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Ultraviolet radiation and melanogenesis

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ABSTRACT

Light radiation is a part of the electromagnetic radiation, and it consists of the ultraviolet (UV) radiation, visible light, and infrared radiation. UV radiation energy is absorbed in the form of photons in biomolecules (chromophores) and induces various cellular reactions, out of which photochemical and photosensitizing are the most significant. In contact with the skin UV radiation incites protection mechanisms: the most important are stratum corneum thickening and melanin synthesis (melanogenesis). Basic role of melanin is absorption and scattering of UV rays and neutralization of free radicals. In this review physical characteristics of UV radiation, its biological effects, and relation to melanogenesis and carcinogenesis are discussed.

KEY WORDS: *Ultraviolet Rays; Melanins; Melanocytes; Skin Pigmentation*

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PHYSICAL CHARACTERISTICS OF ULTRAVIOLET RADIATION

Light is a form of radiant energy omnipresent in the environment and the essential precondition of the survival of man and all the life on the Earth. Light (optical) radiation is a part of the electromagnetic (EM) radiation, situated according to its wavelength between soft x-radiation and radiofrequency radiation. It consists of the ultraviolet (UV) radiation (8.3%), visible light (38.9%), and infrared radiation (52.8%) (1). In the contact with skin light radiation induces numerous photobiological processes actually based on the absorption of the light energy quantum.

UV radiation, as a part of the light radiation, is biologically the most active one, since it has the shortest wavelength (100-400 nM) and thus the highest quantum energy (2). UV radiation behaves as a regular EM radiation and consists of an electric and magnetic field. Physical features of this radiation correspond to its dual nature: wavelike and quantal (2,3). In the contact with an organism both of these aspects are significant. Wavelength enables penetration of UV light into the organism, and quantal features of UV rays induce biological changes within the living cell.

Cellular biological changes depend on the multitude of factors, among which the most important are the UV radiation type, its wavelength and radiation dose. UV radiation has the photon energies in the range 3.1-12.4 eV (2,4). UV radiation wavelengths range from 100 to 400 nM. UV rays below 100 nM wavelengths can induce tissue ionization, but in the natural environment UV rays below 180 nM may exist only in the vacuum conditions (5,6). UV radiation induces numerous harmful effects in men and only a few positive ones (D vitamin synthesis, melanogenesis, photochemical reaction in the retina of the eye) (4).

According to the biological effects they induce, UV rays can be classified into UVA rays (400-320 nM wavelengths - black light region), UVB rays (320-280 nM - skin erythema region), and UVC rays (280-100 nM - germicidal region) (4,5). The ozone layer in the atmosphere (12-42 km thick) absorbs UV rays with wavelengths below 290 nM, and 90% of UV rays with wavelengths 315-290 nM. The total irradiation of the surface of the Earth is 20-60 w/m2 out of the total 550 w/m2 at the outer side of the Earth's atmosphere (1,2). Radiation of the Sun at the surface of the Earth contains 6.3% of UVA and 1.5% of UVB rays. The percentage of loss of UV radiation on its way to the surface of the Earth is influenced by air pollution, air humidity, clouds, and so on. UV irradiation is accomplished through direct and diffuse radiation (UV rays reflected, scattered from the ground and air particles). The intensity of radiation is directly associated with the angle at which UV rays reach the Earth surface and with altitude. The intensity is highest at noon. Around 60% of total daily radiation from the Sun is reached in the period from 10 in the morning to 14 hours in the afternoon. The amount of UV radiation increases by 15% per every 1000 meters of altitude (6% for UVB rays). Thick clouds decrease UV radiation by 10%-80%; the reflection from snowy or icy surfaces increases it by 75%, and from sandy or rocky surfaces by 10%-15%. The reduction of the ozone layer by 1% would induce the increase of non-melanomatous skin cancer incidence by 0.6%-4.6% (1).

BIOLOGICAL EFFECTS OF UV RADIATION

UV radiation energy is absorbed in the form of photons in biomolecules (chromophores); these molecules are then brought to an excited state, usually higher than the previous. During the process, the energy of the excited molecule can be transformed into chemical energy, with accompanying photochemical or photosensitizing reactions. The energy may also be transferred to some other molecule (the acceptor), which similarly undergoes certain changes (2).

UV radiation-induced cellular changes can be beneficial or harmful, associated with free radical release. The form of the photochemical cellular reaction depends on the UV radiation wavelength, molecular structure of the chromophore and specific conditions in the environment where the reaction takes place (5). Absorption spectrum of the cellular molecules is very diverse. Melanin has a broad absorption spectrum, ranging through the whole UV spectrum, through the whole visual part of the light spectrum, and through the part of the infrared spectrum (2,3).

Basic chromophores in the skin cells are DNA, proteins, urocanic acid and melanin, chinones, flavins, steroids, and porphyrins (5,7). Nucleic acids are 220 times more sensitive

to UV radiation compared to proteins. Pyrimidine bases in the DNA chain are especially sensitive. Under the action of UV rays duplicate carbon bonds in the DNA chain are destroyed and pyrimidine base dimers are formed which are responsible for various cellular disorders. The most common dimers are thymine and cytosine. Most of the damage induced in this way in the cell can be repaired owing to the repair enzymes (4,5,8).

OPTICAL FEATURES OF THE SKIN

When UV radiation reaches the skin, reflection, scattering and absorption take place. UV ray penetration into the skin increases with wavelength increase. Most of the UVA rays are reflected and a part penetrates into the deep skin layers, all the way to the subcutaneous tissue. UVC rays are for the most part absorbed by the corneocytes and only 1% penetrates through the epidermis to the epidermal-dermal border.

UV ray penetration depth is reversely proportional to the amount of pigment in the skin. UV ray absorption is increased by the content of urocanic acid (small peptides, nucleoproteins) and aromatic acids (tyrosine, tryptophan, phenylalanine). Urocanic acid originates in the keratinocytes from the process of keratinisation and it is present mainly the superficial layer - *stratum corneum*. It is important for skin moisture maintenance (9). UV radiation in contact with the skin acts to stimulate UV radiation protection pathways. The most important mechanisms of skin protection from UV rays are *stratum corneum* thickening and melanin synthesis by melanocytes (10,11).

MELANOCYTE BIOLOGY AND MELANIN SYNTHESIS

Melanocytes are dendritic cells situated among the keratinocytes of the basal epidermal layer and among the hair matrix cells (12). They are attached o the basal membrane by plates similar to hemidesmosomes. One melanocyte provides melanin for 36 surrounding keratinocytes (melanin unit). Per 1 mm^2 of skin there are 1100-1500 melanocytes and that number is almost the same regardless of the skin type (5,13,14). Melanocytes synthesize melanin out of tyrosine (amino-acid), with the coaction of melanocytic tyrosinase enzymes TRP-1 and TRP-2 (tyrosinase related protein), membrane lysosomal proteins LAMP (lysosome associated membrane protein), and other enzymes.

There are three types of melanin:

- Eumelanin, black-brownish colored
- Pheomelanin, yellow-reddish colored
- Neuromelanin, black colored and present in nerve cells

UV radiation exposure is the stimulant for melanin synthesis, or the synthesis is hormonally stimulated in certain endocrine diseases (5,15,16). UV radiation exposure is the stimulant for eumelanin synthesis. Melanin synthesis commences with the entry of tyrosine into the melanocyte through a specialized transport channel (P-locus). Enzyme tyrosinase catalyses tyrosine all the way to dihydroxyphenylalanine (DOPA) and DOPA chinone. DOPA-to-DOPA chinone oxidation co-factor is TRP1. Further cyclisation produces cyclo-DOPA and DOPA-chrom. With TRP coaction dihydrochinindol-2-carboxyl acid (DHICA) is produced, which with further oxidation produces eumelanin pigment (17-20).

Pheomelanin is synthesized in smaller amounts compared to eumelanin. It is the product of nucleophilic oxidation of L-cystein in the presence of DOPA chinone. L-cystein is an important regulator of melanogenesis. If its level is decreased in the cells, the activity of tyrosinase and eumelanogenesis are incited (21,22). Chemically, melanin is a bipolymer composed of red melanin (which contains sulphur and is soluble at pH 7,2) and black melanin (water-insoluble). The relationship of red and black melanin is genetically determined (4). In the presence of oxygen and under the action of UV rays, black melanin can produce free radicals, which can have harmful effects on melanocytes and fibroblasts in the dermis. Basic role of melanin is absorption and partly scattering of UV rays (in a lesser degree of other light spectrum rays as well). Moreover, melanin neutralizes free radicals since melanin itself is a kind of free radical (4,21).

One of the main factors of proliferation, melanogenesis, adhesion and migration of melanoblasts and melanocytes is stem-cell cytokine factor (SCF), which attaches to the C-kit receptor (CD 117) (9,23,24). Natural melanocytic mitogen and important angiogenesis stimulator and stimulator of fibroblast synthetic activity is basic fibroblast growth factor (BFGF). The most significant source of BFGF in the epidermis is keratinocytes, after being stimulated by UV rays. In addition, to proliferation, differentiation and migration of melanocytes significantly contribute also chemokines (IL-8) and transforming growth factor (TGF), cytokines (endothelin-1 and INF-B). Among the hormones, important is alpha-melanostimulating hormone (aMSH), synthesized and released by UV-stimulated keratinocytes. Melanocortic receptor MC1 (out of the total of six) for this hormone was identified on melanocytes (23,25).

Most of melanine in physiologically normal situation is of mixed type. Melanin produced is transferred from the endoplasmatic reticulum to melanosomes. With the new amounts of melanine transferred, melanosomes are transformed into melanin granules. Keratinocytes phagocytize melanosomes by direct fusion of keratinocyte membranes and melanocytic dendritic projections (16,21).

In addition of melanogenesis stimulation, UV radiation leads to the increase of differentiated melanocytes in the skin by the induction of tyrosinase and TRP1 activity increase without change in their concentration (22,26). Moreover, mitotic melanocyte activity is accelerated. Normal mitotic activity of skin melanocytes not exposed to light radiation is around 30 times less than keratinocyte mitotic activity.

UV RADIATION AND CARCINOGENESIS

In addition to its stimulatory effects on melanogenesis, UV radiation, especially UVB rays, damage melanocytes prolonging the G1 phase of their cellular cycle. Thus the time required for damaged DNA reparation is prolonged too and carcinogenic effect of UV radiation is reduced (27,28). Melanocytes at their disposal have also other numerous mechanisms (such as LAMP1) to neutralize free radicals and other harmful compounds produced in response to UV radiation. Protective effects on irradiated melanocytes also have keratinocytes and fibroblasts (29). The key effect in UV damage protection is the blockade of the cellular cycle while DNA damage is being repaired (30). However, aMSH, in addition to its synergistic effect on melanogenesis, stimulates proliferation of irradiated melanocytes, reducing thus the time required for damaged DNA repair (30,31). UVB rays stimulate the release of aMSH and ACTH from keratinocytes and MC1 receptors for these hormones. These hormones have immunosuppressive action and it is possible that aMSH helps the accumulation of mutations in irradiated melanocytes and their further malignant transformation (32). The invasive behavior of melanocytes is partly the consequence of altered expression and affinity of integrin-receptors for extracellular matrix (32,33).

UV RADIATION AND SKIN TANNING

The immediate effect of skin exposure to UV radiation is the stimulation of melanogenesis, i.e. skin pigmentation (tanning). Skin pigmentation occurs in two phases (4,14):

1. Immediate tanning/pigmentation of the skin,
2. Delayed tanning/pigmentation of the skin

Immediate skin tanning occurs during the UV radiation and it is the consequence of the pigment already existent in melanophages. It occurs only in individuals that constitutionally have at least medium dark complexion. It can be stimulated by UVA and UVB radiation and also by visible light. It is the result of oxidation and redistribution of the existent melanosomes towards peripheral melanocyte dendrites.

The effects of this type of skin pigmentation disappear quickly after the UV exposure; if delayed melanogenesis does not take place the effect may disappear completely (1,13). This type of pigmentation does not significantly contribute to the skin protection from erythema and sunburns. Delayed skin pigmentation is the consequence of increased activity of melanocyte stimulating hormone (MSH) receptor on the surface of melanocytes which stimulate melanogenesis, i.e., the increase of number, size and, amount of pigment in the melanin granules. It lasts longer and plays a significant role in the skin protection from UV rays. Delayed skin pigmentation is induced by UVA and UVB rays. UVA stimulated delayed skin pigmentation is the natural extension of immediate pigmentation and it requires smaller radiation doses. UVA1 rays induce increased melanin levels in the basal layer, and UVA2 rays in all other epidermal layers. UVB stimulated delayed pigmentation occurs three days after radiation exposure and requires larger radiation doses. These rays induce increased activity of melanocytic tyrosinase, branching of the melanocyte dendritic portions, increase of the number and size of melanosomes and melanin granules in the cells and accelerate the transfer of melanin into keratinocytes. In case of uneven resorption, freckled tanning occurs (15,34).

This delayed pigmentation has less importance in skin protection from acute erythema since it occurs later and pigment is predominantly distributed along the basal layer. In that regard, more marked effect has UVB radiation due to diffuse pigment distribution in the epidermis and, moreover, *stratum corneum* is thickened (4,16). Certain antioxidants antagonize skin pigmentation process - they disturb the course of melanogenesis and melanin production by alpha-chinone reduction. Moreover, they transform the black into light brown melanin. Similar effects are expressed by some substances such as magnesium-ascorbil-2-phosphate (VC-PMG). Due to these characteristics, these and similar substances are added to the preparations the purpose of which is to reduce pigmentation and remove sun freckles from the skin (35,36).

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