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Original Article

Leptin alleviates ultraviolet-induced skin photoaging in human skin fibroblasts and mice

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Abstract: Background: To investigate effects of leptin on ultraviolet-induced skin photoaging in human skin fibroblasts (HSFs) and mice and the underlying mechanism. Methods: HSFs in 3-5 subcultures and 50 CD-1 (ICR) mice were randomly divided into blank group, model group and leptin-treated groups. Changes of HSFs and HE stained mouse skin tissue structure exposed to UVA/B irradiation were observed. Senescence-associated β-galactosidase staining of HSFs was determined. Activity of CAT, SOD and GSH-Px and concentrations of MDA, Hyp and LDH were determined by biochemical analysis. Expressions of $p67^{phox}$, PKCε and p66Shc were detected by Western blotting. Results: UV-induced photoaging models were successfully established. In HSFs, compared with blank group, significantly decreased SA β-gal staining rate and concentrations of CAT, SOD, GSH-Px and Hyp but significantly increased concentrations of MDA, LDH and ROS were found in model group; compared with model group, significantly increased concentrations of CAT, SOD, GSH-Px and Hyp and significantly decreased MDA, LDH and ROS were found in leptin-treated groups. Similar trends were observed regarding the concentrations of CAT, SOD, GSH-Px, Hyp, MDA, LDH and ROS in mouse skin tissues. In both HSFs and mouse skin tissues, the expressions of NADPH, $p67^{phox}$, PKCε and p66Shc were significantly up-regulated in model group compared with blank group; while the expressions of $p67^{phox}$, PKCε and p66Shc were significantly down-regulated in leptin-treated groups in comparison with model group. Conclusion: Leptin may prevent skin photoaging by scavenging free radicals, improving antioxidant capacity and enzyme activity, alleviating oxidative damage and promoting collagen synthesis in HSFs and mouse skin tissues.

Keywords: Leptin, human skin fibroblasts, mice, skin photoaging, antioxidant capacity, collagen synthesis, free radicals, mechanism

Introduction

Long-term exposure to solar ultraviolet (UV) irradiation generates serious adverse effects on skin structure and function, and may cause premature skin aging termed as photoaging, immunosuppression and ultimately photocarcinogenesis [1]. Previous evidence revealed that skin photoaging is associated the induced expression of matrix metalloproteinases (MMP) upon chronic UV exposure with functional roles in degrading collagenous extracellular matrix proteins, thus resulting in impaired skin structural integrity [2, 3]. Exposure to UV irradiation attacks keratinocytes and fibroblasts, activating cell surface receptors, initiating signal transduction cascades, resulting in various molecular changes involved with oxidative stress and oxidative photodamage which are considered as a key mechanism underlying photoaging [4, 5]. More specifically, compelling evidence suggested the role of reactive oxygen species (ROS) as mediators of photoaging, and increased ROS production can overwhelm antioxidant defense mechanisms, leading to the damage of cellular DNA, lipids and proteins in the skin [6, 7]. Reduced activity of the major antioxidant enzymes can be caused by UV-induced photoaging, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px), which in turn yields a deficit in skin antioxidative defense system and contributes to photoaging [4, 8].

Leptin is an obesity gene encoded and adipose tissue secreted hormone that can produce a

variety of systemic biological effects after binding to its receptors, and helps to regulate energy balance and participate in immune response [9-11]. It has been evidenced that leptin has an important functional role as an autocrine/paracrine regulator in the promotion of skin wound healing; however, the underlying mechanism has not been fully elucidated [12, 13]. The skin photoaging in human is characterized by impaired wound healing, and oxidative stress is closely related to wound healing, wound tissues are presented with the increase of oxygen free radicals, decreased antioxidant capacity, resulting in oxidative stress injury; while the improvement of the antioxidant capacity can protect the wound tissues and contribute to accelerated wound repair [14-16]. Recent studies documented that leptin participates in the regulation of ROS levels, and may induce oxidative stress; while contrary evidence suggested that leptin can alleviate oxidative damage [17, 18]. Additionally, lipid peroxidation, protein collagen and tissue damage related molecules, like malondialdehyde (MDA), hydroxyproline (Hyp) and lactate dehydrogenase (LDH), as well as $p67^{phox}$, PKCε and p66Shc proteins were demonstrated to be involved in oxidative stress regulation and important experimental indexes in photoaging [19-25]. It has been demonstrated that circulatory levels of leptin apparently interact with the antioxidant system, and leptin could stimulate collagen synthesis, since reduced collagen synthesis, oxidative stress or damage are considered as underlying mechanisms of photoaging, we hypothesized that leptin may alleviate ultraviolet-induced skin photoaging with its antioxidant ability [26-29]. Therefore, the present study established UV-induced human skin fibroblasts (HSFs) photoaging model in human skin fibroblasts and mice, and molecules involved in oxidative stress or damage were detected to investigate effects of leptin on ultraviolet-induced skin photoaging regarding the antioxidant defense mechanisms.

Materials and methods

HSF culture

Normal human skin tissues (protect from light) were provided by the Institute of Skin Damage and Repair, General Hospital of Beijing Military Command PLA, and subcutaneous tissues were removed under aseptic conditions, rinsed, cut into 0.5 cm × 0.5 cm slices; the slices were placed in a culture dish, and digested overnight with 0.25% trypsin (Hyclone, USA); and the separation of epidermis and dermis was performed on the next day. According to explant culture method, HSFs were cultured in dulbecco's modified eagle medium (DMEM) medium (Hyclone, USA) containing 10% fetal bovine serum, penicillin (100 U/ML), 100 mg/ml streptomycin sulfate (GIBCO, USA). Cells were incubated in a 5% CO_2 incubator at 37°C and grown to confluency (80% confluent), and then digested with trypsin and ethylene diamine tetraacetic acid (EDTA) (volume ratio, 1:2; Hyclone, USA). Cell subcultures were performed from 1 growth bottle to 3 (4) growth bottles. After approved by the Ethics committee of General Hospital of Beijing Military Command PLA, written, informed consents were obtained from subjects providing normal human skin tissues.

Cell treatment and UV irradiation

HSFs in 3-5 subcultures were randomly divided into blank group, model group, low leptin concentration group (5 μg/mL) and moderate leptin concentration group (10 μg/mL) and high leptin concentration group (20 μg/mL). In the model group and the leptin groups in different concentrations, UV irradiation was performed with 40 W UV lamps (Beijing Institute of Optoelectronic Technology): 5 UVB lamps (lamp wavelength, 290-320 nm; peak value, 297 nm) and 2 UVA lamps (lamp wavelength, 320-400 nm; peak value, 365 nm) were parallel arranged in a self-made simulated sunlight exposure box. HSFs were exposed to a UV regimen consisting of UVA exposure (8 J/cm^2) and UVB exposure (1510 J/cm^2) for 3 days. After irradiation, HSFs were incubated for 24 h with leptin solution in different concentrations.

Fifty male CD-1 (ICR) of clean grade (weight, 20 ± 2 g) were provided by Beijing Vital River Laboratory Animal Technology Co., Ltd. Mice were randomly divided into blank control group, model group, low leptin concentration group (5 μg/mL) and moderate leptin concentration group (10 μg/mL) and high leptin concentration group (20 μg/mL) (n = 10 per group). Mice were shaved to remove back hair and mice in the model group and leptin group were treated with UV irradiation using the simulated sunlight box

with a UVA dose of 2.92 J/cm^2 and UVB dose of 64.2 J/cm^2 (exposure time, 2 h per time), and measured with UV radiation measuring instrument. Mice were exposed to UV irradiation for 2 h per time every other day. Within the 3rd week, 4 h per time every other day within the 4th-8th week, 6 h per time every other day within the 9th-12th week, 3 times a week at the beginning of the 14th week with increased UVA exposure doses every two weeks. The maximum exposure doses were maintained at 15 J/cm^2. Five min before each irradiation, leptin solution was applied on the hair removal parts until the end of the experiment. All animal experimental processes were performed in compliance with the regulations of the Animal Ethics Committee of General Hospital of Beijing Military Command PLA.

Morphological observation of HSFs and tissue morphology observation of mice skin

Cell culture dishes or culture bottles were placed under inverted microscope (Leica, Germany), the cell growth conditions in the blank group and UV irradiation treated groups were photographed, including cell morphology, size and the degree of convergence, culture liquid color changes, clarity, and contamination; and cell morphology changes were recorded.

Mice were sacrificed 24 h after the last UV exposure, and dorsal skin tissues were collected and fixed with formalin (Qingan chemical industry, Jinan); 48 h later, tissues were embedded into paraffin and sliced for hematoxylin and eosin (H&E, Shanghai Bogu Biological Technology Co., Ltd.) staining. The structural changes were observed under the light microscope (Leica, Germany), and photographed under the system biological microscope (Leica, Germany).

HSF β-galactosidase staining

Senescence-associated β-galactosidase (SA β-gal) staining of HSFs was determined by a β-gal staining kit (Cell Signaling Technology, USA). HSFs in the model group and the blank group were removed from the incubator, and the medium was also removed. HSFs were washed once with PBS solution (2 ml/per well), added with 1 × Fixative solution (1 ml/per well), fixed at room temperature for 10-15 minutes, and then washed with PBS twice. Staining solution (1 ml) was added to each well, and HSFs were incubated overnight at 37°C. On the next day, HSFs were observed and photographed under the optical microscope.

Preparation of mice skin tissue homogenate

The skin tissue specimens (0.5 g) were collected from each mouse, rinsed with ice-cold physiological saline (Shenzhen Ai Jia Medical Devices Co., Ltd.) to remove blood, and dried with filter paper. The tissue specimens were shredded by ophthalmic scissors; the shredded tissues were put into a glass homogenizing tube, and homogenized using a homogenizer (Haimen Botai Experimental Equipment Sales Department) in ice-cold physiological saline (4.5 mL). The skin tissue homogenates were centrifuged at 4000 rpm for l0 min to obtain the supernatant for further usage.

Detection of intracellular reactive oxygen species (ROS) levels

Intracellular ROS levels were determined by a fluorescence probe, 2',7'-dichlorofluorescin diacetate (DCFH-DA, Beijing Solarbio Science & Technology Co., Ltd.). Cells were collected 0.5 h after UV exposure, and the culture medium was replaced by a serum free medium containing DCFH-DA (10 μmol/mL). After incubation in the incubator at 37°C for 20 min, cells were washed with serum free medium (3 times) to remove the DCFH-DA failed to enter into the cells. The average fluorescence intensity of oxidized dichlorofluorescin (DCF) was detected with excitation wavelength at 488 nm and emission wavelength at 525 nm.

CAT, SOD, GSH-Px, MDA, Hyp and LDH detected by colorimetric method

The cell supernatant and mouse skin tissue homogenates were collected, and immediately used to detect the levels of CAT, SOD, GSH-Px, MDA, Hyp and LDH using kit provided by Shanghai Enzyme-linked Biotechnology Co., Ltd. in strict accordance with the manual requirements. Each group was provided with 3 parallel holes, and the average value of triplicate experiments was recorded.

The protein expressions of p67^{phox}, PKCε and p66Shc

Western blotting analyses were performed to detect the protein expressions of p67^{phox}, PKCε

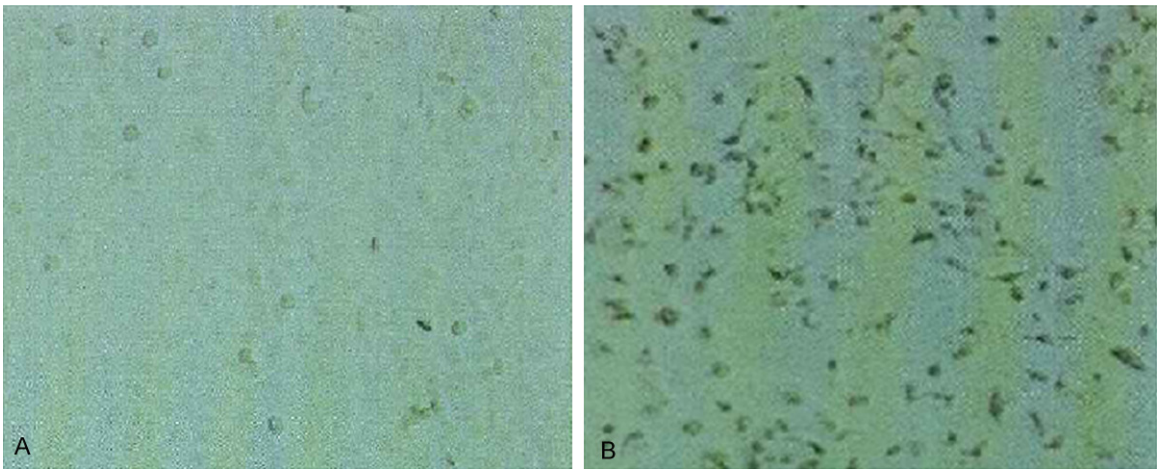

Figure 1. Cell morphology observation in the blank group (A) and the model group (B) under the inverted microscope. (A) Cell morphology in blank group was observed under the inverted microscope, the cell morphology was uniform, and cells were arranged closely and the boundary was clear; (B) The cells were smaller in volume, irregular in shape, larger in intercellular space, and decreased in adherence; there were also a large number of floating cells along with partial cell debris).

and p66Shc. The collected HSF and mouse skin tissue homogenates were lysed for 30 min; lysate was moved to a 1.5 mL centrifuge tube with a transferpettor, and centrifuged at 10000 r/min at 4°C for 20 min; the supernatant was obtained to determine protein concentrations according to the Bradford method; and the protein concentrations were adjusted until basically the same. Samples were put into the 0.5 mL centrifugal tube, and buffer solution was added with a ratio of 1:1; before loading, the samples were boiled in boiling water for 5 min for protein denaturation. Proteins were run on SDS-PAGE and electro-transferred to PVDF membranes, the membranes were blocked with blocking buffer at room temperature for 1 h, incubated with anti-$p67^{phox}$, ant-PKCε and anti-p66Shc antibodies as primary antibodies (dilution, 1:200, Beyotime Biotechnology, China) at room temperature for 1~2 h, washed 3 times with PBS buffer solution (Shanghai Bogu Biological Technology Co., Ltd.) at room temperature, incubated with secondary antibody (Beyotime Biotechnology, China) at room temperature for 1~2 h, further washed 3 times with PBS buffer solution (Shanghai Bogu Biological Technology Co., Ltd.) at room temperature, 3,3'-Diaminobenzidine tetrahydrochloride (DAB; Jackson) was used for color (brown) development. The film was photographed and the relative molecular mass and net absorption of the target bands were analyzed by gel image processing system, and the data were corrected by β-actin.

Statistical analyses

Continuous variables are presented as means ± standard deviation (SD), and compared with *t*-test. Categorical variables were as frequencies and percentages, and compared with chi-square test. SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was applied to statistical analysis. A two-tailed *P* value of 0.05 indicates statistically significant.

Results

Construction of skin photoaging model

Cell morphology in blank group was observed under the inverted microscope, the cell morphology was uniform, and cells were arranged closely and the boundary was clear (**Figure 1A**). In the model group, the cells were smaller in volume, irregular in shape, larger in intercellular space, and decreased in adherence; there were also a large number of floating cells along with partial cell debris (**Figure 1B**); the SA β-gal staining rate of HSFs increased significantly ($P < 0.01$) (**Figure 2**); intracellular ROS concentrations increased significantly; the activity of antioxidant enzymes, such as GSH-Px and SOD, decreased significantly, and the antioxidant system was severely damaged; MDA concentra-

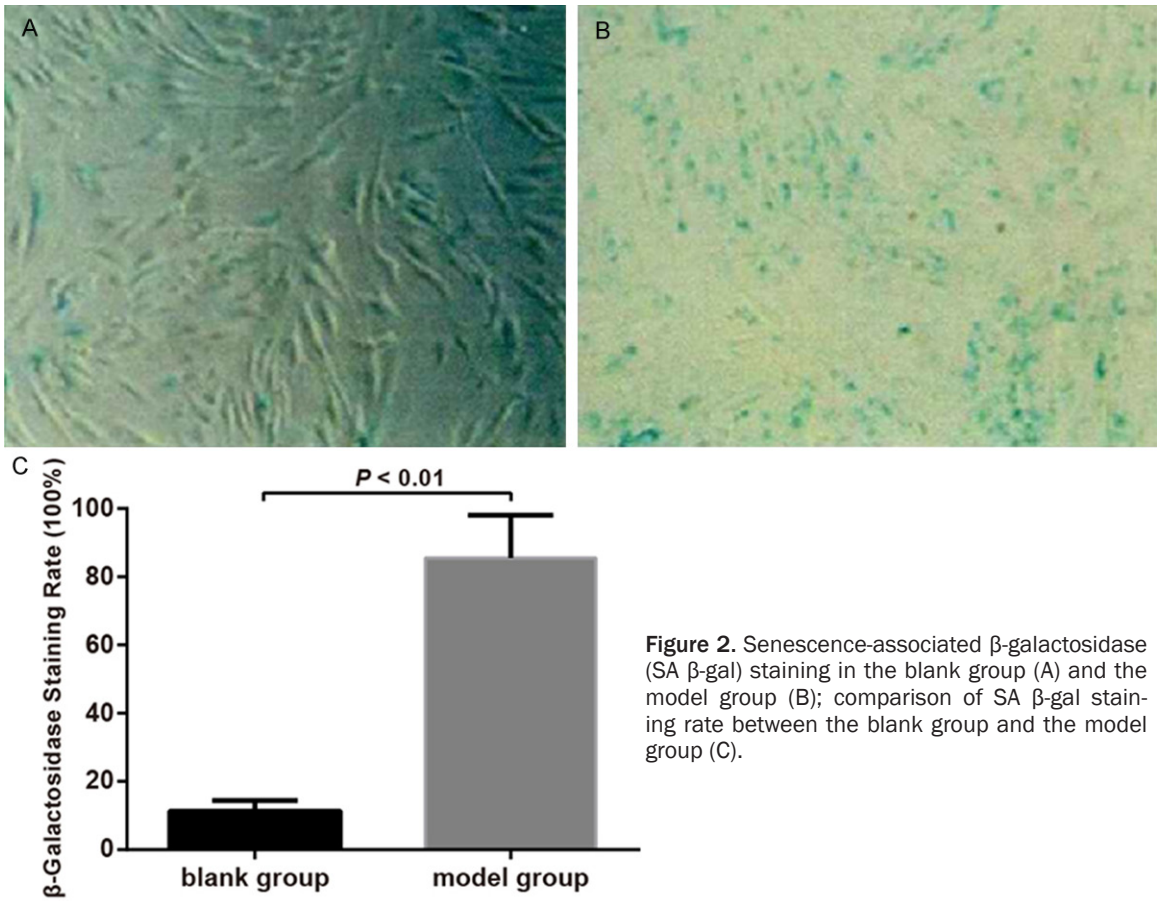

Figure 2. Senescence-associated β-galactosidase (SA β-gal) staining in the blank group (A) and the model group (B); comparison of SA β-gal staining rate between the blank group and the model group (C).

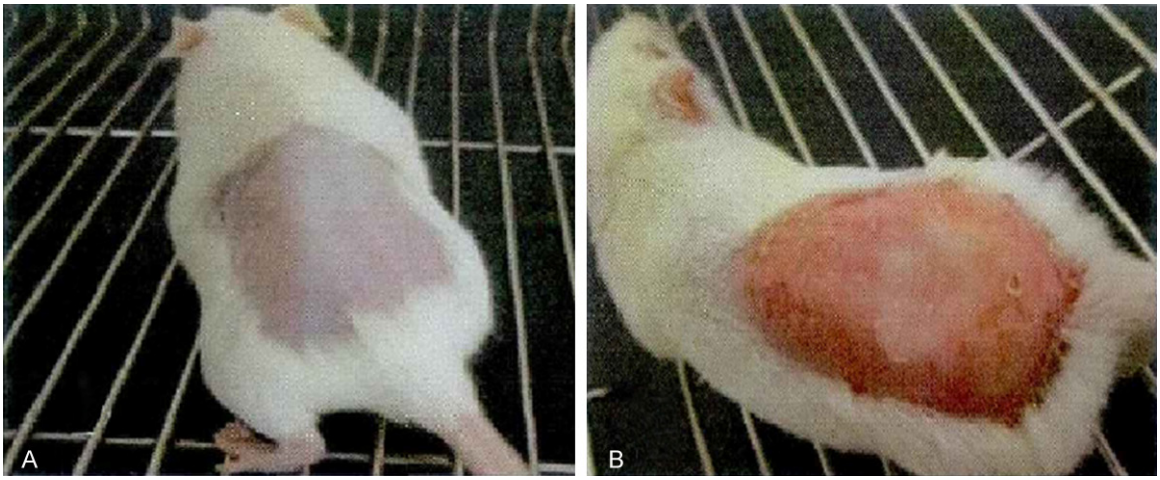

Figure 3. Effects of ultraviolet irradiation on skin tissue of mice in the blank group (A) and the model group (B). (Compared with the blank group, the UV-exposed dorsal area of most mice in the UV-induced model group showed mild erythema formation in early stage, and gradually developed to color deepening, desquamating and thickening; and became rough and hardening, lost elasticity and luster in seriously skin damaged mice).

tions, lipid peroxidation product, increased significantly; the activity of LDH in the culture medium was 2 times higher than that in the blank group, the cell membrane was severely damaged by UV, and Hyp concentrations decreased significantly. These findings suggested that the normal antioxidant ability of HSFs was severely damaged, and the

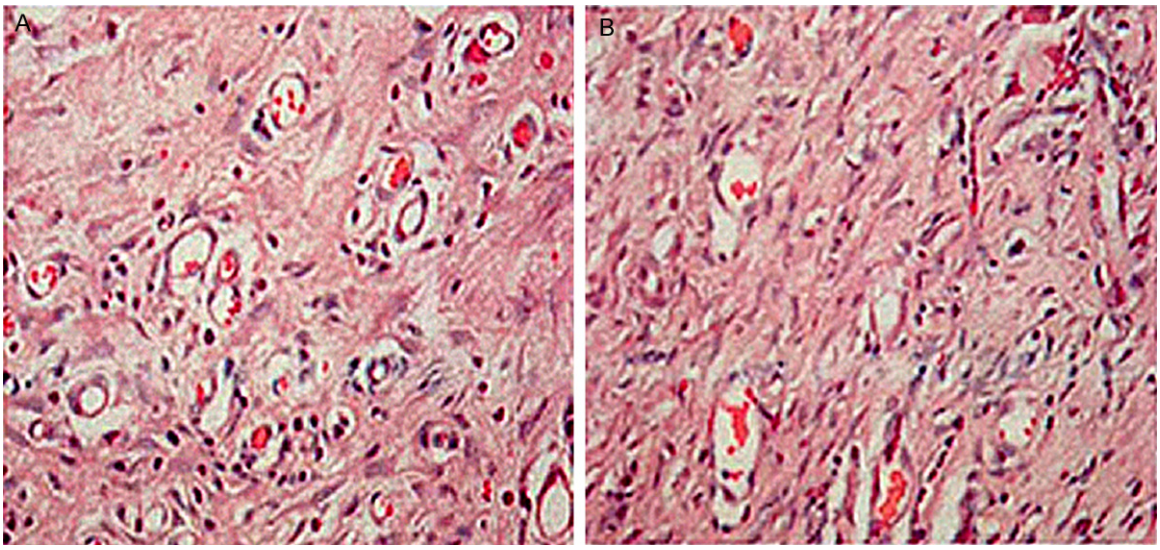

Figure 4. Morphology of mouse skin tissue in the blank group (A) and the model group (B) under light microscope (Under the light microscope (10 × 40), no pathological changes were observed in the blank group, mice in the model group exhibited obvious uneven epidermal thickness and acute injury, clear dermal-epidermal junction, significant dermal proliferation, microcapillary expansion and distortion, vessel wall thickening, inflammatory cell infiltration, irregular hyperplasia of sebaceous glands).

Table 1. Effect of different concentrations of leptin on antioxidant capacity of HSFs exposed to UV irradiation

Group	Blank group	Model group	Low leptin concentration group (5 μg/mL)	Moderate leptin concentration group (10 μg/mL)	High leptin concentration group (20 μg/mL)
CAT (U/mL)	12.61 ± 1.03	4.43 ± 0.75*	6.03 ± 0.88*,#	8.94 ± 0.92*,#	6.57 ± 0.66*,#
SOD (U/mL)	26.39 ± 1.12	13.57 ± 0.12*	21.32 ± 0.58*,#	29.15 ± 0.87*,#	31.04 ± 0.59*,#
GSH-Px (U/mL)	47.31 ± 1.08	21.52 ± 0.43*	37.14 ± 2.48*,#	32.43 ± 4.21*,#	45.59 ± 5.34#
MDA (nmol/mL)	2.84 ± 0.08	3.93 ± 0.24*	2.51 ± 0.11*,#	2.49 ± 0.05#	2.78 ± 0.06#
Hyp (ug/mL)	17.21 ± 0.87	11.78 ± 0.53*	14.33 ± 0.44*,#	17.13 ± 0.61#	15.11 ± 0.58*,#
LDH (U/L)	305.72 ± 15.78	542.23 ± 18.37*	451.12 ± 19.51*,#	425.32 ± 16.17*,#	383.55 ± 14.21*,#
ROS (MFI)	95.29 ± 8.45	182.21 ± 11.43*	128.56 ± 9.98*,#	145.24 ± 8.86*,#	121.59 ± 6.78*,#

*, compared with the blank group, $P < 0.05$; #, compared with model group, $P < 0.05$.

UV-induced HSF photoaging model was successfully established.

Compared with the blank group (**Figure 3A**), the UV-exposed dorsal area of most mice in the UV-induced model group showed mild erythema formation in early stage, and gradually developed to color deepening, desquamating and thickening; and became rough and hardening, lost elasticity and luster in seriously skin damaged mice (**Figure 3B**). Under the light microscope (10 × 40), no pathological changes were observed in the blank group (**Figure 4A**), mice in the model group exhibited obvious uneven epidermal thickness and acute injury, clear dermal-epidermal junction, significant dermal proliferation, microcapillary expansion and distortion, vessel wall thickening, inflammatory cell infiltration, irregular hyperplasia of sebaceous glands, (**Figure 4B**). These findings revealed that the mouse skin photoaging model was successfully constructed.

Effects of leptin on SOD, GSH-Px, MDA, Hyp and LDH in HSFs and mouse skin tissues

In HSF cell supernatant, the levels of CAT, SOD, GSH-Px and Hyp were significantly lower in the model group than those in the blank group (all $P < 0.05$), while the levels of MDA, LDH and ROS increased significantly (all $P < 0.05$). Compared with the model group, the levels of

Table 2. Effect of different concentrations of leptin on antioxidant capacity of mouse skin tissues exposed to UV irradiation

Group	Blank group	Model group	Low leptin concentration group (5 μg/mL)	Moderate leptin concentration group (10 μg/mL)	High leptin concentration group (20 μg/mL)
CAT (U/mL)	10.98 ± 0.87	3.42 ± 0.43*	5.12 ± 0.68*,#	9.24 ± 1.12*,#	6.42 ± 0.77*,#
SOD (U/mL)	68.32 ± 3.43	27.15 ± 2.49*	45.48 ± 3.26*,#	71.98 ± 4.42#	57.58 ± 3.11*,#
GSH-PX (U/mL)	61.23 ± 5.12	9.44 ± 0.56*	19.15 ± 1.32*,#	47.23 ± 5.67*,#	37.61 ± 6.48*,#
MDA (nmol/mL)	2.12 ± 0.13	13.44 ± 1.02*	6.82 ± 1.12*,#	4.88 ± 0.52*,#	7.58 ± 0.99*,#
Hyp (ug/mL)	13.05 ± 1.28	6.44 ± 0.87*	8.42 ± 0.52*,#	9.19 ± 1.31*,#	13.04 ± 1.08#
LDH (U/L)	408.72 ± 15.78	809.23 ± 21.37*	658.44 ± 14.14*,#	511.47 ± 11.23*,#	452.32 ± 9.54*,#
ROS (MFI)	92.93 ± 24.59	171.58 ± 11.51*	92.24 ± 5.58#	71.93 ± 4.12*,#	58.63 ± 4.13*,#

*, compared with the blank group, $P < 0.05$; #, compared with model group, $P < 0.05$.

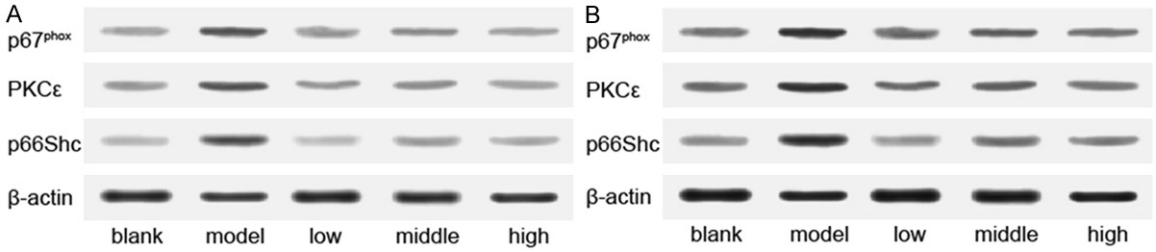

Figure 5. Protein expressions of $p67^{phox}$, PKCε and p66Shc in human skin fibroblasts (HSFs) (A) and mouse skin tissues (B).

CAT, SOD, GSH-Px and Hyp in leptin-treated groups increased significantly (all $P < 0.05$), while the levels of MDA, LDH and ROS decreased significantly (all $P < 0.05$). However, there is still a significant difference in the CAT levels between the leptin-treated groups and the blank group (all $P < 0.01$). The levels of SOD in moderate/high leptin concentration groups were higher than those in blank group, the GSH-Px activity in the high leptin concentration group and Hyp content in moderate leptin concentration group were close to those in the blank group (all $P > 0.05$). There was no significant difference in MDA levels between the moderate/high leptin concentration groups and the blank group (all $P > 0.05$). Intracellular ROS concentrations and LDH concentrations in culture medium in leptin-treated groups were significantly different from those in blank control group (all $P < 0.01$). The SOD and LDH concentrations in the leptin-treated groups were in dose dependent; SOD concentrations increased with the increase of leptin concentrations; LDH content decreased with the increase of leptin concentrations (**Table 1**).

In mouse skin tissues, compared with the blank group, the concentrations of CAT, SOD, GSH-Px and Hyp were significantly decreased in the model group after receiving excess UV irradiation (all $P < 0.05$); while the concentrations of MDA, LDH and ROS were significantly higher in the model group than those in the blank group (all $P < 0.05$). Compared with the model group, the concentrations of CAT, SOD, GSH-Px and Hyp in the mouse skin tissue exposed to UV irradiation were significantly increased after pre-smearing the leptin solution (all $P < 0.01$); while the concentrations of MDA, LDH and ROS in the leptin-treated groups decreased significantly (all $P < 0.05$). Higher SOD concentrations were found in the skin tissues of mice in the moderate leptin concentration group in comparison with the blank group ($P < 0.01$); the concentration of Hyp in the high leptin concentration group was close to that in the blank group, without significant difference ($P > 0.05$). ROS concentrations in low leptin concentration group were close to the blank group, without significant difference ($P > 0.05$) (**Table 2**).

Protein expressions of $p67^{phox}$, PKCε *and* p66Shc *in HSFs and mouse skin tissues*

Compared with the blank group, the expressions of $p67^{phox}$, PKCε and p66Shc in the model

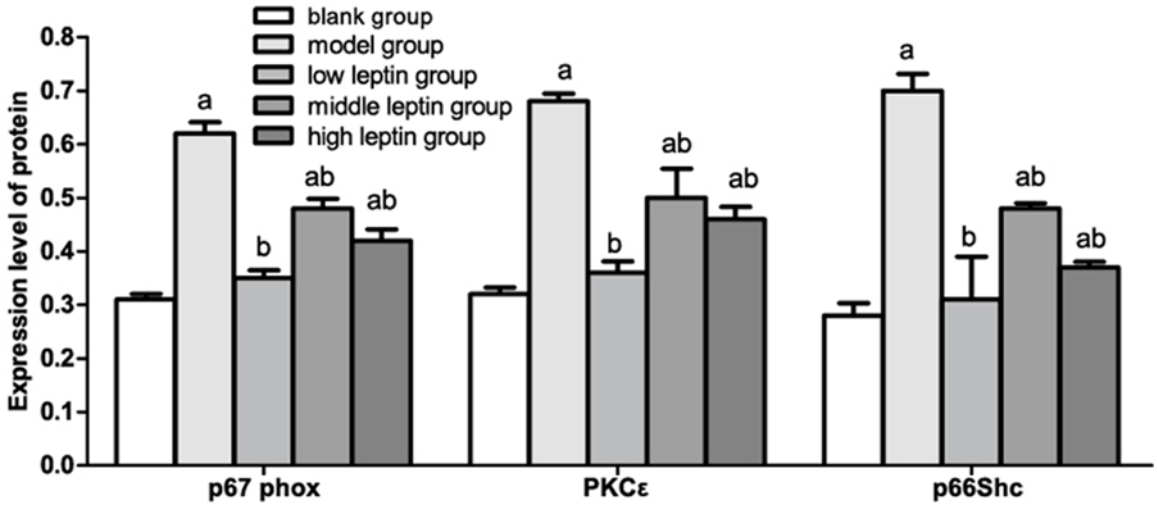

Figure 6. Protein expressions of $p67^{phox}$, PKCε and p66Shc in human skin fibroblasts (HSFs) in blank group, model group and leptin-treated groups; a, compared with the blank group, $P < 0.05$; b, compared with the model group, $P < 0.05$.

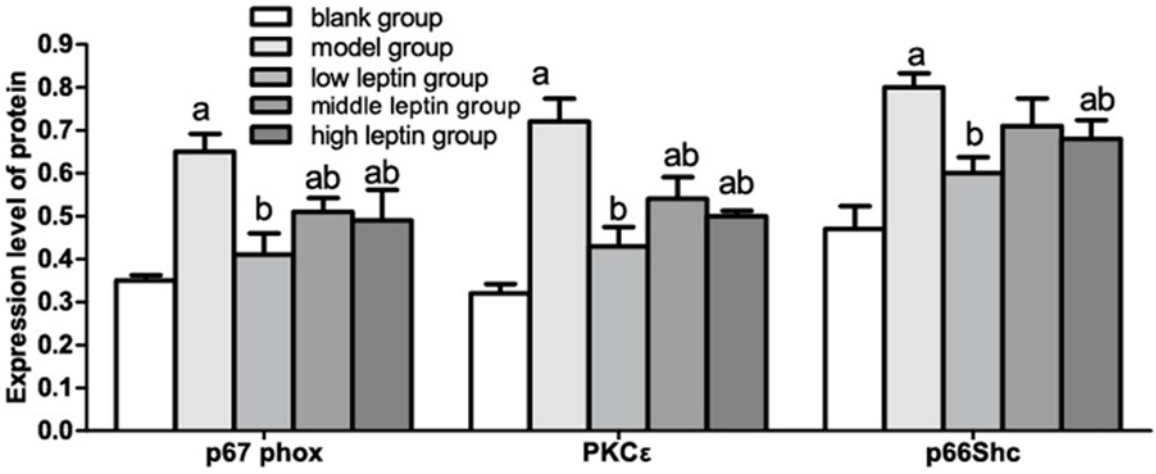

Figure 7. Protein expressions of $p67^{phox}$, PKCε and p66Shc in mouse skin tissues in blank group, model group and leptin-treated groups; a, compared with the blank group, $P < 0.05$; b, compared with the model group, $P < 0.05$.

group was significantly up-regulated in both HSFs (**Figure 5A**) and mouse skin tissues (**Figure 5B**) (all $P < 0.05$); while $p67^{phox}$, PKCε and p66Shc expressions in the leptin-treated groups decreased significantly compared with the model group (all $P < 0.05$). In HSFs, the expressions of $p67^{phox}$, PKCε and p66Shc in the low lectin concentration group were close to the blank group, the expressions of $p67^{phox}$, PKCε and p66Shc in the middle/high leptin concentration groups were significantly higher than those in the blank group (all $P < 0.05$). In mouse skin tissues, $p67^{phox}$ expressions in the low leptin concentration group were close to the blank group ($P < 0.05$), slight differences were detected in the expressions of PKCε and p66Shc between the low leptin concentration group and the blank group, but without statistically significance (all $P > 0.05$). In both HSFs and mouse skin tissues, the expressions of $p67^{phox}$, PKCε and p66Shc in the middle/high leptin concentration group were significantly higher than those in the blank group (all $P < 0.05$) (**Figures 6**, **7**).

Discussion

In both HSFs and mouse skin tissues, significantly lower levels of CAT, SOD, GSH-Px and Hyp, but significantly increased levels of MDA,

LDH and ROS, as well as significantly up-regulated expressions of $p67^{phox}$, PKCε and p66Shc were observed in the model group relative to the blank group, suggesting that antioxidant ability of HSFs was severely damaged in UV-induced photoaging model. In wound healing process caused by UV-induced photoaging, oxidation and oxidation resistance together play roles in the whole process of wound repair, the infiltration of inflammatory cells and the release of inflammatory mediators can produce a large amount of oxygen free radicals in the injured tissues, resulting in oxidative damage through multiple mechanisms [30-32]. According to the free radical theory of aging initially developed by Harman in 1956, ROS increases with aging due to the reduced activity of the antioxidant defense enzymes, such as SOD, CAT and GSH-Px [33]. As previously mentioned, UV irradiation and increased ROS production are associated with photoaging accompanied by PKCε down-regulation and nuclear translocation, CAT decreased in irradiated mice, significantly lower immunoreactivity and protein levels of SOD and GSH-Px were found in the UVB-irradiated skin [4, 6, 34]. In addition, the altered levels of MDA can indirectly reflect the degree of oxidative stress injury, and a previous study revealed an increase in MDA in photoaged skin [4]; LDH is released during tissue damage, increased LDH levels may be triggered by skin exposed to UV radiation (photoaging), Hyp is a major component of the protein collagen, and significantly lower levels of Hyp may indicate a shutdown of collagen synthesis [35]. In human skin, oxidative stress is widely considered a key mechanism underlying the detrimental effects of acute and chronic UV irradiation exposure, and up-regulated expressions of $p67^{phox}$, PKCε and p66Shc may probably through their involvement in oxidative stress regulation [8]. And $p67^{phox}$, PKCε and p66Shc are also demonstrated to be relevant with oxygen radical theory of aging, targeting PKC epsilon may be beneficial in selectively modulating wound healing and fibrotic responses in vivo, and down-regulation of a p66Shc-dependent signaling pathway may be a potential prevention against skin aging and even photoaging [36-39].

HSFs and mice exposed to UV irradiation were treated with leptin to investigate effects of leptin on UV-induced skin photoaging, and findings in the present study revealed that the significantly increased levels of CAT, SOD, GSH-Px and Hyp and significantly decreased levels of MDA, LDH and ROS, as well as down-regulated $p67^{phox}$, PKCε and p66Shc expressions were detected in leptin-treated groups compared with the model group, indicating that leptin may alleviate ultraviolet-induced skin photoaging with its antioxidant ability. The possible explanation for the role of leptin in alleviating ultraviolet-induced skin photoaging may be that leptin may play important biological roles in improving skin defense mechanisms by maintaining antioxidant enzyme activities and modulating mitochondrial oxidative stress with antioxidant and free radical-defusing effects [28, 29]. Previous evidence suggested that binding of leptin at the macrophage cell surface increases is associated with oxidative stress- and PKC-dependent pathways [40]; and leptin deficiency-induced obesity exacerbates UVB-induced inflammatory responses and UVB-induced inflammation-associated skin diseases [41]. Kim E revealed that ultraviolet-induced decrease of leptin and adiponectin in subcutaneous fat may contribute to exacerbation of photoaging process [42]. To gain further insight into the role of leptin in photoaging, mice in the leptin-treated groups were treated with different concentrations of leptin (5, 10 and 20 μg/mL leptin), and the results implied that the effects of leptin on photoaging were in dose dependent.

In conclusion, leptin may prevent skin photoaging by scavenging free radicals, improving antioxidant capacity and enzyme activity, alleviating oxidative damage and promoting collagen synthesis in HSFs and mouse skin tissues.

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Disclosure of conflict of interest

None.

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