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Free Radical Toxicology and Cancer Chemoprevention

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ABSTRACT *: Most reactive oxygen species (ROS) are free radicals and implicated in the development of a number of disease processes including artherosclerosis, neurodegenerative disorders, aging and cancer. ROS are byproducts of a number of in vivo metabolic processes and are formed deliberately as part of normal inflammatory response. On the other hand, ROS are generated either as by products of oxygen reduction during xenobiotic metabolism or are liberated as the result of the futile redox cycling of the chemical agents including several chemical carcinogens. A better understanding of the mechanisms of free radical toxicity may yield valuable clue to risks associated with chemical exposures that leading to the development of chronic diseases including cancer. The molecular biology of ROS-mediated alterations in gene expression, signal transduction and carcinognesis is one of the important subjects in free radical toxicology. Epidemiological studies suggest that high intake of vegetables and fruits are associated with the low incidence of human cancer. Many phytopolyphenols such as tea polyphenols, curcumin, resveratrol, apigenin, genistein and other flavonoids have been shown to be cancer chemopreventive agents. Most of these compounds are strong antioxidant and ROS scavengers in vitro and effective inducers of antioxidant enzymes such as superoxide dismutatse, catalase and glutathione peroxidase in vivo. Several cellular transducers namely receptor tyrosine kinase, protein kinase C, MAPK, PI3K, c-jun, c-fos, c-myc, NFκB, IκB kinase, iNOS, COX-2, Bcl-2, Bax, etc have been shown to be actively modulated by phyto-polyphenols. Recent development in free radical toxicology have provided strong basis for understanding the action mechanisms of cancer chemoprevention.*

Key Words *: Reactive oxygen species (ROS), Xenobiotics, Redox cycling, Carcinogens, Antioxidants, phytopolyphenols, Signal transduction blockade*

I. INTRODUCTION

During the past decades, we have witnessed an explosion of research and understanding of oxygen free radicals or reactive oxygen speices (ROS) and their implication in several pathologic processes (Marnett, 1987). It has come to light that free radical intermediates are not foreign to the normal biology of the cell. ROS are by products of many metabolic processes and are formed deliberately as part of the normal inflammatory response or extracelluar insults (Cohen *et al.*, 1987; Rashbastep and Cederbaum, 1994). To accommodate this, tissue have developed antioxidant defense systems to hold in-check the potential for free radical-mediated tissue damage (Fridovich, 1989; Shull *et al.*, 1991).

An alternate cause for excessive free radical generation is the presence of selected xenobiotics including environmental toxicants and carcinogenic compounds (Goeptar *et al.*, 1995). There exists a rapidly growing list of chemicals which when added to biological systems stimulate several-fold increases in the rate of free radical generation. For the most part, the free radicals are generated either as byproducts of oxygen reduction during xenobiotic metabolism or are liberated as the result of the redox cycling of the chemical agent (Cohen *et al.*, 1987; Goeptar *et al.*, 1995). With the increasing implication of free radical-mediated and oxidative mechanisms of chemical-induced tissue injury, more investigations on this subject are both justified and timely. A better understanding of the mechanisms of free radical toxicity might yield valuable insight and opportunity to managing and preventing the risks associated with the exposures of these chemical toxicants and carcinogens.

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Using experimental carcinogenesis models in animals, it has been well-established that oxidative stress plays a causative role during carcinognesis (Cerutti, 1994; Slaga *et al.*, 1981). Consistent with the involvement of oxidative stress in cancer induction and its subsequent development, efforts are being made to identify naturally-occurring antioxidants which could prevent, slow, and/or reverse cancer induction and its subsequent progresson (Perchellet and Perchellet, 1989; Dragsted, 1998). Many phytopolyphenols including tea catechins, theaflavins, curcumin, apigenin, resveratrol and silymarin are phenolic antioxidants and are effective cancer chemopreventive agents. In addition, these polyphenols have been shown to be effective in blocking several mitogenic signals and in modulating the cellular differentiating pathways. It is obvious that these phytopolyphenols could inhibit the processes of carcinognesis by multiple mechanisms namely antioxidative reaction and signal transduction blockades.

II. FREE RADICAL INDUCES SIGNAL TRANSDUCTION

When eukaryotic cells were exposed at the above-normal levels of ROS are referred to as oxidative stress. This phenomenon occurs frequently in cells exposed to UV light, ionizing radiation or low concentration of H_2O_2 (Devary *et al.*, 1991; Nose *et al.*, 1991), but also upon stimulation of cells with mitogens, cytokines and other ligands for cell surface receptors (Lan and Nathans, 1987). Only high levels of ROS, as produced by stimulated neutrophils, are strictly cytocidal as they cause irreversible damage to DNA, proteins and lipids.

ROS can cause the cellular prooxidant states that have been implicated in a variety of physiological and pathological processes such as inflammation, aging and carcinogenesis. Hydrogen peroxide was reported to have mitogenic effect on quiescent Balb/c 3T3 cells through biochemical processes common to natural growth factors including DNA synthesis, competence family gene expression and protein phosphorylation. Recently, ROS have been shown to induce the immediate early response genes c-fos, c-myc and c-jun (Nose *et al.*, 1991) which can also be induced by tumor promoter TPA (Lamph *et al.*, 1988). Interestingly, c-jun gene is far more responsive to UV than any other immediate early gene than was examined including c-fos (Devary *et al.*, 1991). Ionizing radiation induces c-jun transcription through the formation of ROS and that a protein kinase is also involved in this signaling pathway.

There are strong evidences that hydrogen peroxide stimulate protein kinase activation and protein phosphorylation.

III. INDUCTION OF C-JUN EXPRESSION BY HYDROGEN PEROXIDE THROUGH HYDROXYL RADICAL GENERATION

For more understanding about the action of ROS on cells, we have studied signal pathways responsible for c-jun induction by reagent hydrogen peroxide in NIH3T3 cells (Lee *et al.*, 1996). Hydrogen peroxide can cross cell membrane rapidly, while other ROS such as superoxide anion and hydroxyl radical can not. Once inside the cell, hydrogen peroxide can react with iron or copper ion according to the Fenton reaction to form hydroxyl radical that was suggested to be the most important mediator of hydrogen peroxide action on cells including cytotoxicity and DNA damage (Mello-Filho *et al.*, 1954). In the present work, we have demonstrated that induction of c-jun by hydrogen peroxide is also mediated by hydroxyl radical.

The mechanisms of signal transduction of c-jun induction by hydrogen peroxide are elucidated in NIH3T3 cells by using trapping agents of hydroxyl radical or inhibitors of various protein kinases. Pretreatment of the cell with hydroxyl radical scavenger dimethylsulfoxide (DMSO) abolishes the hydrogen peroxide-induced c-jun expression. Hydroxyl radical generation can be detected and quantified in cells treated sequentially with DMSO and hydrogen peroxide for 30 min separatively by methane sulfinic acid production (Lee *et al.*, 1996). Induction of c-jun by hydrogen peroxide is also dramatically reduced by pretreating the cells with biological antioxidant vitamin E. Protein tyrosine kinase activity of membrane fraction is induced by hydrogen peroxide within 5 min, which can be prevented by DMSO pretreatment. Inhibitor of non-receptor type tyrosine kinase, herbimycin A, has inhibitory effect on hydrogen peroxide-induced c-jun expression while the inhibitor of receptor type tyrosine kinase, tyrphostin 23 or inhibitor of

c-AMP dependent protein kinase, KT5720, has not. Our results suggest that the highly reactive species, hydroxyl radical, is generated after hydrogen peroxide enter cells and mediate the signal responses of hydrogen peroxide including c-jun induction and the activation of $p60^{src}$ tyrosine kinase might be one of the molecular events associated with the c-jun induction pathway (Lee *et al.*, 1996).

IV. INHIBITION OF XANTHINE OXIDASE AND SUPPRESSION OF INTRACELLULAR ROS IN HL-60 BY TEA POLYPHENOLS

The formation of hydrogen peroxide and oxidized DNA bases by human polymorphonuclear neutrophils stimulated with various tumor promoters correlates well with the *in vivo* tumor promoting potencies of the activating chemicals (Frenkel and Chrzan, 1987). A tumor promoter such as TPA enhances the generation of ROS accumulation and decreases the ROS detoxification enzymes in both epidermal and inflammatory cells. It triggers ROS accumulation through activation of xanthine oxidase (XO) or the stimulation of neutrophils which cause NADPH oxidase activation.

The inhibitory effects of five tea polyphenols, namely, theaflavin (TF1), theaflavin-3-gallate (TF2), theaflavin-3,3-digallate (TF3), (-)-epigallocatechin-3-gallate (EGCG) and gallic acid and propyl gallate (PG, a synthetic antioxidant) on XO activity were investigated (Lin *et al.*, 2000). These six antioxidant compounds reduce oxidative stress. Theaflavins and EGCG inhibit XO to produce uric acid and also act as scavengers of superoxide. TF3 acts as a competitive inhibitor and is the most potent inhibitor among these compounds. Tea polyphenols and PG all have potent inhibitory effects (>50%) on TPA-stimulated superoxide production at 20~50 μM in HL-60 cells. The antioxidative activity of tea polyphenols and PG is due not only to their ability to scavenge superoxides but also to their ability to block XO and related oxidative signal transducers (Lin *et al.*, 2000).

V. CANCER CHEMOPREVENTION BY TEA AND TEA POLYPHENOLS

Tea is one of the most popular beverages in the world. It has been shown that both green tea and black tea have antioxidant activity and are able to inhibit tumor induction in experimental animal systems (Wang *et al.*, 1994). In recent years, many animal studies and several epidemiological investigations have suggested that the anti-carcinogenic effects of tea (Katiyar and Mukhtar, 1996). Extracts of green, black and other teas inhibited TPA-induced JB6 cell transformation (Jain *et al.*, 1989). Recent studies in our laboratory have demonstrated that both green tea polyphenol EGCG and black tea polyphenol TF3 suppress the EGF-receptor autophosphorylation and proliferative signals in fibroblast cells (Liang *et al.*, 1997; Liang *et al.*, 1999a) and inhibit the activity of inducible nitric oxide synthase in macrophages (Lin and Lin, 1997; Lin *et al.*, 1999a). Furthermore, TF3 can inhibit the activity of protein kinase C and AP-1 binding in NIH3T3 cells (Chen *et al.*, 1999). EGCG can arrest cell division at the G1 phase through inhibiting the cyclin-dependent kinases 2 and 4 and elevating the cdk inhibitors p21 and p27 (Liang *et al.*, 1999b). On the basis of these findings, we have proposed that the mechanisms of action by tea and its polyphenols on cancer chemoprevention may be through the signal transduction blockade (Lin *et al.*, 1999) and ROS scavenging.

VI. CANCER CHEMOPREVENTION BY CURCUMIN

Curcumin is the major yellow pigment in tumeric and curry. Curcumin has been demonstrated to exert chemopreventive activity in mouse skin, mouse colon and rat mammary tumors (Huang *et al.*, 1988). The action mechanisms of curcumin has been extensively investigated. It is a potent anti-inflammatory agent. It inhibits arachidonic acid metabolism by inhibiting both the lipoxygenase and cyclooxygenase pathways (Huang *et al.*, 1991). Curcumin exhibits strong antioxidant activity, being an effective superoxide scavenger (Sharma, 1976). It inhibits TPA-induced DNA synthesis, demonstrating an inhibitory effect on proliferation (Huang *et al.*, 1988). It also modifies cytochrome p450 and enhances glutathione-S-transferase activity and it may modify the metabolic activation and DNA-binding of polycyclic aromatic hydrocarbon carcinogens by this mechanism.

Curcumin decreases expression of c-jun (Huang *et al.*, 1991), c-fos and c-myc possibly through inhibition of protein kinases (Liu *et al.*, 1993), inhibits ornithine decarboxylase activity and EGF receptor function (Korutla *et al.*, 1995). The activity of xanthine oxidase was inhibited by curcumin (Lin and Shih, 1994) and the TPA-induced 8-hydroxyguanosine in cellular DNA was suppressed by curcumin (Shih and Lin, 1993). Recent studies in our laboratory have demonstrated that curcumin can Induce HSP70 gene expression by modulating calcium ion and cellular p53 protein in colorectal carcinoma cells (Chen *et al.*, 1996); Curcumin can induce apoptosis in immortalized NIH3T3 and malignant cancer cell lines (Jiang *et al.*, 1996; Kuo *et al.*, 1996). It is worthy to note that the normal cell lines such as human foreskin fibroblast and rat embryo fibroblast cells are in-susceptible to this apoptosis induction.

VII. CANCER CHEMOPREVENTION BY OTHER PHYTOPOLYPHENOLS

In addition to the afore-mentioned tea polyphenols and curcumin, several other phytopolyphenols such as genistein (Hawrylewicz *et al.*, 1991), silymarin (Zhao *et al.*, 2000), perillyl alcohol (Gould, 1997), apigenin (Wei *et al.*, 1990), carnosol (Huang *et al.*, 1994) and resveratrol (Jang *et al.*, 1997) have been shown to be effective cancer chemopreventive agents. It is apparent that these phytopolyphenols are strong antioxidants and superoxide scavengers. They also show strong modulating effects on the activities of protein kinases and phosphatases that leading to the control of cellular signal transduction pathways. The action mechanisms that have been discussed in the case of tea polyphenols might be also applicable to these phytopolyphenols.

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