CHAPTER 2

INTRODUCTION

Cancer is one of the most common causes of morbidity and mortality today, with more than 10 million new cases and more than 6 million deaths each year worldwide and is the second leading cause of death in all over world (Greenlee et al., 2000). Epidemiologists estimate that over 80% of human cancer is due to environmental factors. The evidence for oxygen free radical involvement in some types of chemically induced carcinogenesis is now quite strong (Ajitha and Rajnarayana, 2001). Studies suggest that oxidative stress may be causally related to the incidence of many chronic diseases, such as cancer, and that antioxidant nutritional status is inversely related to the occurrence of various cancers. However, there is limited information about possible racial differences in associations between antioxidant nutrients and oxidative stress in healthy persons, and there is a need for improved methodologies to accurately assess self-reported antioxidant nutrient intakes in population-based epidemiologic studies (Jessie, 2000). The major treatment in all forms of cancer is radiology, chemotherapy and immunotherapy. Chemotherapy and radiotherapy are supposed to increase the survival rate of many patients suffering from different forms of cancer. The toxicities of chemotherapy and radiotherapy can adversely affect short and long-term patient quality of life, can limit the dose and duration of treatment, can be life-threatening, and may contribute to both the medical and non medical costs of cancer care (Raber-Durlacher et al., 2000). The ideal chemotherapy and radiotherapy protectant agent would prevent all toxicities, from non–life-threatening side effects like alopecia to irreversible morbidities like hearing loss and neurotoxicity to potentially fatal events (severe cardiomyopathy, severe thrombocytopenia), without adversely affecting the antitumor efficacy of the cancer therapy, and would be easy to administer and relatively nontoxic in its own right.

However, most agents developed to date have a much more narrow spectrum of toxicity protection. For example, the development of serotonin receptor antagonists has led to dramatic improvement in the ability to control chemotherapy related nausea and vomiting. Because their mechanism of action and cellular targets made it highly unlikely that their use would interfere with the antitumor activity of concomitant chemotherapy, and because the toxicity end point (acute nausea and vomiting) is relatively easy to measure, the design of randomized, controlled

trials to assess the clinical efficacy of the serotonin receptor antagonists was straightforward. Evidence-based guidelines have already been developed for their use. Similarly, the development of myeloid and, more recently, thrombopoietic growth factors made it possible to ameliorate myelosuppression. Again, tumor protection, at least in nonhematologic malignancies, has not been a major concern because of the narrow cellular target of these agents (Martee et al., 1999).

2.1 Chemotherapy Associated Oxidative Stress:

Chemotherapy-related toxicity is a major concern in the treatment of patients with solid tumors, particularly in those patients who are cured or achieve prolonged survival. Besides the acute toxicity occurring immediately following the administration of cytostatic agents, e.g., nausea, alopecia, oral mucositis and bone marrow depression, long-term side effects can reduce the quality of life of these patients (Raber-Durlacher et al., 2000). Antineoplastic agents have been shown to produce oxidative stress in patients who receive these drugs during cancer chemotherapy (Faber et al., 1995; Weijl et al., 1998; Sangeetha et al., 1990). This is evident by the elevation of lipid peroxidation products; the reduction of total radical trapping capacity of blood plasma; the reduction in plasma levels of antioxidants such as vitamin E, vitamin C, and β-carotene; and the marked reduction of tissue glutathione levels that occurs during chemotherapy. Those agents that generate high levels of reactive oxygen species (ROS) include the anthracyclines (eg, doxorubicin, epirubicin, and daunorubicin), alkylating agents, platinum coordination complexes (eg, cisplatin, carboplatin, and oxaliplatin), epipodophyllotoxins (eg, etoposide and teniposide), and the camptothecins (eg, topotecan and irinotecan). The anthracyclines generate by far the highest levels of oxidative stress. This is due to their ability to divert electrons from the electron transport system of cardiac mitochondria, resulting in formation of superoxide radicals (Gille and Nohl, 1997), in addition to generating ROS at other cellular sites. In contrast to the above groups of antineoplastic agents, taxanes (eg, paclitaxel and docetaxel), vinca alkaloids (eg, vincristine and vinblastine), antimetabolites such as the antifolates, and nucleoside and nucleotide analogues generate only low levels of oxidative stress. However, all

antineoplastic agents generate some ROS as they induce apoptosis in cancer cells. This is because one of the pathways of drug-induced apoptosis involves the release of cytochrome c from mitochondria (Kaufmann and Earnshaw, 2000). When this occurs, electrons are diverted from the electron transport system to oxygen by NADH dehydrogenase and reduced coenzyme Q10, resulting in the formation of superoxide radicals. Oxidative stress interferes with cellular processes (cell cycle progression and drug-induced apoptosis) that are necessary for antineoplastic agents to exert their optimal cytotoxicity on cancer cells, and modest levels of oxidative stress have been shown to reduce the cytotoxicity of anticancer drugs (Shacter et al., 2000; Lee and Shacter, 1999). Thus, the formation of ROS that occurs when anticancer drugs are administered may diminish the effectiveness of the treatment. In addition, since some side effects caused by antineoplastic agents appear to be prevented by certain antioxidants, administering these supplements during chemotherapy may diminish the development of side effects as well as improve the response to therapy. This contention is supported by many preclinical and some clinical studies (Conklin, 2000; Lamson and Brignall, 1999).

2.2 Mediators of Oxidative Stress

Oxidative stress caused by free radicals has become an area of interest in understanding the process of human disease. Oxidative stress is caused by exposure to reactive oxygen intermediate such as superoxide anion, hydrogen peroxide and hydroxyl radical, which can damage protein, nucleic acid and cell membrane (Aruoma and Halliwell, 1998). Free radicals generated during oxidative stress have many cellular targets, although one of the primary targets is a cellular lipid. Lipid peroxidation of polyunsaturated fatty acids results in the formation of peroxyl and alkoxyl radicals. These primary products of lipid peroxidation, which are highly reactive and relatively short-lived, undergo further reactions to form secondary products of lipid peroxidation that include a variety of aldehydes such as malondialdehyde and acrolein. The aldehydes are more stable than the primary products and can diffuse throughout the cell where they damage cellular components and interfere with cellular functions. Because of their electrophilic character, the aldehydes bind to nucleophilic groups of amino

acids, such as cysteine, lysine, histidine, serine, and tyrosine, which are critical components of enzyme active sites or are necessary for maintaining the tertiary structure of proteins. The binding of aldehydes to proteins, which results in enzyme inhibition and alteration of the structure of cellular receptors, may account for the impact of oxidative stress on the cytotoxicity of antineoplastic agents (Kenneth and Conklin, 2004).

Reactive oxygen species are essential for life because of their role in many vital processes such as signal transduction and the ability of phagocytes to carry out their bactericidal activity. Reactive oxygen species include free radicals, such as hydroxyl and superoxide radicals, which are substances with one or more orbital electrons with unpaired spin states, and non radicals, including hydrogen peroxide and singlet oxygen. Although superoxide is not highly toxic, mitochondrial superoxide dismutase generates hydrogen peroxide from superoxide radicals, and, in the presence of reduced iron or copper, the highly toxic hydroxyl radical is formed via Fenton or Haber-Weiss reactions. The cytochrome P450 monooxygenase system of the hepatic endoplasmic reticulum (microsomes) also generates a substantial amount of ROS in the process of metabolizing a chemically diverse group of compounds that includes most of the drugs that we administer as well as environmental substances. The plasma and nuclear membranes are less active sites of ROS production, and enzyme systems, such as the xanthine-xanthine oxidase system, can also generate ROS. Reactive oxygen species can interact with cellular macromolecules, including DNA, protein, and lipids, and interfere with vital cellular functions. Mutations caused by ROS can result in malignant transformation and the development of cancer. Reactive oxygen species are also implicated in the etiology and progression of many other diseases. Under normal conditions, antioxidant mechanisms, including small-molecular-weight antioxidants and antioxidant enzyme systems, scavenge ROS and protect the organism from the damaging effects of oxidative stress. However, under conditions of excessive oxidative stress, for example, those which occur with the administration of certain drugs, cellular antioxidant mechanisms may be unable to prevent the adverse impact of ROS on critical cellular processes. In cancer cells, processes such as the ordered progression through the cell cycle and intact apoptotic processes are necessary for antineoplastic agents

to exert their optimal cytotoxic activity. Since many antineoplastic agents are capable of producing oxidative stress in biological systems, ROS generated during cancer chemotherapy may interfere with the efficacy of the treatment.

Oxidative stress interferes with many cellular functions, such as cell cycle progression and apoptotic pathways that can reduce the ability of antineoplastic agents to kill cancer cells. The effects are mediated, most likely, by the many aldehydes that result from oxidative stress-induced lipid peroxidation. Since many drugs used for cancer chemotherapy cause oxidative stress, which can interfere with antineoplastic activity, reducing this oxidative stress by administering antioxidants may enhance the effectiveness of the treatment. However, enhancing the cytotoxicity of antineoplastic agents would affect normal cells as well as cancer cells. Thus, although certain antioxidants appear to prevent the development of some chemotherapy-induced side effects (coenzyme Q10 for anthracycline cardiotoxicity and glutathione for cisplatin nephrotoxicity), side effects that result from toxicity to rapidly proliferating normal cells, such as myelosuppression, hair loss, and mucositis (toxicity to enterocytes of the gastrointestinal tract), and may also be enhanced by antioxidants. In this regard, vitamin E has been shown to enhance doxorubicin-induced myelosuppression in mice (Alberts et al., 1978). Certainly, further research is needed to fully elucidate the impact of single antioxidants and antioxidant combinations on the anticancer activity and side effects of individual drugs and the many drug combinations that are in clinical use.

2.3 Antioxidants in Cancer Therapy

While the efficacy of antioxidants during cancer treatment is still being evaluated and clinical trials are ongoing or being set up (NIH, 2004), many cancer patients who are undergoing therapy take antioxidant supplements in an effort to alleviate treatment toxicity and improve long-term outcome. The modulating effects of antioxidants in treatment depend on a wide range of factors, including the metabolic state of the patient, the stage and site of the disease, and the modality being used. Chemotherapeutic agents are not confined to the target tissue; they prevail in the whole body for some time and can interact with and damage a plethora of cellular molecules at different sites for longer periods of time,

depending on metabolism, increasing lipid peroxidation of molecules, reducing antioxidant levels, and enhancing oxidative stress (Sangeetha et al., 1990; Weijl et al., 1998). The primary focus of radiation therapy and chemotherapy is to produce irreversible DNA damage in tumor cells that will prevent their replication and lead to their demise. Another course of action is to alter cellular homeostasis and modify signal transduction pathways, redox state, and disposition to apoptosis. The cellular changes would, ideally, enhance tumor cell killing, largely by apoptosis, and reduce the probability of normal cell death.

2.3.1 Cancer Treatment Reduces Tissue Antioxidants

The development of cancer produces oxidative stress that increases with disease progression (Khanzode et al., 2004; Sangeetha et al., 1990); levels of antioxidants further decrease in response to treatment (Weijl et al., 1998) and therapeutic doses of radiation deplete α-tocopherol in normal cells, increasing their risk of damage (Borek, 1991; Borek, 1997). A decline in tissue vitamin E and selenium, during radiation therapy of breast cancer, and a marked reduction in vitamins A, C, E, β-carotene, and selenium during breast cancer treatment with doxorubicin, would increase normal tissue sensitivity to free radical damage during radiotherapy and treatment with anticancer drugs.

2.3.2 Antioxidants and Tissue Specificity in Therapy

Antioxidant efficacy in tissues depends on the prevailing oxygen partial pressure (P_{O2}) and the nature of the antioxidant, a point that should be considered in antioxidant supplementation during therapy. β-carotene is an effective chain-breaking antioxidant at low oxygen pressure and less efficient at high oxygen pressure, where it may even act as a prooxidant; this may partly explain the lack of efficacy in reducing lung cancer risk in smokers (Virtamo et al., 2003). By contrast, α-tocopherol is an efficient antioxidant in cells with a high oxygen pressure, for example, the lung. The radiation oncologist aims to increase the oxygen content of tumors to enhance the efficacy of cell killing; as P_{O2} differs among tumors, so would the modulating effect of antioxidants in radiation therapy, varying with antioxidant used and the tumor P_{O2} (Young and Lowe, 2001).

2.3.3 Anticancer Drugs

Almost all anticancer drugs work by affecting DNA synthesis; they do not kill resting cells unless those cells divide soon after exposure to the drug. Consequently, the efficacy of anticancer drugs used in chemotherapy is limited by the fraction of actively dividing cells. Most anticancer drugs do not rely on reactive oxygen species, although a few produce free radicals that play a role in treatment; these include bleomycin, which produces superoxide radicals; doxorubicin and cisplatin, but although bleomycin is more toxic to oxygenated cells, similar to x-rays and γ-rays, doxorubicin is preferentially toxic to hypoxic cells. The potential effect of antioxidants in modifying treatment efficacy would depend on the type of drug in use. Antioxidant protection of normal cells, however, would occur, in principle, in all treatments, even when the mechanism of the chemotherapeutic drug is independent of free radical action; antioxidants help maintain the health of normal tissues and protect them from the toxic effects of free radical producing cytokines that circulate in cancer patients and increase with the severity of the disease (Sozen et al., 2004).

2.3.4 Preventing Therapy Induced Injury

While antioxidant vitamins E, C, and selenium may have potential in enhancing the efficacy of cancer treatment by radiotherapy and chemotherapy, they may also protect against side effects to normal tissues that are associated with treatment. Vitamin E (400 IU) and vitamin C (500 mg) protect against proctitis, a painful chronic injury that affects 5% to 20% of patients receiving radiotherapy for cervical and prostate cancer; a clinical trial that combined radiation treatment of head and neck cancer with vitamin E (1000 IU) and pentoxifylline (800 mg) supplementation showed a striking regression of chronic radiation-induced fibrosis, a complication associated with radiation therapy that is difficult to manage clinically, as it does not regress spontaneously (Delanian et al.,1999). A combination of vitamin E and pentoxifylline also reduced fibroathrotic uterine lesions in young women who had been previously irradiated for childhood cancer (Peter and Gottlober, 2002). Several clinical studies have tested the efficacy of antioxidants in ameliorating toxic side effects in chemotherapy, which are of major concern in patients treated for solid tumors. Cisplatin is currently the

most important cytostatic agent for treating a wide range of solid tumors and palliative in metastatic cancers (Wejl et al., 2004). Cisplatin is a free radical producing drug that significantly decreases plasma concentration of antioxidants (Weijl et al., 1998). Nephrotoxicity, loss of high tone of hearing and peripheral neuropathy is major long term side effects in cisplatin treatment. These toxic effects, which are in part irreversible, are largely attributed to free radical damage, reduction in antioxidant levels and oxidant stress, which are induced by cisplatin. Formation of free radicals leading to oxidant stress has been shown to be the major pathogenic mechanism in the toxic side effects of cardiomyopathy in doxorubicin therapy and pulmonary damage in bleomycin treatment (Wejl et al., 1997). A recent randomized, placebo-controlled, double blind study tested the effects of supplementation vitamin E (dl-α-tocopherol acetate 400 mg), vitamin C (ascorbic acid 1000 mg), and selenium (100 μg) compared to placebo in patients with solid tumors treated with cisplatin. While there were no significant differences in nephrotoxicity and ototoxicity between supplemented patients and placebo over one year of chemotherapy that markedly reduced antioxidant levels, patients who achieved the highest plasma concentrations of vit.E, vit C and selenium antioxidants, including those who had a higher antioxidant status from the start, had a significantly smaller loss of high-tone hearing. As vitamin E has shown protection against cisplatin-induced neurotoxicity in another study, the present results are attributed to poor compliance and/or inadequate supplementation, promoting the initiation of a new study with vitamins E, C, selenium, and 8 other antioxidants (Wejl et al., 2004). Other studies have shown that vitamin E (total of 800 mg/d) prevents oral mucositis in doxorubicin treated patients, in some cases with complete resolution (Lopez et al., 1994) and helps attenuate cardiomyopathy in cancer patients treated with doxorubicin and radiotherapy (Wagdi et al., 1996).

Coenzyme Q10 (CoQ10) is a cellular protective lipid that has a vital role in energy production as an electron carrier in the respiratory chain. CoQ10 is also a potent antioxidant and maintains the antioxidant state of vitamin E. CoQ10 is synthesized in human cells under the control of HMG CoA reductase, an enzymatic pathway it shares with cholesterol; thus, patients taking cholesterol reducing statin drugs may be advised to take CoQ10, whose synthesis is decreased during statin treatment. Synthesis of

CoQ10 decreases with aging; as cancer is an age-related disease, oxidative stress, which occurs during the disease and is compounded in older persons, is further enhanced following radiation or anticancer drug treatment, thereby depleting critical levels of CoQ10 that are needed in energy production and antioxidant protection (Borek, 2004). A potential important role for CoQ10 during chemotherapy is the prevention of doxorubicin-induced cytotoxicity, with several studies showing that CoQ10 supplementation may provide a safe and effective way to reduce or prevent cardiotoxicity associated with chronic treatment with anthracyclines (Iarussi et al., 1994).

In addition to the more common nutrient antioxidants, a wide range of phytochemicals, including flavonoids, carotenoids, and organosulfur compounds, are antioxidants and prevent free radical damage by radiation and some chemotherapeutic agents in experimental systems (Borek, 1997). These phytochemicals induce apoptosis in cancer cells; they may have a potential role in adjuvant cancer therapy and may protect normal cells from the acute and long-term effects of free radicals produced in the course of treatment.

Epidemiological studies show that a high intake of antioxidant-rich foods is inversely related to cancer risk. While animal and cell cultures confirm the anticancer effects of antioxidants, intervention trials to determine their ability to reduce cancer risk have been inconclusive, although selenium and vitamin E reduced the risk of some forms of cancer, including prostate and colon cancer, and carotenoids have been shown to help reduce breast cancer risk. Cancer treatment by radiation and anticancer drugs reduces inherent antioxidants and induces oxidative stress, which increases with disease progression. Vitamins E and C have been shown to ameliorate adverse side effects associated with free radical damage to normal cells in cancer therapy, such as mucositis and fibrosis, and to reduce the recurrence of breast cancer. While clinical studies on the effect of antioxidants in modulating cancer treatment are limited in number and size, experimental studies show that antioxidant vitamins and some phytochemicals selectively induce apoptosis in cancer cells but not in normal cells and prevent angiogenesis and metastatic spread, suggesting a potential role for antioxidants as adjuvants in cancer therapy.

Anticancer drug induced toxicities are real obstacles to the successful chemotherapy of anticancer drugs. Administrations of antioxidant agents in animals, but also in clinical studies, have been shown to ameliorate or prevent some of these side effects. Antioxidants are endogenous substance protecting against the assaults by free radicals. Antioxidants are free radical scavengers. The study of antioxidant use in cancer treatment is a rapidly evolving area. Antioxidants have been extensively studied for their ability to prevent cancer in humans. There are possibilities that providing a good antioxidant system to the body can alleviate this oxidative stress. Antioxidants have been shown to dramatically improve the tumor cell damage from pro-oxidative chemotherapy and radiotherapy while protecting the host tissue from damage (Weisburger, 1991). Essentially, the proper selection of nutrients taken before and during chemotherapy and radiotherapy can help make the therapy more of a selective toxin against the cancer.

Antioxidants protect against oncogenic transformation by radiation and free radical–producing anticancer drugs in experimental systems. Antioxidants do reduce the painful side effects of radiation and chemotherapy, thus supporting the beneficial effects of antioxidants in protecting normal cells during treatment and acting as adjutants in the treatment of certain cancers.

2.4 Aims and Objectives

Antioxidant supplementation may prevent the adverse effects of cancer chemotherapy without interfering with their antitumor capabilities, resulting in an improved quality of life for cancer patients. There are several agents reported for their antioxidant activities in various models offering potential as part of intervention in anticancer drug induced oxidative stress. Anticancer drug induced oxidative stress has been reported to be decreased by pretreatment of antioxidants like Vitamin A, C, and E. There are evidences that melatonin, green tea extract, resveratrol (extract of red wine) has also antioxidant effects (Takako et al., 1999; Karim et al., 2001; S devi and Shyamala Devi., 1999; Bardeleben et al., 2002). Recently it was shown that lovastatin potentiate antitumor activity and attenuate cardiotoxicity of doxorubicin (Feleszko et al., 2000).

Green tea, which is a widely consumed drink, has received much attention due to the beneficial biological effects attributable to its excellent antioxidative activity (Guo et al., 1996; Pannala et al., 1997; Yokozawa et al., 1998). Recent reports on tea and human health claim a plethora of therapeutic properties including antilipidaemic, antineoplastic, antidiabetic, antihypertensive, antioxidant and many others (Krishnamoorthy, 1991). Much experimental research has been conducted on the anticarcinogenic properties of mostly green tea extracts and its major constituents (Yang et al., 2000).

Melatonin, N-Acetyl-5-methoxytryptamine, is hormonal product of pineal gland. Melatonin is very potent and efficient endogenous free radical scavenger and affords protection of molecules, especially DNA, from oxidative damage (Poeggeler et al., 1993; Reiter, 1993). Melatonin acts as a primary non-enzymatic antioxidative against the devastating actions of the extremely reactive hydroxyl radical. Melatonin also stimulates glutathione peroxidase activity, which metabolizes the precursor of the hydroxyl radical, hydrogen peroxide, to water (Delius et al., 1988).

Lovastatin is an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase has also been shown to arrest tumor and normal cells in the G1 phase of the cell cycle (Jakobisiak et al., 1991) and has demonstrated antitumor effects in experimental murine models (Rao et al., 1998; Sumi et al., 1992). Agarwal et al. (1999) have shown that pretreatment with lovastatin increased apoptosis induced by chemotherapeutic agents in tumor cells *in vitro*. Lovastatin has also been shown to strengthen the antitumor activity of cisplatin and tumor necrosis factor-α in murine tumor models (Feleszko et al., 1999).

Resveratrol (3,4'-trihydroxystilbene), a natural phytoalexin present in grapes and many other natural sources, has been suggested to play a role in reducing the risk of coronary heart disease and cancer (Kopp, 1998). In addition, resveratrol intake has been reported to have anti-inflammatory and anti-atherosclerosis functions and to modulate hepatic apoliprotein and lipid synthesis, platelet aggregation, and production of antiatherogenic eicosanoids by human platelets and neutrophils. Resveratrol has also been reported to inhibit the development of preneoplastic lesions in carcinogen-

treated mouse mammary organ cultures and the promotional stage of mouse skin carcinogenesis (Jang and Pezzuto, 1999).

Therefore, it would be interesting to find out whether these antioxidants like green tea extract, melatonin, lovastatin and resveratrol have any protective effect against doxorubicin and cisplatin induced toxicity.

Therefore, present work **"Effect of Various Antioxidants on Anticancer Drug Induced Oxidative Stress"** is aimed-

- **To study the antioxidant activity of green tea extract, melatonin, lovastatin and resveratrol by using *in vitro* methods, like DPPH assay, NBT reduction method, nitric oxide scavenging activity and lipid peroxidation.**

- **To study the effect of green tea extract, melatonin, and lovastatin on doxorubicin induced cardiotoxicity and also to study the effect of these drugs on various antioxidant and hemodynamic parameters to justify whether the cardioprotective effect is due to the antioxidant mechanism of action.**

- **To study the effect of green tea extract, melatonin, and lovastatin on cisplatin induced nephrotoxicity and also to study the effect of these drugs on various antioxidant parameters to justify whether the nephroprotective effect is due to the antioxidant mechanism of action.**

- **To study the effect of green tea extract, melatonin, and lovastatin on doxorubicin induced testicular toxicity and also to study the effect of these drugs on various antioxidant parameters to justify whether the protective effect is due to the antioxidant mechanism of action.**

- **To study the effect of green tea extract, melatonin, and lovastatin on cisplatin induced testicular toxicity and also to study the effect of these drugs on various antioxidant parameters to justify whether the protective effect is due to the antioxidant mechanism of action.**

- **To study the effects of resveratrol on doxorubicin induced oxidative stress in DMBA induced mammary gland cancer.**