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ADVERSE EFFECTS OF EXCESSIVE ANTIOXIDANT SUPPLEMENTS AND THEIR UNDERLYING MECHANISMS

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Abstract: *Objectives:* The escalating use of antioxidant supplements has been questioned repeatedly although the potential toxicities of antioxidants has not been adequately assessed nor understood. *Methods:* This work represents a comprehensive insight into the literature seeking potential targets of antioxidants in tissues and cells, with which they would interact to produce their toxic effects. *Results:* Oxidants are cellular components that drive some cellular key metabolic processes, and appear to weaken certain other metabolic pathways in cancer cells. Excess deactivation by antioxidants may enhance cellular survival through abating apoptotic pathways and enhancing energy production from glycolysis. Furthermore, oxidants are important effectors of immune reactivity as well as metabolic activators of the immune cells that carry out anti-microbial and anti-tumoral activities, and abolishing oxidant activity may hinder these vital functions. *Conclusions:* It is hypothesized that the critical balance of oxidants-antioxidants can be disrupted by excessive antioxidant consumption, setting a new state of "antioxidant stress", leading to enhanced growth of cancer cells and to compromised immunity. In addition, the potential molecular mechanisms that may lead to this stress have been thoroughly explored.

Key words: Oxidative stress, anti-oxidative stress, antioxidative supplements, adverse effects.

Introduction

Oxidation implies the transfer of electrons or hydrogen to an oxidizing agent. Oxidation-reduction reactions do not necessarily involve oxygen, but when they do, they may lead to formation of free radicals, so-called reactive oxygen species (ROS) or intermediates (ROI). These may be in the form of superoxide (1), or the highly reactive hydroxyl radicals (2). There are also reactive nitrogen species (RNS), in which nitric oxide (NO) is generated by the action of nitric oxide synthases (3). Within the biological context, these free oxygen radicals have the potential to cause cell or tissue damage. Unusual increases in the oxidative activity, perhaps induced by pathologic insults, may themselves induce further pathologic insults, and the damage inflicted would be due to what is known as 'oxidative stress" (4, 5). This damage, however, may be prevented by natural antioxidants that inhibit further oxidation reactions. Natural cellular buffering of oxidants is maintained by cellular redox buffers, such as glutathione (6) keeping an almost constant "redox homoeostasis" (7, 8). Antioxidants are enzymatic or non-enzymatic effectors that interfere with oxidants by a deactivation process, or by transferring oxidizing agents from membrane-bound locations to aqueous phases of a cell to reduce their deleterious effects (9, 10). Aside from naturally occurring antioxidants, a large number of preparations containing antioxidants activity have been made available for human consumption with claims that they can prevent and cure diseases.

Antioxidants and health

The "benefits of antioxidants" is frequently addressed not only in scientific works, but also in newspapers and social literature as being agents that can treat a vast variety of serious and incurable diseases, and also that they can extend life spans of their consumers (11). The conditions mostly discussed are heart disease, stroke, cancer, arthritis, degeneration of the ophthalmic macula, degenerative nervous diseases such as Parkinson's and Alzheimer's. Other conditions include eclampsia, fertility problems and aging. Vast numbers of preparations containing antioxidants are on sale without medical prescription or advice. The need for antioxidants has been questioned repeatedly. Also questioned have been the claims that antioxidants prevent the accumulation of oxidant molecules thereby helping to prevent their progressive damage in cases of aging, cancer and even

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infections (11-13).

Negative effects of antioxidants

Countering the euphoric advertising blitz about the beneficial potential of antioxidants, a number of medical reports have shown deleterious effects of excessive supplementation with antioxidants. The Dillner group proposed that antioxidants supplements are not helpful, and should be replaced by fruits and fresh foods since they contain the necessary vitamins. Moreover, they concluded that supplements may induce some harm even indirectly since many people take supplements to compensate for unhealthy life styles, in addition to the potential toxicity from excessive antioxidants (14).

In meta-analyses, it was also concluded that cardiovascular disease incidence was not reduced by vitamins supplementation (15, 16). The Cochrane Collaboration compiled tens of clinical and experimental trials that included almost a quarter million people to conclude that antioxidant supplements do more harm than good. With older people, they concluded that people taking beta carotene or any of the vitamins were more likely to die during the study than those who did not. Beta carotene, which previously was alleged to prevent cancer, was found to actually cause more cancer in smokers than those control subjects not taking beta carotene. Furthermore, in the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Trial, researchers reported that men who took beta-carotene had an 18 percent increased incidence of lung cancers and an 8 percent increased overall mortality. However, vitamin E had no effect on lung cancer incidence or overall mortality (17-22). It was further reported that high doses of Alpha-tocopherol are not cancer-preventive (23). Furthermore, in work with skin cancer, antioxidants in general were reported to be more effective in preventing cancer promotion, than its initiation (24). Liver disease was also found not to benefit from vitamins preparations (25). Similarly, in a review of 10 clinical trials involving more than 6000 women with eclampsia, antioxidants, such as vitamins C and E, selenium and lycopene, were found to be ineffective in reducing pre-eclampsia, hypertension, preterm birth or miscarriage (26, 27). Critically ill patients were also recently reported not to benefit from antioxidants, and supplementation with glutamine enhanced patient deaths (28). In January 2013, Nobel Laureate and early co-discoverer of the DNA structure, James D. Watson, proposed that antioxidant supplements "may have caused more cancers than they have prevented" (29). These controversial findings were addressed by Edeas but mechanisms underlying the potential toxicity of antioxidant supplements remain unclear (30).

This work is an attempt to elucidate the possible causes of the controversial findings concerning the role of oxidative reactions in the various metabolic activities in normal and cancer cells and in immune reactivity. This perspective is composed of theoretical interpretations and hypothetical assumptions, based upon the known biochemical roles of ROS in normal and cancer cells, on the role of ROS in normal immune mechanisms and other physiologic functions, generating a hypothesis of these functions as potential targets of antioxidants.

Disturbances of the redox homeostasis

Cellular oxidation-reduction reactions are normally kept under strict control to minimize possibility of a shift. However, the well-described shift is that of increased oxidation, which over time can produce an oxidative stress that induces serious pathologies (31). To prevent oxidative stress, natural antioxidants function in deactivating the excess oxidative potential. These intra-cellular redox homeostatic mechanisms are dominated by glutathione and thioredoxin and to a less extent by low molecular weight antioxidants (8, 31, 32). Oxidative stress is produced when existing homeostatic mechanisms are exhausted, in which case additional antioxidant activity supplied with the food may provide the means for minimising damage. When oxidative status is increased, it is dominated by the output of ROS and/or RNS.

Regarding the controversial view of the harmful effect of antioxidants, a reduction status would prevail when induced by accumulated antioxidants following excessive supplementation over long periods of time. Hence, the term “anti-oxidative stress” as opposed to “oxidative stress”, has been devised and will be used in this text to denote the possible effects of continuous, harmful, and long-term exposure to antioxidants. High levels of antioxidants have been proposed to create a state of oxidation-antioxidation imbalance that could disrupt the physiological activities of ROS and RNS (30).

Effects of antioxidants on cells and tissues

The search into the mechanisms has revealed the presence of mechanisms acting at several potential levels including cellular and molecular levels.

Antioxidants as pathophysiological effectors

It has been reported that high concentrations of antioxidants may inhibit intestinal absorption of certain minerals, especially iron (33, 34). ROS are known to regulate some functions of vascular and cardiac cells (35), possibly through their role in activating cell signalling (36). ROS also regulate cell adhesion, important for differentiation, growth and repair (37). These regulatory functions may be defused by antioxidants once ROS are deactivated.

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Antioxidants and cell signalling

Cyclic guanosine monophosphate (cGMP) requires a hydroxyl radical to be formed (38) and nitric oxide appears to act in biological signalling in vital physiological processes such as blood pressure regulation, smooth muscle activities and neurotransmission (36). The mitogen-activated protein kinase (MAPK) pathways are significantly regulated by ROS. Upon activation, these pathways induce the production of factors that act on DNA repair, stop the proliferation of injured cells, activate the immune system, and participate in inducing inflammatory responses (39). Deactivated radicals and normal physiological functions, through deficient ROS activity may disrupt cell signalling and their dependant functions.

ROS, antioxidants and immunity

ROS is essential for the function of the immune system since a cardinal function of leucocytes is intracellular killing of bacteria by the ROS generated from hydrogen peroxide (40). Immune cells have been reported to produce both superoxide anion and nitric oxide during oxidative burst associated with inflammatory reactions. These oxides may unite forming a pyroxynitrite anion which is necessary for attacking and fragmenting microbial DNA (41, 42).

ROS are also necessary for intra-cellular signalling and regulation, including those of immune cells (7, 38, 43). The signalling stimulation follows induction by large amounts of cytokines, hormones and growth factors (44). And, as stated above, MAPK signalling pathway specifically requires ROS to activate nuclear transcription factors responsible for regulating cytokine production that stimulate the immune system (39). Moreover, the immune response in general, and the activation of T-lymphocytes and macrophages, in particular, are regulated and activated by superoxide radicals and hydrogen peroxide at physiological levels (45, 46). Thus, diminished tissue or cell ROS activity may have detrimental consequences to the immune response.

Immune reactions to infections are potentiated by ROS. Thus, reduced intracellular concentrations of ROS in HIV patients may lead to impaired phagocytic microbicidal functions, predisposing to opportunistic infections (47). Similarly, following experimental malarial infection in normal BALB/c mice, ROS levels in splenic cells increased gradually leading to a reduction in the parasitaemia, whereas in ROS-deficient B10 mice, parasitaemia rose sharply (48). Also, during continued infection, anti-tumour activity of splenic macrophages was increased in infected BALB/c mice, as compared to diminished tumouricidal activity of splenic macrophages from P/J mice which had defective macrophage functions (49).

Since cGMP is known to be activated by ROS, under conditions of "anti oxidative stress" cGMP may be inadequately produced. This "inhibitory" effect may include neutrophils and lymphocytes, leading to mounting weak or non-effective immune responses against potential pathogens. Moreover, neutrophils perform microbial intracellular killing using ROS and RNS. Excess antioxidants may deactivate oxidant activity, leading to failure of the intra-cellular killing. Similarly, antioxidants-induced reduction in cellular ROS may yield weak immune responses against parasites, tumours cells, and other foreign invaders. Part of the mechanism of action of vitamin E involves the reduction of pro-inflammatory cytokines and chemokines (50, 51), probably aided by the deactivation of existing ROS.

Antioxidants and cell metabolism

Antioxidants have the potential to interfere with intracellular metabolic activities and may influence regulatory or functional components. The hypothetical excess of reducing agents may interfere with the normal cellular reactions as outlined below.

Antioxidants and normal cell metabolism

Although potentially damaging, oxidation reactions remain crucial for life, and form an integral part of many metabolic processes (10). Cellular metabolic activities are essentially oxidation-reduction reactions. The respiratory chain is catalysed by dehydrogenases that transfer hydrogen atoms to their substrates in an orderly sequence, until the last reaction, in which oxidases can utilize oxygen as a hydrogen acceptor. These reactions allow the participation of substrates in relevant and equimolar quantities driving the reaction in a fixed direction in the respiratory chain (52, 53).

Elevated concentrations of an external, or even internally produced, reducing agent, such as in antioxidative stress, may alter the reactions microenvironment, thereby creating a tendency to alter the rate and/or direction of the reactions involved, so that the respiratory rate of a normal cell may be gradually abated. Moreover, products yet to be determined, may accumulate in the cell. Other areas where interference may occur are the glycolytic pathway, oxidative phosphorylation and the citric acid cycle. In these chain-pathways, antioxidants may interfere with oxidation-reduction reactions, enhanced by forces of concentration gradients. The persistence of such interferences over long periods may slow the glycolytic process, hinder the assimilation and building of metabolic substrates and products in the citric acid cycle, and reduce energy production. Interconnections among these various pathways may also be influenced. Examples of diversions of reactions driven by external antioxidants have been

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reported with cancer cells as outlined below. In addition to metabolic conversions, the pro-oxidant activity of some vitamins, such as vitamin C has been proposed to generate free radicals, mostly through reduction of metal ions (54, 55).

Antioxidants and cancer cells

It is proposed that cancer cells have increased survival with elevated levels of ROS as they may serve their favoured genetic instability, activate their proliferative potential, and delay senescence. However, excessively high levels of ROS can induce cell death (56, 57).

In cancer cells, the glycolytic pathway is often reduced to what is known as the "Warburg effect", whereby most of the pyruvate produced from glycolysis is converted into lactate (56, 58) with smaller amounts of the pyruvate being recruited into the citric acid cycle. The increased use of glucose and glutamine by a cancer cell, to increase cell mass and energy production, would be expected to produce excessive ROS and consequently increased need for NADPH. During stages of rapid proliferation, some cells may fail to produce sufficient NADPH activity and may thus undergo apoptosis due to ROS activity. Such cells can manage to survive under antioxidative stress (56).

Furthermore, cancer cells with mutant p53 may have defective oxidative phosphorylation, since p53 controls the sestrin genes and regulates the NrF2-transcription factor (nuclear factor-erythroid 2-related factor 2). Lack of sestrins due to defective p53 regulation can increase the ROS load in cells (59-62) with the consequent induction of apoptosis of cancer cells. Again, antioxidants may inhibit this apoptosis by reducing or abolishing ROS (63-67). Apoptosis has been described as one of the hallmarks of tumour phenotypes (68) and in all the above situations, extrinsic antioxidants may salvage potentially dying or apoptotic cells by providing direct protection from excess ROS.

Another potential by which antioxidant stress may provide protection to cancer cells is deactivating ROS in mitochondria. These ROS, when produced, can inflict progressive destruction on mitochondrial structures, thereby reducing the efficiency of oxidative phosphorylation. Furthermore, in the tricarboxylic acid (TCA) cycle, the enzyme aconitase is inactivated by ROS preventing the contribution of glycolysis to the TCA cycle, and such cells become solely dependent on glutminolysis (69, 70). Under antioxidative stress condition, these cells may be allowed to grow with production of more energy.

Another potential mode of action of antioxidative stress in cancer patients may involve inactivation of oxidative-induced cell damage, apoptosis and hypoxia-induced death of cancer cells, initiated by chemotherapeutic agents (71-75). This implies that cancer patients who are undergoing chemotherapy are advised not to take antioxidative supplements. Moreover, it was found out that aerobic glycolysis induced by tumour necrosis factor- α (TNF-α) in the cancer cell line MCF-7 can be reversed by the strong anti-oxidative agent, curcumin (76). In an almost similar way, antioxidants can rescue leukemia cells undergoing glucose starvation and apoptosis induced by apoptosis-inducing factor dependent and caspase-3 dependent mechanisms (77).

The above-mentioned modes of antioxidative stress have been summarised in Tables I and II.

Table 1
Summary of targets and effects of antioxidants on normal functions

• Hindrance of absorption of minerals.
• Cell adhesion is partly regulated by ROS and can be abolished upon their deactivation.
• cGMP formation which requires a hydroxyl radical.
• Neurotransmission, blood pressure regulation and smooth muscle activity regulated by nitric oxide.
• DNA repair and apoptosis of injured cells through MAPK pathway regulated by ROS. Antioxidative stress disallows DNA repair and apoptosis.
• Activation of the immune system and induction of inflammation depend on ROS.
• The intracellular killing of ingested microbes is carried out by ROS.
• Activation of extracellular killing is achieved by superoxide and nitric oxide.
• Abate activity of oxidation generating anti-microbial drugs.
• Attack and fragmentation of microbial DNA during inflammatory reactions through the action of pyroxinitrites.
• Effects on cell integrity of metabolism of normal cells.
• Reversal of TNF-α- induced aerobic glycolysis.
• Pro-oxidant activity of vitamins.

Table 2
Growth enhancement targets of antioxidants on cancer cells

• Excessive ROS production lowers NADPH production which induces apoptosis.
• Defective p53 regulation increases ROS production to induce apoptosis.
• Mitochondrial ROS.
• ROS inactivates the enzyme aconitase of the TCA to prevent linking glycolysis to TCA, so the cancer cell is rendered dependant on glutaminolysis so its' energy production is lowered.
• ROS produced by the action of chemotherapy is necessary for the anti-cancer activity.
• ROS that induces apoptosis following glucose starvation therapy.

Discussion

Antioxidants supplementation is very much like medicinal drugs that may have important curative actions in disease, but at the same time, some medicines

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may exert, especially at higher doses, some un-anticipated side effects. Hence, in spite of the well documented beneficial effects of antioxidants, reports have come in whereby some un-desired effects were encountered. Besides, and especially in cancer management, specific guidelines on the use of antioxidants, are required to be set, since antioxidants may have differential effects on various types of tumours. The best example for this is the experience with vitamin E and beta-carotene which enhanced lung carcinogenesis, but inhibited prostate cancer progression (67). Yet all-trans retinoic acid is another example of a vitamin used as a therapeutic agent for a variety of cancers, but contrary to this, it was reported to enhance the survival of hepatocellular carcinoma (78). Oxidants and antioxidants are natural cellular products, and the simultaneous presence of both is essential for normal cell survival. This co-existence implies that there are limits to their concentrations and efficiencies, and a critical balance needs to be maintained (30). However, potential benefits of antioxidants to normal cells can be shared by abnormal, possibly cancer cells and these potential benefits that antioxidants may offer to cancer cells may explain why cancer cells can improve their own survival in the presence of high antioxidant levels.

Based upon available knowledge in physiology and metabolism, this theoretical analysis shows where oxidants may present benefits to cells and tissues, and speculates upon the consequences when attacked and deactivated by antioxidants. The peculiarities of cancer cell metabolism have been highlighted to show how cancer cells may benefit from antioxidants shift or stress.

In the absence of cancer, normal body cells may benefit from antioxidants, especially when ROS levels rise in disease, by lowering their harmful effects and restoring a lost balance. However, in case of abundance of antioxidants, the likelihood of creating "antioxidative stress" becomes an aggravating factor to an existing pathology, particularly with compromised immunity. This may aggravate situations more in geriatric people whose aging cells would be of marginal integrity. The term "reductive stress" has recently been used to describe the stress linked to endogenous products, aggravating the situations in heart disease (79). Although technically similar, the term "antioxidative stress" may appear more appropriate in indicating the kind and basis of the insult.

A number of metabolic activities may be suppressed by antioxidants in a normal cell, and similar and/or other metabolic points or pathways can be acted upon to enhance survival of cancer cells. Ultimately, it needs to be determined which cell can survive better or be more diversely affected under this new stress.

Antioxidants may hinder the absorption of minerals, interfere with activation of cell signalling and activation pathways, interfere and divert normal metabolic pathways of normal cells, producing weakened cellular respiratory mechanisms, and energy production. If some of these effects are combined over relatively prolonged periods, they may cause adverse health conditions.

If cancer were present, its cells may benefit from the metabolic alterations by the introduced antioxidative stress. This may vary with the type and stage of the cancer involved. Indeed, some reports have emerged recently which highlight the significance of using nitric oxide in cancer therapy, suggesting potential positive roles of nitric oxide in various types of cancer (80, 81).

Antioxidants and the immune system. It has been suggested that the toxic effects of antioxidants are probably dose-dependent (30). The hypothesis regarding immunity and the mechanism of toxicity may involve deactivating the oxidative activity required to activate immune cells (36). In addition, antioxidants may prevent effective inflammatory reactions with high killing ability to foreign invaders, and halting the implementation of physiologic defence mechanisms (80).

The failure of immune cells in particular, to sustain adequate concentrations of ROS due to deactivation by antioxidants may lead to defective intra-cellular signalling, an additional factor for preventing lymphocyte and neutrophil activation by external effector molecules, such as hormones, cytokines and other cellular agonists (45, 46). This may be followed by weak T-cell activation and poor B-cell transformation and antibody productivity, representing a downstream failure. In addition, requirements for normal major histocompatibility complex class I antigen presentation includes the presence of ROS (82). Hence, weak lymphocyte responses may be attributed to defective cellular activation at low ROS activity and/or ineffective antigen presentation. These may allow microbial growth with little or no immune hindrance.

ROS action is essential in mitochondria inducing the expression of interleukin-2 (IL-2) and the CD95 ligand by activated T cells (83), an activity that can diminish under antioxidative deactivation. Also, in mechanisms probably similar to those proposed with cancer cells, anti-microbial activity may be enhanced by utilizing oxidation generating drugs (84, 85), an action reduced in the presence of antioxidative stress.

Other effects may influence other cells during growth, differentiation and repair, as more generalised effects may be anticipated following excessive long-term consumption of antioxidant supplements. Moreover, a progressive and generalized failure can result when a state of chronic "cellular choking" ensues with long-term supplemetation, especially when the oxidation-reduction chain is interrupted, or when cellular demands for ROS cannot be fulfilled by the electron transfer chain. This view is supported clinically by the recent findings that administration of glutamine increased death rate of critically ill patients (28). This increased mortality may be explained by the vulnerability of cells of sick patients to

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unfavourable metabolic alterations leading to the production and accumulation of side products assumed to be detrimental. That these side products were not deactivated by antioxidants implies that they were not of oxidative nature. Extra glutamine would probably participate and hasten the TCA cycle, possibly leading to imbalanced cellular activities with detrimental outcome.

Cancer cells have altered metabolism to augment the needs for its continuous division. Consequently, they would have fewer catalytic enzymes to be targeted or competed for, by antioxidants. If an antioxidant is accepted by the catalyst, it may hinder the glycolytic pathway of the cancer cell involved, possibly causing shortage in energy and apoptosis. Thus, in some cancer cell types, an effective antioxidant can be effective in inducing adverse effects on cancer growth. An example would be lactic dehydrogenase, which, when engaged with an external antioxidant, may fail in the conversion of sufficient quantities of pyruvate into lactic acid, gradually leading to cell death due to energy shortage (57). However, an antioxidant not recognized by the catalytic agent will not induce such an effect, but may find a suitable catalytic agent in a normal cell, thereby exerting an adverse effects on normal cells.

It is hoped that this topic may raise new queries for research to demonstrate the optimum use of antioxidants.

Conclusions

The hypotheses expounded have not been previously presented in a systematic fashion. Criteria for keeping a physiological oxidative - antioxidative balance is advised to prevent or at least minimise potential damage that can aggravate a disease state. Although they are neither toxic, nor carcinogenic, excess antioxidants may shift oxidative stress to an opposite state of "antioxidant stress", whereby immune mechanisms are abated predisposing to infections, and augmenting the growth of tumour cells.

Oxidation is a natural event. ROS are produced for a number of reasons, and removed naturally by existing mechanisms, which when disrupted, can be compensated by antioxidants in a normal diet. A critical balance of antioxidants intake needs to be assessed for the clinical situation.

The mapping of the metabolic points that can be targeted by antioxidants at the cellular level, may suggest that under conditions of poor health, antioxidants in excess, can be harmful to normal cells, can slow the activity of the immune system, and in cancer patients, may exert more harm to normal cells than to cancer cells. Conversely, they may even be useful to cancer cells since in several metabolic points they may reduce the apoptotic effects of ROS.

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