Participation of free radicals in the skin aging process

Udział wolnych rodników w procesie starzenia się skóry

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INTRODUCTION

The aging of the organism, including the skin, has been the subject of research interest for many years. The conducted research concerns mainly the causes and ways of delaying this process, which begins at birth and is the result of metabolic changes taking place in the body which ensure its functioning. The first changes in the structure and functions of the skin are visible between the ages of 20 and 25. The aging process explains many theories, and one of the most popular is the free radical theory of aging, which states that there are reactions induced by free radicals behind this process. Due to the fact that the skin is the outermost layer of the body, it is directly exposed to the harmful effects of external factors. Therefore, it ages much faster than the rest of the organs.

SKIN AGING PROCESS

Skin aging is a physiological, inevitable and multifactorial process, regulated by the influence of exogenous and endogenous factors. Relationships between these factors are extremely important, because each factor may directly influence the aging process or indirectly, affecting others [1, 2]. As a result of the aging process, the biological activity of cells is reduced, regenerative processes are slower, and the response and resistance to oxidative stress are reduced. Moreover, the adaptation properties of the organism controlled by genetic factors are reduced. In other words, the growth of cells is inhibited in the skin and throughout the body, and atrophic processes begin to prevail [2, 3]. Depending on the cause, two main types of skin aging can be

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ABSTRACT

The skin aging process, as well as the whole body is conditioned by intrinsic and extrinsic factors. Reactive oxygen and nitrogen species, whose main source are metabolic processes occurring inside the body, also play an important role in this process. The imbalance between generation of free radicals and their removal is referred to as oxidative stress.

The aim of the article was to present the effect of oxygen and nitrogen species on the skin aging process.

Reactive forms of oxygen and nitrogen perform physiological functions in the body. Abnormalities in their production or removal by the body's antioxidant systems lead to oxidative stress. As a consequence, chronic stress contributes to the acceleration of the aging process and the occurrence of related diseases.

Keywords: *skin aging, free radicals, reactive oxygen species, reactive nitrogen species, oxidative stress*

STRESZCZENIE

Proces starzenia się skóry, jak również całego organizmu uwarunkowany jest czynnikami wewnątrz- i zewnątrzpochodnymi. Istotną rolę w tym procesie odgrywają również reaktywne formy tlenu i azotu, których głównym źródłem są procesy metaboliczne zachodzące we wnętrzu organizmu. Brak równowagi pomiędzy generowaniem wolnych rodników a ich usuwaniem określany jest mianem stresu oksydacyjnego.

Celem artykułu było przedstawienie wpływu wolnych rodników tlenowych i azotowych na proces starzenia się skóry.

Reaktywne formy tlenu i azotu pełnią w organizmie funkcje fizjologiczne. Nieprawidłowości w ich wytwarzaniu lub usuwaniu przez systemy antyoksydacyjne organizmu prowadzą do powstania stresu oksydacyjnego. Przewlekle trwający stres przyczynia się w konsekwencji do przyspieszenia procesu starzenia oraz wystąpienia chorób z nim związanych.

Słowa kluczowe: starzenie się skóry, wolne rodniki, reaktywne formy tlenu, reaktywne formy azotu, stres oksydacyjny

received / otrzymano
23.04.2020
corrected / poprawiono
19.05.2020
accepted / zaakceptowano
01.06.2020

distinguished: extrinsic (exogenous) and intrinsic (endogenous). Exogenous aging is conditioned by the influence of external factors, which include: ultraviolet UV radiation, exposure to cigarette smoke, environmental pollution, improper care and improper diet. Endogenous aging consists of changes resulting from the passage of time, i.e. age-related - chronological aging and hormonal disorders (menopause). Despite the division, both types of skin aging share common biological, chemical and molecular mechanisms, so they are not always distinguishable. These mechanisms overlap and determine the full picture of aging skin [4, 5].

IMAGE OF AGING SKIN

Age-related aging is characterized by the predominance of atrophic processes and muscle flaccidity. On the other hand, extrinsic aging is dominated by hypertrophic processes. Under the influence of the abovementioned egozo- and endogenous factors, changes occur at the level of each layer of the skin: epidermis, dermis and subcutaneous tissue. Over the years, the skin becomes dry, thin, poorly nourished and not very elastic. There are numerous wrinkles on its surface, which depending on the depth can be divided into superficial and deep. They are mainly located around the eye sockets, on the forehead, cheeks and the lower part of the face and neck [6]. The clinical picture of aging skin shows the disappearance of the spinous and granular layers of the epidermis, therefore it becomes thin and prone to damage. The amount of ceramides and sterols in the epidermis is reduced. The process of producing the natural moisturizing factor (NMF) is disrupted, and the content of glycosaminoglycans, mainly hyaluronic acid responsible for optimal hydration, decreases. These processes determine the occurrence of dry skin [7]. Another change in the skin as a result of the aging process is a decrease in the activity of the sebaceous and sweat glands. This causes damage to the skin's hydrolipid layer and increases the TEWL (transepidermal water loss) loss, thus impairing the barrier function of the epidermis [6].There is a reduction in the number and activity of fibroblasts, degradation of collagen and elastic fibers and a change in their mutual arrangement. As a result, the skin's hydration, firmness and elasticity decrease [8]. Another symptom of skin aging is dysfunction of melanocytes, which tend to accumulate in one place. The result is the appearance of stains and discoloration on the skin surface and insufficient protection against UV rays [9]. As a result of reducing the number of capillaries and thickening of their walls, the partial pressure of oxygen in the blood decreases, and the diffusion and osmosis processes are inhibited. This leads to a decrease in the supply of oxygen in the dermis and epidermis, which reduces the exchange of nutrients and disturbs thermoregulation. Moreover, due to the fact that the vessels become brittle, they are often damaged, which is manifested by erythema and telangiectasia on the skin surface [9, 10]. The number of Langerhans cells, which are part of the skin's immune system, also decreases with age. There is a tendency to develop dermatoses and susceptibility to the occurrence of neoplastic changes. The gradual disappearance of the fatty pads in the subcutaneous tissue contributes to the thinning of the skin as a whole [9, 11].

MOLECULAR BASIS OF SKIN AGING

In the course of skin aging, a number of morphological, biochemical, immunological and endocrine changes occur, including the loss of the extracellular matrix (ECM), especially hyaluronic acid (HA), which together with fibrous collagen and elastic proteins is responsible for its elasticity. The disappearance of collagen and elastin fibers is related to the increased expression of matrix metalloproteinases (MMPs), induced, among others, by exposure to ultraviolet radiation or tobacco smoke. MMPs belong to endopeptidases and constitute nearly 20 zinc-dependent proteolytic enzymes, which can be divided into six groups: matrilysins, collagenases, stromelysins, gelatinases, membrane MMPs and unclassified MMPs [12, 13, 14]. Metalloproteinases are involved in many processes in the body. Their main role is to degrade the extracellular matrix (ECM) proteins: collagen, laminin, proteoglycans and fibronectin, which facilitates cell migration and causes the release of growth factors that affect cells. Under physiological conditions, metalloproteinases regulate developmental processes, embryogenesis, and control angiogenesis and wound healing. In addition, they participate in the formation of cell receptors and many immunological and pathological processes [14].

Under physiological conditions tissue metalloproteinases are expressed at a very low level and are closely controlled at many levels of regulation, namely, transcription, post-translational processing, secretion, activation and degradation. It has been revealed that interleukins, interferon and many growth factors contribute to the induction of transcription of metalloproteinase genes, including epidermal growth factor (EGF), keratinocyte growth factor (KGF), hepatocyte growth factor (HGF) factor, vascular endothelial growth factor (VEGF), tumor necrosis factor alpha α (TNF-α) and transforming growth factor β1 (TGF-β1) [15, 16]. Another transcription factor is nuclear factor kappa β (NF-kβ), activated by UV radiation. It affects matrix proteins because it stimulates the transcription of inflammatory cytokines that attract neutrophils, which also contribute to the activation of matrix metalloproteinases (MMP-8) with the simultaneous degradation of extracellular matrix proteins [12].

It has been shown that oxygen free radicals contribute to the activation of matrix metalloproteinases (MMP): collagenase (MMP-1), gelatinase (MMP-2 and -9) and elastase, which break down skin matrix components. Collagenases (MMP-1, -8, -13) have the ability to degrade virtually all

collagen subtypes. A characteristic feature of these enzymes is the ability to hydrolyze the collagen superhelix in about ¾ of the chain length, composed of 3 polypeptide chains twisted around each other. And post-translational modifications (glycosylation and hydroxylation) are responsible for the essential properties of collagen [14]. Gelatinases (MMP-2 and MMP-9) can degrade type IV collagen, laminins, and also show activity against denatured collagen - gelatin. Moreover, MMP-2 digests type I, II and III collagens and is widely expressed in tissues [16].

FREE RADICAL THEORY OF AGING

Oxygen is an element used in the metabolic processes taking place in the cells of aerobic organisms. As a result of a series of enzymatic reactions using oxygen, carbon dioxide and water are produced, and the energy necessary for metabolic processes is released. At the same time, in the process of cellular respiration, harmful particles are formed. Thus, oxygen, which is absolutely necessary for life, paradoxically is also a toxic factor [17].

In 1956 Denham Harman put forward the hypothesis that the primary cause of the aging process in organisms is the accumulation of damage in their cells caused by the activity of reactive oxygen species. This theory is known today as the free radical aging theory. He explains that free radicals generated in the body as a result of physiological processes attach to other cells and damage them. The accumulation of oxidative damage causes impairment of the functions of cells and tissues, which leads to the development of the aging process and related diseases, and consequently to the death of the body. The most exposed to free radicals are proteins, lipids and nucleic acids [18, 19].

CLASSIFICATION AND ROOTES OF FREE RADICALS

Free oxygen radicals are products of the incomplete reduction of the oxygen molecule in the respiratory process and are capable of independent existence. They can be in the form of atoms or molecules. They have at least one oxygen atom and one or more unpaired electrons. Such a chemical structure determines the high reactivity of these molecules, which is caused by the desire to get rid of the excess electron. Therefore, free radicals easily react with the components of cells, leading to the formation of new radicals [20]. Free radicals and other reactive oxygen species are abbreviated as ROS (reactive oxygen species). The group of free radicals also includes nitrogen compounds, RONS (reactive oxygen and nitrogen species) [21].

Reactive oxygen and nitrogen species (RONS) are the second group of compounds with unpaired electrons. RONS includes nitric oxide (•NO) and the nitrosyl cation (NO+), nitrosyl anion (NO-) and peroxynitrite produced from it as a result of metabolic changes (ONOO-). Nitric oxide is a small, highly reactive radical-type molecule that easily reacts with electron acceptors and donors. In the presence of oxygen, it forms toxic nitrogen dioxide ($\bullet NO_2$), while in aqueous solutions it is oxidized to nitrite (NO_2-) [22].

Table 1 *The most common reactive forms of oxygen and nitrogen*

Reactive oxygen species (ROS)	Singlet oxygen $^1O_2$
	Superoxide anion $O_2^{\bullet-}$
	Hydroxyl radical HO•
	Hydrogen peroxide H_2O_2
	Alkoxy radical RO•
	Peroxide radical RO^{2-}
Reactive oxygen and nitrogen species (RONS)	Nitric oxide •NO
	Nitrosyl anion NO^-
	Nitrosyl cation NO^+
	Nitrogen dioxide $\bullet NO_2$
	Nitrile cation NO_2^+
	Peroxynitrite $ONOO^-$

Source: *own study*

Reactive forms of oxygen and nitrogen are found in the air and the water [17]. In the body, however, free radicals are formed as a result of metabolic processes taking place in the body's cells or as a result of the action of external factors. The main endogenous source of free radical generation in the cell is the mitochondrial respiratory chain. About 2-5% of electrons transported through the respiratory chain complexes may leak and enter into one-electron non-enzymatic reactions with oxygen. Instead of water, a superoxide radical is formed to which a second electron can attach. In this way, hydrogen peroxide (H_2O_2) is formed, and after attaching the third electron, the hydroxyl radical (HO •) is generated, which is the most reactive oxidant [23, 24].

The external factors generating free radicals include: ultraviolet and ionizing radiation, cigarette smoke and ultrasound. Moreover, the source of ROS and RONS may also be exposure to high oxygen pressure, the action of chemical compounds (pesticides, ozone and nitric oxide contamination), inflammation (infections, burns), or chronic diseases (cancer, diabetes, alcoholism) [25].

ASPECTS OF OXYGEN AND NITROGEN REACTIVE FORMS

Reactive forms of oxygen and nitrogen are known to act in two ways in the body. Under the conditions of homeostasis, they act as signalers or transmitters, which is important in the proper functioning of the cell. They induce cell differentiation and apoptosis, regulate gene expression, influence the regulation of vascular tone and increase capillary permeability. They also take part in the body's defense processes, secretion of hormones and removal of drugs from the body [20, 26]. Nitric oxide, in addition to regulating blood flow, also has other functions: it inhibits leukocyte adhesion,

platelet aggregation and smooth muscle proliferation. Moreover, it is a mediator of nerve cells of the autonomic nervous system and a mediator of synaptic conduction [27, 28]. Research also indicates the participation of nitric oxide (•NO) in the processes of memory formation, learning and neuronal signaling [22]. Nitric oxide also has an antioxidant effect. It reacts with other free radicals taking part, for example, in the lipid peroxidation process [28]. One of the most important tasks performed by free radicals in the body is participation in the aging process. These molecules determine the death or survival of cells. Low concentration of ROS leads to activation of transcription factors and stimulation of cell differentiation processes. Thanks to this, cells gain the ability to adapt to changed conditions. When exposed to high levels of free radicals, the cell is directed to apoptosis. This mechanism allows for the elimination of damaged cells that could potentially pose a threat to the body (eg. leading to the development of a neoplastic disease) [27].

As already known, low concentrations of ROS and RONS perform physiological functions. On the other hand, exceeding certain concentrations of these molecules in cells causes their damage and, as a consequence, contributes to complete destruction [27]. Under proper conditions, the body maintains a balance between the formation of free radicals and their removal. This equilibrium is called the redox potential. Reactive oxygen species are otherwise known as oxidants. When the number of oxidants is increased in relation to antioxidants, the balance towards oxidation is disturbed, which is referred to as oxidative stress [20, 29].

OXIDATIVE STRESS

Oxidative stress is a constant exposure to the harmful effects of free radicals. Their concentration and the speed of cell-damaging reactions depends on the efficiency of defense mechanisms and the amount of antioxidants in the body. In the case of an increase in the rate of free radical generation under the influence of external or internal factors, or the deficiency of factors protecting the body against ROS and RONS, the reactions induced by free radicals are intensified [10, 17].

The damaging effects of reactive oxygen and nitrogen species can virtually affect all biomolecules, including proteins, lipids, carbohydrates and nucleotides. Chemical modifications within these structures, and above all in the DNA structure, lead, among others, to to mutations or cytotoxic effects [30]. One of the most important biological processes related to the activity of ROS is lipid peroxidation. This process primarily affects the residues of polyunsaturated fatty acids that are part of phospholipids, which are the basic component that builds biological membranes. The lipid peroxidation process consists of many steps which may be assisted by the action of enzymes. The end products of this process include alkanes and alkenes, which change the structure and fluidity of cell membranes, which in turn affects changes in cell integrity. Malondialdehyde (MDA) is another end product of lipid peroxidation. It demonstrates mutagenic and carcinogenic effects, and may also affect the regulation of the rate of cell proliferation [31].

Reactions of strong oxidants with proteins cause modifications of non-amino acid components of complex proteins, i.e. amino acid residues and prosthetic groups, fragmentation of the polypeptide chain or formation of protein aggregates. They are also believed to participate in erythrocyte hemolysis or hemoglobin oxidation. As a result of these modifications, the biological functions of proteins are disturbed or their complete inactivation. Most often it occurs as a result of the activity of the hydroxyl radical, although the oxidation of the thiol groups of proteins may also be contributed by the superoxide anion and hydrogen peroxide. Reactions of ROS with proteins cause not only their oxidation, but also the formation of reducing groups in proteins, capable of reducing cytochrome c and metal ions.

These reactions can lead to the formation of amino acid and protein peroxides, as well as damage to bases and nucleic acids [32, 33]. It has been found that modified, oxidized forms of many proteins accumulate in cells and organisms during aging.

Oxidative DNA damage is mainly caused by direct reactions of the hydroxyl radical (OH•) with DNA nucleotides. Superoxide anion (O_2•-) or hydrogen peroxide (H_2O_2) do not damage the components of nucleic acids. The resulting damage leads to a breakage of a single polynucleotide strand. Products resulting from a single strand break can react with other DNA fragments, causing damage to both strands. DNA damage related to the harmful effects of free radicals may also be caused by the formation of volume aggregates with oxidized proteins and lipids. It is estimated that about 10,000 damages a day occurs in human cellular DNA [34].

Closely related to oxidative stress is nitrosation stress, defined as a state induced by nitric oxide (•NO) or related compounds, leading to the nitrosylation of cysteine thiol proteins (S-nitrosylation) and protein metallocofactors. Nitrosylation refers to the addition of a nitroso group (-NO) to the thiol group or redox active center of a metal ion in a protein. While deregulation of nitrosylation is associated with a number of pathophysiological conditions, well-controlled nitrosylation plays an important role in cell signaling and provides a physiological regulatory mechanism. Continuous exposure to high concentrations of NO may have a cytotoxic effect, leading to damage to many organs [22, 35].

Cells subjected to the destructive influence of oxidants do not remain idle in the face of the changes taking place. Many adaptive reactions of cells have been observed in the human body, manifested by an increase in the concentration of antioxidants and an increase in the activity of enzymes protecting against free radicals [26].

SUMMARY

Reactive forms of oxygen and nitrogen perform physiological functions in the body. However, their excessive production or disturbed removal by the body's antioxidant systems leads to oxidative and nitrosative stress. The effects of chronic stress are modifications of proteins, lipids, nucleic acids and sugars. This contributes to the acceleration of the aging process and the occurrence of related diseases.

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CITE / SPOSÓB CYTOWANIA

Zięba A, Marwicka J. Participation of free radicals in the skin aging process. *Aesth Cosmetol Med*. 2020;9(4):417-421.