Effect of UVA irradiation on proliferation and NO/iNOS system of human skin fibroblast

CHEN Mingliang[1], ZHANG Guiying[2], YI Mei[1], CHEN Xiao[3], LI Ji[1], XIE Hongfu[1], CHEN Xiang[1]

(1. *Department of Dermatology, Xiangya Hospital, Central South University, Changsha* 410008;
2. *Department of Dermatology, Second Xiangya Hospital, Central South University, Changsha* 410011;
3. *Department of Dermatology, First Hospital of Changde, Changde Hunan* 415000, *China*)

Abstract: Objective To investigate the effect of different dosages of ultraviolet A (UVA) on the proliferation and inducible nitric oxide synthase (iNOS)/nitric oxide (NO) system of human skin fibroblasts and to study the mechanism of skin photoaging. **Methods** Fibroblasts from normal skin cultured in vitro were irradiated with 1, 5, and 10 J/cm^2 of UVA, respectively. The proliferation activity, expression of iNOS mRNA/protein and NO production of human skin fibroblasts at different time points after irradiation of different dosages of UVA were measured by MTT assay, RT-PCR, Western blotting, and Griess reaction, respectively. **Results** The survival rate of normal fibroblasts with time increase during 72 h. Low level of iNOS mRNA/protein expression and NO production was detected in normal human skin fibroblasts. But at each time point after 5 and 10 J/cm^2 dosage of UVA, the decrease of cell survival rate and the increase of iNOS mRNA/protein expression and NO production became more significant than those in the control group at the same time and 1 J/cm^2 UVA-irradiated group. There were significant differences ($P<0.01$ or $P<0.05$), which were most significant at 24 h after UVA irradiation. There was significant difference compared with that at 48 h and 72 h after the same UVA dosage ($P<0.01$). **Conclusion** UVA can inhibit the proliferation activity of human skin fibroblasts. It might be related to the up-regulation of *iNOS* gene expression and the over-secretion of NO induced by UVA.

Key words: ultraviolet A (UVA); fibroblasts; survival rate; inducible nitric oxide synthase (iNOS); nitric oxide (NO)

[*J Cent South Univ (Med Sci)*, 2009,34(8):0705-07]

收稿日期(Date of reception) 2009-02-26

作者简介(Biography) CHEN Mingliang, Ph. D., associate professor, mainly engaged in the research of skin photoaging and skin tumor.
通讯作者(Corresponding author) ZHANG Guiying, E-mail: zhangguiying67@yahoo.com.cn

长波紫外线对人皮肤成纤维细胞增殖及NO/iNOS系统的影响

陈明亮[1],张桂英[2],易梅[1],陈潇[3],李吉[1],谢红付[1],陈翔[1]
(1.中南大学湘雅医院皮肤科,长沙 410008;2.中南大学湘雅二医院皮肤科,
长沙 410011;3.常德市第一医院皮肤科,湖南 常德 415000)

[摘要] **目的**:观察不同剂量长波紫外线(UVA)对人皮肤成纤维细胞增殖及诱导型一氧化氮合酶(iNOS)/一氧化氮(NO)系统的影响,探讨皮肤光老化机制。**方法**:用1,5,10 J/cm^{2}剂量UVA照射培养的人原代皮肤成纤维细胞。分别采用四唑盐比色实验(MTT法)、逆转录-聚合酶链反应(RT-PCR)、Western印迹以及Griess反应等技术检测UVA照射后培养不同时间(24,48和72 h)人皮肤成纤维细胞增殖情况、iNOS mRNA和蛋白表达水平及NO生成量。**结果**:正常人成纤维细胞在72 h之内随培养时间延长细胞存活率增加,仅检测到低水平iNOS表达和NO生成,但在5和10 J/cm^{2}剂量UVA照射后各时间点成纤维细胞存活数下降,iNOS mRNA/蛋白表达水平和NO生成量随着UVA剂量的增加升高,与同一时间点对照组和1 J/cm^2 UVA照射组相比,差异有统计学意义($P<0.01$或$P<0.05$),而且以各剂量UVA照射后24 h时间点细胞存活数下降、iNOS mRNA/蛋白表达水平和NO生成量增高最为明显,与同剂量UVA照射后48 h和72 h组相比,差异有统计学意义($P<0.01$)。**结论**:UVA抑制人皮肤成纤维细胞的增殖可能与其诱导*iNOS*基因的表达和NO的分泌有关。

[关键词] 长波紫外线; 成纤维细胞; 存活率; 诱导型一氧化氮合酶; 一氧化氮
[中图分类号] R751 [文献标识码] A [文章编号] 1672-7347(2009)08-0705-07

Ultraviolet (UV) is one of the essential environmental factors that induce inflammation in skin tissue. Ultraviolet A (UVA), though not so strong in biological energy as ultraviolet B (UVB), makes up to 95% of ultraviolet radiation energy in the sunlight and has better penetration capability than UVB, which can cause injury of the cutaneous fibroblasts in the dermis of the skin. Inducible nitric oxide synthase (iNOS), a crucial enzyme involved in inflammation, exists in many kinds of cells of normal skin tissue and exhibits a low-level activity. Once stimulated by inflammatory factors, iNOS can be activated and produce large amount of nitric oxide (NO), which is an active free radical with oxidoreduction trait. NO can react with oxygen-derived free radicals in vivo and generate a more active factor, $ONOO^-$, thus results in cytotoxicity and injury[1-2]. Previous studies have reported that UVB radiation inhibits the proliferation and facilitates the apoptosis of cultured keratinocyte, and induces keratinocytes to synthesize iNOS, which catalyzes NO production and release, and thus contributes to the injury of skin[3-5]. However, no studies on the effect of UVA on iNOS expression and NO production in human fibroblasts have been reported so far. In this study, to discuss the pathogenic mechanisms of UVA-induced skin light-injury, we investigated the effects of UVA radiation on proliferation, iNOS mRNA and protein expression, and NO production of human primary cutaneous fibroblasts.

1 MATERIALS AND METHODS

1.1 Reagents

Dulbecco's modified eagle medium (DMEM) and calf serum were purchased from Gibco Company, USA. Trizol reagent was obtained from Invitrogen Company, USA. MTT reagent was obtained from Sigma-Aldrich Company, USA. Reverse transcription kit was purchased from Ferments Company,

Lithuania. Polymerase chain reaction kit was obtained Tianwei Times Technology Company in Beijing, China. NO reagent kit was purchased from Jiancheng Biochemical Company in Nanjing, China. Rabbit anti-human iNOS polyclonal antibody, rabbit anti-human GAPDH monoclonal antibody, and mouse anti-rabbit HRP-IgG were obtained from Santa Cruz Company, USA.

1.2 Methods

1.2.1 Primary culture of human skin fibroblasts

Human skin fibroblasts isolated from children foreskins after circumcision surgery were routinely cultured in DMEM. Cells were harvested with trypsinase when they got 80% confluence and placed in 6-well plates at a density of 1×10^5 cells/well. After incubated for 48 h, the culture medium was removed. Keratin and vimentin were confirmed by immunocytochemistry. Cells were cultivated successfully and stocked in nitrogen canister. Cells ranged 4 ~ 10 passages were used in our experiments.

1.2.2 Ultraviolet radiation

Cells were irradiated using a UVA desktop equipment (Sigma-Aldrich, USA) which releases UVA wave ranged from 320 to 400 nm. Exposure distance is 15 cm. UVA dosage = UVA radiation intensity × time (s) (single exposure). We set UVA dosages as 1, 5, and 10 J/cm^2 according to the reference[6].

1.2.3 Cell culture and treatment

Fibroblast cells were cultured in 100 mm culture dishs at a density of 2×10^5/mL in a humidified incubator containing of 5% CO_2 at 37°C. Cells were assigned to 4 groups when they got 80% confluence. (1) control group (UVA untreated; 24, 48, and 72 h), (2) 1 J/cm^2 UVA exposure group (24, 48, and 72 h), (3) 5 J/cm^2 UVA exposure group (24, 48, and 72 h), (4) 10 J/cm^2 UVA exposure group (24, 48, and 72 h). Before UVA exposure, the culture medium was removed and a little PBS was added, then cells were place right under the UVA light. After the exposure, fresh medium was added and continue cultured for 24, 48, and 72 h, respectively. The culture medium was centrifuged and the supernatant was collected and stocked for measuring NO content. At the same time, cells were collected for extracting RNA and protein.

1.2.4 Cell viability measurement (MTT assay)

Cells were seeded at a density of 5×10^3 cells/well into 96-well plates. Grouping was the same as 1.2.3. Each sample has 6 repeated wells. Cells in the control group were covered with tin foil paper. MTT solution (5 g/L, 20 μL) was added to each well, and the plate was incubated for 4 h. Subsequently the medium was discarded, and 150 μL of dimethylsulfoxide (DMSO) was added into each well. After shaking for 10 min, the absorbance value at the wavelength of 570 nm (A_{570}) was determined by Microplate EL309 Reader. The survival rate (%) = [(A_{570} of sample − A_{570} of the blank)/(A_{570} of the control − A_{570} of the blank)] × 100%.

1.2.5 RNA extraction and RT-PCR

Total cellular RNA was extracted using TRIzol reagent. cDNA was obtained by RT-PCR. Primer sequences were as follows: iNOS forward 5′-CGGTGCTGTATTTCCTTACGAGGCGAAGAA-3′, reverse 5′-GGTGCTGCTTGTTAGGAGGTCAAGTAAAGG-3′ (259 bp). *GAPDH* forward 5′-TGGATATTGTTGCCATCAATGACC-3′, reverse 5′-GATGGCATGGACTGTGGTCATG-3′ (460 bp). The parameters were pre-denaturation at 94 ℃ for 5 min, cycle times were 1 min at 94 ℃, 1 min at 55 ℃ and 2 min at 72 ℃ for 32 cycles and a final extension at 72 ℃ for 10 min. PCR products (10 μL) were electrophoresed and the gray-scale ratio was calculated using digital gel image analysis system.

1.2.6 Western blot

Equal amounts of denatured cell lysates (40 μg protein) were resolved by 10% sodium dodecyl sulfate polycrylamide gel electrophoresis (SDS-PAGE) and transferred onto nitrocellular membranes (0.45 μmol/L). After incubation in 0.1% Tween-PBS containing 5% milk for 1 h, the membranes were immunoblotted overnight with primary antibodies against iNOS or GAPDH at 1∶1 000 dilution at

4 ℃. Then the membranes washed 5 min with 0.1% Tween-PBS for 3 times and incubated 1.5 h at room temperature with horseradish peroxide (HRP)-conjugated secondary antibody (1∶2 000 dilution).

After washing 5 min with 0.1% Tween-PBS for 3 times, the membranes was exposed on film using enhanced chemoluminescence system. The density ratio of iNOS/GAPDH was quantified by digital gel image analysis system.

1.2.7 NO concentration measurement

The NO content (μmol/L) in the culture medium of each group was measured according to the protocol of the NO testing kit purchased from Jiancheng biochemical company in Nanjing, China.

1.3 Statistical analysis

All experiments were repeated at least 4 times. Data were expressed as mean ± standard deviation ($\bar{x} \pm s$) and analyzed by SPSS13.0 software. One-way ANOVA was used for inter-group comparison and single factor analysis of variance was used for group comparison. $P < 0.05$ was considered statistically difference.

2 RESULTS

2.1 UVA irradiation effects on proliferation of human skin fibroblast

We found that the survival rate of cells treated with 1 J/cm^2 UVA at different time points (24, 48, and 72 h) showed no significant difference compared to the control cells at the same time points. While survival rate of cells treated with 5 or 10 J/cm^2 UVA significant lower than that of the control cells ($P < 0.01$). The survival rate decreased in an UVA dose dependent manner ($P < 0.01$). The survival rate of cells 24 h after exposure to 5 and 10 J/cm^2 UVA is much lower than that of 48 and 72 h ($P < 0.05$, Tab.1).

2.2 UVA irradiation effects on iNOS mRNA expression in human skin fibroblast

At the same time point after various doses of UVA irradiation, the expression of iNOS mRNA was raised with the dose of UVA increasing ($P < 0.05$). While at the same dose of UVA irradiation, the expression of iNOS mRNA at 24 h was higher than that in other time points ($P < 0.01$, Fig. 1, Tab. 2).

2.3 UVA irradiation effects on iNOS protein expression in human skin fibroblast

iNOS protein was poorly expressed in control group at each time point, while expressed in experimental group at each time points. At the same time point, the iNOS protein expression was increased with the dose of UVA increasing ($P < 0.01$). While at the same dose of UVA exposure, the iNOS protein expression at 24 h was higher than that in other time points ($P < 0.01$, Fig. 2, Tab. 3).

2.4 UVA irradiation effects on NO production in human skin fibroblast

NO contents in supernatant at each time point (24, 48, and 72 h) were increased compared to control group ($P < 0.01$). At the same time point, the NO production was increased with the dose of UVA increasing ($P < 0.01$). While at the same dose of UVA exposure, the NO production at 24 h was higher than that in other time points ($P < 0.01$, Tab. 4).

Tab. 1 Survival rate of human fibroblasts after different doses of UVA irradiation at each time points ($\bar{x} \pm s, n = 6$, %)

Groups	24 h	48 h	72 h
Control	81.22 ± 4.92	85.91 ± 5.01	91.61 ± 2.31
UVA 1 J/cm^2	76.61 ± 4.76	81.14 ± 3.85	85.02 ± 4.04
UVA 5 J/cm^2	56.92 ± 4.45**	65.18 ± 5.65**△	73.93 ± 5.16**△
UVA 10J /cm^2	39.08 ± 2.86**##	46.04 ± 1.18**##△	54.40 ± 2.45**##△

Compared with the group of control and 1 J/cm^2 UVA at the same time point, **$P < 0.01$; compared with 5 J/cm^2 UVA at the same time point, ##$P < 0.01$; compared with the 24 h group of the same dose of UVA, △$P < 0.05$.

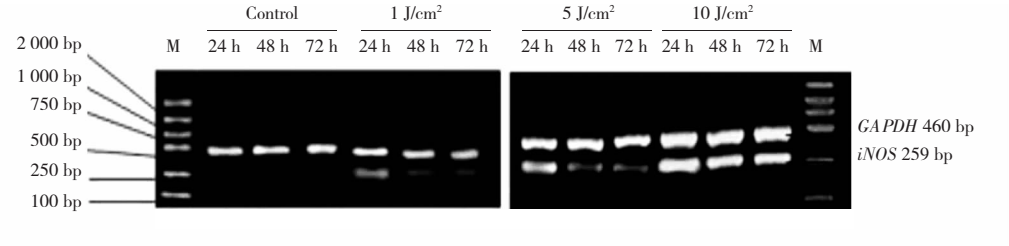

Fig. 1 Fibroblast *iNOS/GAPDH* gene product RT-PCR gel electrophoresis after various doses of UVA irradiation at each time points.

Tab. 2 Fibroblast iNOS/GAPDH mRNA gray-scale ratio after various doses of UVA radiation at each time points ($\bar{x} \pm s$, $n = 4$)

Groups	24 h	48 h	72 h
Control	0.009 ± 0.004	0.018 ± 0.006	0.014 ± 0.008
UVA 1 J/cm²	0.467 ± 0.015	0.313 ± 0.014$^{\triangle\triangle}$	0.122 ± 0.021$^{\triangle\triangle}$
UVA 5 J/cm²	0.910 ± 0.013*	0.530 ± 0.012$^{*\triangle\triangle}$	0.217 ± 0.020$^{*\triangle\triangle}$
UVA 10 J/cm²	1.672 ± 0.024$^{*\#}$	0.968 ± 0.021$^{*\#\triangle\triangle}$	1.017 ± 0.104$^{*\#\triangle\triangle}$

Compared with the group of control and 1 J/cm² UVA at the same time point, $*P < 0.05$; compared with 5 J/cm² UVA at the same time point, $\#P < 0.05$; compared with the 24 h group of the same dose of UVA, $\triangle\triangle P < 0.01$.

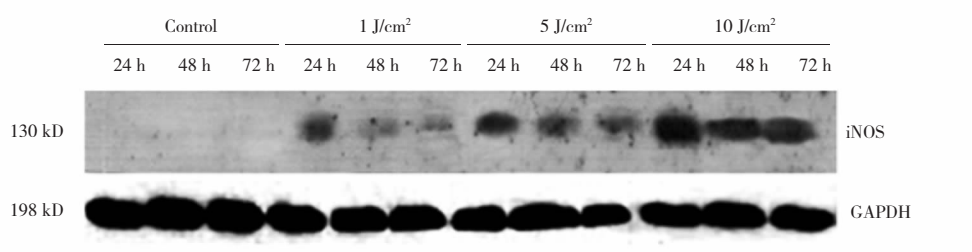

Fig. 2 iNOS Western blot analysis of gel electrophoresis after various doses of UVA radiation at each time points.

Tab. 3 Human fibroblasts iNOS/GAPDH protein gray-scale ratio after various doses of UVA radiation at each time points ($\bar{x} \pm s$, $n = 4$)

Groups	24 h	48 h	72 h
Control	0.012 ± 0.003	0.003 ± 0.000	0.001 ± 0.000
UVA 1 J/cm²	0.163 ± 0.012	0.058 ± 0.008$^{\triangle\triangle}$	0.028 ± 0.001$^{\triangle\triangle}$
UVA 5 J/cm²	0.269 ± 0.016**	0.121 ± 0.009$^{**\triangle\triangle}$	0.051 ± 0.003$^{**\triangle\triangle}$
UVA 10 J/cm²	0.446 ± 0.011$^{**\#\#}$	0.273 ± 0.012$^{**\#\#\triangle\triangle}$	0.237 ± 0.008$^{**\triangle\triangle}$

Compared with the group of control and 1 J/cm² UVA at the same time point, $**P < 0.01$; compared with 5 J/cm² UVA at the same time point, $\#\#P < 0.01$; compared with the 24 h group of the same dose of UVA, $\triangle\triangle P < 0.01$.

Tab. 4 UVA irradiation effects on NO production in human skin fibroblast ($\bar{x} \pm s$, $n = 4$, μmol/L)

Groups	24 h	48 h	72 h
Control	4.39 ± 0.55	5.04 ± 0.16	5.81 ± 0.39
UVA 1 J/cm²	11.05 ± 1.31	7.81 ± 1.03$^{\triangle\triangle}$	6.53 ± 0.64$^{\triangle\triangle}$
UVA 5 J/cm²	24.66 ± 3.58**	14.69 ± 1.56$^{**\triangle\triangle}$	10.84 ± 1.26$^{**\triangle\triangle}$
UVA 10 J/cm²	39.84 ± 3.34$^{**\#\#}$	25.4 ± 3.03$^{**\#\#\triangle\triangle}$	22.15 ± 1.34$^{**\#\#\triangle\triangle}$

Compared with the group of control and 1 J/cm² UVA at the same time point, $**P < 0.01$; compared with 5 J/cm² UVA at the same time point, $\#\#P < 0.01$; compared with the 24 h group of the same dose of UVA, $\triangle\triangle P < 0.01$.

3 DISCUSSION

Fibroblasts are the main cell components for the repairment of injury in skin tissue. Under normal conditions, they are in the resting state. In case of skin injury or pathological lesions, fibroblasts exhibit abnormalities in proliferation and metabolism, as well as changes of gene expression. Therefore, culturing primary cutaneous fibroblasts provides an important tool for studying skin photoaging and other diseases related to fibroblast disfunction.

Aging of the skin is a complex pathophysiological process that influences multi-layers of the skin structure, among which the most obvious changes occur in the dermis layer. And fibroblasts are the most important cell components in the dermis, forming the main structural basis of the dermis function. Studies have validated that UVA radiation can cause injury to nuclear and mitochondrial DNA, induce apoptosis of multi-types of cells and influence the expression of genes such as *ICAM*-1[7] and *MMPs*[8-9], as well as cytokines such as *IL*-6[10] and IL-10[11]. Leccia, et al.[12] observed the enhancement of apoptosis in human cutaneous fibroblasts with UVA radiation treatment by examining cytoplasmic nucleosomes with ELISA methods, which was the first report to verify the apoptosis-inducing effect of UVA on normal human cutaneous fibroblasts. Investigations by domestic researchers[13-16] in the recent years have found that UVA can also cause damage to collagen synthesis in primary-cultured dermis fibroblasts, cause cell crenation, decrease proliferation, induce apoptosis, and induce overexpression of 5-lipoxygenase mRNA in these cells. However, study on the effect of UVA on iNOS expression and NO production in cutaneous fibroblasts has been rare. Moreover, even in the few studies on this problem, the dosage of UVA radiation used was overload, and the observation was not successive. Our results showed that UVA radiation could decrease the proliferation of fibroblasts, induce the overexpression of iNOS, and enhance NO production in fibroblasts. These effects exhibited a correlation with the dosage of UVA radiation and cultured time period post-radiation. These data indicated that UVA radiation could not only damage the proliferation capability of fibroblasts but also activate the iNOS/NO pathway in fibroblasts. The iNOS/NO pathway exists in all kinds of cells in the skin including keratinocytes, melanocytes, Langerhans cells, fibroblasts, and endothelial cells, and participates in inflammation and immune response of the skin. The failure of NO signal cascade is implicated in inflammation, hypertrophy, autoimmune skin diseases, and skin cancer[1]. As a dual-function free radical with both apoptosis-inducing and anti-apoptosis effects, NO exhibits either effect depending on the concentration of NO, the type of target cells, and the pathophysiological environment of the cells. Namely, in low concentration, NO has anti-apoptosis effect, whereas in high concentration, NO induces cell apoptosis by facilitating the generation of $ONOO^-$. In this study, the decrease of proliferation and the enhancement of iNOS/NO system of human skin fibroblast was most significant at 24 h after UVA radiation, which was coincident with the dynamic characteristics of the appearance of sunburn peak at 24 h and continuing 2 ~ 3 d. It indicated that the inhibition of fibroblast proliferation by UVA might be related to the stimulation of iNOS/NO pathway by UVA which causes NO overproduction and leads to apoptosis. Choe, et al.[17] observed that NO donor could increase the expression levels of MMP-1 and MMP-2 by 153% and 243%, respectively in cutaneous fibroblasts, and NOS inhibitor could decrease the synthesis of MMP-1 and MMP-2 in UV-irradiated human cutaneous fibroblasts. This suggested that NO might degraded relevant matrix via promoting the expression of MMP-1 and MMP-2 leading to connective tissue changes observed in photoaging. Greenacre, et al.[18] identified iNOS overexpression and NO overproduction in inflammatory skin of mice, which led to the generation of nitric peroxide, the reaction product of superoxide and NO, and promoted plasma outleakage and inflammatory edema of the skin. Therefore, we conclude that the UVA-induced acute and chronic skin

light-injury might be, at least partly, a consequence of iNOS overexpression and NO overproduction in cutaneous fibroblasts, which inhibits the proliferation and induces the apoptosis of fibroblasts, and increases the expression of MMP-1 and MMP-2, leading to degradation of dermis matrix and thus accelerating the aging of the skin. Our results also suggest that intervention of iNOS or interference of NO synthesis might defer the photoaging of skin, providing experimental evidence for investigating the mechanisms of skin photoaging and for developing novel anti-aging skin care products.

REFERENCES:

[1] Bécherel P A, Le Goff L, Ktorza S, et al. CD23-mediated nitric oxide synthase pathway induction in human keratinocytes is inhibited by retinoic acid derivatives[J]. J Invest Dermatol, 1996, 106(6):1182-1186.

[2] Sawa T, Ohshima H. Nitrative DNA damage in inflammation and its possible role in carcinogenesis[J]. Nitric Oxide, 2006,14(2):91-100.

[3] Chang H R, Tsao D A, Wang S R, et al. Expression of nitric oxide synthases in keratinocytes after UVB irradiation[J]. Arch Dermatol Res, 2003, 295(7): 293-296.

[4] Seo S J, Choi H G, Chung H J. Time course of expression of mRNA of inducible nitric oxide synthase and generation of nitric oxide by ultraviolet B in keratinocyte cell lines[J]. Br J Dermatol, 2002,147(4):655-662.

[5] 杨敏, 王宝玺, 常建民, 等. 中波紫外线对人角质形成细胞 iNOS 的作用[J]. 中国皮肤性病学杂志, 2005, 19(7):401-402.
YANG Min, WANG Baoxi, CHANG Jianmin, et al. Level of iNOS synthase in cultured human keratinocytes effected by UVB irradiation[J]. Chinese Journal of Dermatovenereology, 2005, 19(7): 401-402.

[6] Francz P I, Conrad J, Biesalski H K. Modulation of UVA-induced lipid peroxidation and suppression of UVB-induced ornithine decarboxylase response by all-trans-retinoic acid in human skin fibroblasts in vitro[J]. Biol Chem, 1998, 379(10):1263-1269.

[7] Treina G, Scaletta C, Fourtanier A, et al. Expression of intercellular adhesion molecule-1 in UVA-irradiated human skin cells in vitro and in vivo[J]. Br J Dermatol, 1996, 135(2):241-247.

[8] Naru E, Suzuki T, Moriyama M, et al. Functional changes induced by chronic UVA irradiation to cultured human dermal fibroblasts[J]. Br J Dermatol, 2005,153(Suppl 2):6-12.

[9] Herrmann G, Wlaschek M, Lange T S, et al. UVA irradiation stimulates the synthesis of various matrix metalloproteinases (MMPs) in cultured human fibroblasts[J]. Exp Dermatol, 1993, 2(2):92-97.

[10] Wlaschek M, Heinen G, Poswig A, et al. UVA-induced autocrine stimulation of fibroblast derived collagenase/MMP-1 by interrelated loops of interleukin-1 and interleukin-6[J]. Photochem Photobiol, 1994, 59(5):550-556.

[11] Grewe M, Gyufko K, Krutmann J. Interleukin-10 production by cultured human keratinocytes: regulation by ultraviolet B and ultraviolet A1 radiation[J]. J Invest Dermatol, 1995, 104(1):3-6.

[12] Leccia M T, Richard M J, Favier A, et al. Zinc protects against ultraviolet A1-induced DNA damage and apoptosis in cultured human fibroblasts[J]. Biol Trace Elem Res, 1999, 69(3):177-190.

[13] 雷小兵, 谭升顺, 彭振辉. 长波紫外线对成纤维细胞胶原合成的影响[J]. 中国皮肤性病学杂志, 2000,14(4):223-224.
LEI Xiaobing, TAN Shengshun, PENG Zhenhui. Effect of UVA on collagen of fibroblasts' biosynthesis in vitro[J]. Chinese Journal of Dermatovenereology, 2000, 14(4): 223-224.

[14] 倪建华. 长波紫外线对人皮肤成纤维细胞生长的影响[J]. 上海预防医学杂志, 2004, 16(8): 365-367.
NI Jianhua. Adverse effects of ultraviolet A on the growth of fibroblasts of human skin[J]. Shanghai Journal of Preventive Medicine, 2004, 16(8):365-367.

[15] 王红伟, 尚兰琴, 郝卫东. 长波紫外线致人皮肤成纤维细胞凋亡和活性氧生成的作用[J]. 北京大学学报:医学版, 2003, 35(1):69-73.
WANG Hongwei, SHANG Lanqin, HAO Weidong. The induction of apoptosis and reactive oxygen species in human skin fibroblast by ultraviolet A[J]. Journal of Peking University. Health Sciences, 2003, 35(1):69-73.

[16] 闫言, 王宝玺, 左亚刚, 等. 长波紫外线照射对皮肤成纤维细胞产生白三烯 B4 及 5-脂氧合酶 mRNA 表达的影响[J]. 中华皮肤科杂志, 2005, 38(11): 697-698.
YAN Yan, WANG Baoxi, ZUO Yagang, et al. Experiment study of leukotriene B4 and 5-lipoxygenase expression of skin fibroblast cells after UVA irradiation[J]. Chinese Journal of Dermatology, 2005, 38(11):697-698.

[17] Choe T, Lee B, Park I, et al. Inhibition of matrix metalloproteinase-1 and -2 expression using nitric oxide synthase inhibitors in UV-irradiated human dermal fibroblasts[J]. J Cosmet Sci, 2003, 54(3):229-238.

[18] Greenacre S, Ridger V, Wilsoncroft P, et al. Peroxynitrite: a mediator of increased microvascular permeability?[J]. Clin Exp Pharmacol Physiol, 1997, 24(11): 880-882.

(Edited by GUO Zheng)