Research Journal of Pharmacology 5 (5): 59-67, 2011
ISSN: 1815-9362

Significance of Oxidative Stress and the Role of Anti Oxidants in Cancer

Ramesh Sapariya and Parloop Bhatt
Department of Pharmacology, L.M. College of Pharmacy,
380009 Ahmedabad, Gujarat, India

Abstract: During normal cellular activities, various processes inside of cells produce reactive oxygen species like hydrogen peroxide, superoxideio and hydroxide radical. These radicals when present in high concentration, it damage cellular proteins and lipids or form DNA adducts that promote oxidative stress and carcinogenic activity. Cellular antioxidants may be enzymatic (catalase, glutathione peroxidase, superoxide dismutase) or nonenzymatic (glutathione, thiols some vitamins and metals or phytochemicals such as isoflavones, polyphenols and flavanoids). In normal physiological processes, antioxidants effect signal transduction and regulation of proliferation and the immune response. Reactive oxygen species have been linked to cancer and antioxidants such as vitamin C, vitamin E, selenium, tocopherol, coenzyme Q10, carotenoids and flavonoids have been considered promising therapy for prevention and treatment of this disease, especially given the tantalizing links observed between diets high in fruits and vegetables (and presumably antioxidants) and decreased risks for cancer.

Key words: Hydrogen peroxide, potential, cell, neutrophils, oxygen, India

INTRODUCTION

ROS are widely believed to be involved in the etiology of many diseases as indicated by the signs of oxidative stress seen in cancer. ROS produced as byproducts during the mitochondrial electron transport of aerobic respiration or by oxidoreductase enzymes and metal catalyzed oxidation have the potential to cause a number of deleterious events. It was originally thought that only phagocytic cells were responsible for ROS production as their part in host cell defense mechanisms. Recent research has demonstrated that ROS have a role in cell signaling including; apoptosis; gene expression and the activation of cell signaling cascades (Hancock *et al*., 2001). Stimulated production of ROS was first described in phagocytic cells like neutrophils and macrophages and was named the respiratory burst due to the transient consumption of oxygen. The respiratory burst is performed by a multi-component Nicotinamide Adenine Dinucleotide Phosphate reduced (NADPH) oxidase and is critical for the bactericidal action of phagocytes.

The effect of ROS on cellular processes is a function of the strength and duration of exposure as well as the context of the exposure. The typical cellular response to stress is to leave the cell cycle and enter into G0. With continued exposure and/or high levels of ROS, apoptosis mechanisms are triggered. In cycling cells, p21 is activated in response to stress such as oxidants or oxidative stress and blocks cell cycle progression (Gartel and Radhakrishnan, 2005). Likewise p27 production leads to G1 arrest of cells. In cycling cells, p53 and p21 respond to oxidants by inducing the dephosphorylation of Retinoblastoma (RB).

Exposure to oxidants such as H_2O_2 or nitric oxide also results in dephosphorylation of RB that is independent of p53 or p21. In either case cells are arrested in S-phase. Expression of p27 is controlled in part by the Foxo transcription factors which are known to control the expression of genes involved in cell cycle progression, metabolism and oxidative stress response. For example, mitogenic stimulation by the PI3K/Akt pathway maintains Foxo3a in the cytoplasm but in the absence of stimulation Foxo3a enters the nucleus and up-regulates genes for oxidant metabolism and cell cycle arrest such as p27 (Burhans and Heintz, 2009). Under some conditions Foxo3a can directly activate bim gene expression and promote apoptosis (Gilley *et al*., 2003). Thus, Foxo3a promotes cell survival of cycling cells under oxidative stress by enabling a stress response but induces cell death when conditions warrant. Non-cycling cells such as neurons also have coping mechanisms to oxidative stress that involve Foxo3a. Foxo3a induces expression of the manganese form of SOD in response to oxidative stress (Kops *et al*., 2002). A schematic representation of the effect of ROS on cell cycle is shown in Fig. 1.

Corresponding Author: Parloop Bhatt, Department of Pharmacology, L.M. College of Pharmacy, 380009 Ahmedabad, Gujarat, India

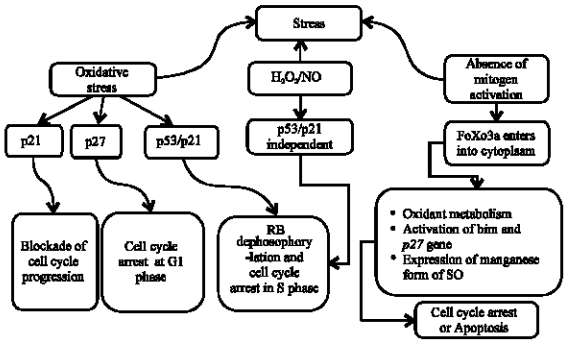

Fig. 1: The effect of ROS on cell cycle progression

MATERIALS AND METHODS

Signal transduction: Mitogenic signaling begins at the surface with the ligand-dependent activation of receptor tyrosine kinases which activate important MAP kinase cascades necessary for proliferation. These oxidative cell cascades lead to the generation of H_2O_2 from several enzyme catalysts including the NADPH oxidases (Park *et al*., 2006). It has been estimated that the production of H_2O_2 at nanomolar levels is required for proliferation in response to growth factors (Burch and Heintz, 2005). Hydrogen peroxide interacts with both the SOS-Ras-Raf-ERK and PI3K/Akt pathways through several mechanisms and in a dose-dependent manner. It has been suggested that small increases of H_2O_2 as a result of Nox1 expression result in increased reentry into the cell cycle while sustained high levels of H_2O_2 lead to cell arrest and eventual apoptosis after prolonged arrest. Peroxidoxins serve as important regulators of H_2O_2 and mitogenic signaling. These thiol-dependent peroxidases are activated and recruited to receptors as part of mitogenic stimulation and serve to limit the effect of ROS-associated stimulation on downstream targets of the mitogen cascade (Choi *et al*., 2005).

Transient fluctuations of ROS levels influence activity of signal transduction pathways leading to cell proliferation or to apoptosis or necrosis, depending on the dosage and duration of ROS and also on cell type. Typically, low doses of ROS can be mitogenic whereas medium doses lead to temporary or permanent growth arrest (replicative senescence); high doses usually result in cell death either by apoptosis or necros is (Holbrook and Ikeyama, 2002). Although, necrosis and apoptosis may be viewed as negative events in terms of cell loss these processes also have positive roles in the down-regulation of immune responses and eliminationof transformed cells (tumor suppression).

ROS in carcinogenesis and cancer etiology: Cancer can be viewed as a number of distinct diseases each defined by different genetic lesions with certain characteristics shared by most cancers and cancer types. These hallmark capabilities are necessary for tumorigenesis as described by Hanahan and Weinberg (2000) as:

- Selfsufficiency in growth signals
- Insensitivity to antigrowth signals
- Evasion of apoptosis
- Limitless replicative potential
- Sustained angiogenesis and
- Tissue invasion and metastasis (Ohshima *et al*., 2003)

ROS and Reactive Nitrogen Species (RNS) participate in these processes, contributing to cancer progression but also playing important roles in endogenous defenses, working to eliminate and controlling the spread of transformed cells. Due to recent increases in the number of cancer patients using antioxidant supplements, a greater understanding of both the damaging and protective actions of ROS in carcinogenesis is crucial to make advances in cancer treatment. Data from large-scale studies concerning supplements in cancer prevention and treatment in humans are often equivocal, reflecting the dual roles of ROS/RNS in malignant diseases.

Genetic alterations: Mutagenic alteration of DNA is an initiating event in carcinogenesis and mutations affecting gene expression and/or function, particularly those of tumor suppressor genes and proto-oncogenes; this can lead to unregulated growth typical of cancer cells (Marnett, 2000). DNA damage can arise from direct interaction of ROS or RNS with nucleic acid bases or the DNA backbone or can be caused by reactive compounds generated by oxidized lipids. One of the easiest assayed and therefore widely studied forms of oxidative DNA damage is the oxidation of guanine residues, resulting in 8-oxo-deoxyguanosine (8-oxo-dG).

These lesions induce G:C to T:A transversions that have been observed in oncogenes and tumor suppressor genes (e.g., p53) known to have significant roles in carcinogenesis. The reliability of commonly used methods to determine levels of DNA oxidation is imperfect and controversial, leading to questions about the importance of oxidatively damaged DNA in cancer and other pathologies (Collins *et al*., 2004) there has been however recent focus on methodological refinement which have improved the usefulness of this biomarker (Cadet *et al*., 2003). Elaborate mechanisms exist for the removal or repair of this damaged DNA which would seem to imply a need to correct this damage and thereby prevent abnormal cell function. Nonetheless, increased amounts of oxidative DNA damage, particularly generation of 8-oxo-dG and 8-oxodeoxyadenosine has been associated with cancers including breast cancer (Malins and Haimanot, 1991) in chronic inflammatory conditions potentially leading to

cancer such as hepatitis and cirrhosis (Shimoda *et al*., 1994) and in gastric tissue from subjects with Helicobacter pylori infections (Farinati *et al*., 1998).

ROS balance: Many groups have noticed increased ROS production in cancer cells that may be due to dysfunctional ROS generators and/or detoxifiers. Aberrant regulation of ROS/ RNS levels is widely believed to have a role in carcinogenesis. Several groups have assessed a number of tumor cell lines in which abnormally high levels of hydrogen peroxide are produced (Szatrowski and Nathan, 1991); other cell lines have been shown to have reduced catalase and glutathione peroxidase levels, suggesting an inability to detoxify H_2O_2 (Oberley and Oberley, 1997). These observed abnormalities in ROS/RNS metabolism also have functional implications as several lines of research have demonstrated that experimental manipulation of ROS levels in cells can affect tumorigenicity. An increase in superoxide dismutase leading to a decrease in superoxide anion and an increase in hydrogen peroxide levels has been correlated with malignancy in breast cancer cell lines (Policastro *et al*., 2004). Another group has demonstrated that reestablishing stable expression of catalase could also revert the transformed phenotype induced in NIH3T3 cells by overexpression of NADPH oxidase (which generated high levels of superoxide anion). The unreverted cells were shown to be able to produce aggressive tumors in an athymic mouse model (Arnold *et al*., 2001; Suh *et al*., 1999).

Tumor metabolism and progression: Besides altered regulation of enzymatic systems involved in generation and detoxification of ROS, several physical characteristics of tumors can also be associated with generation of ROS including glucose deprivation, hypoxia and inflammation arising from infiltrating macrophages. Glucose deprivation observed as a result of the tumor outgrowing its blood supply has also been observed to induce oxidative stress in cultured MCF7 breast cancer cells, triggering MAPK activity in these cells but not in nontransformed cells (Lee *et al*., 1998).

Hypoxia can develop as tumors exceed their blood supply and the chaotic angiogenesis observed in tumors drives cycles of hypoxia and reperfusion, generating ROS. Additionally, ROS themselves have roles in the regulation of genes involved in oxygen sensing (HIF-1) and angiogenesis (VEGF). Macrophage infiltration within the tumor results in ROS production as occurs during inflammation and activated macrophages can produce cytokines such as TNF-a that induce oxidative stress. A cellular environment in which ROS production and detoxification is not properly controlled can lead in turn to improper regulation of downstream molecular events that impinge on proliferation, apoptosis and cell migration all of which have effects on cancer progression.

The impact of aberrant levels of ROS observed in malignant cells can be viewed in the context of normal ROS function which includes mediating the levels and activity of numerous transcription factors and other gene products that can influence cancer cell growth, angiogenesis, invasiveness and metastasis. The role of ROS in signal transduction by the MAPKs (ERKs, JNKs and p38 family members) has been described and the precise modulation of these pathways leads to the proper growth response to mitogens. In normal cells, exposure to higher levels of hydrogen peroxide or superoxide anion leads to increased expression of growth regulated genes and proliferation.

The potential impact of increased ROS on these pathways in cancer cells can be seen in the response of some cancer cell lines to increased (but sublethal) levels of ROS. Sustained activity of MAPK in response to ROS is observed in HeLa cells (Wang *et al*., 1998) and the hyperphosphorylation of JNK and increased activation of AP-1 observed in MCF7 breast carcinoma cells is associated with increased proliferation (Brown and Bicknell, 2001). Activation of ERK1/ERK2 has been seen in multidrug resistant breast cancer cell lines in response to oxidative stress induced by glucose deprivation (Lee *et al*., 1998). A review by Benhar *et al*. (2001) highlights the roles of p38 and JNK in tumorigenesis for a number of cancer types including effects on proliferation and cell cycle progression while an earlier review addresses the activity of oncogenes and tumor suppressor genes (Suh *et al*., 1999).

Gene targets: Cancer researchers have identified numerous oncogenes and tumor suppressor genes; ROS can participate in promoting carcinogenesis mediated by these genes. Transformation by overexpression of oncogenic Ras leads to increased superoxide anion levels produced by Ras/Rac1 mediated activity of inducible NADPH oxidase. This generates high levels of superoxide anions that serve as substrate for processing to hydrogen peroxide by Manganese Superoxide Dismutase (MnSOD) (Irani *et al*., 1997); high levels of MnSOD activity have been observed in highly invasive metastatic tumors (Malafa *et al*., 2000). Increased hydrogen peroxide levels generated by MnSOD appear to cause an increase in AP-1 and/or NFnB activity, aiding tumor invasiveness by upregulation of matrix metalloproteinases and allowing increased degradation of the subcellular matrix which is a key step in metastasis (Behrend *et al*., 2003). Activated

Ras/Rac1 may be required for invasiveness mediated by MnSOD activity because MCF-7 cells that overexpress MnSOD but do not have activated Ras/Rac1 do not show an increased invasiveness (Li *et al*., 1998). Another oncogene involved in development of many cancers is the tumor suppressor p53; its activity also is affected by ROS. In this research, excess nitric oxide in cells leads to an accumulation of p53 protein but the ability of p53 to bind to its consensus DNA sequence is decreased and as a result, tumor suppression activity of p53 is lost (Ohshima *et al*., 2003; Calmels *et al*., 1997). The p53 protein appears to be modified by the highly reactive nitric oxide derivative peroxynitrite produced by excess nitric oxide and superoxide anion.

Cell cycle effects: Other events promoting carcinogenesis such as inhibition of apoptosis, senescence and DNA repair as well as promotion of angiogenesis are affected by ROS. Nitric Oxide (NO) and its derivatives appear to contribute to DNA damage, although evidence that this is integral to transformation is conflicting (Wiseman and Halliwell, 1996; Wink *et al*., 1998; Dhar *et al*., 2003). Nitric oxide has been demonstrated to inhibit the enzymatic activities of DNA repair enzymes such as alkyl transferase and DNA ligase, resulting in increased genotoxic burden on the cell (Laval and Wink, 1994; Graziewicz *et al*., 1996). Nitric oxide also can inhibit caspase 3-like activity, interfering with apoptosis which is designed to eliminate transformed cells (Kim *et al*., 1997). Two other cancer hallmarks, uncontrolled angiogenesis and impaired senescence also are affected by the actions of ROS on transcription factors and other cellular proteins. Changes in oxygen levels regulate activity of the transcription factor HIF-1; this in turn, regulates expression of genes involved in angiogenesis such as erythropoietin and VEGF, thereby contributing to the abnormal angiogenic activity of some solid tumors (Brown and Bicknell, 2001). ROS also have been shown to activate cellular telomerase in endothelial cells, delaying normal replicative senescence (Vasa *et al*., 2000).

Cellular response: Tumor development represents an imbalance between cell proliferation, strictly controlled in healthy cells and apoptosis, used to remove damaged or precancerous cells.

Despite their roles in processes promoting abnormal proliferation and tumorigenesis, ROS also can promote apoptosis in this case, helping to prevent proliferation and the spread of transformed cells. As part of their abnormal proliferation, cancer cells need to evade apoptosis normally triggered by high levels of oxidative stress and cellular damage.

Tumor resistance potentiation: Researchers have long observed that precancerous cells or cells in early stages of cancer are more sensitive to chemotherapy and radiation treatment than are normal cells which often work by generating oxidative stress and inducing apoptosis but this sensitivity is lost as the cancer progresses. The increase in basal ROS levels observed in transformed cells contributes to this by potentiation of SAPK activity (Suh *et al*., 1999).

In potentiated cells, activation of the JNK and p38 pathways is enhanced and induction of kinase activity is more sensitive to lower doses of stress stimuli than in nontransformed cells. As experimental evidence of the effects of potentiation, two cell lines, EGF-transformed NIH3T3 cells and A431 epidermoid cancer cells which show elevated ROS levels and enhanced induction of JNK and p38 pathways also showed greater sensitivity to apoptosis induced by the chemotherapeutic agent cisplatin.

In contrast, the HT29 colon cancer cell line which has lower levels of ROS and JNK and p38 activity in comparison to the A431 cells and transformed NIH3T3 cells is resistant to the cisplatin-induced cell death observed in those other two cell lines (Benhar *et al*., 2001). The enhanced susceptibility of potentiated transformed cells to undergo apoptosis in response to either endogenous or exogenous stress stimuli may be advantageous to the organism because potentially cancerous cells would be eliminated when still at an early stage of transformation. As cancer progresses many tumors become drug-resistant in these tumors an increase in activity of antioxidant defense systems including malondialdehyde formation, superoxide dismutase, glutathione peroxidase and catalase has been observed. This results in decreased ROS and SAPK levels thereby interfering with the apoptotic response to chemotherapeutic agents (Policastro *et al*., 2004). Additionally, resistance to apoptosis can occur as the cell acquires further genetic lesions, e.g., inactivation of tumor suppressor genes or amplification of anti-apoptotic genes such as members of the Bcl-2 family. Overexpression of MAPK phosphatase-1 which down-regulates JNK and p38 has been observed in prostate, colon and other tumors, rendering these cells insensitive to death mediated via the SAPK pathways (Benhar *et al*., 2001). Thus, intracellular levels of ROS may be a key to the understanding of the ability of ROS to participate in both cancer promotion and cancer control or elimination. While cellular damage caused by ROS can be tumor promoting in some transformed cells, elevated levels of ROS lead to enhanced SAPK activity making the cells more sensitive to apoptosis elicited by cancer-fighting therapies.

Overall function of ROS: The multiple (and conflicting) roles of ROS in initiation, progression and metastasis of cancer are also reflected in results from clinical trials which investigated the use of antioxidants in cancer treatment and prevention. To achieve the proper balance between cell proliferation and cell death, the levels of ROS need to be precisely regulated; overzealous antioxidant supplementation could theoretically upset this balance and potentially lead to undesirable effects. As some chemotherapy drugs and radiation regimes kill cancer cells by generating high levels of ROS, antioxidant supplementation could conceivably interfere with some cancer treatments and some evidence for this exists. However, there are also indications that antioxidant supplementation can help to counteract side effects of some chemotherapy and may allow tolerance of higher doses and longer-lasting treatment regimens.

RESULTS AND DISCUSSION

Antioxidants and cancer: The cells of the human body are continuously attacked by ROS generated as natural by products of the normal cellular energy production from daily activities such as exhaustive exercise or from metabolism of xenobiotics. The body has several defence systems to counteract oxidative stress. These comprise endogenous enzymes (including catalase, glutathione reductase and superoxide dismutase), endogenous factors (including glutathione, urate and coenzyme Q) and nutritional factors (principally the antioxidant nutrients, especially β-carotene and other carotenoids, vitamin C, vitamin E and selenium) or phytochemicals such as isoflavones, polyphenols and flavanoids.

The dietary intake of antioxidants is thought to play a major role in this network. Antioxidant is a widely used term that is difficult to define clearly in biological systems we use the term antioxidant to broadly denote any substance that prevents oxidation of biomolecules either directly by scavenging reactive oxygen species or indirectly by upregulating the antioxidant defense or DNA repair systems. The indirect antioxidant effect may be evoked by xenobiotics or components in vegetables that are not scavengers or even considered harmful, e.g., isothiocyanates are oxidants that stimulate cellular antioxidant proteins and detoxification enzymes (Zhang, 2005). Antioxidants such as vitamin C and E, carotenoids and flavonoids have been identified in many natural food products (Halliwell, 1996).

Natural products also contain mixtures of other antioxidants and bioactive substances with unknown antioxidant properties. The antioxidant activity can be tested *in vitro* or in animal experimental models but this is associated with a number of uncertainties when extrapolating to humans. The most relevant way to explore antioxidant effects in humans is supplementation trials although this often is restricted to the use of surrogate tissues such as White Blood Cells (WBC) and urine.

Epidemiological data link vitamin C intake with reduced risk of several cancers, especially oral cavity, esophagus, stomach and to a lesser extent, colon and lung (Block , 1991; Byers and Guerrero, 1995). Likewise, the epidemiological evidence clearly shows a strong inverse association between the intake of β-carotene and the risk of several cancers, especially lung and stomach (Van Poppel and Goldbohm, 1995).

Some attention has been paid to other carotenoids. Epidemiological studies have reported that β-carotene has an inverse association with cancer of a similar strength to that seen for β-carotene (Greenwald and McDonald, 1999). The 1st large-scale European trial to be completed was the β-Tocopherol, β-carotene cancer prevention study in Finland.

About 29,000 male smokers aged 50-69 took part in a randomised placebo-controlled trial with β-tocopherol (50 mg day^{-1}) alone, β-carotene (20 mg day^{-1}) alone, β-tocopherol and β-carotene together or a placebo. Supplementation continued for up to 8 years at the end of which it was found that the incidence of lung cancer was significantly higher (by 18%) in those who had received β-carotene. In the USA, the CARET trial (β-Carotene and Retinol efficacy trial) looked at >18000 smokers, former smokers and asbestos workers (Omenn *et al*., 1996). They were given 30 mg day^{-1} of β-carotene and 25000 IU of retinol or a placebo for an average of 4 years. Lung cancer incidence was again, significantly elevated (by 28%) in the supplemented group.

The physicians health study also in the USA, tested β-carotene (50 mg on alternate days against placebo) in healthy male physicians (11000 in each group) (Hennekens *et al*., 1996). Almost 90% were non-smokers at the start of the study which lasted for 12 years. Incidence of lung cancer and number of deaths from cancer did not vary significantly between the groups. Lycopene, a carotenoid present in tomatoes has attracted much attention recently; it shows a strong inverse relationship with several types of cancer, especially prostate, lung and stomach (Giovannucci, 1999).

A weaker association has been described for lutein (Greenwald and McDonald, 1999). There is some evidence of protection against cancer by supplemental β-carotene based on early endpoints. One study reported significant reversal of leukoplakia, a precancerous oral lesion (Garewal *et al*., 1990). Similarly another study observed

partial regression of precancerous changes of the stomach (Bukin and Draudin-Krylenko, 1999). In addition, to vitamin C and carotenoids there are other antioxidants in food that may have anti-carcinogenic action. Animal experiments have demonstrated that selenium functions as an antioxidant (Vinson *et al*., 1998). There is much evidence from international correlation studies and from animal experiments that selenium is protective against cancer (Greenwald and McDonal, 1999). Data from the health professionals follow-up study indicate a strong inverse association between selenium status and risk of prostate cancer (Yoshizawa *et al*., 1998). One controlled intervention study has been carried out and this reported a dramatic 50% fall in total cancer mortality using a supplement of 200 μg day^{-1} (i.e., several times greater than the RDA) (Clark *et al*., 1996). Turning to vitamin E the epidemiological evidence is inconclusive for a protective role in cancer though this antioxidant does appear to be negatively associated with colorectal adenomas (Greenwald and McDonald, 1999). In the ATBC cancer prevention study a dose of 50 mg day^{-1} apparently reduced the incidence of prostate and colorectal cancer by 36 and 16%, respectively (Heinonen *et al*., 1998). Vitamin E (400 IU day^{-1}) caused partial regression of precancerous changes of the stomach (Bukin and Draudin-Krylenko, 1999).

One interpretation of these seemingly contradictory findings is that vitamin E becomes anticarcinogenic only at pharmacological doses (i.e., when the intake is several times greater than the US RDA (8-10 mg day^{-1}). Supplemental vitamin E at a dose of 60-800 ID day^{-1} reportedly improves immune reaction in elderly subjects (Meydani *et al*., 1997) while a single dose of 1 g helped prevent oxidative damage of DNA (Panayiotidis and Collins, 1997). In Linxian county in china an area with a very high incidence of oesophageal/gastric cancer almost 30,000 subjects aged 40-69 were randomly assigned to intervention groups to receive various combinations of retinol, zinc, riboflavin, niacin, vitamin C, molybdenum, β-carotene, vitamin E and selenium (Blot *et al*., 1993). At the end of the 5 years supplementation period, significantly lower cancer mortality resulting mainly from lower cancer rates among those receiving supplementation with β-carotene, vitamin E and selenium was seen. In the Suvimax study (Hercberg *et al*., 2004), 13,000 French men and women received modest daily supplementation with vitamin C (120 mg), vitamin E (30 mg), β-carotene (6 mg), selenium (100 lg) and zinc (20 mg) or a placebo for about 7.5 years.

A significant protective effect against cancer incidence was seen in men (relative risk 0.69 in the supplemented group) but not in women. The sex difference may be explained by the lower baseline antioxidant status in men. Finally, disappointing results were found in a randomized trial with α-tocopherol as a supplement given to patients with head and neck cancer during and after radiation therapy with the aim of decreasing the incidence of second primary cancers (Bairati *et al*., 2005).

The incidence of such cancers was higher in patients receiving a-tocopherol during the three year supplementation period (compared with the placebo group) but lower after supplementation ceased and overall cancer-free survival was similar in both groups after 8 years of follow-up. Coenzyme Q10 and α-tocopherol have beneficial effect in cervical intraepithelial neoplasia and cervical cancer.

CONCLUSION

Cancer is a wide spread, complex, multi-factorial and multi-istage disease with a number of molecular alterations involved in each stage (namely initiation, promotion and progression) of its development. Cancer can be viewed as a number of distinct diseases each defined by different pathways with certain characteristics shared by most cancers and cancer types. Root cause for tumorigenesis may be ≥1 of these self sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis. ROS and Reactive Nitrogen Species (RNS) participate in these processes, contributing to cancer progression but also playing important roles in endogenous defenses, working to eliminate and controlling the spread of transformed cells. Oxidative stress (a state of persistent generation of ROS over-whelming cellular defenses) has long been known to contribute to human carcinogenesis. Such contribution has been shown to regulate altered carcinogenic gene expression at the genetic as well the epigenetic level by very distinct mechanisms.

The cells of the human body are continuously attacked by ROS generated as natural by products of the normal cellular energy production from daily activities such as exhaustive exercise or from metabolism of xenobiotics. The body has several defence systems to counteract oxidative stress. These comprise endogenous enzymes (including catalase, glutathione reductase and superoxide dismutase), endogenous factors (including glutathione, urate and coenzyme Q10 and nutritional factors (principally the antioxidant nutrients, especially β-carotene and other carotenoids, vitamin C and E and selenium) or phytochemicals such as isoflavones, polyphenols and flavanoids. All these antioxidants can

halt the progression of the disease and they also have disease preventive effect. For optimal treatment in clinical practice a longitudinal management plan should be defined for each individual patient with established cancer including the goals of treatment.

The choice of particular antioxidants depends on the type of the disease, stage of the disease and response given by the patient to that particular treatment. However, it is difficult to predict how patients will respond the line therapy. Patients not responding to initial therapy are candidates for therapy change including combination strategies. Due to recent increases in the number of cancer patients using antioxidant supplements, a greater understanding of both the damaging and protective actions of ROS in carcinogenesis is crucial to further advances in cancer treatment.

REFERENCES

Arnold, R.S., J. Shi, E. Murad, A.M. Whalen and C.Q. Sun *et al*., 2001. Hydrogen peroxide mediates the cell growth and transformation caused by the mitogenic oxidase Nox1. Proc. Natl. Acad. Sci. USA., 98: 5550-5555.

Bairati, I., F. Meyer, M. Gelinas, A. Fortin and A. Nabid *et al*., 2005. A randomized trial of antioxidant vitamins to prevent second primary cancers in head and neck cancer patients. J. Natl. Cancer Inst., 97: 481-488.

Behrend, L., G. Henderson and R.M. Zwacka, 2003. Reactive oxygen species in oncogenic transformation. Biochem. Soc. Trans., 31: 1441-1444.

Benhar, M., I. Dalyot, D. Engelberg and A. Levitzki, 2001. Enhanced ROS production in oncogenically transformed cells potentiates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase activation and sensitization to genotoxic stress. Mol. Cell Biol., 21: 6913-6926.

Block, G., 1991. Vitamin C and cancer prevention: The epidemiological evidence. Am. J. Clin. Nutr., 53: 270S-282S.

Blot, W.J., J.Y. Li, P.R. Taylor, W. Guo and S. Dawsey *et al*., 1993. Nutrition intervention trials in Linxian, China: Supplementation with specific vitamin/mineral combinations, cancer incidence and disease-specific mortality in the general population. J. Natl. Cancer Inst., 185: 1483-1492.

Brown, N.S. and R. Bicknell, 2001. Hypoxia and oxidative stress in breast cancer. Oxidative stress: Its effects on the growth, metastatic potential and response to therapy of breast cancer. Breast Cancer Res., 3: 323-327.

Bukin, Y.V. and V.A. Draudin-Krylenko, 1999. The Role of Carotene and Vitamin E in the Treatment of Early Gastric Premalignant Lesions: Biochemical and Clinical Aspects. In: Antioxidants in Human Health and Disease, Basu, T.K., N.J. Temple and M.L. Garg (Eds.). CAB International, Wallingford, Oxfordshire, UK., pp: 235-248.

Burch, P.M. and H.H. Heintz, 2005. Redox regulation of cell-cycle re-entry: Cyclin D1 as a primary target for the mitogenic effects of reactive oxygen and nitrogen species. Antioxidants Redox Signaling, 7: 741-751.

Burhans, W. and N. Heintz, 2009. The cell cycle is a redox cycle: Linking phase-specific targets to cell fate. Free Radic. Biol. Med., 47: 1282-1293.

Byers, T. and N. Guerrero, 1995. Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. Am. J. Clin. Nutr., 62: 1385S-1392S.

Cadet, J., T. Douki, D. Gasparutto and J.L. Ravanat, 2003. Oxidative damage to DNA: Formation, measurement and biochemical features. Mutation Res./Fundam. Mol. Mech. Mutagen., 531: 5-23.

Calmels, S., P. Hainaut and H. Ohshima, 1997. Nitric oxide induces conformational and functional modifications of wild-type p53 tumor suppressor protein. Cancer Res., 57: 3365-3369.

Choi, M.H., I.K. Lee, G.W. Kim, B.U. Kim and Y.H. Han *et al*., 2005. Regulation of PDGF signalling and vascular remodelling by peroxiredoxin II. Nature, 435: 347-353.

Clark, L.C., G.F. Jr. Combs, B.W. Turnbull, E.H. Slate and D.K. Chalker *et al*., 1996. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: A randomized controlled trial. Nutritional Prevention of Cancer Study Group. J. Am. Med. Assoc., 276: 1957-1963.

Collins, A.R., J. Cadet, L. Moller, H.E. Poulsen and J. Vina, 2004. Are we sure we know how to measure 8-oxo-7,8-dihydroguanine in DNA from human cells? Arch. Biochem. Biophys., 423: 57-65.

Dhar, A., J.M. Brindley, C. Stark, M.L. Citro, L.K. Keefer and N.H. Colburn, 2003. Nitric oxide does not mediate but inhibits transformation and tumor phenotype. Mol. Cancer Ther., 2: 1285-1293.

Farinati, F., R. Cardin, P. Degan, M. Rugge, F.D. Mario, P. Bonvicini and R. Naccarato, 1998. Oxidative DNA damage accumulation in gastric carcinogenesis. Gut, 42: 351-356.

Garewal, H.S., F.L. Meyskens, D. Killen, D. Reeves and T.A. Kiersch *et al*., 1990. Response of oral leukoplakia to Î²-carotene. J. Clin. Oncol., 8: 1715-1720.

Gartel, A.L. and S.K. Radhakrishnan, 2005. Lost in transcription: p21 repression, mechanisms and consequences. Cancer Res., 65: 3980-3985.

Gilley, J., P.J. Coffer and J. Ham, 2003. FOXO transcription factors directly activate bim gene expression and promote apoptosis in sympathetic neurons. J. Cell Biol., 162: 613-622.

Giovannucci, E., 1999. Tomatoes, tomato-based products lycopene and cancer: Review of the epidemiological literature. J. Nat. Cancer Inst. 91: 317-331.

Graziewicz, M., D.A. Wink and F. Laval, 1996. Nitric oxide inhibits DNA ligase activity: Potential mechanisms for NO-mediated DNA damage. Carcinogenesis, 17: 2501-2505.

Greenwald, P. and S.S. McDonald, 1999. Antioxidants and the Prevention of Cancer. In: Antioxidants in Human Health and Disease, Basu, T.K., N.J. Temple and M.L. Garg (Eds.). CAB International, Wallingford, Oxon, UK., pp: 217-234.

Halliwell, B., 1996. Commentary oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. Free Radical Res., 25: 57-74.

Hanahan, D. and R.A. Weinberg, 2000. The hallmarks of cancer. Cell, 100: 57-70.

Hancock, J.T., R. Desikan and S.J. Neill, 2001. Role of reactive oxygen species in cell signalling pathways. Biochem. Soc. Trans., 29: 345-350.

Heinonen, O.P., D. Albanes, J. Virtamo, P.R. Taylor and J.K. Huttunen *et al*., 1998. Prostate cancer and supplementation with Î±-tocopherol and Î²-carotene: Incidence and mortality in a controlled trial. J. Natl. Cancer Inst., 90: 440-446.

Hennekens, C.H., J.E. Buring, J.E. Manson, M. Stampfer and B. Rosner *et al*., 1996. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. N. Engl. J. Med., 334: 1145-1149.

Hercberg, S., P. Galan, P. Preziosi, S. Bertrais and L. Mennen *et al*., 2004. The SU.VI.MAX study: A randomised, placebo-controlled trial of the health effects of antioxidant vitamins and minerals. Arch. Int. Med., 164: 2335-2342.

Holbrook, N.J. and S. Ikeyama, 2002. Age-related decline in cellular response to oxidative stress: Links to growth factor signaling pathways with common defects. Biochem. Pharmacol., 64: 999-1005.

Irani, K., Y. Xia, J.L. Zweier, S.J. Sollott and C.J. Der *et al*., 1997. Mitogenic signaling mediated by oxidants in Ras-transformed fibroblasts. Science, 275: 1649-1652.

Kim, Y.M., R.V. Talanian and T.R. Billiar, 1997. Nitric oxide inhibits apoptosis by preventing increases in caspase-3-like activity via two distinct mechanisms. J. Biol. Chem., 272: 31138-31148.

Kops, G.J., T.B. Dansen, P.E. Polderman, I. Saarloos and K. Wirtz *et al*., 2002. Forkhead transcription factor FOXO3a protects quiescent cells from oxidative stress. Nature, 419: 316-321.

Laval, F. and D.A. Wink, 1994. Inhibition by nitric oxide of the repair protein O6-methylguanine-DNA-methyltransferase. Carcinogenesis, 15: 443-447.

Lee, Y.J., S.S. Galoforo, C.M. Berns, J.C. Chen and B.H. Davis *et al*., 1998. Glucose deprivation-induced cytotoxicity and alterations in mitogenactivated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells. J. Biol. Chem., 273: 5294-5299.

Li, J.J., N.H. Colburn and L.W. Oberley, 1998. Maspin gene expression in tumor suppression induced by overexpressing manganese-containing superoxide dismutase cDNA in human breast cancer cells. Carcinogenesis, 19: 833-839.

Malafa, M., J. Margenthaler, B. Webb, L. Neitzel and M. Christophersen, 2000. MnSOD expression is increased in metastatic gastric cancer. J. Surg. Res., 88: 130-134.

Malins, D.C. and R. Haimanot, 1991. Major alterations in the nucleotide structure of DNA in cancer of the female breast. Cancer Res., 51: 5430-5432.

Marnett, L.J., 2000. Oxyradicals and DNA damage. Carcinogen, 21: 361-370.

Meydani, S.N., M. Meydani, J.B. Blumberg, L.S. Leka and G. Siber *et al*., 1997. Vitamin E supplementation and *in vivo* immune response in healthy elderly subjects: A randomized controlled trial. J. Am. Med. Assoc., 27: 1380-1386.

Oberley, T.D. and L.W. Oberley, 1997. Antioxidant enzyme levels in cancer. Histol. Histopathol., 12: 525-535.

Ohshima, H., M. Tatemichi and T. Sawa, 2003. Chemical basis of inflammation-induced carcinogenesis. Arch. Biochem. Biophys., 417: 3-11.

Omenn, G..S, G.E. Goodman, M.D. Thornquist, J. Balmes and M.R. Cullen *et al*., 1996. Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N. Engl. J. Med., 334: 1150-1155.

Panayiotidis, M. and A.R. Collins, 1997. *Ex vivo* assessment of lymphocyte antioxidant status using the comet assay. Free Radic. Res., 27: 533-537.

Park, H.S., D. Park and Y.S. Bae, 2006. Molecular interaction of NADPH oxidase 1 with âPix and nox organizer 1. Biochem. Biophys. Res. Commun., 339: 985-990.

Policastro, L., B. Molinari, F. Larcher, P. Blanco and O.L. Podhajcer *et al*., 2004. Imbalance of antioxidant enzymes in tumor cells and inhibition of proliferation and malignant features by scavenging hydrogen peroxide. Mol. Carcinog., 39: 103-113.

Shimoda, R., M. Nagashima, M. Sakamoto, N. Yamaguchi and S. Hirohashi, J. Yokota and H. Kasai, 1994. Increased formation of oxidative DNA damage, 8-hydroxydeoxyguanosine, in human livers with chronic hepatitis. Cancer Res., 54: 3171-3172.

Suh, Y.A., R.S. Arnold, B. Lassegue, J. Shi and X. Xu *et al*., 1999. Cell transformation by the superoxide-generating oxidase Mox1. Nature, 401: 79-82.

Szatrowski, T.P. and C.F. Nathan, 1991. Production of large amounts of hydrogen peroxide by human tumor cells. Cancer Res., 51: 794-798.

Van Poppel, G. and R.A. Goldbohm, 1995. Epidemiologic evidence for Î²-carotene and cancer prevention. Am. J. Clin. Nutr., 62: 1393S-4028S.

Vasa, M., K. Breitschopf, A.M. Zeiher and S. Dimmeler, 2000. Nitric oxide activates telomerase and delays endothelial cell senescence. Circ. Res., 87: 540-542.

Vinson, J.A., J.M. Stella and T.J. Flanagan, 1998. Selenium yeast is an effective *in vitro* and *in vivo* antioxidant and hypolipemic agent in normal hamsters. Nutr. Res., 18: 735-742.

Wang, X., J.L. Martindale, Y. Liu and N.J. Holbrook, 1998. The cellular response to oxidative stress: Influences of mitogen-activated protein kinase signalling pathways on cell survival. Biochem. J., 333: 291-300.

Wink, D.A., Y. Vodovotz, J. Laval, F. Laval, M.W. Dewhirst and J.B. Mitchell, 1998. The multifaceted roles of nitric oxide in cancer. Carcinogenesis, 19: 711-721.

Wiseman, H. and B. Halliwell, 1996. Damage to DNA by reactive oxygen and nitrogen species: Role in inflammatory disease and progression to cancer. Biochem. J., 313: 17-29.

Yoshizawa, K., W.C. Willett, S.J. Morris, M.J. Stampfer, D. Spiegelman, E.B. Rimm and E. Giovannucci, 1998. Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. J. Natl. Cancer Inst., 90: 1219-1224.

Zhang, Y., 2005. Cancer-preventive Isothiocyanates: Measurement of human exposure and mechanism of action. Mutat. Res., 555: 173-190.