Acta Med Kinki Univ Vol. 35, No. 2 57-65, 2010

Free radicals, antioxidants and cancer chemotherapy

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Abstract

Free radicals play a dual role as both beneficial and deleterious species, since they can be either helpful or harmful to the organism. They are products from normal cellular metabolism, or from external sources like pollution, medication, and radiation. The accumulation of excessive amount of free radicals in the body generates redox imbalance, a phenomenon referred to as oxidative stress. This deleterious process is involved in development of chronic and degenerative diseases such as cancer, rheumatoid arthritis, cardiovascular and neurodegenerative illnesses. In order to check the activities of free radicals in vivo, antioxidant systems have evolved, either naturally generated (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants). This review deals with free radicals and their beneficial and deleterious effect on cellular activities, role of antioxidants (glutathione) in the maintenance of cellular redox homeostasis; atention is focused on free radical-linked pathogenesis of cancer, and a discussion is also devoted to antioxidant supplementation in cancer patients undergoing chemotherapy.

Key words: oxidative stress, cancer, chemotherapeutic drugs, dietary antioxidants

Introduction

Oxygen is one of the elements, which are indispensable for life. Cells use oxygen to generate energy in adenosine triphosphate production by the mitochondria and as a consequence, free radicals are created. These by-products of cellular redox process are generally reactive oxygen species (ROS) as well as reactive nitrogen species (RNS) and are result from normal cellular metabolism.

ROS and RNS are well recognized for playing a dual role as both deleterious and beneficial species, since they can be either harmful or beneficial to living systems.[1] The "two-faced" character of ROS is clearly substantiated and the delicate balance between their two antagonistic effects is an important aspect of life. At low and moderate levels ROS and RNS exert beneficial effects on cellular responses and immune function. At high concentrations, however, they are mediators of oxidative stress that can damage all cell structures.[1-9] Oxidative stress plays a major role in the development of chronic and degenerative diseases such as cancer, arthritis, cardiovascular and neurodegenerative disorders. The organisms have evolved several mechanisms to counteract this deleterious process by antioxidants-naturally produced or externally supplied through foods and/or supplements. Endogenous and exogenous antioxidants can prevent and/or repair injuries caused by ROS and RNS and thus enhance the immune defense and decrease the risk of cancer and degenerative diseases.[10,11]

The theory of free radicals has been known for more than fifty years, however, only in the last

two decades there has been an intensive research of their roles in the development of diseases and ageing, and the health protective effects of antioxidants. This review deals with free radicals and their beneficial and deleterious effect on cellular activities, role of antioxidants in the maintenance of cellular redox homeostasis; attention is focused on free radical-linked pathogenesis of cancer, and on the topic of heated debate concerning antioxidant supplementation in cancer patients undergoing chemotherapy.

"Two faced" character of free radicals

Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals.[12] This unpaired electron(s) usually gives them a considerable degree of reactivity. Free radicals include hydroxyl (OH•), superoxide ($O_2^{\bullet -}$), nitric oxide (NO•), nitrogen dioxide (NO_2•), peroxyl (ROO•) and lipid peroxyl (LOO•). Hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), and lipid peroxide (LOOH) are not free radicals and are generally called oxidants, but they can easily lead to free radical reactions in living organisms.[6] Biological free radicals are thus highly unstable molecules that have electrons available to react with various organic substrates such as lipids, proteins and DNA.

It seems somewhat paradoxical that both gases oxygen and nitrogen, which underlie essential life processes, also engenders derivatives that can be deleterious to life. The delicate balance between beneficial and harmful effects of free radicals is a very important aspect of living organisms and is achieved by a mechanism called "redox regulation". This process protects living organisms and maintains "redox homeostasis" by controlling the redox status in vivo.[3] Free radicals are generated either from endogenous or exogenous sources. Endogenous ROS and RNS are produced as a result of immune cell activation, inflammation, mental stress, excessive exercise, ischemia, infection, cancer, and ageing. Exogenous ROS and RNS are produced as a consequence of air and water pollution, cigarette smoke, alcohol, heavy or transition metals (Cd, Hg, Pb, Fe, As), certain drugs (cyclosporine, gentamycin, bleomycin), industrial solvents, cooking (smoked meat, used oil), and radiation.[9] After penetration into the body by different routes, these exogenous compounds are decomposed or metabolized into free radicals.

Beneficial actions of ROS and RNS occur at low/moderate concentrations and involve physiological roles in cellular responses against infectious agents and in the function of a number of cellular signaling systems. Phagocytes (neutrophils, macrophages, and monocytes) release free radicals to destroy invading pathogenic microbes as part of the body's defense mechanism. In various types of nonphagocytic cells (fibroblasts, endothelial cells, vascular smooth muscle cells, cardiac myocytes, and thyroid tissue) the production of free radicals plays a key role in the regulation of intracellular signaling cascades. These include regulation of vascular tone, monitoring of oxygen tension in the control of ventilation and erythropoietin production, signal transduction from membrane receptors in various physiological processes,[3] and also induction of a mitogenic response.[1]

At high concentrations, however, free radicals can be important mediators of damage to cell structures. When the generation of ROS/RNS exceeds cellular adaptive and repair capacities a phenomenon called oxidative stress occurs, in which biological molecules such as nucleic acids, proteins, and membrane phospholipids become damaged through oxidative reactions.[4–9] The imbalance between the generation of ROS/RNS and their neutralization by cellular antioxidant system leads to the failure of normal cellular functions. The hydroxyl radical is known to react with all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone. Oxidative damage to DNA leads to the formation of different oxidative DNA lesions which can cause mutations. Permanent modification of genetic material represents the first step involved in mutagenesis, carcinogenesis, and ageing. Hydroxyl radical and peroxynitrite in excess can also damage cell membranes and lipoproteins by a process called lipid peroxidation. This reaction leads to the formation of malondialdehyde (MDA) and conjugated diene compounds, which are cytotoxic and mutagenic. Lipid peroxydation occurs by a radical chain reaction, i.e. once started, it spreads rapidly and affects a great number of lipid molecules. Proteins may also be damaged by ROS/RNS, leading to structural

changes and loss of enzyme activity. If not regulated properly, oxidative stress can induce a variety of chronic and degenerative diseases such as cancer, autoimmune disorders, rheumatoid arthritis, cardiovascular and neurodegenerative diseases.[13]

Antioxidants

In order to check the activities of ROS/RNS in vivo and maintain cellular redox homeostasis, antioxidant systems have evolved, either naturally generated (endogenous antioxidants), or externally supplied through foods (exogenous antioxidants). Endogenous compounds in cells can be classified as enzymatic antioxidants as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx) and non-enzymatic antioxidants, also divided into metabolic and nutrient antioxidants. Metabolic antioxidants belonging to endogenous antioxidants are produced by metabolism in the body, such as glutathione (GSH), lipoid acid, L-arginine, coenzyme Q10, melatonin, uric acid, bilirubin, transferrin, etc. Nutrient antioxidants, belonging to exogenous antioxidants, are compounds which cannot be produced in the body and must be provided through foods or supplements, such as vitamin E, vitamin C, carotenoids, trace metals (selenium, manganese, and zinc), flavonoids, polyphenols and others.[1,4,7,10]

The cell cycle is characterized by fluctuations in the redox status, mediated in particular by intracellular changes in GSH concentrations.[14–16] GSH is the major intracellular antioxidant and it is highly abundant in the cytosol, nuclei and mitochondria.[17] Besides being a cofactor of several detoxifying enzymes against oxidative stress such as GPx, GRx and others, GSH scavenges hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of GPx. It is also able to regenerate the most important antioxidants, vitamins C and E back to their active forms.

Oxidative stress and cancerogenesis

Under physiological conditions the redox state of the cells is kept within a narrow range, similar to the manner in which a biological system regulates its pH. Under pathological conditions, however, the redox state can be altered to lower or higher values. Generally, a more reducing environment of the cell, maintained by elevated levels of glutathione, stimulates proliferation and a slight shift towards a mildly oxidizing environment initiates cell differentiation. A further shift towards a more oxidizing environment leads to apoptosis and an intense oxidizing effect induces necrosis.[18,19] Cancer is characterized by a more reducing environment of the cell and can be considered as a disturbed balance between cell proliferation and cell death shifted mostly towards cell proliferation.[15] The role of oxidative stress at various stages of carcinogenic process and the process of apoptosis is outlined in Fig. 1.[2]

The depletion of intracellular glutathione is just one of the factors involved in the commitment to undergo apoptosis. Free radicals can also alter many intracellular signaling pathways including protein phosphatases, protein kinases, and transcription factors, suggesting that their effects might also be through actions on signaling pathways. In fact redox signaling is a regulatory process in which the signal is delivered through redox reactions. Redox signaling requires that the state of redox balance is disturbed either by an increase in ROS formation or a decrease in the activity of antioxidant systems. Lipid peroxidation products, released during oxidative stress, such as MDA, have been suggested as modulators of signal pathways related to proliferation or apoptosis, two processes implicated in cancer development.[20]

Mitochondria are the organelles mainly

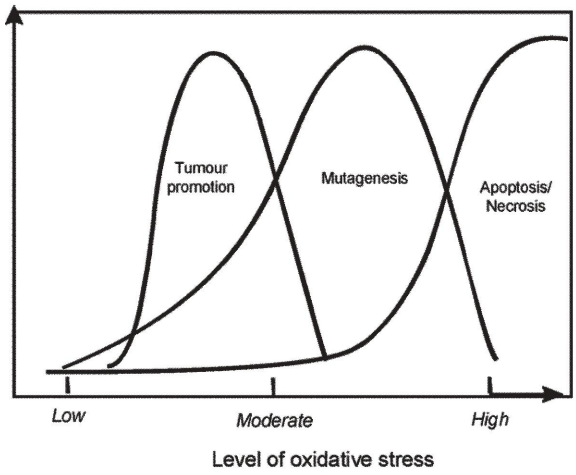

Fig. 1 Dose dependent relationship between the level of oxidative stress and the tumour promotion, mutagenesis and apoptosis/necrosis.

involved in the redox status of the cell. They can perform multiple cellular functions as energy production, cell proliferation, and cell death.[21] Mitochondria contain their own genetic material, mitochondrial DNA, which is maternally inherited and which is much smaller than the nuclear genome. It has been hypothesized that mitochondrial DNA plays a decisive role in carcinogenesis. This is connected with the fact that mitochondria are the major site for the generation of cellular oxidative stress and play a key role in mediating apoptosis. Damage to mitochondrial DNA is therefore an important contributor to carcinogenesis.[22]

Oxidative stress induces a cellular redox imbalance which has been found to be present in various cancer cells compared to normal cells and the redox imbalance thus may be related to oncogenic stimulation.[23] The intracellular redox state is thus a key determinant of cell fate, such as cell survival, proliferation, differentiation, and apoptosis, as well as selective clonal expansion of the initiated cells. The cumulative production of ROS is common for many types of cancer cells that are linked with altered redox regulation of cellular signaling pathways. The exact mechanisms, however, by which redox status induces cells to proliferate or to die, and how oxidative stress can lead to processes evoking tumor formation are still under investigation. The profound knowledge of these mechanisms can conveniently be used in the development of targeted cancer-preventive and therapeutic strategies.

Oxidative stress is not only linked to carcinogenesis but also to sensitivity or resistance of cancer cells to anticancer drugs.[10] The involvement of ROS in induction of apoptosis of various cancer cells, especially drug resistant cancers is well known.[24–26] The ability of a therapeutic agent to induce apoptosis of cancer cells often depends upon the ability of cancer cells to generate ROS. Drug resistant cancers usually show very low levels of ROS. This might be due to high intracellular GSH levels[27] and enhanced activities of antioxidant enzymes like GPx, CAT and SOD.[28] GSH is also required for phase II detoxification reactions, for example glutathione S-transferase requires GSH for the conjugation of electrophilic drugs and xenobiotics.[29] High levels of GSH have therefore been implicated in drug resistant tumors.[30]

Antioxidants and cancer chemotherapy

Dietary antioxidants as well as endogenous antioxidant mechanisms can help maintain an appropriate balance between the desirable and undesirable cellular effects of ROS. However, any health-related effects of interactions between dietary antioxidants and ROS likely depend on the health status of an individual. As disease prevention and disease treatment are different processes, the potential effectiveness of antioxidants is not expected to be the same although the basic mechanisms might be similar. If the free radicals actions and dietary antioxidants actions are different in normal cells and tumor cells, it is important to clarify whether these differences might have certain consequences of using antioxidant supplements along with standard chemotherapy. Therefore, clinical studies of antioxidant supplementation and changes in oxidative status have been carried out in healthy individuals and patients undergoing disease therapy (chemotherapy).

The use of antioxidants during cancer therapy is currently a topic of heated debate. Does the administration of antioxidants during cancer chemotherapy affect antineoplastic efficacy or only the development of side effects? The answer of this question depends on the properties of the individual antioxidants, the mechanism of action of the antineoplastic drugs, and the mechanism whereby antineoplastic agents cause their side effects. Additionally, the impact of chemotherapy-induced oxidative stress upon antineoplastic efficacy and the role that ROS may play in drug-induced apoptosis need to be elucidated.

Chemotherapy agents can be divided into several categories: alkylating agents (e.g., cyclophosphamide, ifosfamide), antibiotics which affect nucleic acids (e.g., doxorubicin, bleomycin), platinum compounds (e.g., cisplatin), mitotic inhibitors (e.g., vincristine), antimetabolites (e.g., 5-fluorouracil), camptothecin derivatives (e.g., topotecan), biological response modifiers (e.g., interferon), and hormone therapies (e.g., tamoxifen). The drugs most noted for creating cellular damage by initiating free radical oxidants are the alkylating agents, tumor antibiotics and the platinum compounds.

Chemotherapy has been associated with increased oxidative stress, which may further deplete tissue antioxidant levels. Faber and

colleagues[31] measured lipid peroxidation, plasma GSH and GPx activity and plasma micronutrient levels in patients with cancer before and after chemotherapy. The concentration level of lipid peroxidation products in the plasma of cancer patients was higher than in controls, and the level increased still more after chemotherapy. These results indicate that cancer patients had increased oxidative stress at presentation, which was further aggravated by chemotherapy. They had also lower plasma levels of GSH, GPx, selenium and zink.

Proponents of antioxidant use argue that co-administration of antioxidants may provide benefit when given along with chemotherapy.[32,33] During cancer chemotherapy, oxidative stress-induced lipid peroxidation generates numerous electrophilic aldehydes that can attack many cellular targets. These products of oxidative stress can slow cell cycle progression of cancer cells and cause cell cycle checkpoint arrest, effects that may interfere with the ability of

Table 1 Effect of various antioxidants combined with chemotherapeutic drugs.

Antioxidant	In vitro studies	Animal studies	Human studies
Vit. A	Increased cell differentiation andd more sensitive to DOX[39], Increased cytotoxic effect (VIN[39])	Decreased toxicity* (MT)[40]	Increased therapeutic effect (TAM[41,42])
Beta carotene		Increased therapeutic effect (CYC, DOX, ETO)[43]	
Vitamin C	Increased cytotoxic effect (DOX[44], CIS[44], TAX[44], VIN[45])	Increased therapeutic effect and decreased toxicity* (DOX[46])	
Vitamin E	Increased cytotoxic effect (DOX[47])	Increased therapeutic effect (MT[47], CYC[48])	Decreased neurotoxicity (CIS[49])
Selenium		Decreased toxicity* (DOX[50,51], CYC[52]), Increased therapeutic effect (CYC[52], DOX[53])	Decreased toxicity* (CIS[54])
Coenzyme Q10		Increased therapeutic effect (TAM[55])	Decreased toxicity* (DOX[56])
Melatonin		Decreased toxicity*, no change in therapeutic effect (CYC[57])	Decreased toxicity* (EPI[58]) Increased therapeutic effect (TAM[59]), Increased survival, Decreased toxicity* (CIS+ETO)[60,61]
Glutathione (GSH)			Reduced toxicity, increased therapeutic effect (CYC+CIS)[62,63]
Quercetin	Increased cytotoxic effect (CIS[64,65], DOX[66])	Increased therapeutic effect (CIS[64,65])	
Whey protein	Enhanced cytotoxicity of baicalein[67]	Decreased toxicity* (DOX)[68]	Render tumor cells more vulnerable to chemotherapy (CYC+ MT+ 5FU)[69]

* : "decreased toxicity" refers to healthy tissue

Abbreviations of antitumor drugs : VIN-vincristine ; CYC-cyclophosphamide ; CIS-cisplatin ; TAM-tamoxifen ; DOX-doxorubicin ; ETO-etoposide ; TAX-paclitaxel ; MT-methotrexate ; 5FU-5-fluorouracil ; EPI-epirubicin ; BUS-busulphan.

anticancer drugs to kill cancer cells. The aldehydes may also inhibit drug-induced apoptosis by inactivating death receptors and inhibiting caspase activity, which would also diminish the efficacy of the treatment. The use of anti-oxidants during chemotherapy may enhance therapy by reducing the generation of oxidative stress-induced aldehydes. The antioxidant's ability to quench aldehydes would actually accelerate the cytotoxic effects of chemotherapy, thereby improving its efficacy.[34]

For some supplements, activities beyond their antioxidant properties, such as inhibition of topoisomerase II or protein tyrosine kinases, may also contribute.[33]

A reasonable amount of data supports a beneficial effect for supplementation with high doses of antioxidants used in combination with conventional cancer therapy.[35,36] Antioxidants at high doses induce differentiation, proliferation inhibition and apoptosis, without producing similar effects on most normal cells in vitro and in vivo. The growth inhibiting effect of these agents on cancer cells may not involve antioxidant action, but changes in expression of genes and proteins. However, it was also indicated by the same authors, that low doses of antioxidants may be detrimental to cancer therapy.

According to the opponents of using antioxidants during cancer chemotherapy anticancer drugs rely in part on their ability to cause oxidative damage to the replicative machinery of cancer cells, and antioxidants might conceivably interfere with this therapeutic process.[1,2]

All antioxidants cannot be viewed as equal when evaluating their potential impact on cancer chemotherapy, and the individual antioxidants cannot be expected to have the same effect on the activity of all chemotherapeutic agents. Some of the potentially favorable impacts of antioxidant nutrients on chemotherapy are shown in Table 1.

Unfortunately, at present, there are no widely accepted standards to identify and document adverse events that arise from the use of dietary antioxidants along with chemotherapy. The clinical implications of drug/ antioxidant interactions depend on a variety of factors such as dose, frequency, and timing of antioxidant intake, dosing regimen, route of drug administration, and therapeutic range. Some of the antioxidants are complex mixtures of many molecular entities and generally contain a number of biochemicals, each of which may have some pharmacological effect. They may exert modulating effect (synergistic or antagonistic) on physiological activity and may have the potential to interact with various classes of drugs. Such interactions include induction or inhibition of metabolizing enzymes and drug efflux proteins.

In the excellent review of Seely et al., 2007 detailing interactions of antioxidants with respect to pediatric cancer and chemotherapy, it was proposed a strategy of "approach".[68] The suggested method avoids possible interactions based on the elimination half-lives of both chemotherapy and natural antioxidants. Basically, if a potential interaction is suspected, the strategy requires stopping administration of the antioxidant supplement within 3-5 half-lives before the start of chemotherapy and restarting it only after 3-5 half-lives of the chemotherapy have elapsed. Passage of at least 3 elimination half-lives ensures that more than 80% of the compounds are excreted, thereby decreasing the chance of pharmacodynamic interaction. The use of antioxidant supplements between rounds of chemotherapy would be thus possible, without risking potentially negative interactions.

Conclusion

Most metabolic processes involve free radical production. At low or moderate concentrations ROS and RNS are involved in the maturation of cellular structures, in host defense system and in the function of a number of cellular signaling mechanisms. However, when produced in excess, they generate oxidative stress, which can seriously affect cell membranes, proteins, lipids and DNA. If not regulated properly by the antioxidant systems of the organism, oxidative stress can induce a variety of chronic and degenerative diseases including cancer. The involvement of free radical overproduction and altered redox homeostasis in cancer suggests that antioxidant supplementation represents a promising approach for synergistic treatment together with the conventional chemotherapy. Through the scientific application of antioxidants many of the side effects of conventional cancer chemotherapy can be prevented or ameliorated by protecting healthy tissues from cytotoxic effects of chemotherapeutic drugs. Also, various dietary components may enhance the actions of chemotherapeutic agents. However, many questions about the effect of antioxidant supplements

on the activity of anticancer drugs in cancer patients remain unsolved or controversial. Further research is needed before this supplementation could be officially recommended as an adjuvant therapy. Reliable clinical studies involving an extensive number of patients must be conducted for studying especially the long-term impact of antioxidants upon the efficacy of cancer chemotherapy. The future development of synergistic and protective scientifically proved strategies, including dietary antioxidants along with conventional chemotherapy, might improve quality of life for the people facing a cancer challenge.

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