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Antioxidants: Friend or foe?

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ABSTRACT

Reactive oxygen species are the intermediates that are formed during the normal metabolic process which are effectively neutralized by the antioxidant system of the body. Any imbalance in this neutralization process causes oxidative stress which has been implicated as one of the cause in diseases such as Alzheimer's disease, cardiovascular disorders, cancer *etc*. Research has enabled the use of antioxidants as therapeutic agents in the treatment of various diseases. Literature also puts forth the negative effects of using antioxidants in the treatment of diseases. This review is a compilation of both the beneficial and detrimental effects of use of antioxidants in the treatment of diseases such as cancer, cardiovascular diseases, diabetes and oral diseases.

1. Introduction

Reactive oxygen species (ROS) are those that include reactive oxygen ions and peroxides and cause detrimental effects at high concentrations to biomolecules such as DNA, RNA, protein and lipids, leading to pathological conditions in humans. In normal conditions, ROS are produced as necessary intermediates and involve as secondary messengers in cell signalling in picomolar concentrations. Excessive production of ROS and its uncontrolled regulation lead to detrimental effects [1]. Singlet oxygen is the most reactive one and all other ROS can be formed from singlet oxygen involving a single step electron transfers. A superoxide radical is one electron reduction of the singlet oxygen whereas hydrogen peroxide, another deleterious ROS, is one electron reduction of the superoxide radical. In living cells, hydrogen peroxide plays a vital role in defence against some infections as well as in the cellular signalling in kinase related pathways that are associated with cell migration and proliferations [2]. ROS in living cells include hydroxyl radicals, occurring commonly through fenton type reactions, nitric oxide and the nitric oxide derived peroxynitrite, hypochlorous acid which acts as a precursor for many strong oxidizing species. ROS are mainly produced in the organelles such as mitochondria, peroxisomes and endoplasmic reticulum and mainly through mitochondrial respiratory complex during high rates of ATP production, oxidation of fatty acids and detoxification of xenobiotic processes respectively [3]. A balance in the production of ROS and its scavenging by antioxidants determines a healthy system. The disruption in this balance is what leads to oxidative stress.

Some of the common targets for ROS include nucleic acids, carbohydrates, proteins and lipids. Malondialdehyde, a toxic molecule regarded as a biomarker of oxidative stress, is one of the examples of the end products from the oxidation reactions involving lipids [4]. Whereas the amadori rearrangement of Schiff base products as observed with the glycated haemoglobin is another example of advanced glycated end products. Level of glycated haemoglobin is used as a marker for diabetic conditions [5]. ROS interacts with proteins, and causes several reactions that lead to peptide cleavage and amino acid oxidation causing protein damage. DNA oxidation leads to DNA strand breaks and ROS interaction with mRNA can lead to defective protein translations [6].

Antioxidants are those molecules that are involved in the scavenging of these reactive species causing oxidative stress. They are defined as those substances that could prevent the oxidation of substrate at low concentrations [7]. Oxidative stress

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and its detrimental effects can be prevented through the intake of naturally occurring antioxidants. Antioxidants act as free radical scavengers and can prevent oxidative reactions that lead to various diseases. The majority of exogenous antioxidants are from the plants, the phytochemicals. Several classes of phytochemicals exist with antioxidant potential which is based on their unique structural rearrangements [8].

Antioxidants have a wide range of effects in various disease conditions and help to prevent the onset of such conditions. Natural antioxidants that occur in an organism could fight against the oxidative stress that occurs through various physiologic processes. These include antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and reduced glutathione which are endogenous antioxidants. In particular, glutathione (GSH) plays a central role in defence against oxidative stress. GSH channels through its oxidized form oxidized glutathione through the enzymes such as glutathione peroxidase and glutathione reductase to neutralize ROS. Further, antioxidants from diets such as vitamin C, vitamin E and vitamin A are taken exogenously for their protective effects against ROS. One of the beneficial effects of antioxidants is their role in cellular signalling apart from its free radical scavenging property. Antioxidants such as catechins have been found to alter the signalling modulating transcription factors such as AP-1 and NFκB in endothelial cells [9].

2. Antioxidants and cancer

The ability of antioxidants to protect the cells from ROS formed the basis of its production in food supplement industries. Increase in scientific literature with regards to its beneficial effects also increased its wide acceptance among the general public. However, current research in cancer shows two different aspects of antioxidants. Antioxidants are both beneficial as a treatment strategy for cancer patients [10] and at the same time implicated with deleterious effect of increasing the cancer cell progression [11].

On the brighter side, the ROS scavenging potential of antioxidants is very crucial in several cancer types in advanced stages owing to the fact that ROS promotes cell migration and invasion in metastatic cancer cells [12]. The harmful effects of electrophiles or ROS at the molecular level are countered by Keap1 and Nrf2 related signalling cascades. Keap1 acts as a sensor for these oxidative changes and Nrf2 in turn helps in the transcription of key antioxidant enzymes that scavenge ROS. In parallel to the Keap1, thioredoxin reductase 1 also regulates Nrf2 in order to maintain the balance between oxidants and reductants [13]. Thereby treatments that involve potential trigger of this Keap1-Nrf2 signalling cascade might confer advantage in treating metastatic tumour conditions. Supplementation of vitamin C, one of the most abundant dietary antioxidant, has been found to protect against oxidative damage induced by cigarette smoking thereby reducing the risk associated with cancer [14]. In another study, it is shown that mitochondrial superoxide dismutase, an antioxidant enzyme that scavenges the superoxide anion, prevents cancer development [15].

However studies opposing these trends keep increasing, denoting a fact that the scavenging of ROS is in fact deleterious to cancer patients rather than preventing the risk. It is particular to note that some antioxidants do behave as pro-oxidants under certain circumstances [16]. Also treatment for lung cancer using β-carotene, a potential antioxidant, in lung cancer has to be abandoned owing to the instability of β-carotene molecule in the oxygen rich environment in the lungs of cigarette smokers [17]. Another study mentioned that the β-carotene should be used cautiously owing to its highly reactive carotenoid radical formation during the scavenging mechanism of free radicals. The study showed that the carotenoid radical form which is scavenged through vitamin C could have aggravating effects on UV-carcinogenesis with varying levels of vitamin C [18]. In another study, N-acetylcysteine (NAC), a medication that works by increasing the glutathione levels and vitamin E, a powerful antioxidant that scavenges peroxyl radical, was shown to accelerate the tumour progression in a B-RAF and K-RAS induced lung cancer in murine models. The study shows that these antioxidants promote tumour progression by reducing ROS levels and in turn reduce p53 expression levels that could have induced apoptosis leading to the cell death. The fact that inactivated p53 showed similar effect of promoting tumour as with the supplements of NAC and vitamin E confirmed that these antioxidants confer these deleterious effects in cancer through the ROS-p53 axis [19]. The implication of the study is that when antioxidants are taken by healthy individuals they confer beneficial effects, but when there is an onset of tumour formation, high doses of antioxidants should be prevented so as to stop the increased proliferation of tumour cells. It is also noteworthy that the antioxidants used as cream could turn unstable through the reactions associated with UV-irradiation thereby leading to deleterious effects [20]. These facts raise considerable questions regarding antioxidant therapy. One possible solution could be the intake of selected antioxidants according to the disease progression and employing the usual diet comprising such antioxidants rather than the intake as direct supplements.

3. Antioxidants and cardiovascular diseases

Cardiovascular diseases (CVD) are one of the major types of non-communicable diseases which account for majority of deaths worldwide. Several studies have shown the association between CVD and ROS and the beneficial effects of antioxidant nutrients against these ROS. One study has shown the beneficial effect of antioxidants against ROS in preventing atherosclerosis, which is one of the major complications that give rise to CVD [21]. It is shown that oxidative stress is a contributing factor to atherosclerosis [22]. Oxidation of low density lipoprotein is one of the major steps in atherogenesis and it was shown that antioxidants help to prevent atherosclerosis by blocking this oxidation of low density lipoprotein along with other protective mechanisms [23]. One recent study showed the antioxidant effect of the drug Probucol in reducing ROS and protecting endothelial progenitor cells against oxidized low density lipoprotein, thereby playing an important role in preventing atherosclerosis. This study involved C57BL/6 mice models and showed that the decreased levels of superoxide dismutase, circulating mononuclear cells and epithelial progenitor cells by the injection of oxidative low density lipoprotein were all restored by the antioxidant effect of Probucol [24]. During treatments associated with myocardial infarction, reperfusion to ischemic regions is associated with increased ROS production. This leads to impairment in the function of the heart [25]. Further hypoxia and increased ROS lead to a decrease in the levels of antioxidant enzymes further

aggravating the oxidative burst [26]. These detrimental effects of ROS on CVD can be countered by antioxidant supplements which could prevent these diseases [27].

Antioxidants owing to their protective effects against ROS are thus widely considered to offer protection against diseases. However ROS should not be considered completely deleterious for its varying intracellular signalling roles. ROS is involved in a number of signalling pathways in which they alter the intracellular redox status and involve oxidation of regulatory proteins. These redox sensitive proteins are involved in transcriptional activities. These which include protein kinases such as Src, protein kinase C, phosphatises such as PTEN, G-proteins such as ras, RhoA involved in cardiovascular functions are all activated by ROS. Mitogen activated protein kinases are also indirectly activated by ROS through Src and protein kinase C [28–30]. It is also important to note that ROS is involved in regulating redox sensitive transcription factors such NFκB, implicating an important role in cell signalling associated with cardiovascular functions [31]. Particularly, ROS can play a dual role depending upon the signals received, for example apoptosis triggered by TNF-α in endothelial cells is mediated through ROS with NFκB as the major transcription factor involved in the activation of genes associated with inflammation and endothelial dysfunction [32]. ROS produced during ischemic preconditioning can in turn prevent apoptosis by upregulating Bcl-2 [33]. This shows the putative roles played by ROS in various conditions. Disruption of the redox status by supplementation of antioxidants might thus affect these intracellular signalling processes. This warrants a systematic approach for the use of antioxidants based on the nature and type of disease involved thereby harbouring the positive aspect of antioxidants and omitting their deleterious effects as observed with some diseases.

Major drawback for use of antioxidants is the lack of clinical evidence. It is also argued that antioxidant supplement could disturb the normal homeostasis associated with the role of ROS in various physiologic processes [1]. Study also exists where there were no effect associated with antioxidants in CVD. One such study which is a meta-analysis study of 13 randomised controlled trials evaluated the effect of vitamin E in the prevention of stroke. The study concluded that the administration of vitamin E showed no benefit in preventing stroke of any type including ischemic stroke, hemorrhagic stroke, fatal stroke and non-fatal stroke [34]. Other meta-analysis studies showed that vitamin E and beta carotene have no effect on the cardiovascular mortality and morbidity, and concluded that vitamin E is not warranted for routine use [35,36]. One of the reasons for the failure is the non-specific accumulation of antioxidants in sites other than the compartments in which ROS levels are elevated. One possible strategy to overcome this involves the use of antioxidants conjugated to triphenylphosphonium cations by which these antioxidants could accumulate in the mitochondrial matrix several folds when compared to cytosol, thereby increasing the efficacy of antioxidants to treat oxidative stress associated with CVD [37]. However human studies are yet to be performed with such strategies.

4. Antioxidants and diabetes

An increasing body of evidence suggests that diabetes is associated with oxidative stress with insufficient endogenous antioxidant defence systems [38,39]. Oxidative stress plays a major role in the pathogenesis of complications associated with diabetes. For example, the superoxide production contributes to endothelial dysfunction in diabetes mellitus as observed in one of the studies. This study revealed important roles for nitric oxide synthase, protein kinase C, NAD(P)H oxidases in the vascular superoxide production in diabetes mellitus [40]. Increased levels of mitochondrial ROS is associated with increased expression levels of receptors for advanced glycated end products. It is also evidenced that oxidative stress is the major source for diabetic complications such as diabetic nephropathy [41].

Antioxidants have been found to defend against the oxidative burst associated with diabetes and in turn help in reducing the hyperglycaemic state in diabetes. Medicinal plants have found its use in diabetic complications owing to its high antioxidant content. It is noted in several studies that these plants and their extracts have provided less toxicity compared to the currently available drugs for diabetes. In one study a natural plant extract boosted the endogenous antioxidant defence systems and decreased the levels of ROS in rat models of type II diabetes thereby protecting the various organs from the oxidative damage during diabetic conditions [42]. It is also found that the overexpression of the antioxidant enzyme catalase had therapeutic potential in a diabetic-induced cardiomyocyte dysfunction. The study showed that the catalyse had a positive effect in removing the enhanced levels of ROS, preventing apoptosis and also attenuating the diabetic induced cell signalling associated with Akt, forkhead transcription factor and silent information regulator [43]. The potential use of antioxidant vitamins, such as vitamin E and vitamin C, has been shown in one study where these antioxidants protect against diabetic nephropathy, which is one of the major diabetic complications associated directly with oxidative stress. This study which was performed in diabetic models of Wistar rats showed that the kidney glomeruli have decreased levels of malondialdehyde formation and augment the levels of antioxidant enzymes thereby improving the kidney function from oxidative stress [44].

The beneficial effects of antioxidants in experimental studies confirm its potential use as a treatment for diabetes. However clinical data is not solid. Supplementation of antioxidants is not always beneficial as observed in a recent study involving NAC in human subjects. NAC is the precursor for glutathione and helps in increasing the GSH levels. NAC supplementation in subjects with type 2 diabetes had little or no effect on the increased blood glucose levels and other oxidative stress markers such as GSH, GSH/oxidized glutathione ratio, TBARS, urine F2α isoprostanes. Conversely it suggested that high doses of NAC in fact could be detrimental by increasing the blood glucose levels [45]. In randomized controlled trials, it is found that the supplementation of vitamin E, vitamin C and beta carotene did not show any significant effect on the prevention of type II diabetes. Though not statistically significant, there was a slight decrease in the diabetes risk for women who received vitamin C, and conversely a slight increase in the trend for the risk of diabetes was observed in the treatment groups of vitamin E [46]. Most of the clinical trials failed to show the protective effects of antioxidants with an exception for the treatment with α-lipoic acid in diabetes [47]. A possible reason could be attributed to the population under study in clinical trials where it observed that diabetic patients with high oxidative stress responded well to vitamin E treatments. Also a standard measure to compare the baseline oxidative stress for all patients under study is not available thereby contradicting effects of antioxidant vitamins in diabetes management has been observed.

5. Antioxidants and other diseases

Oxidative stress also plays a major role in the development of alcoholic liver diseases. In one study, curcumin, the active principle from turmeric, and its synthetic analogue were shown to decrease the oxidative stress as observed through decreased lipid peroxidation and increase in the antioxidant status in albino Wistar rat models of alcoholic liver disease thereby concluding a putative role for curcumin and its analogue in protection against the oxidative stress [48].

In fertility, the oxidative stress that causes the DNA strand breaks in sperm cells could lead to miscarriage and abnormal embryonic development with increased defects in the offspring [49]. This could be prevented with the supplementation of antioxidants that could counter these effects. It was found that men with oral supplementation of antioxidants were associated with a significant increase in live birth rate. Also it was shown that a significant increase in the pregnancy rate was found associated with the antioxidant supplementation and found to have no evidence of harmful side effects [50].

However, the effect of exogenous antioxidants could also lead to decreased antioxidant production within the body. In a particular concentration, the antioxidants could also behave as pro-oxidants causing deleterious effects. This is evident in studies where the antioxidants were found to cause adverse effects when given in high doses [51]. It is found that unlike male fertility, female fertility does not improve with antioxidant supplement. Besides, antioxidant supplement do not show any benefit in the prevention of miscarriages occurring through oxidative stress [52].

One of the major organs more susceptible to oxidative damage than most other organs is the brain. Oxidative stress has been linked to several central nervous system disorders [53,54]. One key protein that plays a role in the regulation of redox signalling is thioredoxin, Trx1. These thioredoxins have been associated with protective effects in nerve cells [55]. It is also shown that ebselen, a compound with glutathione peroxidase like activity exerts neuroprotective effects [56]. However clinical findings are lacking to show the potential use of antioxidants for the treatment of neurodegenerative diseases [57].

A major limitation for the failure of antioxidants is the lack of standard route of administration and non-optimal dosages. Moreover, antioxidants themselves act as pro-oxidants in some cases and lead to aggravated states of the disease. Also the lack of availability of the antioxidants in the specific compartment of the cells where there is increased levels of ROS leads to failure of treatment strategies based on these supplements. A possible scenario for the failure of antioxidants in clinical trials could be attributed to the population under study in which the subjects enrolled were those who had established disease state and could not harbour the preventive effects of antioxidants in reducing the risk of the disease. Antioxidant based therapies could yield successful outcomes when focused at specific sites rather than as a whole [58].

6. Antioxidants in dentistry

6.1. Periodontal disease

Gingivitis and periodontitis together constitute periodontal disease which is one of the most widespread chronic conditions that affects people worldwide [59]. ROS has been implicated in the pathogenesis of periodontal disease [60]. The leukocytes in response to the chronic inflammation release the reactive species which is responsible for the oxidative damage to the gingival and the periodontal tissues and the alveolar bone [61]. It has also been seen that the oxidative stress plays a key role in the pro-inflammatory cascades that are responsible for the tissue damage in the other inflammatory conditions associated with periodontitis [62]. The biological substances with potent antioxidant capacity that have been studied in periodontal disease include Vitamin C, vitamin E, carotenoids, polyphenols, bilirubin, GSH, uric acid, and melatonin [63].

6.2. Dental caries

One of the most common oral problems encountered in day to day clinical practice is dental caries and its associated morbidities such as pain and swelling. It has been observed that certain components present in green tea such as epigallocatechin-3-gallate have found to reduce the risk of development of dental caries and plaque formation by its scavenging effect. Clove, containing eugenol which is an enzyme activator of antioxidant action, helps in reducing the tooth ache [64]. Antioxidant rinses, mouth washes, irrigating solutions, ingredients in tooth paste are some of the products that are under research for common dental diseases and mucosal diseases [65].

6.3. Potentially malignant oral disorders

Potentially malignant oral disorders comprise a group of clinical presentations that carry a risk of cancer development [66]. Leukoplakia, erythroplakia, oral bubmucous fibrosis, oral lichen planus are some of the disorders that come under this category. It has been observed that ROS plays an important role in the development of cancer in patients with potentially malignant oral disorders [67]. Phytochemicals and antioxidant vitamins have found to induce apoptosis in cancer cells but do not affect the normal cells as shown by experimental studies [68]. Therefore the use of antioxidants early in therapy for potentially malignant oral disorders can prevent the malignant transformation or delay its onset [65]. The human studies that have been conducted so far could not effectively prove whether antioxidants could become a hindrance to therapy by protecting the cancer cells from free radical damage. Though there are benefits in using antioxidants especially in the early stages, in high risk cases and advanced lesions its usefulness needs to be ascertained [69,70].

7. Conclusion

Antioxidants have been continually investigated for their health benefits in terms of their scavenging potential of free radicals. Nevertheless simultaneous research findings reveal the dark side of antioxidants which could be detrimental to human health. A proper balance in the existing treatment with antioxidants should exist based on the accumulating evidence of the recent research reports. The various aspects of antioxidants both as a preventive and causative risk factor in various human diseases as explained in this review suggest a multitude of factors have to be considered in the prescription of antioxidants as a preventive measure to decrease the risk of certain diseases. Combinatorial therapy using antioxidants is one of the ways to

harbour the beneficial effects of antioxidants and to reduce the risk of the aggravating diseases. Also tailoring the antioxidants to specific locations associated with oxidative outburst could be potentially useful to treat various diseases associated with oxidative stress. The negative cases observed for few antioxidants should not be generalized to all antioxidants. Exploration of the mechanism of action and the optimization of concentrations to be administered according to the physiological sites might help in improving the treatment efficacy of antioxidants making them more of a friend than a foe.

Conflict of interest statement

The authors declare that there is no conflict of interests.

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