Research Article

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Antioxidants an Epigenetics Regulator for the Prevention of Diseases and Aging Process

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Abstract

During metabolism, oxygen consumption involves the constant generation of free radicals and reactive oxygen species (ROS). There are many enzymatic and non-enzymatic antioxidant defense systems in the body that remove these toxic species. Cardinal amongst, are enzymes such as superoxide dismutase (SOD), catalase (CAT), which are involved in this detoxification process. Current research has shown several herb extracts in animal models and human, with potentials to restore changes in other target engagement biomarkers of hepatic enzyme like aspartate aminotransferase (AST), alanine aminotransferases (ALT), alkaline phosphatase and acid phosphatase. In the genome, epigenetic events such as DNA methylation, acetylation, plays essential role in regulating gene activity bearing, aberrant epigenetic landscape in cancerous cells are an established phenotype, showing a causal relationship of epigenes in cancer. The de-methylation of DNA at the CpGs site on DNA strands using combination of Phyto-medicine and foods high in flavonoids had all been proposed as vital in altering epigenetic events. Although cellular targets for phyto-active compounds are largely unclear, flavonoids and other phytochemicals have shown relevant functionality in diverse cellular processes. ROS attack DNA readily, generating a variety of DNA lesions, leading to bases and strand breaks. In the case of double strand breaks (DSBs), they are devastating to normal cell physiology as they enhance cell death with attendant diseases and concomitant aging phenomenon. Moving forward, concerted research efforts are required for successful intervention with phytomedicine on a larger scale, which could boost immune reactions, reduce ROS attach and enhance cellular processes in disease preventions and aging management.

Keywords: Epigenetics, ROS, Phytochemicals, Methylation, Acetylation, Double Strand Break

Introduction

Oxidative stress is a harmful condition that occurs due to excess of ROS and/or a decrease in antioxidant levels, causing tissue damage of physical, chemical or psychological nature (Halliwell, 2007). The term ROS comprises of the superoxide anion (O_2•−), hydroxyl radical (OH•), hydrogen peroxide (H_2O_2) and several others. They act as the intermediate during the breakdown of molecular oxygen products bearing all aerobic organisms constantly engaged in the breakdown of oxygen and produce ROS in the process. Also they are being routinely formed during cell metabolism, due mainly to their importance in pathogen defense during respiratory burst [1]. When certain leukocytes come into contact with bacteria or fungi, NADPH oxidase, an enzyme on their outer membrane, is activated to rapidly produce large amounts of superoxide, which effectively kills the pathogen [2].

ROS and Reactive Nitrogen Species (RNS) are the product of normal cellular metabolism having both deleterious and beneficial effects in the body, for instance, the NOX isozymes, are a signaling molecule and are known enzyme family with the sole purpose of producing ROS, an indication that at least certain ROS are physiologically essential [3]. Moreover, they regulate numerous cellular processes as for example, Nitrogen Oxide (NO) with a half-life of 30s is a highly diffusible gas and it is a free radical that is generated from L-arginine metabolism, acting as a second messenger regulating a number of physiological processes including; neurotransmitters, platelet adhesion and vascular permeability [4].

However, because these free radicals using various mechanisms such as; lipid peroxidation, which destroys cell structures, lipid, proteins and nucleic acids, hence they can unleash their side effects such as, damage to cell membranes with the release of intracellular components, leading to further tissue damage [1]. Oxygen derived free radical reactions have been implicated in the pathogenesis of many human diseases including Neurodegenerative disorder, Cardiovascular disease, Autoimmune disease, hemoglobinopathy such as sickle cell disease, Tumors and cancer as well as aging via the activation of oncogenes or inactivation of tumor suppressor genes [5].

Cancer prevention using antioxidant approaches has been suggested to offer a good potential in providing important fundamental benefits to public health and is being adopted in some cases by health practioners and researchers as key strategy for inhibiting, delaying or even reversal of the process of carcinogenesis [6].

The administration of several herb extracts in animal models and humans, has been shown to restore changes in target engagement biomarkers of hepatic enzyme like AST, ALT, alkaline phosphatase and acid phosphatase [7]. Many factors are implicated in the intracellular release of molecules some of which, are responsible for the generation of reactive oxygen species (ROS), leading to DNA double strand break (DSB), causing oxidative DNA damage that could trigger signaling cascades leading to the stimulation of cell growth, glutathione depletion, lipid peroxidation, protein cross liking, culminating ultimately in oxidative-induced cell death. Neutrophils can also provide reactive oxygen and nitrogen species that can contribute to DNA damage and genomic instability. The damage by oxidants and other genotoxins agents play a role in the development and generation of cancer [5] bearing, in order to maintain DNA fidelity, cells must overcome multiple challenges as genotoxics agents that threaten genome stability. Moreover, altered metabolic pathway involving NADPH oxidase, tumor-associated macrophages delivering sub-lethal oxidative stress and an inadequate tumor vascular network may all contribute to such cellular transformation [4].

Generally, the biological importances of ROS are dependent on their quantities, chemical nature, subcellular and tissue localization/location, the rates of their formation and degradation. This mini-review looks at how ROS affects epigenetic signature, DNA damage with the attendant health consequences and how such could/are mitigated with antioxidant from natural sources.

Discussion

Antioxidant for cellular maintenance

The body has developed several endogenous antioxidants as defense systems and they are classified into two groups such as enzymatic and non-enzymatic. The enzymatic defense systems include superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), whilst the non-enzymatic defense system include vitamin E, vitamin C and reduced glutathione (GSH), which is the cellular antioxidant [2].

The non-enzymatic antioxidants can also be further divided into metabolic antioxidants and nutrient antioxidants [8]. Metabolic antioxidants are the endogenous antioxidants, which are produced by metabolism in the body such as lipoic acid, glutathione, L-arginine, melatonin, uric acid, transferrins [8]. Nutrient antioxidants belongs to the exogenous antioxidants, as they cannot be produced in the body but are provided through diets or supplements e.g. trace metals (selenium, manganese, zinc), flavonoids, omega-3 and omega-6 fatty acids etc. [9]. However, in some recent cases Omega 6 (n-6 poly unsaturated fatty acids) PUFAs, found in dietary fat from meat, have been generally attributed to be pro-inflammatory as they give rise to arachidonic acid, which can result in the production of both pro- and anti-inflammatory eicosanoids such as prostaglandins and leukotrienes [10]. Vitamin E and C are the non-enzymatic antioxidants, which exists within normal cells as well as they can be supplied through diet.

Antioxidant products have found increasing usage as natural products for anti-sickling in the treatments of diseases including SCD and cancer. Such usage is connected with the assumption that the medicinal plants are natural and safe. Research into antisickling properties of medicinal plants has been promising because many active phytochemicals have been isolated and characterized. Therapy using phytomedicines has proven to not only reduce crises for SCD patients, but also have been found to reverse sickling [11,12]. The effectiveness of medicinal plants as anti-sickling drug was demonstrated decades ago with root extract of Fagara xanthoxyloides [12,13].

The uses of plants, natural products are thoughts to be beneficial in protecting against oxidative stress-induced damage. They are less toxic compared to synthetic compounds used at their optimum protective dose levels [14,15]. Thus, the interests have always existed in the development of potential drug of plant origin, being a good source of potent but non-toxic anti-oxidant [16]. Antioxidants of plant origin include vitamin E, C, selenium, phenolic compounds, carotenoids and flavonoids [see 11 and references therein]. Earlier studies in the laboratory indicated that oral administration of carotene and plant extract of spinach [14], amaranths and linseed [14], to Swiss albino mice protects various tissues against oxidative stress induced by radiation. The mechanism of actions of these plants was earlier postulated [16], to includes the activation of metabolizing enzymes that detoxify carcinogens, the suppression of DNA adduct formation, the inhibition of the production of reactive oxygen species, the regulation of cell-cycle arrest and the induction of apoptosis [17].

Most recently we studied the effects of the plants (garlic, onion and ginger) extracts on oxidative stress using specific biochemical enzymes such as, aldenylate oxidase (AO), super oxide dismutase (SOD), catalase (CAT), sulphite oxidase (SO). Based on the comparative study on the effects of the extracts; we deduced that garlic and onions were more potent than ginger, albeit; the results suggests each extract confer some degree of radio-protective effects combined with anti-oxidative properties [7].

In all many naturally occurring antioxidants such as garlic acts on several fronts in blocking several disease including cancer e.g. prostate cancer growth as it inhibits polyamines needed for cell division, increasing breakdown of testosterone, that is needed for prostate cancer growth and reducing prostate specific antigen (PSA) levels, a prostate cancer marker. Apoptosis induced by garlic-derived organo sulphur compounds (OSC) essentially involves the mitochondrial pathway [18]. The mitochondrial pathway requires the release of cytochrome c from the mitochondria into the cytosol through the permeabilization of the outer mitochondrial membrane [19]. Studies showed that the biochemistry of these effects consist of an early impairment of cell proliferation with a severe arrest and accumulation of cells in G2/M phase followed by apoptosis via the mitochondrial pathway. These events is mediated by the formation of specific channels and is carried out by the two pro-apoptotic members of the B-cell lymphoma 2 (Bcl-2) family, the Bcl-2-associated X (Bax) and Bcl-2 homologous antagonist killer (Bak) proteins [19].

Epigenes and cellular maintenance

Genomic regulation by epigenetic events such as DNA methylation, play an essential role in regulating gene activity. Cellular

differentiation is a good example of biological process that is well connected to epigenetic events. Cell differentiation for instance, is tightly related to the establishment of the correct epigenetic status visa viz the proper epigenetic maintenance. Epigenetic abnormalities alter gene expression, counteracting regular differentiation and cell physiology that could ultimately lead to aging processes and diseases [20].

Evidence from cancer cells showed aberrant epigenetic landscape, suggesting causal relationship between epigenome and cellular processes in the form of cell proliferation, identity maintenance and general genomic instability [21]. Moreover, during embryonic development, changes in methylation are thought to lead to disrupted expression of some important regulatory genes, and where such are sustained, the accumulated changes in DNA methylation could increase risk of later cancer development [22]. It is also noted that, CpG islands in the proximity of tumor suppressor genes (TSG) are frequently methylated in various cancers, inducing TSGs transcriptional repression and promoting cancer progression, as specific patterns of histones H3 and H4 acetylation and methylation are thought to be associated with numerous cancer subtypes [5,23].

In the last couple of years Neri, et al. 2012 reported that c-myc, a key regulator of embryonic stem (ES) cells pluripotency maintenance, is directly involved in the transcriptional upregulation of all components of Polycomb repressive complex 2 (PRC2) as c-myc binds PRC2 (transcriptional repressors) subunits promoters and induces the acetylation of histones H3 and H4, an epigenetic modification involved in genes transcriptional activation [24].

Other than genetic modifications, specific drugs have been found to induce the restoration of "normal" cellular pathways, which in turn promote cellular senescence or apoptosis, which can reverse epigenetic alterations. Hence, epigenetic changes are excellent target candidates for chemotherapeutic intervention in cancer and could be a veritable target for anti oxidative agents, which are of natural sources e.g phytomedicine [16]. Moreover, the de-methylation of DNA at the CpGs site on DNA strands using combination of Phyto-medicine and foods high in flavonoids had been proposed as vital in altering histone acetylation, modulating gene expression. Unfortunately cellular targets for phyto-active compounds remain largely unclear, although flavonoids and some other phytochemicals, have been used to target functionally diverse cellular processes and they do help to modulate the activity of a large number of downstream genes [16].

Several Histone deacytylase (HDAC) and DNA Methyltransferase (DNMT) inhibitors are already available as putative anticancer drugs, and several clinical trials are underway [25]. Because a number of proteins and transcription factors other than histones are also modified by acetylation, there are wide ranges of biological effects caused by HDAC inhibition, which remain unknown. However, the main result of HDAC inhibition is hyperacetylation and, consequently, gene expression [5,11,16]. Apart from the effects observed on gene transcription, evidence is accumulating to show that HDAC inhibitors influence chromatin stability, mitosis, and DNA repair mechanisms [26,27].

DNA Damage and repair

In order to maintain DNA fidelity, cells must overcome multiple challenges that threaten genome stability including environmental toxicants [28]. Causes of DNA damages can be divided into spontaneous actions emanating from ROS attack on the DNA strands and or environmentally-induced factors. Spontaneous DNA damages are usually caused by intracellular metabolism stress emanating from excess ROS, or formed during genetically programmed processes such as V (variable), D (diversity), and J (joining) (V(D) J) recombination in developing vertebrate lymphocytes or meiotic recombination in germ cells [29]. Genomic integrity is particularly important where germ cells are generated, as they provide the blueprint for the next generation hence, cells have multiple DNA repair pathways that repair Double Strand Breaks (DSBs) caused by various genotoxic agents. These agents contain both physical factors, such as ultraviolet (UV), visible light and ionizing radiation, environmental metals [28], as well as chemical factors, such as benzopyrene, alkylating agents and aflatoxins. These DNA damages can lead to single base mutation or more deleterious chromosomal lesion. The cellular response to oxidative DNA damage includes, DNA repair, cell cycle arrest and apoptosis.

The various types of DNA damage include aberrant base or nucleotide modifications, single strand DNA (ssDNA) breaks, and chromosomal lesions caused by double strand breaks (DSBs). Amongst these, DSBs are known to be the most cytotoxic bearing, when left unrepaired, DSBs will affect genome integrity by causing mutations, chromosomal deletions/ translocations, aging etc, because there is no intact complimentary template to repair the damaged strand [4,29]. There are two mechanisms by which DSBs can be repaired i.e.; the non-homologous end joining (NHEJ) and homologous recombination (HR) [29]. These two pathways differ in their functional enzymes, the repair efficiency and also the cell cycle phases where they are active. For the NHEJ the breaks are repaired by direct ligation of the exposed ends by the enzymes involved such as Ku binding element (within the promoter), hence NHEJ is efficient but error prone.

DNA bases are sensitive to ROS oxidation, especially guanine because it has a low redox potential [30]. Thus, the formation of 8-oxoG the most abundant, is also one of the most well characterized DNA breaks due to the effects of ROS [4,31]. Estimate has it that ~180 guanines are oxidized to 8-oxoG per mammalian cell per day [32], and 8-oxoG is a highly mutagenic miscoding lesion that can lead to G:C to T:A transversion mutations [32]. Accelerated aging process due to ROS has been observed and reported in mice defective in DNA repair mechanisms, such as Nucleotide Excision Repair (NER) and DSB repair, telomere maintenance and mitochondrial genome replication [33].

Conclusion

DNA damage accumulation leads to genomic instability with cancer and age-associated disorders being the resultant effects. Thus, the genetic damage emanating from exposure to ROS as discussed poses serious deleterious consequences on genome stability. ROS via its continuous attack on protein, lipids and DNA are thought to be involved in producing the changes in diseases and its associated aging process. They seems to affect cancer cell proliferation through modification of histone acetylation with, its ability to target multiple pathways, including the cell cycle machinery and thus, regulate gene expression and proliferation.

AST and ALT are enzymes directly associated with conversion of amino acid to keto acid. AST and ALT activities are used, as indicator

of hepatic damage as elevated activities of AST and ALT in the serum is a common laboratory finding in some forms of cancer e.g. colon cancer, especially with liver metastasis. Thus, phyto-compound that targets and regulate these biochemical parameters could be useful in the management of cancer with its multifaceted consequences.

Research in the field of ROS-biochemistry is slowly evolving with large volume of theory and hypothesis yet to be tested. However, enzymatic tools investigating ROS via xanthine oxidase and its elimination via SOD have helped to facilitate additional research in a number of areas of molecular biology and pathology. Therefore, concerted biochemical/molecular research efforts are required, to drive a successful phytochemical (with anti-oxidative properties) intervention on a larger scale, with its potential to boost other immune events due to several feedback mechanisms that tends to be associated with diseases and aging process. Thus, the recent development of a web-based platform (Enalos Cloud) for accurate virtual screening of small molecules is a welcome development. However, moving forward, there will be need to incorporate the rapid screening of phyto-compound to this platform as it may leap frog research in this vital area, with potentials to address high cost of patients care especially in resource poor economy with an added advantage of it being less toxic compared to synthetic drugs used at their optimum protective dosage.

Conflicts of Interest

I declare that there are no conflicts of interests

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