BIOMOLECULAR MECHANISM OF ANTIOXIDANT ACTIVITY ON AGING PROCESS

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Abstract

Over the recent decade, attention have been focused on the pathologic role of free radicals in a variety of diseases, which are most related to aging process. Free radicals are normally produced as a by-product of cellular metabolism. In normal conditions nature has supported human body with a large number of antioxidant potential. Elusive balance exists between free radical generation and antioxidant defense system at cellular level to cope with oxidative stress, which prevents the occurrence of disease. Factors tilting the balance in favor of excess free radicals generation lead to widespread oxidative tissue damage and diseases. Therefore, trouble starts when there is an excess of free radicals and the defense mechanism lags behind.

Aging is a continuation of development which is influenced by genetically programmed phenomena. Completion of various genetic programs and the duration of life are linked to a metabolic potential which is itself a genetically determined sum of energy expenditure. The rate, at which metabolic potential is reached, is linked to the rate of metabolism and the level of oxidative stress. In aerobic cells, partially reduced oxygen species are produced in an uncontrolled fashion and do not play any useful physiological function. The principle tenet of the free radical hypothesis in aging is that molecular damage is the underlying cause of aging and oxygen radicals and derivatives induce most of the damage sustained by cells during aging.

Cells exert control not only on their level of antioxidant defense but also on their rate of oxidant production. Defense systems against oxidative damage are classified as preventive antioxidants, which suppress the formation of free radicals, radical-scavenging antioxidants, which scavenge radicals to inhibit chain initiation and break chain propagation, repair and de novo enzyme, which repair the damage and reconstitute membranes, and adaptation, which generate appropriate antioxidant enzymes and transfer them to the right time and in the concentration. Studies have found evidence of oxidative damage to macromolecules (DNA, lipids, protein), and mitochondria, that support the hypothesis that oxidative injury might directly cause the aging process. Although a causal role for oxidative stress in the aging process has not been clearly established, this does not preclude attempts to reduce oxidative injury by supplementing antioxidants as a means to reduce morbidity and perhaps increase the healthy, useful life span of an individual.

MEKANISME BIOMOLEKULAR AKTIVITAS ANTIOKSIDAN PADA PROSES *AGING*

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Abstrak

Pada dekade belakangan ini perhatian banyak ditujukan terhadap peran radikal bebas pada berbagai patogenesis penyakit termasuk proses aging. Radikal bebas secara normal merupakan hasil sampingan metabolisme sel. Dalam keadaan normal, tubuh manusia telah dilengkapi dengan potensi antioksidan yang cukup banyak. Keseimbangan yang sulit terdeteksi terjadi antara produksi radikal bebas dengan sistem pertahanan antioksidan pada tingkat sel untuk mengatasi stres oksidativ. Adanya faktor yang mendorong pergeseran keseimbangan kearah produksi radikal bebas yang berlebih akan menyebabkan kerusakan berbagai jaringan dan penyakit. Oleh karena itu, masalah akan mulai muncul saat mekanisme pertahanan tertinggal dibanding dengan kelebihan produksi radikal bebas.

Aging merupakan kelanjutan dari perkembangan yang dipengaruhi fenomena yang terprogram secara genetik. Berbagai pengaruh genetik dan usia terkait dengan potensi metabolsme yang merupakan resultan penggunaan energi yang ditentukan secara genetik. Kecepatan laju metabolisme terkait dengan tingkat stres oksidativ. Pada sel aerob, molekul oksigen yang tereduksi sebagian dihasilkan dalam keaadaan tidak terkontrol yang tidak memiliki fungsi fisiologis. Prinsip dari hipotesis radikal bebas pada aging adalah bahwa kerusakan molekuler merupakan dasar dari aging dan oksigen radikal serta derivatnya menginduksi kebanyakan kerusakan sel yang berlangsung selama aging.

Sel memiliki kontrol bukan saja terhadap tingkat pertahanan antioksidan, namun juga pada produksi oksidan. Sistem pertahanan terhadap kerusakan oksidativ dalam tubuh digolongkan sebagai antioksidan preventif, yang menekan pembentukan radikal bebas, antioksidan penangkap radikal, yang mencegah inisiasi rantai radikal dan memutuskan pembentukan rantai radikal, perbaikan dan enzim de novo, yang memperbaiki kerusakan dan merekonstruksi membran, serta sistem adaptasi, yang menghasilkan enzim antioksidan yang sesuai dan mentrasfernya ke tempat dan saat yang tepat. Banyak studi yang telah menggambarkan kerusakan oksidativ terhadap berbagai makromolekul seperti DNA, lipid, protein, dan juga mitokondria, yang mendukung hipotesis bahwa kerusakan oksidativ dapat berperan langsung pada proses aging. Meskipun peran stres oksidativ pada proses aging belum tergambarkan secara pasti, hal ini tidak perlu menghalangi upaya untuk menekan kerusakan oksidativ melalui suplementasi antioksidan sebagai upaya menurunkan morbiditas dan mungkin meningkatkan kebugaran, yang akan bermanfaat bagi kehidupan.

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General aspects of geriatric medicine

As a part of a remarkable revolution in human biology, the average life-span has increased beyond 65 years in most human populations, and the fastest-growing age group is the oldest. In the United States since 1950, the age group 65 years and older has grown from 8 % to 13 % of the general population. The record life-span is held by a recently deceased French Woman, Jeanne Calment, who reached 122 years and 4 months. Despite impairments of vision and hearing, she appeared to be cognitively intact. It is predicted that by 2020 a total 50 million will live to be at least 65 years of age, i.e., an increase by another 50 % (Figure 1). Thus aging has become a world-wide issue affecting all nations, irrespective of their economic development.

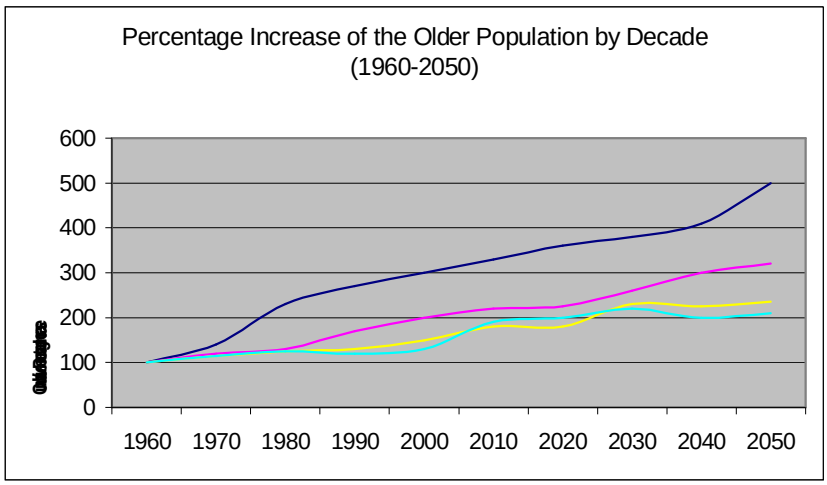

Figure 1. Past and Projected increases in the elderly by decade. (from U.S. Bureau of the Census, Current Population Reports, P25-1092. Population Projections of the U.S. by Age, Sex, Race, and Hispanic Origin. U.S. Government Printing Office, Washington DC, 1992)

Society often classifies everyone over 65 as “elderly,” but most authorities consider the field of geriatrics to apply to persons over 75-even though this too is an arbitrary definition. Of all the people who have ever lived to age 65, more than two-thirds

are currently alive. Although the implications of this startling statistic are usually viewed in demographic and economic terms, the impact of age on medical care is also substantial and requires significant alterations in the approach to the older patient. Shakespeare probably characterized aging best in his elegant description of the seven ages of man. It begins at the moment of conception, involves the differentiation and maturation of the organism and its cells, at some variable point in time leads to the progressive loss of functional capacity characteristic of senescence, ends in death.

Many fears about aging are related to the myth that everything declines with aging. Even genetically identical mice experience heterogeneity in their physiologic responses. The normal heterogeneity of aging is further compounded in a clinical setting by the diseases acquired during a lifetime and the increased number of medications taken by older patients. With age, there are physiologic and structural alterations in almost all organ system. Aging in individuals is affected to a great extent by genetic factors, diet, social conditions, and occurrence of age-related diseases, such as atherosclerosis, diabetes, and osteoarthritis. In addition, there is good evidence that aging-induced alterations in cells are an important component of the aging of the organism. Aging changes appear to be very diverse and subject to numerous environmental and genetic influences. Aging processes thus show a great deal plasticity and potential for modification.

The biology of aging

A number of cells functions decline progressively with age. Cellular aging could represent the progressive accumulation over the years of sub-lethal injury that may lead to cell death or at least to the diminished capacity of the cell to respond to injury. Although several mechanism have been proposed to account for cellular aging, recent concepts center on two interrelated process; the existence of a genetically determined clock that times aging, and the effects of continuous exposure to exogenous influences that result in the progressive accumulation of cellular and molecular damage. In addition to the importance of timing and a genetic clock, cellular life span may also be determined by the balance between cellular damage resulting from *metabolic events* occurring within the cell and counteracting molecular responses that can repair the damage.

Senescent cells have a decreased capacity for uptake of nutrients and for repair of chromosomal damage. The morphologic alterations in aging cells include irregular and abnormally lobed nuclei, pleomorphic vacuolated mitochondria, decrease endoplasmic reticulum, and distorted Golgi apparatus. Oxidative phosphorylation by mitochondria is reduced, as is synthesis of nucleic acid and structural and enzymatic proteins, cell receptors, and transcription factors. Concomitantly, there is a steady accumulation of the pigment lipofuscin, which, as we have seen, represents a product of lipid peroxidation and evidence of oxidative damage; advance glycation end product, which result to a non enzymatic glicosylation and are capable of cross linking adjacent proteins; and abnormally folded proteins.

Many mechanism previously postulated to mediate aging have not been borne out, including *the somatic mutation theory, free radical theory, cell-aging theory, apoptosis theory, immune theory, and neuroendocrine theory,* which *a*ccount for a wide range of physiologic changes. However, the biological changes are clearer than the mechanisms that mediate them. The theories differ in the emphasis placed on increased damage (e.g., by free radicals, oxidation, or glycation) versus deficient repair and about the mechanism that might mediate each. Although such theories are attractive, it remains unclear whether the described abnormalities are the cause or the result of senescence.

Much more might be said about the mechanism of cellular aging, but it suffices here to say that these mechanisms involve both programmed events in cell proliferation and differentiation – such as *telomere* shortening and the activity of clock genes – and the consequences of progressive environmental injury overwhelming the cell's defense mechanisms. Oxidative free radical damage to proteins, lipids, and DNA as well as post-translational modifications of proteins (e.g., nonezymatic glycation) are two well-studied examples of such exogenously induced effects. Failure in the ability to repair oxidative injury or repair DNA damage appears to be particularly important in cell aging and may contribute to the premature aging of cells in certain disorders.

Free radicals

Molecules are composed of atoms and electrons, and electrons are present generally in pairs. However, under certain conditions, molecules have unpaired electrons

and as such they are called free radicals. Thus, the term free radical is used to describe chemical species, which have unpaired electrons. Since unpaired electrons usually seek other electrons to become paired, free radicals are in general reactive and attack other molecules, although some radicals are not reactive but stable enough to have long life. Examples of reactive free radicals are the hydroxyl (HO^{*}) and alkoxyl (LO^{*}) radicals, while the nitric oxide ($^{*}NO$), vitamin E (tocopheroxyl), and vitamin C (dehydroascorbate) radicals are examples of stable radicals.

Active oxygen or reactive oxygen species (ROS) and related species, as parts of free radicals, play an important physiological role and, at the same time, they may exert toxic effects as well. The active oxygen species are essential for production of energy, synthesis of biologically essential compounds, and phagocytosis, a critical process of our immune system. They also play a vital role in signal transduction, which is important for cell communication and function. On the other hand, there is now increasing evidence which shows that these active oxygen species may play a causative role in a variety of diseases including heart disease and cancer, and aging. Both active oxygen species and antioxidants are double-edged swords and the balance of their beneficial and toxic effects is determined by the relative importance of many competing biological reactions.

Free radicals and other oxygen-derived species are constantly generated in vivo, both by “accidents of chemistry” and for specific metabolic purposes. Exposure to oxidant molecules issued from the environment (pollution, radiation), nutrition, or pathologies can generate reactive oxygen species (ROS for example, H2O2, O2-,OH). These free radicals can alter DNA, proteins and/or membrane phospholipids. The reactivity of different free radicals varies, but some can cause severe damage to biological molecules, especially to DNA, lipids, and proteins. The set of intracellular and extracellular conditions that leads to chemical or metabolic generation of reactive species is termed “oxidative stress”. Oxidative stress is thus defined as a disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses. The susceptibility of oxidative stress is a function of the overall balance between the factors that exert oxidative stress and those that exhibit antioxidant capability.

Depletion of intracellular antioxidants in acute oxidative stress or in various diseases increases intracellular ROS accumulation. This in turn is responsible for several

chronic pathologies including cancer, neurodegenerative or cardiovascular pathologies. Thus, to prevent against cellular damages associated with oxidative stress it is important to balance the ratio of antioxidants to oxidants by supplementation or by cell induction of antioxidants. Antioxidant defense systems scavenge and minimize the formation of oxygen-derived species, but they are not 100% effective. Hence, diet-derived antioxidants may be particularly important in diminishing cumulative oxidative damage and helping us to stay healthier for longer. Repair systems exist to deal with molecules that have been oxidatively damaged. The biological efficacy of oxidants is based on highly regulated equilibrium between the production of oxygen radicals and the counteracting defense mechanisms of antioxidant scavenging systems and repair enzymes for the elimination of the degraded bioproducts. Imbalance of this finely tuned, sophisticated equilibrium can result in oxidative stress unleashing a cascade of pathological processes.

Free Radical Theory of Aging

Rapid developments in free radical biology and molecular technology have permitted exploration of the fee radical theory of aging. Over the recent years, researchers have focused their attention on the pathologic role of free radicals in a variety of diseases, among which the most important are atherosclerosis, cancer, and diabetes, which are most related to aging process. Studies have found evidence of oxidative damage to macromolecules (DNA, lipids, protein), and data in transgenic Drosophilia melanogaster support the hypothesis that oxidative injury might directly cause the aging process. Additional links between oxidative stress and aging focus on mitochondria, leading to development of the mitochondrial theory of aging. However, despite the number of studies describing the association of markers of oxidative damage with advancing age, few, if any definitively link oxidative injury to altered energy production or cellular function. Although a causal role for oxidative stress in the aging process has not been clearly established, this does not preclude attempts to reduce oxidative injury as a means to reduce morbidity and perhaps increase the healthy, useful life span of an individual.

The free radical theory of aging proposed by Harman hypothesizes that the degenerative changes associated with aging might be produced by the accumulation of

deleterious side reactions of free radicals generated during cellular metabolism. Oxygen free radicals, particularly superoxide, hydroxyl, and peroxyl radicals, are the most commons radicals generated during metabolism and could contribute to aging via several mechanisms. Oxyradicals can also be generated *in vivo* by environmental exposure to prooxidant toxicants like cigarette smoke and smog. Oxyradical-induced DNA cross-links could lead to somatic mutations and loss of essential enzyme expressions. Oxidation of sensitive sufhydryl groups could cause cellular damage to mitotic and cytoplasmic microtubules. Membrane lipid peroxidation could destroy the integrity of subcellular organelles. Macro-molecular cross-links of connective tissue could impede nutrient diffusion and impair tissue viability. While cellular antioxidants effectively quench many reactive oxygen species and damage that does occur is usually repaired rapidly, only a small fraction of unrepaired lesions allowed by inadequate antioxidant defenses could contribute to aging and the pathogenesis of chronic diseases. Although supporting evidence keeps the free radical theory of aging both reasonable and attractive, several basic predictions are not observed, particularly the fact that supplementary antioxidants do not lengthen the maximum life span of mammals appreciably. However, rigorous experimental testing of this approach with a full complement of antioxidants has not been conducted.

Pryor has suggested that the free radical hypothesis of aging be reproposed with free radicals considered as involved in the etiology and development of chronic diseases that are most life-limiting, i.e., with radicals implicated in processes that shorten life below the maximum life span. Indeed, free radicals appear to play an important role in the initiation and/or promotion of Alzheimers disease, atherosclerosis, cancer, cataract, Parkinsonism, rheumatoid arthritis, and other chronic diseases common among older adults. Nonetheless, it is important to recognize that tissue injury and many diseases may themselves generate ROS such that oxidant formations is an epiphenomenon that makes little contribution to the progression of the condition. However, increasing antioxidant defenses may prove to be a useful intervention, if not necessary to slowing the rate of the aging process, for reducing the associated risk of chronic disease.

Aging is a continuation of development and is thus influenced by genetically programmed phenomena. Completion of various genetic programs and the duration of life

are linked to a metabolic potential which is itself a genetically determined sum of energy expenditure. Nevertheless, the rate at which metabolic potential is reached is linked to the rate of metabolism and the level of oxidative stress both of which are influenced by epigenetic stimuli. The current version of the free radical hypothesis postulates that partially reduced oxygen species are produced in aerobic cells in an uncontrolled fashion and do not play any useful physiological function. The principle tenet of the free radical hypothesis is that molecular damage is the underlying cause of aging and that O2-radicals and derivatives induce most of the damage sustained by cells during aging. The damage accumulated during aging is a secondary effect rather than a direct cause of senescence. Cells exert control not only on their level of antioxidant defense but also on their rate of oxidant production. Aging is the terminal stage of development, and as such is influenced genetically. It is also postulated that a definite sum of energy is required to complete the genetic programs associated with aging. Thus, the rate of aging is linked to the rate of oxidative stress. Oxidative stress is one of the factors which appears to govern changes in gene expression during differentiation and it is suggested that it causes alterations in gene expression during aging. Free radicals promote aging by affecting specific genetic programs and the incidental damage they inflict in cells is only a by-product of this process.

Aging and Antioxidants

Substantial experimental evidence indicates a role for oxygen free radicals in the aging process and in the development of chronic diseases common among the elderly. Experimental, clinical and epidemiological studies are converging to reveal a reduced risk of age-related conditions like atherosclerosis, cataract, cancer, and other chronic diseases in groups with high antioxidants vitamin status. Diets characterized by high intakes of fruits and vegetables, particularly those rich in antioxidant nutrients, are similarly associated with a lower prevalence of these conditions.

Investigations into the relationship between antioxidants and aging have largely focused on how oxidative processes change with age. Mitochondria, key organelles in aerobic metabolism and a major source of ROS, have served not only as a useful model but perhaps a central functional point as mitochondrial dysfunction may be a principal

underlying event in aging. Mitohondrial DNA, which does not contain histones, appears to be particularly sensitive to oxidative modification. The accumulation of mitochondrial DNA mutations may lead to decreased gene expression, a decline in oxidative phosphorylation, inefficient electron transport, and increased oxidant flux. Ames *et al* have shown that mitochondrial DNA damage is greater in old than in young rats and significantly greater as well than in nuclear DNA. Increased oxidative damage in mitochondria with age may result from increased oxidant generation and/or decreased antioxidant defenses.

In healthy conditions nature has endowed human body with enormous antioxidant potential. Subtle balance exists between free radical generation and antioxidant defense system to cope with oxidative stress by various enzymes and vitamins at cellular level which prevent the occurrence of disease. However, factors tilting the balance in favor of excess free radicals generation lead to widespread oxidative tissue damage and diseases. Therefor, trouble starts when there is an excess of free radicals and the defense mechanism lags behind. Overwhelming production of free radicals in response to exposure to toxic chemicals and aging may necessitate judicious antioxidants supplement to help alleviate free radical mediated damage.

Conclusions

Active oxygen and related species play in important physiological role and, at the same time, they may exert toxic effects as well. The active oxygen species are essential for production of energy, synthesis of biologically essential compounds, phagocytosis, and signal transduction. Active oxygen species, however, may play a causative role in a variety of diseases including heart disease and cancer, and aging. Antioxidants, which suppress such oxidative damage play an important role in aerobic organisms.

Free radical production occurs continuously in all cells as part of normal cellular function. However, excess free radical production originating from endogenous or exogenous sources might play a role in many diseases. Antioxidants prevent free radical induced tissue damage by preventing the formation of radicals, scavenging them, or by promoting their decomposition. A growing amount of evidence indicates that increasing with age

imbalance between pro- and antioxidants is implicated in a premature aging and a variety of diseases.

A better understanding of the complex relationships between antioxidants and aging within the context of the free radical theory of aging should be promoted by looking at several facets of this interaction. First, it is important to understand how antioxidant status change with age in people, particularly for consideration of revising dietary requirements and proffering recommendations for food fortification and supplementation. Second, the only established model demonstrating a direct relationship between nutrition and aging is that of caloric restriction.

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