ISSN 2079-0570, Advances in Gerontology, 2022, Vol. 12, No. 2, pp. 143–148. © Pleiades Publishing, Ltd., 2022.

AEDG Peptide Prevents Oxidative Stress in the Model of Induced Aging of Skin Fibroblasts

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Received September 9, 2021; revised September 25, 2021; accepted September 29, 2021

Abstract—Ultraviolet (UV) radiation is one of the main environmental factors leading to oxidative stress and accelerated skin aging. AEDG peptide, a regulator of pineal gland functions, has demonstrated geroprotective and antioxidant effects in in vivo and in vitro studies. The aim of this research was to evaluate the effect of AEDG peptide on the expression of genes encoding enzymes of the antioxidant system (NQO1, SOD1, CATALASE, and TRXR) in human skin fibroblasts in a model of accelerated aging induced by UV radiation. The expression of *SOD1* and *TXNRD1* genes during UV-induced aging of dermal fibroblasts increases by 2 and 1.7 times, respectively. This can be considered as a defense mechanism against oxidative stress caused by UV radiation. Photoaging does not influence the expression of the *NQO1* and *CATALASE* genes in the culture of skin fibroblasts. AEDG peptide promotes the expression of the *SOD-1*, *NQO1*, and *CATALASE* genes in dermal fibroblasts exposed to UV radiation by 2.7, 2.6, and 3.2 times, respectively. AEDG peptide can stimulate the expression of these genes via the Keap1/Nrf2 signaling pathway. AEDG peptide might be potentially effective in preventing the accelerated aging of dermal fibroblasts.

Keywords: AEDG peptide, photoaging, oxidative stress, gene expression, dermal fibroblasts

DOI: 10.1134/S2079057022020096

INTRODUCTION

Skin is one of the organs most prone to accelerated aging under exposure to adverse environmental factors. Barrier and immunological skin functions are essential for maintaining organism homeostasis. Therefore, an important task in gerontology and dermatology is to investigate the molecular and cellular mechanisms of accelerated skin aging and search for effective and safe methods of geroprotection [1, 8]. Dermal fibroblasts are responsible for the synthesis and remodeling of extracellular matrix elements, providing the integrity of skin and its barrier function.

During ontogenesis, human skin undergoes morphological, structural, and biochemical changes [6]. Open skin areas are more exposed to exogenous factors (meteorological, ultraviolet (UV) radiation), resulting in hyperkeratosis, telangiectasias, and dyspigmentation, which can provoke the development of neoplasms and involutive skin changes (photoaging). The main signs of photoaging include actinic keratosis, solar lentigo, elastosis, hyperpigmentation, melisma, wrinkling, decreased elasticity, and soft tissue loss.

One of the reasons for accelerated skin aging is long-term exposure to UV radiation. UV radiation induces the formation of ROS, DNA damage, and disturbed cellular homeostasis, followed by the activation of an inflammatory cascade, development of immune suppression, and pathological remodeling of the extracellular matrix. DNA damage by UV radiation plays an important role in the induction of photocarcinogenesis and photoaging [7]. Changes originating from UVB radiation mainly affect the epidermis and the upper dermis layer [28]. The process of skin fibroblast aging induced by UV radiation is associated with increased ROS generation and proteasome inhibition with further activation of autophagy. Inactivation of the proteasome system in dermal fibroblasts under UV radiation is caused by the generation of singlet oxygen, protein oxidation, and activation of transcription factors. As a result, a senescence-associated secretory phenotype (SASP) of skin fibroblast and autophagy induction is developed [2].

ROS provoke various dermal diseases, particularly dermatitis of different etiology, vitiligo, psoriasis, acne, neoplasms, and accelerated aging [19]. In the physiological conditions, skin cells produce antioxidant enzymes to protect from ROS, e.g., heme oxygenase 1 (HO-1), superoxide dismutase (SOD), or

catalase [3]. The synthesis of substances inactivating ROS is regulated by nuclear E2-related factor 2 (Nrf2) connecting with antioxidant response elements of gene promoters. ROS can damage DNA, lipids, and proteins, and modify the regulation of gene transcription in skin fibroblasts. In particular, ROS-induced fibroblast apoptosis affects the production of collagen, elastin, and hyaluronic acid, leading to wrinkling, low turgor and skin elasticity, and impaired barrier function [11, 22].

Certain short peptides show antioxidant and geroprotecive properties [18, 24]. These peptides include AEDG, the regulator of pineal functions. AEDG peptide facilitates increase in the lifespan of various organism species. On the molecular-cellular level, the geroprotective effect of this peptide is expressed in the increase of telomere length in fibroblasts and lymphocytes of humans and elevation of the proportion of transcribed euchromatin [12, 13, 15]. AEDG peptide increases the level of antioxidant enzymes (SOD, glutathione peroxidase, and glutathione-S-transferase) in the blood, liver, and brain of rats [18]. The antioxidant properties of AEDG peptide are more prominent in old rats than in young ones [9]. Furthermore, AEDG peptide can exert an indirect antioxidant effect by intensifying the synthesis of pineal melatonin, which declines with age [10].

The aim of this study was to evaluate the influence of AEDG peptide on the expression of genes encoding antioxidant enzymes (*NQO1*, *SOD-1*, *CATALASE*, and *TRXR*) in skin fibroblasts of humans on the model of UV radiation-induced accelerated aging.

MATERIALS AND METHODS

The study was conducted on skin fibroblasts of a 45-year-old woman excised from the periauricular face region. Samples were obtained during circular face-lift surgery. After sampling, the skin was treated in sterile conditions with dispase II solution (Gibco, United States) at the concentration of 2.4 U/mL for 18 h at 4°C. Afterwards, the epidermis was mechanically separated from the dermis. To derive a cellular suspension, the dermis was dispersed and placed into collagenase type I solution (Gibco, United States) in DMEM medium (Gibco, United States). The culture medium consisted of DMEM medium, 10% fetal bovine serum (Gibco, United States), 1% L-glutamine (Biolot, Russia), 1.5% HEPES buffer, and penicillin-streptomycin solution (Biolot, Russia). After five days the primary culture formed a monolayer and was then subcultured at a 1 : 3 split ratio. Trypsin-versene solution (Gibco, United States) was used to remove cells from the scaffolding. The cell concentration was 50000 cells per 1 mL of medium for passage zero. The cells were grown until passage three. When 80% of the monolayer was reached, the cells were divided into six groups: group 1—control (intact cells); group 2—control + control GGGG peptide, 400 ng/mL; group 3—control + AEDG peptide, 400 ng/mL; group 4—radiation; group 5—radiation + control GGGG peptide, 400 ng/mL; group 6—radiation + AEDG peptide. GGGG peptide was chosen as the control since the biological activity of short peptides is known to depend on the amino acid composition and their sequence in the peptide chain. The cells were cultured with peptides for 24 h. Cells of groups 4–6 were exposed to UV radiation of a Bio-Link Crosslinker BLX 312/365 device (Vilber Lourmat, Germany). The total radiation dose was 20 J/cm^2 for UVA and 200 mJ/cm^2 for UVB [5]. The duration of radiation was 24 h. At such dose of radiation, the cell viability was 70–74%, referring to the results of toluidine blue staining and MTS test. The peptides were added to dermal fibroblasts exposed to UV radiation. The control cell cultures (groups 1–3) were grown under the same conditions except for the exposure to radiation.

Total RNA was extracted from the skin fibroblasts using fixation with RNeasy Mini Kit for RNA stabilization (Qiagen LLC, United States). RNA extraction was performed with RNeasy MiniKit (Qiagen LLC, United States), according to the manufacturer protocol. The first cDNA strand was synthesized with Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific Inc., United States), using 100 ng of RNA per 20 μL of reaction mix. The resulting cDNA was used directly as a matrix for quantitative PCR at the rate of 1 μL per 24 μL of the reaction mix. PCR was conducted with the help of the QuantiFast SYBR Green PCR Kit (Qiagen LLC, United States). Oligonucleotide primers were designed with the NCBI Primer-Blast online service. The primer pairs were selected so that one of the primers matched with two adjacent exons. The expression of the genes encoding antioxidant proteins (*SOD-1*, *NQO1*, *Catalase*, and *TXNRD1*) were identified with real-time PCR. Quantitative PCR was performed in the presence of SYBR Green I fluorescent intercalating dye using the QuantiFast SYBR Green PCR Kit (Qiagen LLC, United States) and CFX96 Real-Time PCR Detection System (BioRad Laboratories, United States) thermal cycler. Statistical analysis of the results was conducted in the automated mode of CFX Manager Software. GAPDH mRNA was used as an internal standard. Its concentration was taken as 1 in all samples.

SOD-1, *NQO1*, *Catalase*, and *TXNRD1* genes were chosen for the analysis as the proteins they encode play an important role in the antioxidant defense of skin fibroblasts from photoaging. The NQO1 enzyme (NAD(P)H—quinone oxidoreductase 1) resides in cytosol, catalyzes two-electron reduction of quinone compounds, and prevents the production of semiquinone free radicals and ROS, thus protecting the cell from oxidative stress. Hyperexpression of the *NQO1* gene in different cell types can be observed under the action of oxidants and heavy metals [4]. Superoxide

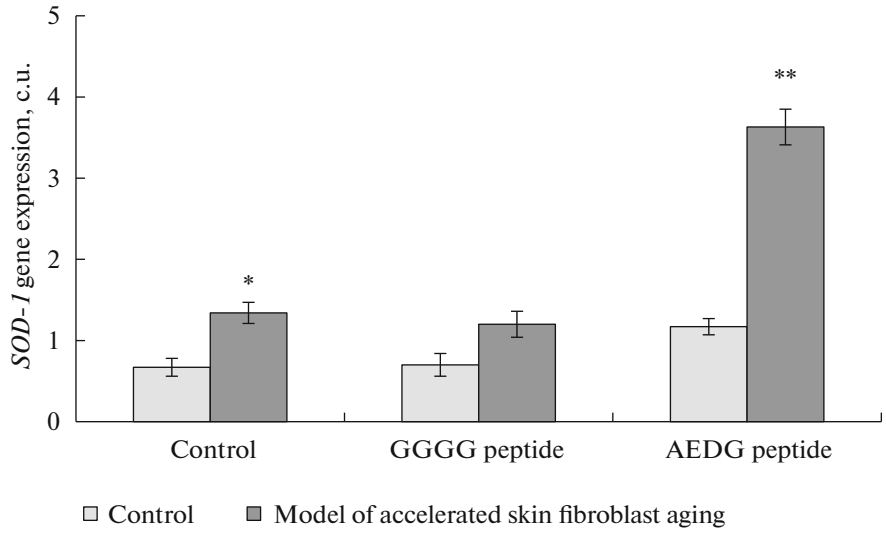

Fig. 1. The influence of AEDG peptide on *SOD-1* gene expression in human skin fibroblasts in the control and model of accelerated aging induced by UV radiation. Here and Figs. 2–4: * $p < 0.05$ compared to the control group; ** $p < 0.05$ in the model of accelerated aging of skin fibroblasts compared to the control group.

dismutase 1 (*SOD-1*) is an antioxidant enzyme that protects intracellular space from superoxide anions, catalyzing their conversion into molecular oxygen and hydrogen peroxide. Expression of the gene encoding the SOD-1 enzyme of the antioxidant system decreases with aging in dermal fibroblasts of humans. Intensification of *SOD-1* expression in human skin fibroblasts is one of the response mechanisms to the action of oxidative stress [20]. SOD-deficient mice demonstrated dermal thinning, defective migration and proliferation of fibroblasts, and accelerated skin aging [17]. It was found that age-related slowing of wound healing is associated with suppressed *SOD-1* expression in dermal fibroblasts, which can lead to the elevation of ROS level in cells and DNA damage [26]. Therefore, the induction of SOD-1 synthesis is considered as one of the ways to prevent accelerated skin aging. Catalase is an enzyme belonging to the oxidoreductase class and a member of the antioxidant system of cells; it performs an antiperoxidative function. This enzyme catalyzes the degradation of hydrogen peroxide into water and molecular oxygen produced during biological oxidation. Age-related increased synthesis of catalase and *SOD-1* has a protective role, preventing from DNA damage in skin fibroblasts under the action of oxidative stress. Compensatory elevation of catalase production in dermal fibroblasts was detected in accelerated skin aging under UV exposure [25]. Thioredoxin reductases (TR, TrxR) are flavoprotein enzymes connecting the NADPH domain with the site containing a redox-active disulfide bond. TrxR1 defends skin fibroblasts from the oxidative stress provoked by hydrogen peroxide [27].

Statistical analysis of the results included the calculation of mean values, standard deviation, and confidence interval for each sampling, conducted using the Statistica 6.0 software. The type of distribution was determined with the Shapiro–Wilk test. The statistical homogeneity of several samplings was assessed with the Kruskal–Wallis test. Pairwise intergroup comparison was conducted with the Mann–Whitney U test for multiple comparisons. The critical significance level of the null hypothesis on the absence of differences was taken as 0.05.

RESULTS AND DISCUSSION

The action of UV radiation on skin fibroblasts provoked a twofold increase in *SOD-1* gene expression compared to intact cells (Fig. 1). The addition of AEDG peptide to cultures of dermal fibroblasts in the control group and during induced aging was associated with the enhancement of *SOD-1* gene expression by 1.7 and 2.7 times, respectively. It should be emphasized that the influence of AEDG peptide on *SOD-1* gene expression was more prominent in the model of accelerated cell aging than in the intact cultures. GGGG peptide did not affect *SOD-1* gene expression in skin fibroblasts in normal conditions and under the action of UV radiation (Fig. 1). Induced aging did not result in statistical changes of *NQO1* gene expression compared to the control. AEDG peptide induced increased *NQO1* gene expression in the control fibroblast cultures by 1.8 times and in UV-exposed cells by 2.6 times. The control GGGG peptide did not affect the expression of the studied gene in the control dermal fibroblasts and those with accelerated aging (Fig. 2).

Expressions of the *CATALASE* gene in intact skin fibroblasts and models of accelerated aging did not statistically differ. AEDG peptide increased the

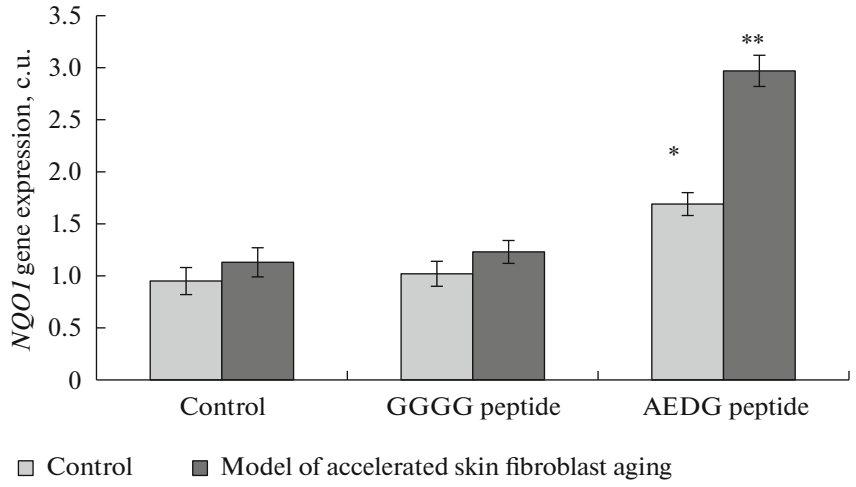

Fig. 2. The influence of AEDG peptide on *NQO1* gene expression in human skin fibroblasts in the control and model of accelerated aging induced by UV radiation.

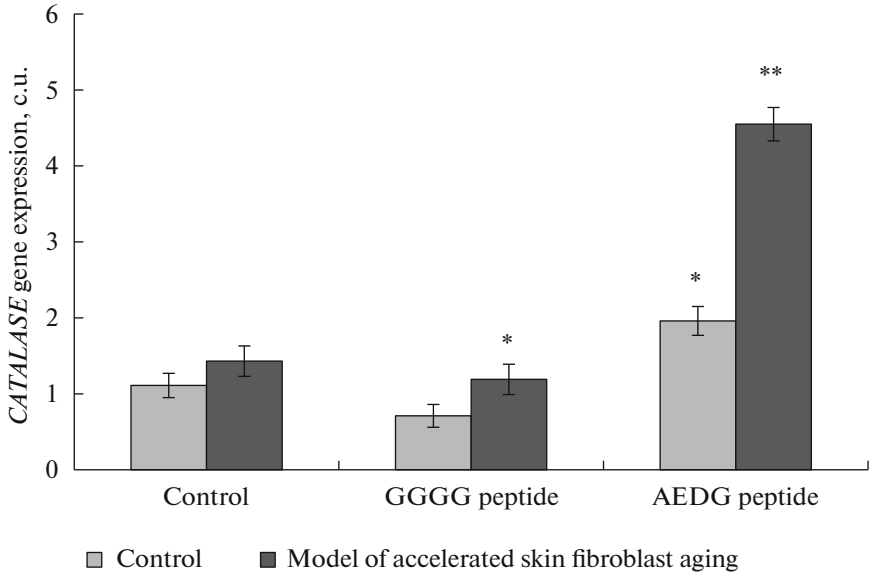

Fig. 3. The influence of AEDG peptide on *CATALASE* gene expression in human skin fibroblasts in the control and model of accelerated aging induced by UV radiation.

expression of the *CATALSE* gene in the control cultures and UV-exposed dermal fibroblasts by 1.7 times and 3.2 times, respectively. The control GGGG peptide did not influence the *CATALASE* gene expression in skin fibroblasts (Fig. 3).

Induced aging of dermal fibroblasts increased the expression of the *TXNRD1* gene by 1.7 times compared to the intact cells. AEDG peptide did not significantly affect the expression of the *TXNRD1* gene in the control fibroblast cultures and in the cultures with accelerated aging. GGGG and AEDG peptides did not alter the expression of the studied gene in the control cultures and fibroblasts exposed to UV radiation (Fig. 4).

According to some sources, natural aging is associated with the suppression of antioxidant cell function, seen as the decreased synthesis of the *SOD-1*, *NQO1*, and catalase enzymes [23]. Other researchers believe that compensatory elevation of the SOD-1 protein and catalase synthesis is possible during normal aging, preventing DNA damage of senescent cells by free radicals [20, 25].

Accelerated aging of skin fibroblasts induced by UV radiation is associated with the activation of the production of antioxidant enzymes, which is a compensatory-adaptive reaction of cells to stress [7]. AEDG peptide enhanced the expression of the *SOD-1*, *NQO1*, and *CATALASE* genes encoding the antioxi-

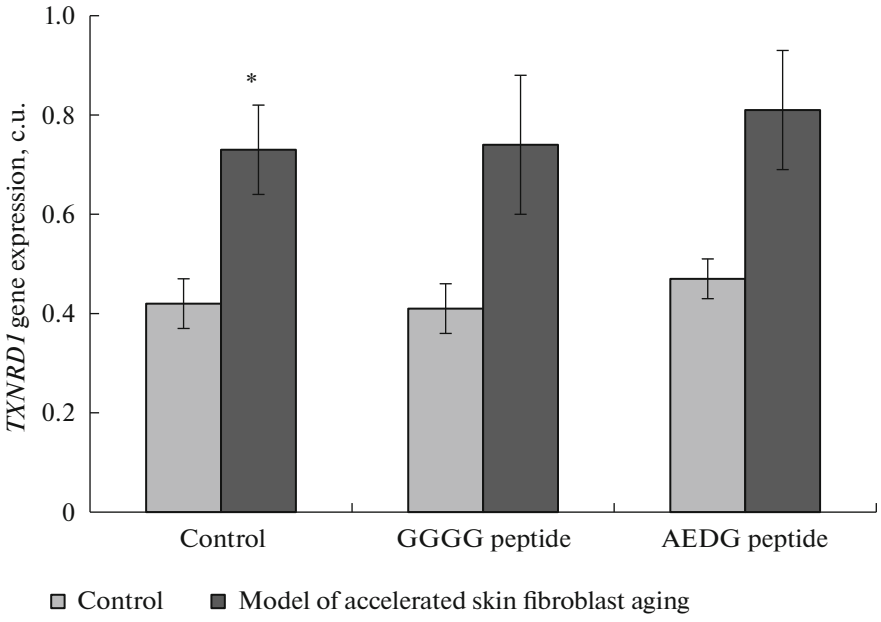

Fig. 4. The influence of AEDG peptide on *TXNRD1* gene expression in human skin fibroblasts in the control and model of accelerated aging induced by UV radiation.

dant enzymes by 2.7, 2.6, and 3.2 times, respectively, indicating its antioxidant and geroprotective effect in skin fibroblasts exposed to accelerated aging under the conditions of UV radiation. Our data are consistent with the antioxidant and geroprotective properties of the studied tetrapeptide discovered earlier [12, 13, 15, 18].

It can be proposed that AEDG peptide affects the Keap1/Nrf2 signaling pathway that maintains homeostasis and regulates the antioxidant status of cells, including the synthesis of *SOD-1*, *NQO1*, and catalase enzymes. This system is regulated by the interaction between the Nrf2 transcription factor and intracellular Keap1 regulator. Furthermore, Nrf2 can modulate the expression of certain antioxidant genes, such as *HO-1*, *SOD-1*, *NQO1*, *TRX*, and *CATALASE*. Apparently, Nrf2 plays a different role in the regulation of induced *SOD* expression in different cell types [29]. Nrf2 and Trx proteins have also been identified as protective factors for cardiovascular diseases. It has been found that the Nrf2 protein stimulated the expression of Trx, thus protecting myocardial cells against apoptosis [21]. Probably, AEDG peptide induces the activation of the Nrf2 signaling pathway by regulating the expression of the *SOD-1*, *NQO1*, and *CATALASE* genes, rather than *TRX*. The proposed mechanism of regulation can underlie the antioxidant action of AEDG peptide on dermal fibroblasts of humans exposed to oxidative stress induced by UV radiation. In addition, the influence of AEDG peptide on the expression of the *SOD-1*, *NQO1*, and *CATALASE* genes can be achieved by direct binding of AEDG peptide with the promoter zones of the mentioned genes [14] or with increased availability of these genes for transcription, provided by the interaction between tetrapeptide and the H1/3 and H1/6 histone proteins [16].

CONCLUSIONS

The geroprotective and antioxidant effect of AEDG peptide for human skin fibroblasts is seen in elevated expression of genes encoding antioxidant enzymes: *SOD-1*, *NQO1*, and catalase. AEDG peptide can directly bind with the promoters of these genes or regulate their expression via the Keap1/Nrf2 signaling pathway. Intensification of antioxidant enzyme production by skin fibroblasts under the action of AEDG peptide is one of the possible means of preventing skin photoaging.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement of compliance with standards of research involving humans as subjects. Patient signed an informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

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Translated by E. Sherstyuk