Antioxidants Suppress Apoptosis[1,2]

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EXPANDED ABSTRACT

KEY WORDS: • *antioxidants* • *apoptosis* • *cancer therapy*

There is a well-documented association between increased consumption of antioxidants and decreased incidence of cancer (1–4). These epidemiological studies are supported by animal-model and cell-culture studies correlating oxidative DNA damage to the process of carcinogenesis (5,6). For these reasons, antioxidant supplements are often recommended as part of a cancer prevention diet (7,8). However, the generation of excess levels of reactive oxygen species is important for activation of internal cell programs for cell suicide (apoptosis) that are important protection mechanisms that kill cancer cells (9,10). Also, this mechanism is critical for effective cancer chemotherapy and radiation treatment (10,11). Perhaps, before cancer patients supplement their diets, suppression of apoptosis by antioxidants needs to be considered.

Apoptosis occurs when internal monitors recognize damage or malfunction and initiate signaling cascades that eventually activate caspases and endonucleases that kill the cell (12–15). One of the important functions of apoptosis is the elimination of preneoplastic and neoplastic cells (16–18). In most forms of cell suicide, the signaling cascade utilizes reactive oxygen species as essential intermediate messenger molecules (19–23). This is the reason that antioxidants are capable of inhibiting apoptosis. Antioxidants such as α-tocopherol, which partition into the lipid compartment of cells, or *N*-acetylcysteine, a free radical scavenger that partitions into the aqueous phase of the cytosol, can delay or inhibit apoptosis (24,25). Thus, it is reasonable to suggest that removal of antioxidants from the diet might enhance apoptosis, and thereby inhibit tumor growth.

We observed a reduction in brain tumor size in the TgT (121) transgenic mouse model, which spontaneously develops brain cancer, when these mice were fed diets depleted of antioxidants; there was enhanced apoptosis within tumors (26). Recently, colleagues extended this observation to another cancer type, breast cancer (27). Using a transgenic mouse model of mammary tumorigenesis with defined rates of tumor growth and lung-targeted metastasis, they determined that dietary antioxidant depletion inhibited tumor growth and diminished metastasis. Compared with control mice fed a standard diet, mice fed an antioxidant-depleted diet exhibited tumor-targeted generation of reactive oxygen species; the number of apoptotic cells in tumors increased 5-fold, and the percentage of tumor cells undergoing mitosis decreased by half. The mice fed the antioxidant-depleted diet had more small primary tumors and fewer large primary tumors than did controls, and they also had <30% of the number of lung metastatic tumor foci compared with mice fed the control diet.

Cells contain endogenous antioxidant enzymes (e.g., catalase, superoxide dismutase, and glutathione peroxidase), and many, but not all, human cancer cell types have decreased antioxidant enzyme levels compared to their normal tissue counterparts (28–30). The concentrations of free oxygen radicals are reportedly higher in malignant cells than in normal cells (31,32). Thus, some cancer cells may be more sensitive to generated reactive oxygen species, and this may be a useful difference that can be exploited when seeking to kill cancer cells but spare normal cells. Even a moderate increase in the accumulation of oxygen radicals in malignant cells of animals fed an antioxidant-poor diet could increase reactive oxygen species to the critical level required for progression of apoptosis (21–23). Conversely, even modest quenching of oxygen radicals by dietary antioxidants could block completion of apoptosis.

Antioxidants, by preventing oxidant-mediated damage to diverse targets (DNA, RNA, proteins, and lipids), may play a protective role in healthy individuals with no existing cancer cells that must be eliminated; however, by inhibiting apoptosis, these same antioxidants may exert a cancer-promoting effect in cancer patients and in individuals with precancerous DNA changes. Inhibition of apoptosis by antioxidants may explain why, in several studies in heavy smokers, vitamin E and β-carotene enhanced carcinogenesis in the lung (33) (where, presumably, precancerous lesions caused by smoking

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predated antioxidant treatment) but decreased carcinogenesis in the prostate (34) (where, presumably, smoking had not caused precancerous lesions that predated antioxidant treatment). Thus, though early administration of antioxidants may prevent the initiation and progression of cancer by quenching the action of potentially mutagenic reactive free radicals, administration of antioxidants subsequent to a mutagenic event may effectively intercept free radicals that are critical in promoting apoptosis. This imbalance may allow the rate of proliferation in tumors to exceed the capacity for apoptosis. It seems reasonable to suggest that the potential risks and benefits of high-dose antioxidants need to be considered on a case-to-case basis, and indiscriminate use of antioxidant dietary supplements should be avoided.

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The Benefits and Hazards of Antioxidants: Controlling Apoptosis and Other Protective Mechanisms in Cancer Patients and the Human Population

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ABSTRACT

Cellular oxidants, called reactive oxygen species (ROS), are constantly produced in animal and human cells. Excessive ROS can induce oxidative damage in cell constituents and promote a number of degenerative diseases and aging. Cellular antioxidants protect against the damaging effects of ROS. However, in moderate concentrations, ROS are necessary for a number of protective reactions. Thus, ROS are essential mediators of antimicrobial phagocytosis, detoxification reactions carried out by the cytochrome P-450 complex, and apoptosis which eliminates cancerous and other life-threatening cells. Excessive antioxidants could dangerously interfere with these protective functions, while temporary depletion of antioxidants can enhance anti-cancer effects of apoptosis. Experimental data are presented supporting these notions. The human population is heterogeneous regarding ROS levels. Intake of exogenous antioxidants (vitamins E, C, beta-carotene and others) could protect against cancer and other degenerative diseases in people with innate or acquired high levels of ROS. However, abundant antioxidants might suppress these protective functions, particularly in people with a low innate baseline level of ROS. Screening human populations for ROS levels could help identify groups with a high level of ROS that are at a risk of developing cancer and other degenerative diseases. It also could identify groups with a low level of ROS that are at a risk

of down-regulating ROS-dependent anti-cancer and other protective reactions. Screening populations could provide a scientifically grounded application of antioxidant supplements, which could significantly contribute to the nation's health.

Key words: oxidants, antioxidants, apoptosis, cancer

Key teaching points:

• The human population is heterogeneous in relation to levels of ROS, as well as to almost all other features.

• People who over-generate ROS are at high risk for developing cancer, cardiovascular diseases, cataracts and other degenerative diseases because of the oxidative damage to cell constituents (DNA, proteins, lipids, etc) and cell structures.

• People with a low level of ROS might be in danger of harboring low activity of highly important protective reactions. These include apoptosis, which deletes precancerous, cancer, virus-infected and other cells threatening human health; phagocytosis, which fights infectious microorganisms; and detoxification reactions provided by cytochrome P-450 complexes. The ROS are essential triggers and mediators of all these protective reactions. Consequently, a low ROS level limits the activity of these protective reactions.

• Antioxidants protect people with a high level of ROS, whereas antioxidants might be detrimental in people with a low level of ROS by further decreasing the activity of ROS-dependent protective mechanisms.

• Screening the human population for ROS levels could provide a scientifically well-grounded, controlled application of antioxidants and might significantly contribute to improvement of human health.

INTRODUCTION

Progress in understanding the deleterious effects of reactive oxygen species (ROS) on cell components and structures has led to the development of protective antioxidant supplements. The supplements are used to protect cell structures from oxidative damage and people from cancer and other ROS-dependent morbid conditions. However, accumulating data demonstrate that ROS, depending on dose, not only act as damaging entities, but also carry out some important beneficial functions. ROS are mediators, triggers or executioners of essential protective mechanisms such as apoptosis, phagocytosis and detoxification reactions. Among these mechanisms, apoptosis, which eliminates precancerous and cancerous, virus-infected and otherwise damaged cells, is particularly important. Increase of ROS concentration by depletion of antioxidants enhances apoptosis and thereby inhibits tumor growth. Excessive antioxidants decrease ROS level, inhibit apoptosis and suppress the elimination of cancer cells induced by anticancer drugs.

This review illustrates the notions outlined above with experimental data. To ensure effective defense against

ROS-induced damage, while maintaining the ROS level which promotes apoptosis and other protective mechanisms, it is important to obtain answers to the following questions: a) How heterogeneous is the human population regarding the ROS level? b) Are people with very high and very low ROS levels at risk for developing cancer or other degenerative diseases? c) How high is the ROS level at which cells are damaged? d) How low is the ROS level unable to maintain apoptosis and other ROS-dependent protective functions? e) What is the optimal level of ROS, which induces minimal oxidative damage to cell structures but promotes cell protective mechanisms that eliminate precancerous, cancerous and other "bad" cells? It would also be necessary to establish optimal doses of antioxidants capable of coping with high and low levels of ROS. Answers to these questions could be provided through screening of the human population for ROS level and monitoring alteration of ROS under different doses of antioxidants. These studies might help to develop optimal regimens of antioxidants for different population groups that would be capable of preventing cancer, cardiovascular diseases, cataracts, and other ROS-dependent morbid conditions by maintaining optimal levels of protective reactions.

BACKGROUND

Cellular Oxidants and Antioxidants

Cellular oxidants, derivatives of oxygen, which are often called reactive oxygen species (ROS), are constantly produced in our cells (Fig. 1). Among cellular ROS, the most aggressive entities are superoxides and hydroxyl radicals [1]. There are a few main sources of ROS in our body. ROS are generated by mitochondria (Fig. 2) via the release of electrons from the electron transport chain and the reduction of oxygen molecules to superoxides ($O_2^{\bullet}$). Superoxides, through the reaction catalyzed by superoxide dismutase (SOD), are transformed into the much less reactive hydrogen peroxide moiety (H_2O_2). However, when hydrogen peroxide interacts with ions of transition metals such as iron or copper, the most reactive ROS, hydroxyl radicals ($OH^{\bullet}$) are formed (Fenton reaction). Other sources of ROS, located in the endoplasmic reticulum, are cytochrome P450 complexes (Fig. 3), which generate superoxides to metabolize toxic hydrophobic compounds [2]. Important sources of ROS are phagocytes (Fig. 3), which produce superoxides, hydrogen peroxide and hydroxyl radicals to kill infectious microorganisms [3] and cancer cells [1,4].

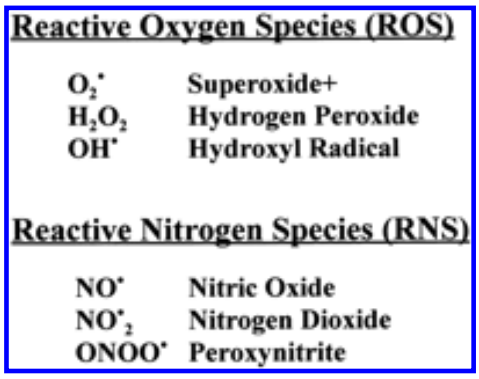

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Fig. 1. The main reactive oxygen species (ROS) that are constantly generated in living cells.

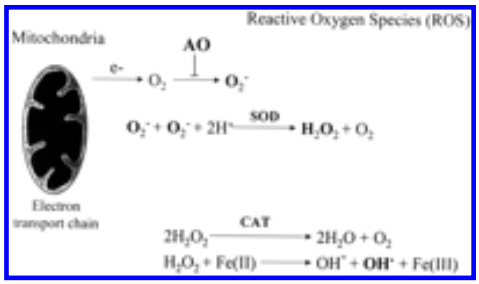

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Fig. 2. The generation of ROS by mitochondria. Electrons released from mitochondria reduce oxygen molecules, thereby producing such ROS as superoxides (O_2^-). Superoxide dismutase (SOD) catalyzes H_2O_2 formation from superoxides. H_2O_2 might be deactivated by catalase (CAT). However, when H_2O_2 reacts with iron or copper ions, hydroxyl radicals (OH•), the most reactive form of ROS, are produced. Excessive antioxidants (AO) can inhibit production of O_2^- and other ROS.

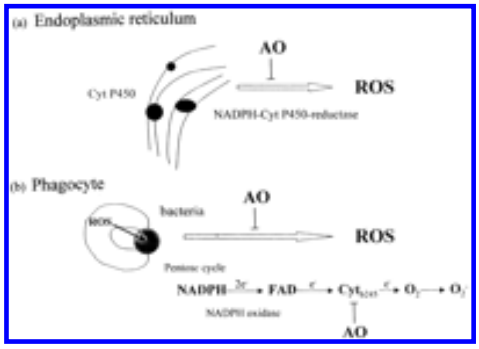

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Fig. 3. (a) ROS generated by microsomal monooxygenases, which have cytochrome P450 as a central link. Oxidation is the way to transform hydrophobic toxic substances, drugs, steroids etc., and thereby remove them. Excessive antioxidants can inhibit this protective function.

(b) ROS generated by phagocytes kill infectious microorganisms and cancer cells. Excessive antioxidants can inhibit this protective mechanism.

Production of ROS is essential for a number of biochemical reactions involved in the synthesis of prostaglandins, hydroxylation of proline and lysine, oxidation of xanthine and other oxidative processes [1]. Numerous data demonstrate that ROS are capable of oxidizing cell constituents such as DNA, proteins, and lipids, thereby incurring oxidative damage to cell structures. Excessive oxidation leads to impairment of cell

functions and development of morbid conditions [1,5]. Besides ROS, cells also generate reactive nitrogen species (RNS) such as nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$) and peroxynitrite ($ONOO^{\bullet}$) (Fig. 1) [6]. Nitric oxide and nitrogen dioxide carry out a number of physiological functions. Excessive $NO^{\bullet}$, $NO_2^{\bullet}$ and $ONOO^{\bullet}$ damage cell constituents.

An array of powerful cellular antioxidants protects cells from excessive oxidation (Fig. 4). Among the endogenous antioxidants that scavenge ROS are glutathione, ubiquinol, bilirubin, uric acid, albumin and others. Potent antioxidant enzymes such as superoxide dismutase and catalase protect our cells from oxidative damage by inactivating ROS. Metallothioneins, ferritin, transferrin and ceruloplasmin eliminate ions of transition metals, which are capable of catalyzing the formation of hydroxyl radicals through the Fenton reaction [7,8] (Fig. 1).

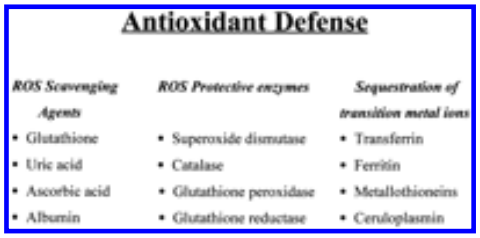

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Fig. 4. Principal cellular antioxidants that scavenge or inactivate excessive ROS and thereby protect cells from oxidative damage.

There is good evidence that endogenous antioxidants do not completely remove ROS in animal and human cells. This raises the question of why, despite the existence of a powerful cellular system of antioxidants, the short-living ROS are not removed entirely and are permanently present in cells. The reasonable explanation for this phenomenon is that continuously produced ROS are needed to perform some important biological functions [1]. Seemingly, the cells are tuned to remove excessive ROS and to leave the required level of oxidants.

The Beneficial Functions of ROS

Indeed, ROS play a crucial role in a few lifesaving biological mechanisms. Phagocytic cells protect us from deadly microorganisms, killing them by producing an avalanche of ROS. When neutrophils and other phagocytic cells engulf bacteria, they greatly increase consumption of oxygen ("respiratory burst"), which is rapidly transformed to ROS that kill the dangerous intruders. NADPH supplies electrons, required for the reduction of oxygen and the formation of ROS (Fig. 3). In turn, $NADP^+$ receives electrons from the pentose cycle pathway by NADPH oxidase through cytochrome b_{245} [9]. Importantly, by a burst of ROS, phagocytes kill not only invading bacteria [3,9], but also cancer cells [1,4]. Excessive antioxidants scavenge these beneficial ROS and can thereby interfere with the protective functions of phagocytes [10].

Detoxification reactions, ensured by the cytochrome P450 family, are dependent on the integrity of the microsomal ROS-generating system. NADPH and NADH supply reducing equivalents for the reduction of

cytochrome b_5 and cytochrome P450 (Fig. 3). The latter oxidizes hydrophobic toxic substances, steroids and drugs, transforming them into hydrophilic ones, which are removed from the body. In view of the pivotal role of ROS in the functioning of the cytochrome P450 complex, it is reasonable to suggest that excessive antioxidants could interfere with this important cell function. Data support this suggestion [11].

ROS are essential mediators of apoptosis (Fig. 5), which eliminates cancer and other cells that threaten our health [12–17]. Excessive antioxidants interfere with this highly important protective mechanism [18–21], as also described in this paper.

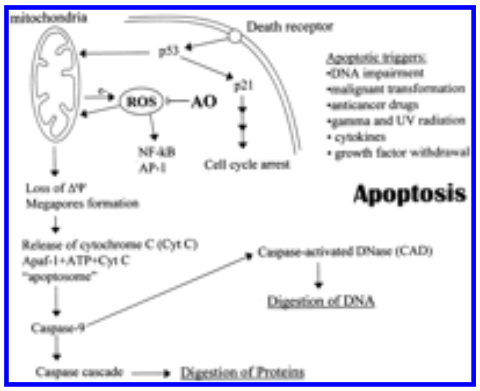

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Fig. 5. Schematic representation of apoptosis. ROS generated by mitochondria are essential mediators of apoptosis. Together with cytochrome C, Apaf-1, and ATP, released from mitochondria, ROS activate proteolytic enzymes, termed caspases, which promote deoxyribonuclease, and thereby destroy targeted "bad" cells.

It seems plausible that ROS generation is prevented from being entirely suppressed by endogenous antioxidants because of their important beneficial functions. Seemingly, endogenous antioxidants might be regulated to scavenge ROS to a certain level, but not more. The remaining oxidants are required for carrying out apoptosis, phagocytosis, detoxification and certain other biochemical reactions. Oxidative modification of DNA is also not entirely repaired in healthy animals, despite the existence of potent enzymatic machinery for the repair of DNA [22].

Are moderate oxidative modifications of DNA required for our well being? It seems reasonable to suggest that oxidatively modified DNA might be required to induce mutations necessary for the selection of the fittest to survive in the changing environment, thereby preventing extinction of the population. Industrial pollution as a source of mutations has existed for no more than 150–200 years. Perhaps all living beings should have independent mechanisms for inducing a certain level of mutations. It is also possible that this is the reason why the enzymatic machinery of DNA-repair is adjusted to remove most, but not all, oxidatively modified promutagenic nucleotides from DNA. A mechanism for maintaining a certain level of endogenous antioxidants might exist. This would prevent their excessive accumulation, which in turn might abundantly scavenge ROS and interfere with beneficial ROS-dependent mechanisms.

APOPTOSIS AND CANCER

Apoptosis, sometimes called "a guardian angel" or "cell policeman," is a cell suicidal altruistic mechanism targeted to selectively eliminate cancerous and other cells that threaten our health and life. The sacrifice of the "bad" cells occurs to save the integrity and life of the whole organism [12,13]. Apoptosis is carried out by a multistage chain of reactions in which ROS act as triggers and essential mediators [12–15]. Recently, it became evident that mitochondria play a critical role in apoptosis [16]. Schematically, apoptotic signals, which arise in cancer cells, promote accumulation of the p53 protein that triggers the release of ROS, cytochrome C and a few other regulators from mitochondria. The latter activate a cascade of proteolytic enzymes, called caspases, that digest a number of pivotal cell proteins and promote a caspase-activated deoxyribonuclease (Fig. 5). Cleavage of the critical proteins and DNA results in apoptotic cell death. Importantly, most anticancer drugs and radiation kill cancer cells by inducing apoptosis [17–21]. Mutations in the p53 gene make cancer cells resistant to apoptosis and, accordingly, to anticancer drugs [13].

Because of the pivotal role of ROS in triggering apoptosis, antioxidants can inhibit this protective mechanism by depleting ROS [18–21]. This is why antioxidants could interfere with the therapeutic activity of anticancer drugs that kill cancer cells by apoptosis. Our data demonstrate that, indeed, apoptosis induced in human breast cancer cells by cisplatin, a widely applied anticancer drug, is accompanied by an increase in ROS generation (Fig. 6). We have further demonstrated that the powerful antioxidant alpha-tocopherol inhibits ROS generation (Fig. 6) and apoptotic death of breast cancer cells induced by cisplatin (Fig. 7) [21]. It appears that antioxidants might inhibit the therapeutic activity of anticancer drugs in patients [20].

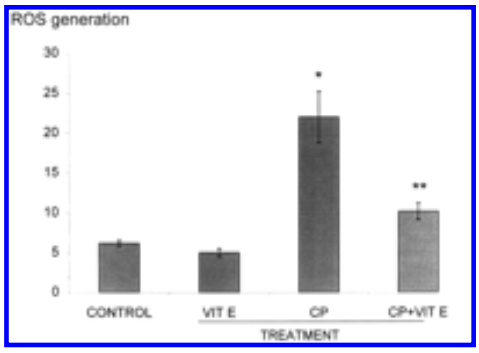

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Fig. 6. The anticancer drug, cisplatin, kills breast cancer cells by the induction of apoptosis. The antioxidant vitamin E inhibits the cisplatin-induced apoptotic death of cancer cells by scavenging ROS that are essential for carrying out apoptosis (see Fig. 7). MCF-7 breast cancer cells were grown in Eagle's MEM in 6-well plates (4 x 10^4 cells per well) and incubated for 24 hours with 15 μM cisplatin. Vitamin E (15 μM) was added to the medium simultaneously with cisplatin or separately. Apoptosis was determined by TUNEL assay and morphological cell patterns [31].

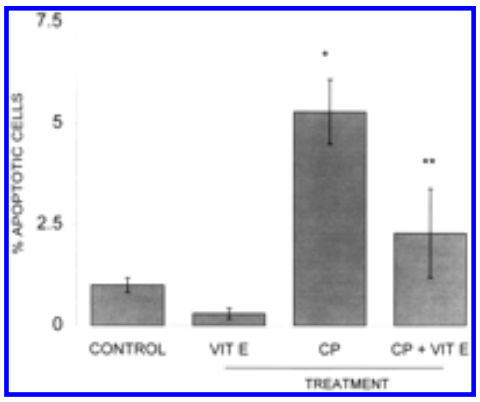

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Fig. 7. The antioxidant vitamin E inhibits cisplatin-induced ROS generation in cancer cells. The conditions of the experiment were as in Fig. 6. ROS generation was determined using the avidin-FITC which reacts specifically in apoptotic cells with 8-oxo-deoxyguanine, the biomarker of ROS generation, as described in reference [23].

We reasoned that if depletion of ROS by antioxidants suppresses apoptosis, then a rise in ROS concentration could enhance the apoptotic death of cancer cells. The concentration of ROS can be increased by enhancing ROS generation or by depleting antioxidants. We tried to increase ROS accumulation by depleting antioxidants. Experiments to verify this reasoning were performed at the University of North Carolina at Chapel Hill [23]. Transgenic mice developing brain tumors were fed a diet depleted of antioxidants, while control mice were fed a standard diet. The antioxidant-depleted diet significantly increased ROS concentration in brain tumors that, in turn, led to a dramatic increase in apoptotic death of brain tumor cells (Fig. 8). Because of intensive apoptosis, a sharp decrease in tumor volume resulted (Fig. 9). Quantitative evaluation of the changes in brain tumor size is presented in Table 1. Importantly, an enhancement of apoptosis was not observed in normal tissues of animals fed the antioxidant-depleted diet. Neither weight loss nor changes in behavior or pathology of normal tissues were found in mice fed the antioxidant-depleted diet for four months [23]. Similar results were obtained in transgenic mice developing mammary tumors. The data indicate that antioxidants scavenging ROS can interfere with cancer cell-killing apoptosis and vice versa: an increase of ROS concentration could enhance apoptosis, thereby selectively removing cancer cells. Currently, clinical studies are in preparation to verify this presumption.

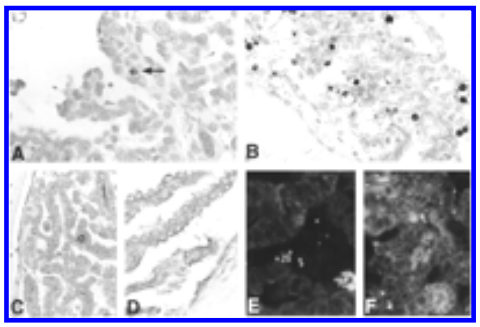

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Fig. 8. Increased oxidative stress results in enhanced apoptosis in the brain tumors of mice fed an antioxidant-devoid diet. The distribution of TUNEL-positive (black label, arrow) apoptotic cells in brain tumor for control (A) and in mice on the antioxidant-devoid diet (B) is shown. Oxidized guanine residues (8-oxo-Gua), biomarkers of ROS generation, were detected in brain tumors, using specific monoclonal antibodies (C and D) or an avidin-FITC conjugate (E and F). By both methods, cells in tumors of antioxidant-depleted mice (D and F) exhibit higher levels of 8-oxo-Gua residues than do cells in the tumors of control brains (C and E).

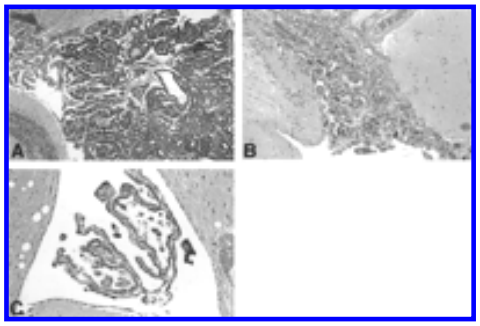

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Fig. 9. The tumors of mice fed an antioxidant-depleted diet are reduced in size. Compared with tumors in mice fed a standard diet (A and B), the tumors in mice fed an antioxidant-depleted diet (C) were significantly smaller. Magnification: A= 60x; B and C= 120x.

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Table 1. Alterations in Apoptosis Rate and Brain Tumor Size Induced in TgT121 Transgenic Mice by Antioxidant-Depleted Diet

DAMAGING EFFECTS OF ROS AND PROTECTION BY ANTIOXIDANTS

Numerous *in vitro* experiments demonstrate that ROS damages DNA, inducing premutagenic modifications of nucleotides and promoting oxidation of proteins and lipid peroxidation [1,24–26]. Data support the notion that increased formation of ROS may play an important role in carcinogenesis, atherosclerosis, diabetes, emphysema, cataracts and neurodegenerative diseases [1,5,7,24–29]. Manifestations of an increased level of ROS are detected in most of these morbid conditions. However, in many cases it is not easy to discriminate whether an increase in ROS is the cause or a consequence of the disease. Our experiments clearly demonstrate the detrimental effect of excessive ROS in animals. Using Wistar rats, an OXYS rat strain with an inherited over-generation of hydroxyl radicals was developed [26]. An increase in oxidative impairment of DNA proteins and lipids is characteristic of OXYS rats compared with control rats. Development of cataracts, emphysema, scoliosis, cardiomyopathy and manifestations of carcinogenesis in the form of a sharp increase of liver preneoplastic foci were observed in OXYS rats [26–28]. Low life span and poor breeding are innate features of OXYS rats. These data support the view that over-generation of ROS can be a cause of many degenerative diseases and premature aging [1,5,7,24].

If the high level of ROS is the cause of degenerative diseases in OXYS rats, then application of antioxidants could presumably protect the impaired functions from oxidative damage. We have shown that an impairment

of long-term memory is characteristic of OXYS rats. Memory and some cognitive functions are impaired in people with ROS-promoted neurodegenerative diseases. The integrity of N-methyl-D-aspartate (NMDA) receptors, Na/K-ATPase, and brain protein SH-groups is required for carrying out cognitive functions. We determined the level of these neurochemical processes in OXYS rats and the protection of these functions by a few selected antioxidants such as butylated hydroxytoluene (BHT), emoxipine and carnosine [29]. Studies found that BHT protects rat brains from the oxidative alteration of NMDA receptors and Na/K-ATPase, but does not protect SH-groups. Emoxipine protects rat brains from oxidative impairment of SH-groups, but not NMDA receptors and Na/K-ATPase. Carnosine protects all these neurochemical functions from oxidative damage [29]. Importantly, the data demonstrate that various antioxidants are targeted to protect different neurochemical functions. It seems plausible that combinations of such targeted antioxidants might provide more efficient protection of different functions against oxidative damage than randomly combined antioxidants.

SHOULD WE TAKE OR AVOID ANTIOXIDANTS TO PREVENT THE DEVELOPMENT OF CANCER?

If excessive ROS cause degenerative diseases of aging, particularly cancer and atherosclerosis, can we protect people from these diseases and aging by giving them antioxidants? Alternatively, if antioxidants interfere with highly important protective mechanisms, particularly apoptosis, is it safe to take antioxidants? It seems that there is no simple unequivocal answer. First, we should attempt to answer the question: how efficient are antioxidants in humans for cancer prevention? The answer to this question appears controversial. No reduction in the incidence of lung cancer among male smokers was found in a large randomized, double-blind trial of daily supplementation with alpha-tocopherol or beta-carotene alone, both alpha-tocopherol and beta-carotene, compared with placebo. An even more discouraging and unexpected finding from this Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) trial was the higher incidence of lung cancer and mortality among the male smokers who received beta-carotene [30,31]. This trial was followed by the Carotene and Retinol Efficacy Trial (CARET) that examined the effect of a combination of beta-carotene and vitamin A (retinol) on the incidence of lung cancer among smokers and workers exposed to asbestos [32]. An increase in lung cancer observed in the antioxidant-supplied group resulted in the premature stopping of the study.

The above data led to a tendency to deny protective effects of antioxidant supplements against cancer [33–35], despite findings from numerous studies supporting their protective effects [36–41]. The Chinese Cancer Prevention Study found lower gastric and esophageal cancer rates and a reduction in mortality among people whose daily diets were supplemented with beta-carotene, vitamin E and selenium for more than 5 years [36]. A strong correlation was found between a higher intake of antioxidants and a lower incidence of lung cancer in nonsmokers [37]. Vitamin E and beta-carotene lowered the rates of occurrence of gastric cancer [38]. A high intake of vitamin E reduced the risk of colon cancer [39]. Data from the ATBC trial that demonstrated a high incidence of lung cancer among male smokers found that vitamin E decreased the incidence of prostate cancer [31]. Diets rich in vegetables and fruits that contain a variety of antioxidants clearly have cancer-

preventive effects [41–45]. However, the possibility that some non-identified natural compounds in these foods could also contribute to their cancer-protective activity cannot be ignored. Patients with low baseline beta-carotene levels were at increased risk of prostate carcinoma compared to those with high beta-carotene levels. Further, people with low plasma concentrations of beta-carotene benefited from supplementation with beta-carotene, in contrast to people with a high level of this compound in the plasma [40].

Possible explanations for these contradictory findings are outlined below:

1. Antioxidants can protect healthy people, but they also can harm people who, being smokers, are constantly exposed to chemical carcinogens in tobacco. Lung tissues of heavy smokers unavoidably harbor numerous mutagenized precancerous cells. ROS-dependent apoptosis targets these precancerous and cancerous cells for deletion. Antioxidants, which scavenge ROS, suppress apoptosis and prevent the apoptotic death of precancerous and cancer cells, thereby potentially promoting the development of lung cancer. In addition, antioxidants scavenge excessive ROS, but do not remove numerous chemical carcinogens, which appear in lungs as a result of cigarette smoking. This is seemingly why antioxidants can prevent lung cancer in non-smokers [37]. In non-smokers, lung cancer is induced predominantly by constantly generated ROS, whereas in smokers, chemical carcinogens induce this disease. At the same time, smokers were protected by alpha-tocopherol and beta-carotene against prostate cancer. The prostate, unlike the lungs, does not experience the heavy pro-mutagenic effect of tobacco smoke. Therefore, the prostate obviously does not harbor the multitude of mutagenized precancerous cells as in lung tissues. Prostate DNA most probably is modified by ROS and, to a much lesser extent, by cigarette carcinogens. Antioxidants in the prostate, by scavenging constantly generated ROS, could prevent their carcinogenic effect, thereby reducing the incidence of prostate cancer.
2. The effect of antioxidants could depend on their initial levels in the body. Only in rare cases have baseline levels of antioxidants been measured, despite the importance of this information [40]. Data demonstrate that baseline antioxidant levels could influence decisions regarding the intake of antioxidants.
3. The cancer-preventive effect of antioxidants depends on the baseline level of ROS in cells, which is largely determined by the rate of ROS generation and antioxidant defense. Antioxidants could be efficient in individuals with a high level of ROS and non-efficient or even cancer promoting in people with a low level of ROS. The reason for the negative effect of antioxidants could be inhibition of ROS-dependent cancer-protective apoptosis and phagocytosis. However, ROS levels, and therefore the expected activity of antioxidants, have yet to be measured in people before intake of antioxidant supplements is recommended.
4. The human population is heterogeneous in all inherited features [46,47] and the diversity of the population regarding the levels of constantly generated ROS is hardly exceptional. Obviously, there are people with an innate high level of ROS who are at a high risk of developing cancer, degenerative diseases and premature aging. At the same time, there are groups of people in the population with a low level of ROS, who also are in danger because of poor functioning of apoptosis and other ROS-dependent protective mechanisms. The heterogeneity of the human population regarding ROS levels depends on the rate of ROS production and on the activity of endogenous antioxidants. The heterogeneity of the human population can probably be described by the normal distribution curve where the extremes are "ROS hyper-producers" from one side and "ROS hypo-producers" from the other side of the curve. Therefore, requirements for protective antioxidants may differ among individuals. Antioxidants are reasonable to apply under a control of ROS levels, in accordance with the

rate of ROS accumulation data. An excessive intake of antioxidants can be as harmful as a lack of these protective entities. Unfortunately, widely advertised, poorly controlled application of antioxidants can lead to unwanted consequences to our health. Only recently have non-invasive methods for the control of ROS level or for studying the protective effects of antioxidants and pro-oxidants became available [43]. Screening human populations for innate levels of ROS could identify low and high "ROS-producers" and help determine true requirements for antioxidants. Screening could help justify decisions regarding intake of antioxidant supplements as well as food sources of antioxidants.

5. Currently, antioxidants are applied in mostly empirically determined combinations and concentrations. It is assumed that combinations of water-soluble and lipid-soluble antioxidants in these supplements are sufficient to meet all body requirements. However, in reality, the situation is far more complex. We have determined that different antioxidants protect various memory-related neurochemical functions oxidatively impaired in OXYS rats that over-generate ROS [25]. Seemingly, some antioxidants have a targeted protective effect, which provides the opportunity for optimal scientifically grounded combinations of these compounds.

There are data showing that some antioxidants might have pro-apoptotic anticancer effects. This property was discovered in alpha-tocopherol succinate, an analog of alpha-tocopherol, but not in alpha-tocopherol and alpha-tocopheryl acetate applied in comparable doses [48–50]. Moreover, alpha-tocopherol inhibited pro-apoptotic activity of alpha-tocopheryl succinate. The succinate moiety of alpha-tocopheryl succinate played an indispensable role in promoting apoptosis by the alpha-tocopherol analog [48]. However, alpha-tocopherol at very high doses is capable of promoting apoptosis in cancer cells, most probably due to the damaging effect of an excess of this compound [51]. Individual antioxidants at defined doses might prevent tumor growth by inducing cell differentiation, promoting transforming growth factor-beta, inhibiting protein kinase C activity, suppressing transcription factors, and by other mechanisms not related to ROS inhibition [52,53].

Some trials have failed to demonstrate protective or anticancer properties of combinations of antioxidants, vitamins E, C, A and beta-carotene, commonly found in supplements [25]. However, diets rich in a multitude of certain vegetables and fruits have been found to be protective against cancer and cardiovascular diseases [39–41]. A few well-controlled studies demonstrate that consumption of diets enriched in a variety of fruits and vegetables from diverse botanical families significantly reduces oxidative damage to DNA and other cellular constituents, thereby preventing the development of cancer and cardiovascular diseases [42–45].

CONCLUSION

The data discussed in this review show that the biological effects of antioxidants in humans are controversial. Depending on the oxidative status of cells, antioxidants can be protective against cancer or cancer promoting. Since ROS induce oxidative carcinogenic damage in DNA, antioxidants can prevent cancer in healthy people harboring increased levels of ROS. However, since ROS in moderate concentrations act as indispensable mediators of cancer-protective apoptosis and phagocytosis, in people with a low ROS level, an excess of antioxidants can block these cancer-preventive mechanisms and

promote cancer. An excess of antioxidants, which interferes with apoptosis, also can be cancer-promoting in people who are constantly exposed to the effect of environmental carcinogenic factors (tobacco smoke, industrial pollutants), which result in a high accumulation of pre-cancerous and cancerous cells. In cancer patients, an excess of antioxidants can interfere with the therapeutic activity of anticancer drugs, which kill cancer cells by ROS-dependent apoptosis. It is becoming increasingly clear that the beneficial effect of antioxidants can be achieved if these factors are taken into account.

The human population is heterogeneous regarding the ROS level. Screening the human population regarding innate or acquired ROS levels can provide the necessary information about individual oxidative status. High doses of antioxidants can reduce the ROS level in people who over produce ROS and protect them against cancer, cardiovascular diseases, cataracts and other ROS-dependent morbid conditions. For people with a low ROS level, high doses of antioxidants can be deleterious, suppressing the already low rate of ROS generation and the ROS-dependent cancer preventive apoptosis. Screening and monitoring the human population regarding the ROS level can transform antioxidants into safe and powerful disease-preventive tools that could significantly contribute to the nation's health.

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Cancer, Apoptosis and Reactive Oxygen Species: A New Paradigm

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Abstract

Apoptosis or cellular suicide is one of the most important means of eliminating precancerous and cancerous cells from the body. Cellular apoptotic execution is usually modulated by levels of electronically modified oxygen derivatives serving as the effector stimulus initiating subsequent cellular death. Studies have shown that antioxidants can block apoptosis in many tumorous or neoplastic cell types. Caution should be exercised to prevent creating insufficient electronically modified oxygen derivative (EMOD) levels and to avoid the injudicious use of antioxidants, especially in subjects with compromised immunity or with cancerous or precancerous conditions. Allowance of cellular proliferation may represent an EMOD insufficiency state and an EMOD insufficiency syndrome may explain clustering of common diseases, such as cancer, atherosclerosis, diabetes and obesity. In short, sufficient prooxidant levels can induce cancer cell apoptosis, which can be blocked or nullified by certain antioxidants.

Introduction

An accumulating body of evidence favors the involvement of intracellular reactive oxygen species at some point during apoptotic execution. [1-5]

The critical role of cellular redox status in the regulation of death signaling has been demonstrated. [6-9] Investigators have shown that intracellular increase in H2O2 was a critical effector mechanism during drug-induced apoptosis of human tumor cells. [4] This increase in H2O2 was responsible for early cytosolic acidification, thus creating an environment conducive for caspase activation.

Most of the past studies seem to imply that the mitochondrial burst of H2O2 is likely to be a downstream effector mechanism for the execution signal. Hydrogen peroxide (H2O2) is considered to be a mediator of most forms of apoptotic cell death. [5]

Many investigators have demonstrated the critical role of intracellular H2O2 in rendering the cytosolic milieu permissive for efficient apoptotic execution. [4, 5, 10] I believe that this strongly indicates that H2O2 is one of the essential agents for cellular killing.

Tumor cell hypoxia

Otto Warburg was the first scientist to implicate ground state oxygen in cancer, [11] although he was later derided for it. It appears that EMODs, not ground state oxygen, are primary effectors for modulation of cellular apoptosis.

A pro-oxidant intracellular milieu is an invariable finding in cancer cells and has been shown to endow cancer cells with a survival advantage over their normal counterparts. [12, 13] However, I believe that it is this very feature which allows us to have selectivity for cancer cell killing.

Most human tumors develop regions of chronically or transiently hypoxic cells during growth. [14] Clinical studies have shown that metastatic spread is associated with hypoxia in the primary tumor. [15, 16]

Hypoxia (oxygen tension [pO_2] value 10 mmHg) is associated with lower overall and disease-free survival, greater recurrence, and less locoregional control in head and neck carcinoma, cervical carcinoma and soft-tissue sarcoma. Tumor hypoxia is associated with adverse clinical outcomes and reduced patient survival. Tumor hypoxia is a therapeutic concern since it can reduce the effectiveness of radiotherapy, some O_2-dependent cytotoxic agents, and photodynamic therapy. [17]

EMODs

Both radiotherapy and photodynamic therapy generate electronically modified oxygen derivatives (EMODs) within treated carcinoma cells and they are felt to be essential for induction of apoptosis of the neoplastic cells. At least 127 genes and signal transducing proteins have been reported to be sensitive to reductive and oxidative (redox) states in the cell. [18]

Evidence from both animal and human studies indicates that exercise may reduce the risk of breast cancer. Among eleven human studies that took into account many of the established risk factors for breast cancer, eight reported a decrease in the risk of breast cancer in pre-menopausal, post-menopausal or all women with high levels of physical activity compared to women with low levels of activity. [19] With exercise, there is a consequent increase in oxygen consumption of up to 10 to 15 fold and a concomitant increase in EMOD production.

The chemotherapeutic agents doxorubicin, mitomycin C, etoposide and cisplatin are superoxide generating agents [20]. The anti-estrogen tamoxifen, increasingly used alongside other breast cancer therapies, has also been shown to induce oxidative stress and increased apoptotic-inducing EMOD levels within carcinoma cells in vitro. [21]

Testosterone deficiency, through castration, increases EMODs and this is the best treatment for prostate cancer, which has resulted in cases of long term total remission of the cancer. Administration of testosterone to the castrated group resulted in decreases in EMODs, which would "allow" for continued growth of prostate cancer. Additionally, antioxidant enzymes are restored by testosterone administration, which would result in a further deficiency state of EMODs and a more favorable environment for cancer survival and growth. [22]

When normal androgenic status is disrupted, such as under the condition of castration-induced deprivation, EMODs are increased in the prostate via up-regulation of Nox-dependent EMOD anabolism and down-regulation of a number of key antioxidant enzyme EMOD scavengers. All of these factors help to increase EMODs, which will aid in controlling neoplastic growth, and thus, it has been found that castration is the best treatment for prostatic cancer. This data strongly supports my Unified theory, which states that EMODs are normally of low toxicity and are essential secondary cellular messengers.

This data may relate to the results from the Finasteride Prostate Cancer Prevention Trial, which demonstrated that androgen-blockade at the cellular level lowers prostate cancer risk but increases the prevalence of high-grade cancers. [23]

Many human cancer cells overproduce hydrogen peroxide. High levels (up to 0.5 nmol/hr/10^4 cells) of hydrogen peroxide are constitutively released from a wide range of human tumor cells. I believe that this makes the tumor cell more vulnerable to increases in EMODs and creates selectivity for PDT and cancer therapy. In other words, adding a specified amount of EMODs to neoplastic and normal cells can induce

apoptosis in a cancer cell and not do so in a normal cell. This establishes a unique therapeutic site for selectivity in the killing of tumorous cells, without doing harm to normal cells.

On September 12, 2005, Mark Levine's group published online for PNAS results showing that, "Pharmacologic ascorbic acid concentrations selectively kill cancer cells: Action as a pro-drug to deliver hydrogen peroxide to tissues." [24]

Dehydroascorbic acid (DHA) may be the key to vitamin C therapy. Dr. Benade et al. at the National Cancer Institute found that in cultures vitamin C selectively destroyed cancer cells by generating excess intracellular H_2O_2. [25] Ascorbic acid and ascorbic acid salts are preferentially toxic to tumor cells, which are thought to be related to intracellular generation of hydrogen peroxide. [26, 27]

I believe that the cancer cells have higher levels of EMODs, due to low levels of antioxidants and antioxidant enzymes, and it therefore takes a smaller amount of additional EMODs to reach apoptotic levels than for normal cells to reach apoptotic levels. This may be the trigger point of selectivity for toxicity to cancer cells without harming normal cells.

Myeloperoxidase (MPO) deficiency

EMOD insufficiency levels appear to be related to increased risk of neoplasia.

Myeloperoxidase is a heme-containing enzyme that catalyzes the reaction between hydrogen peroxide and chloride ions, producing hypochlorite or hypochlorous acid (HOCl), a potent oxidation agent. [28] Evidence from a number of investigators indicates that individuals with total MPO deficiency show a high incidence of malignant tumors. [29]

There appears to be a high incidence of malignancy in patients with complete MPO deficiency, suggesting a relationship between a defective MPO system and neutrophil-mediated tumor cell cytotoxicity. [30] Patients with cancer of the larynx show a deficiency of neutrophil myeloperoxidase. Activity of myeloperoxidase in neutrophils from patients with gastric carcinoma is slightly elevated. [31]

Decreased MPO activity in PMN from acute myeloid leukemia (AML) patients may contribute to the increased susceptibility to infections and that in the pre-remission phase of the disease it may account for approximately 15% of the infections. [32]

A complete lack of myeloperoxidase (MPO) was demonstrated in a boy suffering from acute myeloid leukemia during the acute phase of the disease and after a remission was achieved. This indicates a possible connection between MPO deficiency and leukemia. [33] Also, mice deficient in myeloperoxidase have somewhat increased atherosclerosis. [34] I believe that this follows the pattern of disease clustering in the EMOD insufficiency syndrome.

Antioxidants

EMOD insufficiency levels, secondary to antioxidants, also appear to be related to increased risk of neoplasia.

A study on human gliomas cells demonstrated that overexpression of CuZnSOD can inhibit tumor cell growth. [35] Again, H_2O_2 may be generated to sufficiently high levels such that it demonstrates antineoplastic properties, in accordance with my Unified theory. To the contrary, in tumor cells, the activity of CuZnSOD is usually low. [36]

Catalase deficiency in humans was first documented by Dr. Takahara in 1946. [37] Japanese acatalasemic patients are phenotypically normal with the exception of an increased tendency in development of progressive oral gangrene, presumably as a result of tissue damage from H2O2 produced by peroxide-generating bacteria such as streptococci and pneumococci as well as by the phagocytic cells at the sites of bacterial infection. [38]

Mice null for the Gpx1 and Gpx2 genes appear normal under normal housing conditions, although they tend to be more sensitive to oxidative stress. More recently, knockout mice for catalase were generated, and these mice null for catalase appear normal as well. [39] Animals null for CAT and Gpx1 and Gpx2 develop normally. [40] This argues strongly for the low toxicity of EMODs, which is an integral part of my Unified Theory (available at www.thepundit.com). [43, 44]

I believe that it is of utmost importance to consider the fact that animals null for CAT and Gpx1 and Gpx2 appear to develop normally and live normal lives. This must weaken the argument that EMODs are extremely toxic and causative of up to 100 pathophysiologies.

The results show that (1) the increased liver antioxidant capacity of CAT and Gpx in male mice might be a sign of oxidative stress; (2) the increase in CAT and Gpx activities in male mice is strongly correlated with incidence of hepatic tumors; (3) the significantly increased SOD activity in tumor-bearing mice might have induced damage with accumulated hydrogen peroxide. [41]

The free radical theory

Denham Harman [42] proposed in 1956 the "free radical theory," speculating that damage to aerobic organisms occurs due to harmful free radical production of oxidative products. Subsequently, these alleged damaging derivatives of oxygen, which were termed either oxygen free radicals or "reactive oxygen species (ROS)", were defined as being deleterious and harmful. For greater accuracy, I will use the term "electronically modified oxygen derivatives (EMODs)" to replace the less accurate term of reactive oxygen species.

Unfortunately, the overly optimistic predictions based on the free radical theory have repeatedly failed to scientifically support the free radical theory. [43-47] The Harvard School of Public Health web site summed it up this way, "The evidence accumulated thus far on antioxidant vitamins isn't promising. Randomized trials of vitamin C, vitamin E, and beta-carotene haven't revealed much in the way of protection from heart disease, cancer, or aging-related eye diseases (website accessed 2/09/06). I present in great detail the essential task of EMODs for the normal functioning of aerobic cells and their crucial role as secondary cellular messengers in my e-books, [43-47] which are available in "The Howes Selective World Library of Oxygen Metabolism at www.thepundit.com.

The free radical theory erroneously stated that diseases and the aging process resulted from the "stochastic" accumulation of oxidative damage purportedly caused by EMODs, from sources such as the environment and from normal by-products of cellular metabolism. [48-50] Contrary to the free radical theory of aging, which argues that EMODs are uncontrolled, EMODs are under strict metabolic control. There is a compartmentalization of oxidative events, which strongly suggests EMODs' crucial role influencing and modulating physiological stimuli, signaling mechanisms, and functional homeostasis. [51, 52]

Tests of effect of vitamin E and other antioxidant vitamins or their combinations on clinical manifestations of cardiovascular disease, cancer and diabetes, have consistently shown that commonly used antioxidant vitamin regimens (vitamins E, C, beta carotene, or a combination thereof) do not significantly reduce overall cardiovascular events, diabetes or cancer in studies such as HOPE, [53] GISSI, [54] ATBC, [55] Hennekens study, [56] Omenn's study, [57] Brown's study, [58] MRC/BHF, [59] Vivekananthan's meta-study, [60] Miller's meta-study. [61]

Antioxidants actually appear to cause harm and in some studies they may increase overall mortality, which is discussed in a 2005 nutrition and supplement review in JAMA. [62] Yet, one should keep in mind the fact that certain vitamin supplements may be beneficial for some people, such as those with a deficiency state, pregnant women, women of childbearing age, and people with restricted dietary intakes.

Apoptosis is a form of cell death necessary to make way for new cells and to remove cells in which the DNA has been damaged to the point of cancerous change. Thus, it is believed that one of the most important functions of apoptosis is the elimination of preneoplastic and neoplastic cells. [63] In most forms of cellular suicide, the signaling cascade requires EMODs as essential intermediate messenger molecules. [64]

Inhibition of apoptosis by antioxidants may explain why, in several studies in heavy smokers, vitamin E and β-carotene enhanced carcinogenesis in the lung. [65] Increased formation of EMODs also accompanies apoptosis induced by most, if not all, other stimuli, [66] and free radical scavengers often nearly always delay such apoptosis. [67-76]

Davies [77] has shown that cellular division or cell death is EMOD concentration dependent, when utilizing the EMOD, H2O2. Cellular responses go from proliferation, to arrest, to apoptosis.

Conclusion

The primary means that the human body has to rid itself of precancerous and cancerous cells is by electronically modified oxygen derivative-induced apoptosis. This crucial process can be blocked or negated by either small molecule antioxidants or by antioxidant enzymes. Cellular proliferation, cellular arrest and cellular suicide appear to be modulated by relative concentrations of electronically modified oxygen derivatives. Cautious use of antioxidants may be appropriate for individuals with tumorous or preneoplastic growths. EMODs appear to be gaining an increasingly important role in the modulation of cellular proliferation and cellular death. EMODs offer a therapeutic site in the selective killing of neoplastic cells, without causing harm to normal cells. Indeed, the potential of therapeutically increasing EMOD levels in combating disease offers the possibility of a promising opportunity.

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- Lancet. 1996 Aug 24;348(9026):488-9.
- Lancet. 1996 Aug 24;348(9026):489.
- Lancet. 1996 Oct 26;348(9035):1165; author reply 1167.
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- Lancet. 1996 Oct 26;348(9035):1167-8.
- Lancet. 2000 Dec 2;356(9245):1933.

Calcium-channel blockade and incidence of cancer in aged populations.

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BACKGROUND: Calcium-channel blockers can alter apoptosis, a mechanism for destruction of cancer cells. We examined whether the long-term use of calcium-channel blockers is associated with an increased risk of cancer. METHODS: Between 1988 and 1992 we carried out a prospective cohort study of 5052 people aged 71 years or more and who lived in three regions of Massachusetts, Iowa, and Connecticut USA. Those taking calcium-channel blockers (n = 451)

were compared with all other participants (n = 4601). The incidence of cancer was assessed by survey of hospital discharge diagnoses and causes of death. These outcomes were validated by the cancer registry in the one region where it was available. Demographic variables, disability, cigarette smoking, alcohol consumption, blood pressure, body-mass index, use of other drugs, hospital admissions for other causes, and comorbidity were all assessed as possible confounding factors. FINDINGS: The hazard ratio for cancer associated with calcium-channel blockers (1549 person-years, 47 events) compared with those not taking calcium-channel blockers (17225 person-years, 373 events) was 1.72 (95% CI 1.27-2.34, p = 0.0005), after adjustment for confounding factors. A significant dose-response gradient was found. Hazard ratios associated with verapamil, diltiazem, and nifedipine did not differ significantly from each other. The results remained unchanged in community-specific analyses. The association between calcium-channel blockers and cancer was found with most of the common cancers. INTERPRETATION: Calcium-channel blockers were associated with a general increased risk of cancer in the study populations, which suggested a common mechanism. These observational findings should be confirmed by other studies.

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