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Chronic inflammation accelerates skin ageing: Involvement in oxidative stress and degradation of extracellular matrix in dermal cells

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Introduction:

Inflammation refers to a variety of factors such as infection or non-infection. It is a series of protective responses that cause body tissue damage through direct or indirect immune mechanisms, and the clinical manifestations are red, swollen, hot, painful and itchy [1-3]. In the process of human aging, due to the imbalance of the immune system in the body caused by external factors, the overexpression of pro-inflammatory factors such as TNF-α, IL-6, etc., long-term stimulation of inflammatory factors can lead to chronic inflammatory aging with low concentrations in the body. Thereby increasing the incidence of aging diseases, such as Alzheimer's, diabetes, Parkinson's disease, osteoporosis, etc. [4-6]. For example, in previous studies on cardiovascular disease, it was found that continuous stimulation with 10 ng/mL TNF-α The expression of cellular senescence-related genes p21 and p16 after vascular endothelial cells was significantly up-regulated compared with the control group, indicating that the inflammatory factor TNF-α successfully induced vascular endothelial cells into the senescence phase [7]. At present, the current researches at home and abroad focus on the relationship between inflammation and aging diseases, but the related research on inflammation and skin aging is relatively scarce, so the impact of inflammatory factors on human skin needs to be discovered urgently.

Materials & Methods:

Cell culture

HSF cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% (v/v) fetal bovine serum and 1% streptomycin–penicillin. Moreover, cells were preserved in the 5% CO_2 incubator at 37℃.

3D skin model and treatment

The 3D skin model was purchased from EPISKIN, and the treatment was refered to the procotal supplyed by EPISKIN.

Histology and immunostaining

Samples were fixed in 10% neutral formalin and treated for histology. Five-micrometer paraffin sections were stained with hematoxylin, eosin, and saffron. Melanin was colored by Fontana-Masson (FM) staining. The sections were labbelled with anti-TNF-α antibody (Abcam, Beijing, China) coupled to goat anti rabbit IgG (Alexa Fluor[R] 488) (Beyotime Biotechnology, Nanjing, China).

COL-I and MMP-1

HSF cells in logarithmic growth phase were seeded in a 6-well plate at a density of 50,000 cells/mL, and incubated in a 37° C, 5% CO2 incubator for 24 h. Then add TNF-α solution for 24 hours. Remove the culture medium from the wells, wash twice with PBS, add 200 uL of PMSF-containing lysate to each well for 1 min, and centrifuge at 10,000 r/min for 5 min. Subsequently, the COL-I ELISA kit and MMP-I ELISA kit was used to detect the COL-I and MMP-I content in the cells and corrected with protein.

Redox system analysis

HaCat and HSF cells in logarithmic growth phase were seeded in a 6-well plate at a density of 50,000 cells/mL, and incubated in a 37° C, 5% CO2 incubator for 24 h. Then add TNF-α solution for 12 h. Remove the culture medium from the wells, add PBS to wash twice, add 1 mL of 20 uM DCFH-DA to each well, incubate at 37° C for 30 min, wash twice with DMEM solution, and add 1 mL of PBS. Observe the fluorescence under an inverted fluorescence microscope and take pictures for subsequent processing.

After the TNF-α was incubated with HSF for 12 h, the cells were washed twice with PBS, add 200 uL of PMSF-containing lysate to each well for 1 min, and centrifuge at 10,000 r/min for 5 min. Subsequently, the GSH-Px kit and CAT kit was used to detect the GSH-Px and CAT content in the cells and corrected with protein.

Statistics

For replicate experiments with numerical values, an analysis of variance was performed using Tukey comparison test.

Results & Discussion:

The effect of UVA on inflammatory cytokines in keratinocytes

Cell senescence is accompanied by the generation of senescence-associated secretory phenotype (SASP). SASP includes pro-inflammatory cytokines (such as TNF-α, IL-1β, etc.), growth factors, chemokines, etc. In this section, UVA was used to stimulate keratinoctes, and then the differences in the expression of TNF-α and IL-1β, which are related to senescence, were detected in the cells. The results showed that the expression levels of TNF-α and IL-1β in cells stimulated by UV were 1.18±0.14 and 1.16±0.09 times that of the control group, respectively.

The effect of UVA on 3D skin equivalent

After the ultraviolet light exposed to 3D skin model, HE staining results showed that the epidermis and dermis became thinner and the boundary between the epidermis and dermis was not obvious (Figure 1B, red arrow). Immumohistochemical staining results showed that TNF-α in the dermis increased significantly after the stimulation (Figure 1B, red arrow).

The effect of inflammatory cytokines on collagen synthesis in HSF

The effects of different concentrations of TNF-α on the content of COL-I in HSF cells are shown in Figure 2A. As shown in Figure 2A, after 20, 100, and 200 ng/mL TNF-α acted on HSF cells for 24 h, the content of type I collagen per protein was 2.740, 2.270, and 1.595 mg, respectively, compared with 3.00 mg in the control group.

The effects of different concentrations of TNF-α on the MMP-I content in HSF cells are shown in Figure 2B. As shown in Figure 2B, after 20, 100, and 200 ng/mL TNF-α acted on HSF cells for 4 h, the content of MMP-I in unit protein was 3.10, 3.12, 3.10 ng, respectively.

The effect of inflammatory cytokines on redox system in HSF

The effects of different concentrations of TNF-α on the content of reactive oxygen species in HSF cells are shown in Figure 3. As shown in Figure 3A, after 20, 100, and 200 ng/mL TNF-α acted on HSF cells for 12 h, the fluorescence intensity per unit cell increased compared with the control group, with significant differences and a certain degree of concentration-dependent, indicating that TNF-α acting on HSF cells can effectively induce the increase of intracellular reactive oxygen species. After 20, 100, and 200 ng/mL TNF-α acted on HSF cells for 12 h, the content of GSH-Px and CAT in unit protein was lower than that in the control group, and there was a significant difference.

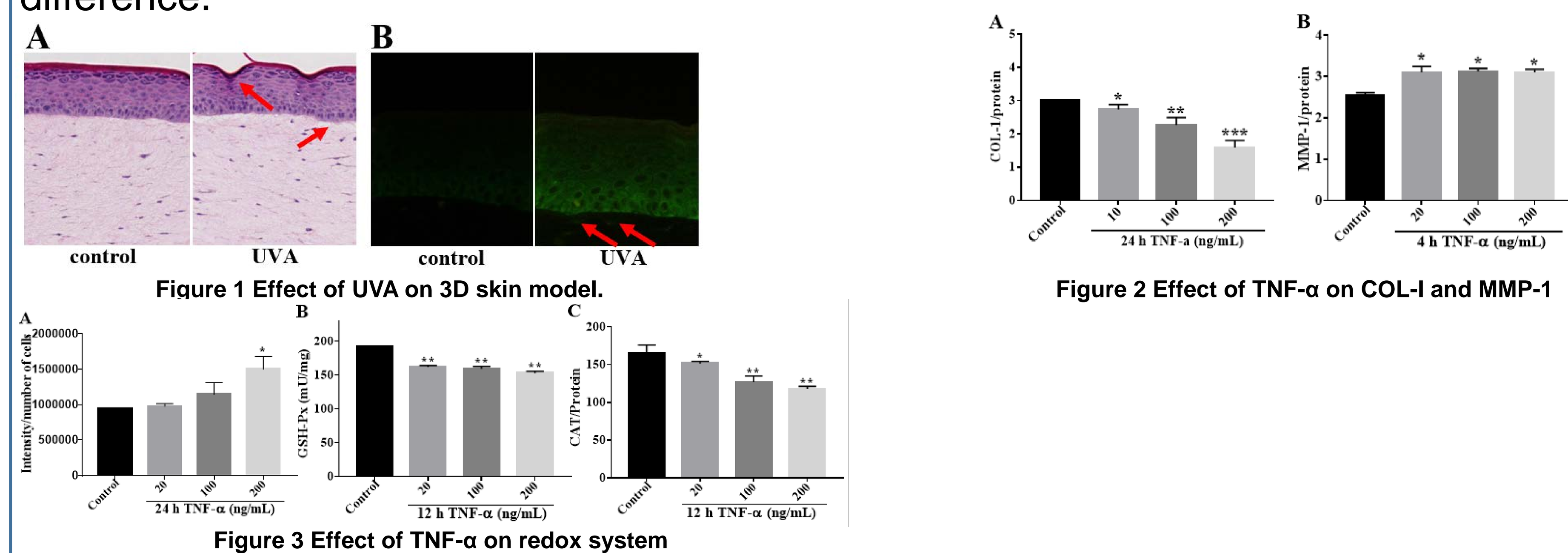

Figure 1 Effect of UVA on 3D skin model.

Figure 2 Effect of TNF-α on COL-I and MMP-1

Figure 3 Effect of TNF-α on redox system

Conclusions:

Taken together, inflammation and the resulting accumulation of reactive oxygen species (ROS) play an important role in the aging of human skin in vivo and chronic inflammation accelerates skin ageing. We thought anti- inflammation is not only important for anti-ageing, but also is new trend in skin care and sun protection.

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References:

[1] Li Zhiliang, Feng Suying (2013) Adverse skin reactions caused by tumor necrosis factor alpha antagonists and countermeasures. Chinese Journal of Dermatology, (8): 610-613.

[2] Shen Kuiya, Wu Xinan (2016) Co-influence of IL-17 and TNF-α on the expression of IL-17R, p-p38 MAPK and inflammatory factors in HaCaT cells. Anhui Medicine, 20(7): 1292-1295.

[3] Yao Lu, Luo Wen, Gu Hua (2011) The effect of TGF-β1 on the inflammatory factors IL-1α, IL-6 and TNF-α in artificial skin photoinjury. Dermatology and Venereology, (5): 252-255.