathione peroxidase enzyme is a well-known first line of defence against oxidative stress, which in turn requires glutathione as a cofactor. Among the many functions of glutathione, it is involved in the generation of the nucleotide precursors of DNA via the reduction of ribonucleotides to deoxyribonucleotides\(^ {146}\). GPx catalyses the oxidation of GSH to GSSG at the expense of \(H_2O_2\). By its selenium (Se)-dependency, GPx can be divided in to two forms; Se-dependent GPx and Se-independent GPx. The former is a tetramer of MW 84000 with high activity toward both \(H_2O_2\) and organic hydroperoxides. It is found in both cytosol (70%) and mitochondria (30%) of various tissues. Iodoacetate, cyanide and \(O_2^+\) are considered as inhibitors of this enzyme\(^ {147}\). The gene encoding Se-dependent GPx is located at chromosome 3p13-q12, while the genes encoding Se-independent GPx are found to be on 6p12.2 and 11q-13ter\(^ {132}\). Since selenium is an integral component of GPx, the measurement of this enzyme has been used as a functional index of selenium level, GPx activity being reduced in selenium deficiency\(^ {148}\). Low levels of selenium have been associated with a high risk of cardiovascular diseases and cancer in humans\(^ {149}\).

GPx catalyses the oxidation of GSH to GSSG. This reaction occurs at the expense of \(H_2O_2\). Plasma GPx activity was found to be significantly elevated with respect to the controls in breast cancer patients regardless of clinical stages and menopausal status\(^ {26}\). The reason of higher GPx activity in breast cancer patients may be in response to higher productions of ROMs and increased activity of SOD in those patients. Whether there is any correlation of GPx and SOD in breast cancer patients is unknown. However, reports support the fact that higher SOD activity may be responsible, in part, for higher GPx activity in breast cancer patients. It is found that overexpression of the Cu–Zn–SOD gene in transfected cells can induce GPx activity\(^ {150}\). There is an overexpression of the cDNA for the major cytoplasmic glutathione peroxidase isoenzyme, GSH Peroxidase 1 (GSHPx-1), in human MCF-7 breast cancer cells\(^ {151}\), which may be the cause of higher level of GPx in breast cancer. Di Ilio et al.\(^ {152}\) reported higher GPx activity in human breast tumour tissue than non-tumour tissue. Therefore, higher GPx may be an indicator of malignancy. There are also many contrasting reports suggesting its effective in patients with regard to antioxidant activity. Junod\(^ {28}\) demonstrated that the increased GPx activity of endothelial cell had no protective effect against the hyperoxia-induced inhibition of DNA synthesis\(^ {28}\). The human breast adenocarcinoma cell line MCF-7WT showed low GPx activity. The cell line transfected with a plasmid that contains the cDNA for human...
Catalase (CAT; EC: 1.11.1.6)

Catalase is an enzyme, which is present in most cells, and catalyses the decomposition of hydrogen peroxide to water and oxygen. CAT is a heme-containing protein. The mechanism of the action is:

$$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$$

CAT is found to act 10^4 times faster than peroxidase. It is localized mainly in mitochondria and in subcellular respiratory organelles. CAT is present in peroxisome (80%) and cytosol (20%). It has 240,000 molecular weight and consists of four protein subunits, each containing a heme Fe(III)-protoporphyrin group bound to its active site. The gene encodes human CAT found on chromosome 11p13 (ref. 132).

Catalase catalyses the decomposition of H₂O₂ to H₂O and O₂. Most of the in vitro studies suggested that this antioxidant functions as promotion/ transformation inhibitor in carcinogenesis. CAT is found to reduce SCE levels resulting from treatment with H₂O₂ (ref. 39). GPx and CAT were found to be important in the inactivation of many environmental mutagens. Plasmid DNA strand scission caused by xanthine/XO has been reported to be prevented by both SOD and CAT enzymes. CAT also prevented chromosomal aberration caused by hypoxanthine/XO in Chinese hamster cells. It inhibited the onset of spontaneous neoplastic transformation in mouse fibroblast and epidermal keratinocytes. In BALB/c3T3 cells, SOD and CAT can inhibit TPA (12-O-tetradecanoyl-phorbol-13-acetate)-enhanced transformation.

Plasma CAT activity in breast cancer patients was significantly decreased irrespective of clinical stages and menopausal status when compared with controls. The decreased CAT activity in the present study may be due to higher ROMs production, especially O₂·. Reports suggested that O₂· itself affected directly the CAT activity. It has also been reported that while CAT is inactivated by ·OH, GPx and SOD are considerably less affected by the ROMs (ref. 160). Increased rate of ROMs production frequently elicits, as a response, an increase in the level of antioxidants. Under high rate of free radicals input, the enzyme inactivation prevails and the enzymatic activities are reduced leading to autocatalysis of oxidative damage process. There are reports that many tumours appear to have a decrease in the expression of antioxidant enzymes. In breast cancer tissue CAT activity was found decreased, while the activities of SOD and GPx were elevated. CAT activity was also reported to be lower in tumour than in normal tissue. Analysis of carcinoma cells from human breast and colon revealed significantly lower activity of peroxisomal enzymes, such as CAT, urate oxidase, and fatty acyl CoA oxidase, than in the adjacent healthy mucosa. Zigman et al. suggested that ·OH may be involved in the decreased lens CAT activity elicited by UV-A exposure. Moreover, an inverse relationship was reported between such enzyme activities and tumour grade. Ray et al. study showed maximum depression in CAT activity in stage II (70.1%) breast cancer patients as compared with the controls. The exact cause of the maximum depression in CAT activity is not clear. However, in this study Ray et al. have found the maximum elevations in O₂· production (91.6%) and MDA level (42.5%) in stage II. As MDA is an index of LPO caused by mainly ·OH radicals, it may be suggested that the cause of maximum depression in CAT activity in stage II may be due to the maximum generation of O₂· and ·OH in breast cancer patients. Sun hypothesized that anti-tumour function of antioxidants may be more convincing if the gene or cDNA for the enzyme transfected into cells to elevate endogenous levels of the enzyme.

Antioxidative vitamins

Antioxidative vitamins have a number of biological activities such as immune stimulation, inhibition of nitrosamine formation and an alteration of metabolic activations of carcinogens. They can prevent genetic changes by inhibiting DNA damage induced by the ROMs. Luputescu indicated that cancer cells synthesized an increased amount of DNA, RNA and proteins as compared to normal cells which may be controlled by the administration of vitamins. Another case-control study suggested inverse association of vitamins A, C and E and the risk of breast cancer. Several studies have also observed inverse associations between β-carotene, α-tocopherol, and vitamin C.
and breast cancer risk. The major protective function of the vitamins against cancer is the scavenging of ROMs.

**Vitamin A (CAS# 11103-57-4)**

Vitamin A is a fat-soluble vitamin, which is essential for growth, maintenance of visual function, reproduction and differentiation of epithelial tissue. The naturally occurring preformed vitamins include the compounds retinol and its esters, retinylaldehyde and retinoic acid. Vitamin A occurs mainly as the alcohol (retinol) in plasma and circulates as a 1:1:1 complex with two hepatically synthesised proteins, retinol binding protein (RBP) and transthyretin (or thyroxine-binding pre-albumin). The amount in the circulation remains almost constant as the body stores decline during a period of deficiency, until the liver stores become too low to maintain this normal circulating level in the plasma.

Vitamin A is reported to play a vital role in suppressing carcinogenesis by increasing immunity to tumours through several mechanisms. Vitamin A deficiency has been associated with a higher incidence of cancer and increased carcinogenesis. A number of epidemiological studies have shown that low dietary intakes of vitamin A or carotenoids were correlated with the increased incidence of mortality from lung or breast cancer. A significant inverse association between vitamin A precursor, β-carotene and carotenoids, and the risk of breast cancer has been established. Enzymes, such as cytochrome P450IIIC8, have shown to be involved in vitamin A metabolism. This may participate in maintaining the delicate balance between retinol concentrations that promote cellular integrity, opposing the development of cancer, and those that cause cellular toxicity.

Experiments with laboratory animals suggested that vitamin A deficiency may enhance susceptibility to certain forms of chemical carcinogenesis. Vitamin A deficiency promotes carcinogenesis, but paradoxically, an excess of vitamin A may have a similar effect. The association between vitamin A and the risk of breast cancer is controversial. DeCarli et al. noted that foods providing large amount of retinol increase the risk of cancer of the oesophagus.

Vitamin A and its metabolites play a crucial role in regulating the differentiation and proliferation of epithelial cells. Both natural and synthetic analogues of vitamin A have been shown to be effective in suppressing micro nucleated cells, reversing oral leukoplakia, and preventing new and recurrent lesions in subjects with oral leukoplakia, as well as in reducing the occurrence of head and neck cancer. Vitamin A derivatives (retinoids) are potent regulators of embryogenesis, cell proliferation, epithelial cell differentiation and carcinogenesis. Retinoids are vitamin A-related compounds that have been found to prevent cancer in animals and humans. Retinoids have been shown to suppress the growth and prevent the development of breast cancer in animals. These agents suppress tumorigenesis in carcinogen-treated rats and in
The major function of vitamin E is its role as an antioxidant, which plays an important role in various stages of carcinogenesis through its contribution to immune competence, membrane and DNA repair, and decreasing oxidative DNA damage. Vitamin E can directly scavenge ROMs. It is the major lipid-soluble antioxidant present in all cellular membranes which protects against LPO. Vitamin E can directly act with a variety of oxy radicals, including the peroxy radical (ROO·), CCl₃·, •OH, O₂⁻ and singlet oxygen. The major function of vitamin E is its role as a physiological membrane bound antioxidant, protecting cell membrane lipids from oxidative damage initiated by ROMs. Vitamin E donates hydrogen from the 6 position of its chromol ring to free radical. The ROMs subtract H atom from the PUFA in the cell membrane. The fatty acid radical formed reacts with oxygen that recycles to form more peroxy radicals in a chain reaction. The phenolic hydroxyl group of tocopherol reacts with an organic peroxy radical to form the corresponding organic hydroperoxides and the tocopheroxyl radical. It can reduce nitrite by inhibiting the production of carcinogenic nitrosamines and nitrosomides. It may also potentiate the immune response.

In vitro studies showed that vitamin E can prevent oxidation of DNA by inhibiting activated neutrophils. Vitamin E can protect the conjugated double bond of β-carotene from oxidation. The sparing action of tocopherol on β-carotene was described in vivo in humans by Urbach et al. Vitamin E can protect against many of the symptoms of selenium deficiency. These sparing as well as synergistic actions are thought to result from the ability of both tocopherol and selenium-dependent GPxs to decrease the production of LPO.

AH: Vit E-O · + AH → Vit E-OH + A

The lipophilic antioxidant, α-tocopherol has been shown to protect LDL from oxidation. α-tocopherol has additional biological effects, including effects at the intracellular level. It inhibits protein kinase C and may also play a role in preventing activation of intracellular redox-sensitive signal transduction pathways such as NF-κB. Via these mechanisms, α-tocopherol may influence cellular functions such as cell proliferation, platelet aggregation, cellular superoxide and cytokine production, and adhesion molecule expression. Protein kinase C-dependent generation of O₂⁻, e.g. induced by phorbol 12-myristate 13-acetate (PMA), have reported to be inhibited by addition of α-tocopherol. Cachea et al. suggested that in human monocytes preincubated with α-tocopherol, reduced protein kinase C activity results in impaired assembly of NADPH-oxidase. After supplementation with α-tocopherol, van Tits et al. found a decreased capacity of PMNs to generate O₂⁻ in response to PMA. The concentration of α-tocopherol in plasma and LDL had increased 2-3-fold and the α-tocopherol content of leukocytes may have increased to the same extent. In a study by Devraj et al., a similar increase in plasma α-tocopherol led...
to a 2.5-fold increase in monocyte \( \alpha \)-tocopherol content. Concomitantly, release of ROMs by monocytes in response to LPS was reduced. Several reports described the effects of \( \alpha \)-tocopherol on cytokines. \( \alpha \)-tocopherol has been shown to inhibit PMA-induced IL-1B expression in the human monocytic leukaemia cell line THP-1 and LPS-induced activation of rat Kupffer cells \textit{in vitro}^{204}.

Ray \textit{et al.}^{41} have shown lower vitamin E concentration in breast cancer patients. Gerber \textit{et al.}^{205} also reported significantly lower vitamin E levels in breast cancer cases than in controls. However, studies also suggested direct associations of dietary intake of vitamin E and breast cancer\(^{206}\). Basu \textit{et al.}^{207} however did not find any significant association between vitamin E intake and breast cancer risk. London \textit{et al.}^{208} suggested a decreased risk of breast with increased vitamin E intake from food sources. E circulates in blood predominantly with the LDL-C fraction. Ito \textit{et al.}^{209} found that serum vitamin A and vitamin E levels among breast cancer cases were not significantly different from those in postmenopausal healthy controls, but were higher than those in controls.

**Vitamin C (CAS # 50-81-7)**

Vitamin C (ascorbic acid) is an important water-soluble antioxidant in biological fluids and an essential micronutrient required for normal metabolic functioning of the body\(^{209}\). It readily oxidises to dehydroascorbic acid (Fig. 7). Human have no ability to synthesise vitamin C due to mutation in the gene coding for L-gulonolactone oxidase, an enzyme required for the biosynthesis of vitamin via the glucuronic acid pathway\(^{210}\). Thus, vitamin C is obtained through the diet. The vitamin is especially plentiful in fresh fruit, in particular, citrus fruit and vegetables\(^{211}\). The molecular mechanisms of the antiscorbutic effect of vitamin C are not completely known. Vitamin C is co-factor for several enzymes involved in the biosynthesis of collagen, carotene, and neurotransmitters\(^{212}\).

Vitamin C has been implicated for steroid metabolism in adrenal. Hydroxylation of aromatic drugs and carcinogens by hepatic cytochrome P450 is also enhanced by reducing agents such as vitamin C\(^{213}\). The temporal order of antioxidant consumption in human with blood plasma exposed to a constant flux of aqueous peroxyl radicals is vitamin C > bilirubin > uric acid > vitamin E. Plasma devoid of vitamin C, but no other endogenous antioxidant, is extremely vulnerable to oxidant stress and susceptible to peroxidative damage to lipids\(^{214}\). Vitamin C readily scavenges ROMs, ozone, ONOO\(^{-}\), NO\(_2\), NO, and hypochlorous acid\(^{215}\). Vitamin C neutralizes ROMs, and reduces oxidative DNA damage and genetic mutations\(^{216}\). It has also been reported that vitamin C may enhance host immunological functions\(^{216}\). Epidemiological studies have indicated an inverse association between vitamin C intake and the risk of cancers\(^{217}\). It can prevent carcinogenic nitrosamine formation in cancer, which is another protective function of vitamin C\(^{218}\). It can also protect lipid and lipoprotein against oxidative damage\(^{219}\). Vitamin C can act as a co-antioxidant by re-generating \( \alpha \)-tocopherol from the \( \alpha \)-tocopherol radical produced during scavenging of ROMs\(^{220}\). In addition, vitamin C may reduce carcinogenesis through the stimulation of immune systems, where cytotoxic T lymphocytes, macrophages, and natural killer cells can lyse tumor cells\(^{211}\). Vitamin C has also been shown to regenerate urate, glutathione, and \( \beta \)-carotene \textit{in vitro} from their respective one-electron oxidation product, i.e. urate radicals, glutathyl radicals, and \( \beta \)-carotene radical cations\(^{221}\). Vitamin C may modulate the activity of hydroxymethylglutaryl-CoA reductase, the rate-limiting enzyme in the biosynthesis of cholesterol\(^{222}\). Although vitamin C also reacts rapidly with \( \cdot \text{OH} \) (rate constant \( >10^9 \text{L.mol}^{-1}.\text{s}^{-1} \)), it is nevertheless unable to preferentially scavenge this radical over other substrates\(^{223}\). The reason for this is that \( \cdot \text{OH} \) is extremely reactive and combines indiscriminately with any substrate in their immediate environment at a diffusion-limited rate\(^{224}\). Vitamin C may prevent the formation ONOO\(^{-}\) by reaction with \( \text{O}_2^- \) and may help to release NO from endothelial cell by preventing the oxidation of LDL\(^{225}\). For this reaction high concentrations of vitamin C are required because of large difference in rate constants (\( 10^5 \text{ L.mol}^{-1}.\text{s}^{-1} \) at pH 7.4 for \( \text{O}_2^- \) with vitamin C compared with \( 10^9 \text{ L.mol}^{-1}.\text{s}^{-1} \) for \( \text{O}_2^- \) with NO).

Vitamin C may protect cell against carcinogenesis through several mechanisms in addition to inhibition of DNA oxidation. DNA oxidation, as determined by 8OhdG in cells, is increased in case of oxidative stress and is correlated with reduced plasma concentration of the antioxidative vitamin C and E\(^{226}\). One potential mechanism is chemoprotection against mutagenic compounds such as nitrosamines. \( N \)-Nitroso compound undergo activation by cytochrome P450-dependent enzymes and have been implicated in gastric and lung cancer\(^{217}\). Vitamin C can protect host cells against harmful oxidants released into the extracellular medium. Vitamin C may also affect the
production of immune proteins such as cytokines and antibodies as well as complement components. Ray et al. showed significantly lower plasma vitamin C level in breast cancer patients. The exact cause of lower vitamin C in these patients is not known. There are reports that $O_2^-$ plays an important role in the oxidation of vitamin C. Ray et al. in another study showed that production of $O_2^-$ was significantly higher in breast cancer patients. Thus higher $O_2^-$ production may be one of the possible causes of low plasma vitamin C in breast cancer patients. There are reports suggesting that vitamin C may prevent the formation of ONOO- by reaction with $O_2^-$ and may help to release NO from endothelial cell. Serum vitamin C has been shown to decrease in colon, breast, and stomach cancers in many studies. Thangaraju et al. also reported a significantly decreased concentration of serum vitamin C among breast cancer patients. Decreased vitamin levels were associated with increased LPO in the serum in patients with gastric carcinoma. Supplementation of vitamin C (100mg/d), vitamin E (200mg/d) and B-carotene (25mg/d) for 20 weeks significantly decreases endogenous oxidative DNA damage in human lymphocytes. A study also reported a positive correlation between vitamin A, E, and C intake and the risk of breast cancer. The intake of B-carotene, vitamin C and vitamin E in relation to breast cancer risk in a case-control study in Greece was assessed in 820 women with histologically confirmed breast cancer compared with 1548 control women. Among postmenopausal women, there was no association between any of the micronutrients evaluated and risk of breast cancer. However, among premenopausal women, B-carotene, vitamin C and vitamin E were inversely associated with breast cancer risk.

Conclusion

ROMs, such as superoxide anions ($O_2^-$) hydrogen peroxide (H$_2$O$_2$), and hydroxyl radical ($OH$), malondialdehyde (MDA) and nitric oxide (NO) are involved in initiation, promotion and progression of carcinogenesis. Their increased productions may cause cellular and molecular damage leading to LPO as well as mutations of tumour suppressor genes or the genes of antioxidant enzyme. $O_2^-$, H$_2$O$_2$ and $OH$ are reported to be involved in higher frequencies of chromosomal aberrations such as SCEs and CBGs. MDA is believed to produce DNA adduct which are responsible for cancer progression. The formation of ROMs in cancer is found to be higher as compared to the normal healthy controls. The exact cause of these higher ROMs production is not clear. One of the major sources, such as XO is found to be higher in cancer patients. These higher production of ROMs may also result from higher phagocytic activity, which is one of the potent sources of ROMs production. The higher ROM production may also be indicated by the higher MDA production in cancer patients. NO, at high concentration, kills tumour cells, and, at low concentration, it promotes tumour growth and metastasis. NO level are usually found to be higher in cancer patients. However, NO level was inversely correlated with the severity of breast cancer.

Antioxidant enzymes such as SOD, GPx, and CAT can directly counter the oxidant attack and may protect cells against LPO and DNA damage. SOD inhibits $OH$ production. SOD has been shown to inhibit nuclear transcription factor in human cancer cell that acts as antiproliferative agent. SOD also act as anticarcinogens, and inhibitor at initiation and promotion/ transformation stage in carcinogenesis. SOD activity is found to be significantly higher in cancer patients, including breast cancer. Higher SOD activity may be because of higher ROMs production. GPx is another antioxidative enzyme which catalyses to convert H$_2$O$_2$, to H$_2$O. The most potent enzyme is CAT. GPx and CAT are important in the inactivation of many environmental mutagens. CAT is found to reduce the SCE levels. CAT activity is found to be significantly lower in the cancer patients than in the controls. This inactivation of CAT may be due to higher $OH$ production, which may adversely affect the activity of CAT. Antioxidative vitamins such as vitamin A, E, and C also have a number of biological activities such as immune stimulation, inhibition of nitrosamine formation and alteration of metabolic activations of carcinogens. They can prevent genetic changes by inhibiting DNA damage induced by the ROMs. Vitamin E and C were found to be significantly lower in cancer, particularly in breast cancer. The elevation in ROM production and decreased levels of antioxidants in cancer indicate oxidative stress, which may be the cause of LPO, DNA damage, and mutation leading to higher pathology of cancer patients. Therefore, antioxidant therapy may be helpful in the treatment of human cancer.

References


37 Birnboim H C, DNA strand breaks in human leukocytes induced by superoxide anion, hydrogen peroxide and tumor promoters are repaired slowly compared to breaks induced by ionizing radiation, *Carcinogenesis*, 7 (1986) 1511.


Thangaraju M, Vijaayakalakshmi T & Sachdanandam P, Effect of tamoxifen on lipid peroxide and antioxidative system in postmenopausal women with breast cancer, Cancer, 74 (1994) 73.


Maxwell SRJ, Prospect for the use of antioxidant therapies, Drug, 49 (1994) 345.


