,,VICTOR BABEŞ" UNIVERSITY OF MEDICINE AND PHARMACY TIMIŞOARA
FACULTY OF MEDICINE
Department XIV -MICROBIOLOGY

PhD STUDENT
BOTA SANDA IZABEL

SUMMARY OF PhD THESIS

CONTRIBUTIONS REGARDING THE PREVENTION OF PHOTOAGING EFFECT BY GROWTH FACTORS AND ANTIOXIDANTS THERAPIES

Scientific Coordinator
PROF. UNIV. DR. CAIUS SOLOVAN

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CONTENT

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INTRODUCTION

Across the world, throughout time humans have been obsessively seeking for eternal youth. This fascination of defeating death and maintaining the youthful appearance intrigued many cultures for centuries. Cleopatra was bathing in milk. In the 4th century BC, Alexander the Great found a river with healing properties. Ponce de Leon, the first governor of Porto Rico was seeking for the Fountain of Youth in the 14th century AD. Elizabeth Bathory, a Hungarian Countess from the 16th century drenched herself in virgins' blood. Not least, the former Prime Minister of India, Morarji Desai was drinking regularly his own urine in order to maintain eternal youth. Regardless of these desperate search and ways to applying them there are all empty promises of youth and fortunately will have to rely on scientific research to bring us closer in finding the fountain of youth.

The quest for eternal youth is far from over. Today's society has not forgot about the quest for immortality. There is an increase demand for products that claim to postpone the aging process. Numerous studies in the past decades have shown an increase alertness about the process, management, treatment and prevention of aging and photoaging. This rapidly expanding journey for youth is probably due to the increase of the human average lifespan. According to World Health Organization (WHO) the global average life expectancy has increased by 5.5 years in the last 15 years. With each research we hope that we are getting closer in finding a treatment for this *new age* disease. Consumers desire skin care products, either locally applied or taken systemically, that can change the destructive appearance induced by UV radiation and give them the look that matches their mental age and not chronological age.

Aging of skin is associated with various well known changes in its structure and function from thinning of epidermis to decrease barrier function all the way to deterioration of epidermal immune response. Many cumulative damaging changes control in various forms the aging process from radiation to dietary and even genetic and hormonal intervention. Skin aging is influenced by a combination of extrinsic and intrinsic factors that leads to destruction of the structure and function of skin. Various skin aging theories that trigger natural aging mechanisms and contribute to age related alterations have emerged in recent years. These include oxidative stress theory of free radicals, thelomere shortening and cellular senescence, mitochondrial dysfunction, and ultraviolet (UV) radiation.

The importance of finding clinical solutions in age related deficiency of skin function caused by intrinsic and extrinsic factors is great. The demand for anti-aging products indicates an infatuation with appearing younger and living longer. While esthetic industry offers "dreams in a jar", and promote a multitude of anti-aging products, but there is no scientific confirmation that any of these means actually reverse or delay ageing. Most products on the market only target the effect of the aging process, rather than preventing ageing itself, but consumers are not aware of these differences, thus it is our responsibility to educate the end consumers to realize the limitation of these antiaging products.

The aim of this PhD thesis is to:

-present the relationship between photoaging and and photoprotective mechanisms

-establish new strategies to selectively target the skin's capacity to withstand optimal regeneration

-analyze the potential capability of antioxidants in protecting the skin against the harmful, negative effects of the UV radiation
-analyze the potential capability of platelet rich plasma and its cytokines in protecting the skin against the harmful negative effects of the UV radiation
-examine diverse growth factors (TNF-α, TGFβ 1, and FGF2) levels in PRP concentrates of diverse skin phototype in stimulating cellular regeneration
-to significantly improve our understanding of skin's abundant regenerative capacity based on skin phototype.

1.1 SKIN AGING

Aging of the skin is a complex process that involves two individual aspects: extrinsic aging and intrinsic aging. Various factors that influence skin's aging are genetics, environmental exposure, metabolic processes and hormonal changes. Chronological aging describes our genetic programs and all the damages that take place over an entire life. Intrinsic aging is played by hormonal changes and extrinsic aging includes environmental exposures that damages skin structures. Even though all of these factors act together on alteration of dermal and epidermal structures, function and expressiveness, UV radiation, without a doubt, is the primary major liable determinant of skin aging. The rate of aging in chronological aging can be greatly influenced by personal and environmental factors, particularly UV exposure

With ageing, structural and functional progressive degenerative changes occur. Through this process it affects the skin in numerous ways. Skin becomes thinner with a limited capability of withstanding mechanical strength There are two defined types of skin aging. The one caused by the genetic expression, hormonal changes and the passing of time, which is called intrinsic or chronological aging. The other type of the aging process is due to environmental factors and is known as extrinsic aging.

1.2 THEORIES OF AGING SKIN

1.2.1 Free radical theory

The free radical or the oxidative stress theory, originally described by Denham Harman in the 1950's states that oxidative cellular damage accumulated throughout the years is the main contributor in the skin aging cascade. The cellular damage is associated with the free radicals of reactive oxygen species (ROS) formation induced by DNA damage, inflammation, immune suppression and intracellular lipid peroxidation. Accumulated changes in molecular structure, especially proteins, produce the basis in cellular aging.

ROS formation can activate cellular senescence prematurely which contributes to not only skin aging but to other age-associated disorders. This cellular senescence is induced as a protective response of high levels of reactive oxygen species. Skin's immune system has complex defense mechanism in order to deal with destructive environmental factors, but chronic, repetitive, and excessive exposure to these factors can overwhelm this system generating oxidative stress and cellular damage

1.2.2 Telomere shorting and cellular senescence

Telomeres, repetitive DNA sequences present at the ending point of chromosomes are important in protecting and regulating the chromosomal activities. When replication of DNA occurs, during cellular division, a RNA primer helps it to get started. Since this primer doesn't attach to the very end of the DNA strand, the new copy of the template DNA has a missing end

section of DNA strand. Therefore, as cellular division continues, the end section of DNA strand gets shorter each time duplication takes place. The shortening of telomere DNA inhibits cellular division. When telomere shortening reaches a critical level, cellular division and DNA synthesis stops causing cellular senescence, thus contributing to changes in aging.DNA. Cellular senescence is the irreversible growth arrest of individual mitotic cells. The main mechanism of aging of mitotic tissue is thought to be due to the gradual accumulation of senescent cells.

1.2.3 Mitochondria theory

Mitochondrial DNA accumulates mutations with age, while these lead to physiological decline in aging. Simultaneously oxidative stress also plays an important role in the mitochondrial destruction since it triggers DNA mutation provoked by reactive oxygen species. Accumulation of ROS exposure in time alters MMPs and induces mitochondrial DNA mutation through encoding errors. A vicious cycle starts when dysfunctional mitochondria DNA together with reactive oxygen species leads to an excessive production of ROS. Thus it is safe to claim that cumulative DNA damage is a well-documented consequence of repeated ultraviolet radiation. A dysfunctional mitochondria is the aging trigger not only on skin but in other age-associated disorders.

I.3 PHOTO-AGING

Photo-aging characterizes the changes in histologic, clinical and morphologic function of skin that's habitually exposed to UV radiations. It is a part of chronic sun destruction that's superimposed on intrinsic or chronological aging. Aging due to sun exposure accounts for greatest undesired changes on skin appearance. Contrary to sun tanning, which reveals an increase melanin production within hours or days, after enough sun light exposure, photoaging occurs gradually over years to chronic intense exposure of UV radiation. The damaging effects of UV on the skin are both direct and indirect. The extent of damage depends on the amount of UV radiation exposure Ultraviolet rays can damage cellular and extracellular components in the skin.

UV radiation activates mitogen-activated protein kinase (MAPK) pathway that stimulates activation of activator protein 1 (AP-1). Activator protein 1 regulates metalloproteinase gene expression which has a significant effect on the dermal extracellular matrix. The MMP genes induced by ROS boosts the degradation of collagen and elastic tissue formation by deactivation the naturally occurring tissue inhibitors of MMPs.

During the UV radiation process, ROS formation activates cellular surface receptors that triggers receptor-initiated signaling, protein oxidation, mitochondrial damage and telomere DNA damage response. Reactive oxygen species trigger the release of proinflammatory cytokines and growth factors. DNA byproducts induced by UV, lead to cellular mutation that ultimately result in photoaging of the skin.

II.2 DEGENERATIVE CHANGES IN PHOTO-AGING

In photo-aged skin, the degree and depth of damage is dependent on the amount of the ultraviolet radiation exposure. The most remarkable histological change of the skin is the pronounced flattening of the dermo-eidermal junction, occurring as a result of dermal papillae loss, and the great accumulation of elastotic material, which is the result of a capricious production of fibrilin and elastin with a degradation of elastic fibers, in the upper and mid dermis.

Degradation of these elastic fibers arises from dermal fibroblasts and infiltration of inflammatory neutrophils in response UV light radiation. There is also a significant increase in the space between fiber bundles due to the thinning of fibers and an increase in fiber proteins chaos.

III.3 DEFENSIVE AND PREVENTIVE MECHANISMS OF PHOTO-AGING

The major cause of skin aging is a lifetime of accumulation damage from oxidative stress, DNA damage and immunosuppression The desired therapeutic anti-aging effect of the skin is a continuous process that combines various methods of biorevitalization, rejuvenation, augmentation and restauration of individual skin layers. Numerous strategies in managing the reversal of dermal and epidermal signs of skin aging have been studied in the last years. One major way of overcoming this process is prevention.

3.1 Sun protection

Proper photoprotection is an integral part of management of photoaging. This can be obtained by minimizing sunlight exposure during the peak hours of 10am to 4pm, by seeking shade, using protective clothing and eyewear. Topically applied sunscreen lotions is an integral part of the strategy of photoprotection. Studies have shown that the regular use of high SPF broad-spectrum sunscreens protects against photodamage at the cellular level. The use of sunscreens (SPF) can significantly reduce the amount of ultraviolet rays that reaches the skin, thus reducing the harmful effects of solar UV radiation on skin

3.2 Antioxidants

When talking about the need to protect the skin from sun-induced damage, everybody thinks immediately about sunscreens, which are the gold standard for photoprotection, However, numerous studies demonstrate mechanism by which antioxidants play an important role in prevention of photo-aging by counteracting the deleterious effects of oxidative stress. Topical antioxidants ointments appear as an alternative to sunscreens in preventing photoaging and other types of skin damage induced by acute or long-tern sun exposure.

Low molecular weight antioxidants were shown to be efficient in preventing oxidative stress in various models. Although antioxidants can be supplied to the skin through diet and oral supplementation, physiological processes related to absorption, solubility, transport and metabolism limit the amount of the active form that can be delivered to the skin. Therefore, direct application has the advantage of targeting the antioxidants to the area where they are needed, provided they are applied in a formulation that is able to prevent their deactivation by undesirable oxidation and to facilitate their absorption into the skin.

SPECIAL PART

I. Statistical analisis of sun exposure and photoprotection

The main intent of the study was to examine the characteristic tanning manner and UV protection pattern in medical students, which have a supposedly higher educational level. Their approach, awareness and knowledge of UV radiation and effectiveness of sunscreens protection was also tested. At the same time, analysis between students from rural area versus students from urban regions on their understanding about the matter was observed.

A lacking knowledge on sun exposure and protection among university students from Timisoara is notice. Even though sunscreen was the preferred protective method of protection, only participants with lighter skin color and frequent sunburns showed an increase use of sunscreen protection, while females, with a photo-type IV and V are more inclined to engage in intentional artificially tanning to stay in the sun for a longer time and not to use sunscreen.
Thus people in this area need to be more educated on risk factors and sun protection methods. Systemized educational campaigns should be promoted in order to emphasize the importance of sun protective ways in order to change and improve people's quality and quantity of life, especially for those who are at a higher risk, women and lighter skin photo-type.

In the end, based on the evidence, one can assume that common sense is the most important tool when it comes to sun exposure and sun protection. It is certainly clear the impact of regular sunscreen use and other sun protective measures use on cutaneous cancers and skin aging

II. Analyzing the antioxidants efficacy

The purpose of this study was to assess the involvement of the combination between superoxidismutase, catalase and selenium on photo-aging. Thirty female patients with skin photo-types II, III and IV (Fitzpatrick) were included in this study. They were divided into three groups: participants in the first group receive a systemic administration of the enzymatic-antioxidant combination, the second group also received a systemic administration but they had to use a various tanning system during this study, and the third group received only a topical administration on sun exposed areas of the superoxidismutase, catalase and selenium combination. Participants' antioxidant capacity of the blood was evaluated as well as clinical and histological changes that occurred during this study

Although the intrinsic aging is a subtle disintegrating process with an anticipated end result, photo-aging it's a blunt factor of the surroundings, is neither assumed nor unavoidable. This destructive mechanism is a cumulative process that's influenced by the degree of UV exposure

and the skin type which we need, because we somehow can to a degree, prevent it. Studies have shown that systemic intake of antioxidants can provide photo-protection and delay or even prevent, to an extent, photo-aging. In our study we observed small but worthy significant changes that we cannot overlook. Over a 6 month trial period we demonstrated clear benefits of using enzymatic antioxidants systemically and topically. Clinical improvement was seen in the treated groups, smoother skin and a better tolerance to sun exposure.
Due to the fact that there's an expected extended lifespan, photoaging will be even a greater concern in the future. It's essential to inform about the effects of ultraviolet radiation and attain a balance among its beneficial and harmful effects

III. In vitro evaluation of the photoprotective role of oral antioxidants on human fibroblasts

The purpose of this study was to analyze the extent to which the administration of PRP(obtained from healthy volunteers and volunteers who previously consumed systemic antioxidant), acts on fibroblasts and exracellular matrix components, in the case of UVB exposure. To do this we performed two expermiental batches, using human fibroblasts in vitro culture. Thus, the evaluation of the therapeutic potential of PRP (PRP-batch) compared whti the PRP obtained form people who were given exogenously supplimantation with antioxidants (PRP-O) was obsered. Cells were treated with different concentration of PRP and PRP-O, subseqeuntly exposed to UV. In parallel, control batches not exposed to UV were used. At different time periods (24h, 48h, and 72h, respectively). We analized the effect of PRP on cellular phenotype, cellular proliferation as well as NFkB transcription factor and type 1 collagen synthesis, matrix metalloproteinases MMP1 and MMP3. We also compared the antioxidant capacity of the two types of PRP.

In our study we demonstrated the following:

- For each comparison regarding the 4 studied concentrations, significant statistical differences were observed ($p < .05$). For each concentration, the antioxidant activity (DPPH) was higher for the experimental group. Also, with each increase in concentration C1,C2,C3,C4, the DPPH becomes higher.
- All groups tested, except for the C1 at 24hr, between experimental group and PRP vs PRP-O and C3 at 24hr, showed significant result, meaning that overall there are significant differences in proliferative activity between groups, experimental (PRP) and control (PRP-O) and control for each of the 3 measurments of time and for each concentration studied.
- With the exception of C1 concentration, most analyzes showed insignificant differences between PRP vb PRP-o. Although significat differences ere found in some concentrations and at same hours. These are isolated cases and overall no significant effect was identified. In most analyzes, differences appeard between PRP-O and the control groups
- Except for C1 concentration at 24 hr, the analysis doesn not indicate significant differences between the PRP group and PRP-O. Between PRP-O and control 1, except C4 all the analysys identified significant differences.

- The values of metalloproteinases, MMP1 and MMP3, were increased in the cellular culture medium exposed to UVB, but not incubated with PRP or PRP-O (M2). In the experimental groups, the values of these markers were significantly lower compared to M2. At the same time, we observed that MMP1 and MMP3 had similar values to control M1, not exposed to UVB, which suggests the important role of growth factors contained in PRP. On the extracellular matrix components. The antioxidants administered orally did not have a significant influence on the expressino of the metalloproteinases studied.

IV. Bioactivation of growthfactors to determine concentration of TNFα, TGF and PDGF in various skin phototypes

The aim of this study was to detect and quantify the amount of diverse growth factors and cytokines in PRP concentrates in diverse skin photo-type of healthy young individuals using enzyme-linked immunosorbent assay (ELISA). The platelet rich plasma was prepared by using a double spin method and then activated with sodium citrate that stimulates platelet degranulation and the release of growth factors.

In our study we did not find a significant correlation between the number of white blood cells, platelets and the level of cytokines studied. Also, concentrations of TNFα, TGFβ and FGF2 were relatively the same for all three skin photo-types studied. The levels of TGFβ and FGF2 are determined by the acquiring procedure and do not depend on the number of platelets. Some studies reported values that could be measured in ng/ml (21), however in our study the values obtained, regardless of skin type, were much lower. Similar results were also reported by the study by Pochini et al. Values obtained for FGF2 were similar to reports from other similar studies, being undetectable or very low. (21

We demonstrated that activated factors in PRP product is not dependent on skin phototype at levels similar to other studies, suggesting that other factors in time contribute to the imbalance of those cytokines that reflect in aging of the skin or even franc pathologic conditions