Influence of intense pulsed light on the secretion of TGF-β_1 in cultured human fibroblasts and intervention of JNK inhibitor

LU Jianyun, YU Xiaofeng, KANG Jian, OUYANG Zehui,
GENG Xuerui, XIANG Yaping, HUANG Jinhua

(*Department of Dermatology, Third Xiangya Hospital, Central South University, Changsha* 410013, *China*)

Abstract: **Objective** To determine the influence of intense pulsed light (IPL) on the secretion of TGF-β_1 in cultured human fibroblasts and the intervention of JNK inhibitor. **Methods** The callan foreskin fibroblasts were cultured and divided into 2 groups. In the IPL treatment group, cells were irradiated with IPL with fluences of 0 (negative control), 10, 18, 27, 36, and 36 J/cm^2 × 2 (irradiated with IPL with fluences of 36 J/cm^2 twice). In the IPL + inhibitor group, cells were irradiated with IPL with fluences of 36 J/cm^2 after incubation with the inhibitor SP600125 for 2 h. TGF-β_1 in the culture supernatant was evaluated 48 h after the irradiation using enzyme-linked immunosorbent assay. **Results** Compared with the negative control, TGF-β_1 in the culture supernatant decreased at the IPL irradiation of 10, 18, 27, and 36 J / cm^2, whereas TGF-β_1 increased at the IPL irradiation of 36 J/cm^2 × 2. In the IPL + inhibitor group, the concentration of TGF-β_1 in the culture supernatant decreased compared with the controls ($P<0.05$). **Conclusion** IPL can suppress the secretion of TGF-β_1 at the lower fluence and promote the secretion at a higher fluence. JNK inhibitor may play an inhibitive role when IPL regulates the TGF-β_1 secretion in cultured human fibroblasts. IPL may stimulate TGF-β_1 secretion of the fibroblast cells in human skin via JNK signal pathway.

Key words: intense pulsed light; fibroblasts; JNK; transforming growth factor β_1

强脉冲光对成纤维细胞分泌 TGF-β_1 的影响及 JNK 抑制剂的干预作用

鲁建云, 于小峰, 康健, 欧阳泽辉, 耿雪瑞, 向亚平, 黄进华

(中南大学湘雅三医院皮肤科, 长沙 410013)

[**摘要**] **目的**:研究强脉冲光(intense pulsed light, IPL)照射对皮肤成纤维细胞分泌 TGF-β_1 的影响及 JNK 抑制剂 SP600125 的干预作用。**方法**:利用包皮环切术切除的包皮组织体外分离、培养原代成纤维细胞。然后,将成纤维细胞分为两组试验:第 1 组为 IPL 治疗组,用能量密度分别为 0(阴性对照),

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Biography LU Jianyun, doctorial degree, associate professor, mainly engaged in the research of laser beauty.

Corresponding author LU Jianyun, E-mail: kateljy@yahoo.com.cn

10,18,27,36 和 36 J/cm^2 ×2(能量密度为 36 J/cm^2 的 IPL 照射两次)的 IPL 照射;第 2 组为 IPL + 抑制剂组,包括 IPL(对照)和 IPL + SP600125(JNK 抑制剂)两亚组,在加入抑制剂 2 h 后,用能量密度为 36 J/cm^2 的 IPL 照射。48 h 后,采用 ELISA 法检测两组细胞培养上清液(culture supernatants, CS)中 TGF-β_1 的浓度。**结果**:IPL 治疗组 CS 中 TGF-β_1 的浓度在 10,18,27 及 36 J/cm^2 分别与阴性对照组相比较均减少,而在 36 J/cm^2 × 2 时与阴性对照相比较增高;IPL + 抑制剂组 CS 中 TGF-β_1 的浓度 IPL + SP600125 组与对照相比较减少($P < 0.05$)。**结论**:强脉冲光在较低能量密度时抑制皮肤成纤维细胞 TGF-β_1 的分泌,较高能量密度时能促进 TGF-β_1 的分泌;在 IPL 影响成纤维细胞分泌 TGF-β_1 的过程中,JNK 抑制剂起抑制作用,IPL 可能通过 JNK 途径上调 TGF-β_1 的分泌。

[**关键词**] 强脉冲光; 成纤维细胞; JNK; TGF-β_1

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Intense pulsed light (IPL) is a continuous, multi-wavelength non-coherent light, with the wavelength range of 500-1 200 nm. The therapeutic technology of non-stripping, non-invasive skin rejuvenation of IPL under low energy density is called photorejuvenation[1], which is a new technology in the medical cosmetology field in recent years and is extensively applied. However, its molecular biological mechanism and signaling pathway for treatment is rarely reported at home and abroad. In this study, we studied the influence of IPL on TGF-β_1 secreted by in vitro cultured fibroblasts and the intervention of JNK inhibitor.

1 MATERIALS AND METHODS

1.1 Main materials, reagents, and instruments

Foreskin of circumcision for healthy young men was from Department of Urology, Third Xiangya Hospital of Central South University. Trypsin was purchased by Sigma Co, USA. DMEM high glucose medium was purchased by Hyclone Co, USA. Fetal bovine serum was from Tianjin Hao Yang Biological Manufacture Co., Ltd. TGF-β_1 ELISA kit was from Wuhan Boster Biological Engineering Co., Ltd. JNK / MAPK pathway inhibitor SP600125 (10 mg liquid, HPLC purity > 98%) was from Sigma Co, USA. IPL Queen photorejuvenation equipment was produced by Wuhan Miracle Laser Technology Co., Ltd. Microplate reader was produced by Bio-TEK Instruments, Inc.; Electrophoresis meter was from Bio-Rad Inc. USA. ImgeQuant 350 from GE Healthcare Co, USA.

1.2 Methods

1.2.1 Primary fibroblast culture

Tissue pieces were placed in a small beaker, soaked for about 1 min in 75% alcohol, and then rinsed for 2-3 times. After the bloodiness was removed, *D*-Hanks solution containing double resistance (100 U/mL of streptomycin and 100 U/mL penicillin) was added until the rinse water became clear and transparent; and then tissues were transferred to a culture dish to shear the fat and connective tissues and cut them into pieces as possible. Several small pieces were suctioned to put in a culture flask with a suction bend and were placed at the bottom of the culture flask. The tissue corium surface contacted to the bottom of the culture flask; and the distance between the pieces should be 0.5 cm so 20-30 pieces could be placed at the bottom of a 25 mL culture flask. Then a small amount of culture liquid was dripped to the bottle, and vertically put into an incubator at 37℃. After 2-4 h, the culture flask was slowly reversely placed, cultured in still; and on the 2nd day, a small amount of culture liquid was added to the bottle and the culture liquid was replaced every 3 days. The result was observed under an inverted microscope. Fibroblasts could be separated out from the surrounding of tissue pieces 3-5 days later; after one month, the cell coverage could be as high as 80%; and then cells were passaged by trypsin digestion. In this test, 3rd to

8th generations of fibroblasts were used.

1.2.2 Grouping and determination of IPL energy density

The cultured fibroblasts were divided into IPL treatment group and IPL + inhibitor group. IPL treatment group: 0 (negative control), 10, 18, 27, 36, and 36 J/cm^2 × 2 (irradiation twice with the energy density of 36 J/cm^2 IPL). The IPL + inhibitor group: IPL (control); IPL + SP600125. In this test, common therapeutic parameters in clinic for IPL irradiation were selected, with wavelength of 560-1 200 nm, 3 pulses (2.6, 4.2, and 3.6 ms of pulse width), delay of 30 and 40 ms. In the pre-test, MTT method was used to determine the subcellular toxin dose of IPL, and the results showed that, within the energy density range, it had no toxic reaction on the fibroblasts. The cells in the IPL + inhibitor group were subject to higher energy density (36 J/cm^2) irradiation, mainly because the concentration of TGF-β_1 in culture supernatants began to rise when the IPL energy density was 36 J/cm^2 in our preliminary experiment. In addition, in a mouse test, we discovered that the minimal erythema dose (32 J/cm^2) was also close to the energy.

1.2.3 Determination of TGF-β_1 concentration of supernatant in 2 groups with ELISA method

The fibroblasts at the logarithmic growth period were diluted with DMEM culture solution containing 10% fetal bovine serum, and then inoculated to a culture dish of 3 cm diameter, 2 mL with 7×10^5 cells per dish. Cells grew to 70% - 80% 1-2 days later. The DMEM culture solution containing fetal bovine serum was replaced, and subject to synchronization treatment for 24 h. The IPL group was subject to IPL irradiation with the energy density of 0 (negative control), 10, 18, 27, 36, and 36 J/cm^2 × 2; the IPL + inhibitor group was subject to the inhibitor treatment 2 h before irradiation, and then IPL irradiation with the energy density of 36 J/cm^2. After irradiation was over, a complete new culture medium was replaced, and continuously cultured for 2 d in the incubator. The culture supernatants of cells in various groups were collected, then a TGF-β_1 ELISA test kit was used to test the TGF-β_1 concentration of culture supernatants of each group.

1.3 Statistical analysis

SPSS13.0 software was used for statistical analysis of the test data which were expressed with mean ± standard deviation ($\bar{x} \pm s$). Dunnett-t test was used for group comparison. When $P < 0.05$, there was a statistical difference between the groups.

2 RESULTS

2.1 TGF-β_1 concentration of culture supernatants in the IPL treatment group after IPL irradiation

As shown in Figure 1 the relative concentration of (ratio of every group and negative control) TGF-β_1 in culture supernatants in the IPL group with fluences of 10, 18, 27, and 36 J/cm^2, decreased about 30%, 20%, 80%, and 17%, respectively, compared with the negative control group. The TGF-β_1 content of culture supernatants in the IPL group increased about 45% compared with the negative control group.

2.2 TGF-β_1 concentration of culture supernatants in the IPL + inhibitor group after IPL irradiation

As shown in Figure 2, the TGF-β_1 content of culture supernatants in the IPL + inhibitor group was (13.20 ± 0.98) pg/mL for IPL + SP600125, significantly decreased compared with (20.50 ± 1.78) pg/mL of the control group ($P < 0.05$).

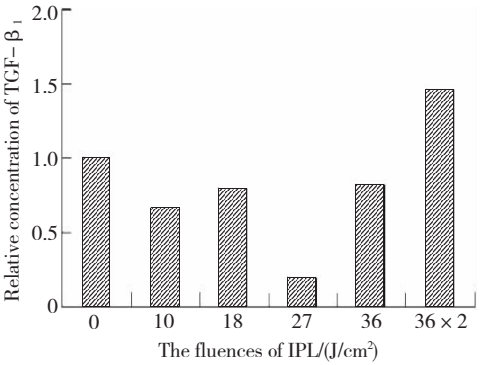

Fig. 1 Relative concentration of TGF-β_1 in culture supernatants of IPL group.

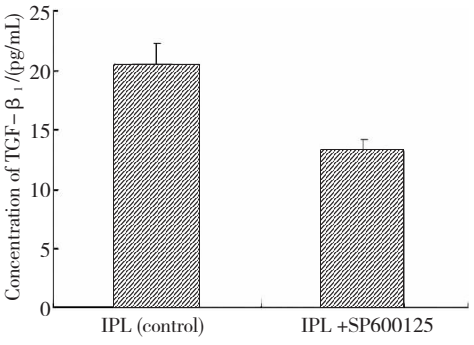

Fig. 2 Concentration of TGF-β_1 in culture supernatants of IPL + inhibitor group.

3 DISSCUSSION

The photobiological and photochemical effect is the main functional mechanism of IPL. When these wavelengths of light radiate on the skin, they are absorbed by skin target tissues such as melanin, collagen fibers, and (or) hemoglobin, to convert into heat energy and produce photothermy; and the "interior blast effect" or "selective photothermolysis"[2] occur. The color spots become fading and even disappear after the melanin was crushed and exuded from skin which are removed out from the kidney. The hemoglobin is denatured and coagulated after heating without or less impact on the surrounding normal tissues, which finally produce vascular occlusion and improve telangiectasia symptoms[3]. In addition, IPL can also promote collagen synthesis and re-arrangement. The thermal effect of IPL can stimulate fibroblast cells to convert into fiber cells and to secrete Collagen I. Meanwhile, the heat energy could also obviously shorten the Type I collagen and reduce skin wrinkles[4]. Therapeutic effect of IPL is associated with appropriate energy density which cause moderate erythematous response in the skin. The exact mechanism of clinical effect of IPL is still unknown.

This study showed that lower than 36 J/cm^2 energy density of IPL irradiation could inhibit the secretion of TGF-β_1 by fibroblasts. However, it could promote its secretion at the energy density of 36 J/cm^2 × 2. Wong, et al.[5] irradiated the fibroblasts at 20, 50, and 75 J/cm^2 of energy density in the model of 570 nm, three-pulse, pulse width 7 ms, delay of 70 ms. They found the secretion of TGF-β_1 presented dose-dependent manner. It could significantly promote the secretion of TGF-β_1 at the energy density of 50 and 75 J/cm^2. Our result is similar to them. It is also the putative explanation why therapeutic effect of IPL is associated with appropriate energy density which cause moderate erythematous response in the skin. The specific molecular mechanism still needs further studies.

TGF-β_1, a TGF-β superfamily member, is a kind of cell factor that regulates cell proliferation, differentiation, apoptosis, adhesion, movement, and ECM generation. Accumulated evidence has shown that TGF-β_1 is an important factor to cause fibrillation and promote collagen proliferation. Bettinger, et al.[6] found that, during the scar proliferation and tissue fibrillation pathological process of the fibroblasts, TGF-β_1 can accelerate the proliferation and division of cultured fibroblasts, and promote the Collagen I gene expression and protein synthesis[7]. These studies supported that, after IPL irradiation, the construction of extracellular matrix (ECM) was improved by increasing the secretion of TGF-β_1.

This study also revealed that the secretion of TGF-β_1 from fibroblasts significantly decreased after

adding JNK inhibitors, which indicate that JNK inhibitor plays the inhibitory effect during the process of IPL affecting the secretion of TGF-β_1 from fibroblasts, and IPL may up-regulate the secretion of TGF-β_1 through JNK pathway. Therefore, the mostly studied mitogen-activated protein kinase (MAPK) signaling pathway is a protein kinase cascade pathway, and c-Jun N-terminal kinase is a common pathway in the eukaryotic cells, which plays important role in the cell growth, development, proliferation, and cell malignant transformation. Previous studies have shown that both P38 and JNK were activated to promote the renal fibrosis during human renal fibrosis process[8]. TGF-β_1 could up-regulate the expression of connective tissue growth factors by activating JNK during the traumatic corneal fibrosis [9]. Ultrasound could activate JNK and erk to promote the healing of wounds[10]. All these studies suggest that JNK is closely related to fibrosis and wound healing. However, whether the fibroblasts secrete TGF-β_1 through activating JNK or other signaling pathway during the IPL reaction is to be further studied.

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