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Effects of Elastin Peptide on Antioxidative Defence of Photoaging Mice

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Abstract: Ultraviolet (UV) radiation in sunlight is the main exogenous factor underlying the aging of skin. The objective of the present study was to create a hairless mouse model of photoaging via the application of UVA+UVB irradiation for 14 weeks. To study the protective effects of the oral administration of elastin peptide, the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), and malondialdehyde (MDA) level in the serum of irradiated mice were measured. Pretreatment with different doses of elastin peptide before UV irradiation could effectively promote the SOD, GSH-Px and CAT activities in serum of mice and reduce the expression MDA level. In conclusion, elastin peptide can effectively repairing cells by removing superoxide free radicals from UV damage. This study proved the possibility of elastin peptide as a component for inhibiting wrinkle formation which was induced by photoaging.

1. Introduction

The aging of skin in response to the long-term exposure to sunlight is referred to as photoaging; the main factor involved in this type of aging is exposure to ultraviolet (UV) radiation in sunlight [1]. Epidemiological studies have shown that 80-90% of facial aging is caused by chronic exposure to ultraviolet radiation [2]. UV light can be divided into UVA (320-400 nm), UVB (275–320 nm), and UVC (230-275 nm). Due to the blocking effect created by the Earth's atmosphere, UVC is almost completely absorbed by the ozone laye [3, 4]. Thus, the ultraviolet rays that are incident on the Earth's surface are mainly composed of UVA and UVB forms. UV light not only has a cumulative effect on the photoaging of skin; the ability of repairing skin damage will inevitably decrease with age. Therefore, how to effectively prevent photoaging and repair existing skin damage has become a major research hotspot.

Elastin is a protein mainly exists in elastic tissues such as cervical ligament, blood vessel, lung and skin [5]. Elastin peptide (EP) is the hydrolysate of elastin, which has a variety of biological behaviors inducing cell adhesion, migration, proliferation, differentiation and apoptosis [6, 7]. Liu et al. [8] found that the administration of EP could significantly increase the content of hydroxyproline and water in skin, and significantly improve epidermal proliferation and the apoptosis of fibroblasts in

photoaged skin. In this study, a photoaging mouse model was first established by the application of continuously UVA+UVB irradiation. Then, in order to study the effect of EP on antioxidant defense system of photoaged mice, the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT), and malondialdehyde (MDA) level in the serum of irradiated mice were detected. The results may provide a theoretical basis for the application of EP in skin photoaging repair and skin care.

2. Materials and Methods

2.1 Chemicals, reagents and kits

Elastin peptide supplied by Beijing Semnl Biotechnology Co., Ltd. (Beijing, China). SOD detection kit, MDA detection kit, GSH-Px detection kit and CAT detection kit were purchased from Jiangsu Edison Biotechnology Co., Ltd (Jiangsu, China)

2.2 Experimental animals and feeding conditions

Sixty BALB/C nude mice weighing 15.00-19.00 g were obtained from Laboratory Animal Center of Peking University (license No.: SCXK (Jing) 2016-0012). The mice were kept in a conventional animal room (temperature 19-23ºC; relative humidity 40-65%; 12:12 h light/dark cycle) of Beijing Union University. All experimental protocols were reviewed and approved by the Animal Bioethics Committee, Beijing Union University, China.

2.3 Model establishment and dose selection

After 7 days of adaptive feeding, mice were randomly divided into 5 groups as follows (n = 12/group): blank control group, model group, EP low-dose group, EP medium-dose group, and EP high-dose group. At the beginning of irradiation, the blank group was not irradiated; the other four groups were irradiated in the same manner. First, a UV irradiator (UVA 340-80 W: wavelength range, 320-400 nm; wave crest, 340 nm; UVB 313-80 W: wavelength range, 300-320 nm; wave peak, 313 nm) was placed 30 cm upper the mice. The intensities of UVA and UVB light were measured by a UV irradiatometer (Photoelectric Instrument Factory of Beijing Normal University, Beijing, China). The minimum amount of erythema was 31 MJ/cm^2 and irradiation was carried out 6 times each week. The initial irradiation dose was 20 min/day, increasing by 10 min to 30 min/d each week. Then the dose was maintained at 120 min/d for 12 weeks until the appearance of the skin showed typical signs of aging (i.e., desquamation, erythema, and wrinkles), which demonstrated that the model of photoaging had been successfully created. Each group received an oral gavage one hour before irradiation to deliver treatments in appropriate amounts. The model group was given distilled water every day, and the concentrations of EP given to the low, medium, and high dose groups were 1.5, 5.0 and 10 mg/animal/day, respectively.

2.4 Biochemical Studies

At the end of the experiment, mice were culled by cervical dislocation and blood samples were collected to determine the biochemical values. The blood was immediately placed into Eppendorf tubes infiltrated by heparin sodium, and centrifuged at 3000 g for 10 minutes. The upper serum was transferred to a new 0.5 mL Eppendorf tube, and quickly stored in the refrigerator at - 80℃ for subsequent detection. Measurements of MDA levels, SOD, GSH-Px and CAT activities were performed corresponding detection kits according to the manufacturer’s protocols.

2.5 Data analysis

Data were tested to ensure that variances were homogenous and normally distributed. Data were compared using the student's t-test and one-way analysis of variance (ANOVA test). These tests were performed in Statview statistical software (Brainpower, Calabasas, CA). Significant differences are denoted as follows: *p < 0.05, ** p < 0.01, and *** p < 0.001.

3. Results

3.1 Determination of SOD activity in serum of mice

Fig. 1 showed the variation of SOD activity in serum of different group.

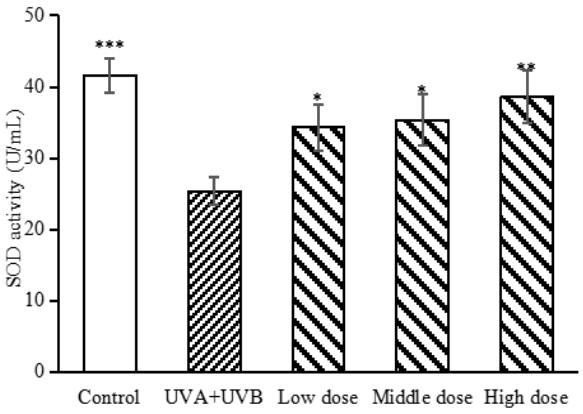

Figure 1: The SOD activity in serum of mice.

For comparisons between UV-irradiated controls and test article groups, * indicates ρ<0.05, ** indicates ρ<0.01, *** indicates ρ<0.001

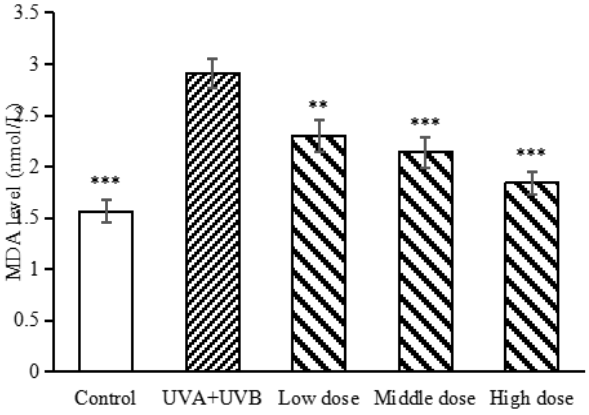

Figure 2: The MDA level in serum of mice.

For comparisons between UV-irradiated controls and test article groups, ** indicates ρ<0.01, *** indicates ρ<0.001

SOD is a kind of metal binding enzyme that catalyzes the conversion of superoxide anion radical into H_2O_2 and O_2, so as to play the role of antioxidant and protect cells from damage. SOD is an important free radical scavenger in organisms, and its activity reflects the ability of the body to scavenge oxygen free radicals. Fig. 1 shows that after UVA+UVB irradiation, the activity of SOD in serum of model group mice is significantly lower than that of blank group (ρ<0.001). After intragastric administration of EP, the activity of SOD in serum of experimental animals increased significantly (ρ<0.05), of which the effect of high-dose EP group was the most significant (ρ<0.01), indicating that EP can significantly improve the decrease of SOD activity caused by UV irradiation, so as to effectively improve the antioxidant capacity of organisms and reduce the damage of superoxide anion free radicals to the body.

3.2 Determination of MDA content in serum of mice

Fig. 2 showed the variation of MDA content in serum of different group.

MDA is the end product of peroxidation reaction between free radicals and lipids. Its content reflects the degree of lipid peroxidation in organisms, and then reflects the degree of cell damage. MDA is cytotoxic and can cause a series of cell metabolism, dysfunction and even death. It is often used as an important reference index for the degree of tissue lipid peroxidation damage. Fig. 2 shows that after UVA+UVB irradiation, the MDA level in serum of model group mice was significantly higher than that of blank group (ρ<0.001), and after intragastric administration of EP, the level of

MDA in serum of experimental animals decreased significantly ($\rho<0.01$), in which the middle dose group and high dose group of EP had the most significant effect on the decrease of MDA content in serum ($\rho<0.001$), indicating that EP can significantly improve the lipid peroxidation caused by UV irradiation, so as to effectively reduce the damage of MDA and its analogues to organisms.

3.3 Determination of GSH-Px activity in serum of mice

Fig. 3 showed the variation of GSH-Px activity in serum of different group.

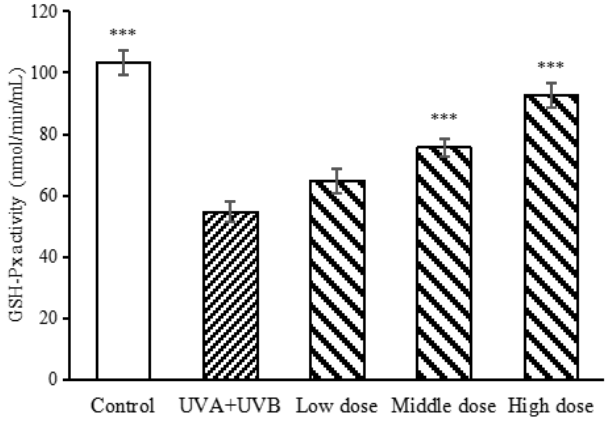

Figure 3: The GSH-Px activity in serum of mice.

For comparisons between UV-irradiated controls and test article groups, *** indicates $\rho<0.001$

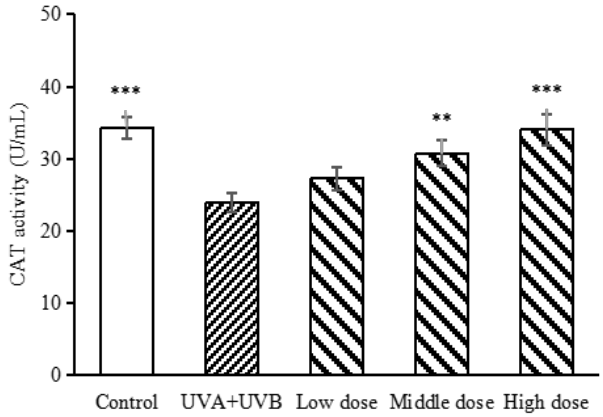

Figure 4: The CAT activity in serum of mice.

For comparisons between UV-irradiated controls and test article groups, ** indicates $\rho<0.01$, *** indicates $\rho<0.001$

GSH-Px is an important member of the antioxidant system in organisms. It uses glutathione as reducing agent to remove hydrogen peroxide and other peroxides in the body, so as to maintain the normal physiological function of the body and delay aging [9]. Fig. 3 shows that after UVA+UVB irradiation, the GSH-Px activity in the serum of the model group was significantly lower than that of the blank group ($\rho<0.001$). After intragastric administration of EP, the level of GSH-Px in serum of experimental animals increased to a certain extent, among which the middle dose group and high dose group of EP had the most significant effect ($\rho<0.001$), indicating that EP can significantly improve the decline of GSH-Px activity caused by UV irradiation, so as to effectively improve the antioxidant capacity of organisms and reduce the damage of free radicals to the body.

3.4 Determination of CAT activity in serum of mice

Fig. 4 showed the variation of CAT activity in serum of different group.

CAT is one of the key enzymes in the biological defense system. Its main function is to remove excess H_2O_2 [10]. It plays an important role in preventive protection and reducing inflammatory response. Fig. 4 shows that after UVA+UVB irradiation, the CAT activity in the serum of the model group was significantly lower than that of the blank group ($\rho<0.001$). After intragastric administration of EP, the CAT activity in serum of experimental animals increased to a certain extent, among which the effect of high-dose EP group was the most significant ($\rho<0.001$), indicating that EP can significantly improve the decline of CAT activity caused by UV irradiation, so as to effectively remove excess H_2O_2 in the organism and reduce the damage of H_2O_2 to the organism.

4. Discussion

Long term sunlight exposure will lead to or accelerate skin aging, which will not only weaken the barrier function of the skin, but also prone to skin diseases [11]. With the gradual thinning of the atmospheric ozone layer, there are more and more opportunities for ultraviolet rays to reach the surface and irradiate the human body, and the skin photoaging damage is more and more serious, and even lead to various skin diseases. How to effectively prevent the skin aging caused by photoaging and repair the existing skin damage has become a major research hotspot at present. UVA and UVB not only cause skin photoaging, but also have synergistic effects. In the past, we mainly focused on the aging damage of UVB to skin, and often ignored the role of UVA. In the process of modeling, UVA and UVB are superimposed in order to be closer to the actual situation. The results showed that UVA+UVB could significantly reduce the activities of SOD, GSH-Px and CAT in the serum of experimental animals ($\rho<0.001$), which significantly increased the content of MDA ($\rho<0.001$), indicating that the modeling is successful. SOD, GSH-Px and CAT are three important enzymes for scavenging peroxides in organisms. They can reduce the damage caused by free radicals formed by ultraviolet irradiation, while MDA can reflect the degree of lipid peroxidation and indirectly reflect the degree of cell damage. The results showed that elastin peptide could significantly increase the activities of SOD, GSH-Px and CAT in serum of photoaging mice, and significantly reduce the content of MDA.

5. Conclusions

In conclusion, EP can effectively reduce the damage degree of mouse skin caused by ultraviolet radiation, improve the ability of skin to resist ultraviolet radiation, and provide a certain theoretical basis for the application of EP in skin care and improving skin photoaging. With the further study of EP, it is expected to reveal more effects of EP on skin photoaging.

Conflict of interest

All contributing authors declare no conflicts of interest.

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