Cancer cell research 8 (2015) 197-200

www.cancercellresearch.org Article

The Influence of paeoniflorin on P21 expression in the course of HaCat cell photoaging

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Abstract: **To investigate the protective effect of Paeoniflorin on UVB-induced apoptosis of HaCat cells. HaCat cells were divided into eight groups, including group A: control group; group B: UVB irradiation group; group C: 0.25mg/L Paeoniflorin experimental group; group D: 0.5 mg/L Paeoniflorin experimental group; group E: 2.5 mg/L paeoniflorin experimental group; group F: 5 mg /L paeoniflorin experimental group; group G: 10 mg/L paeoniflorin experimental group and group H: 12.5 mg/L paeoniflorin experimental group. In addition to the group A, the other groups were irradiated by UVB. The apoptosis rate was analyzed by the flow cytometry. RT-PCR was used to detect the expression levels of P21mRNA. Compared with the group B(4.840±0.236), the apoptosis rate of group C~group F(0.423±0.179, 1.127±0.235, 2.570±0.239, 3.137±0.347) was significantly reduced. The differences were statistically significant (P <0.05); while compared with the group B, the apoptosis rates of the group G and the group H were not statistically significant (P> 0.05); Compared with the group B (1.040±0.007), the expression of P21mRNA of group C~group H (0.440±0.015, 0.551±0.013, 0.848±0.027, 0.850±0.052, 0.923±0.035, 0.936±0.041) was significantly decreased, and the differences were statistically significant (P <0.05). From this experiment, it can be speculated that paeoniflorin have a protective effect on UVB-induced apoptosis of HaCat cells in a certain concentration (0.25 mg/L ~ 5 mg/L), and it can reduce the expression of P21mRNA of Hacat cells.**

Keywords: Paeoniflorin; UVB; HaCat cell

Received 5 November 2015, Revised 22 November 2015, Accepted 25 November 2015

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1. Introduction

UVB (wavelength range, 290–320 nm) radiation in sunlight is the major environmental factor causing skin photoaging [1]. UVB causes DNA damage, erythema, hyperpigmentation and skin cancer. UVB irradiation also induces lipidperoxidation, which is a related free radical process, and can lead to cell death.

In the process of skin photo aging, UVB activates p53, which is well-known to up-regulate p21 depending on UVB doses. P21, a p53-inducible protein, plays an important role in cell cycle, DNA repair, and apoptosis [2-4].

Paeoniflorin (Pae) is the main active monomer of Chinese herbaceous peony. Research shows that paeoniflorin not only has tonic effect, but also can adjust the immune function of the body. The proliferation of the cell can be promoted by the Paeoniflorin.

In the present study, Paeoniflorin exhibits a variety of types of biological activity, including anti-inflammatory, analgesic and anti-oxidative properties [5-6]. HaCaT cells are relevant to UV-induced initiated keratinocytes, and this study yields new insights into the molecular and cellular responses of premalignant epidermal cells to additional UVB damage.

Because normal skin harbors numerous clones of p53-mutated keratinocytes, we used HaCaT cells as a photo aging cellular model. We will investigate the effect of UVB radiation on p21 and its molecular mechanisms and function in human HaCaT keratinocytes.

This study established a model of cellular apoptosis by UVB irradiating HaCat cells and take paeoniflorin as light protective agent. It can detect the effect of paeoniflorin on apoptosis and expression of P21mRNA in the process of UVB-induced HaCat cells damage.

2. Materials and methods

2.1. materials and instruments

HaCaT was kindly provided by the Qingdao University Medical School. 95% paeoniflorin was kindly provided by Ningbo Liwah Pharmaceutical Company. UVB type ultraviolet radiation meter is produced by the photoelectric instrument factory of Beijing Normal University.

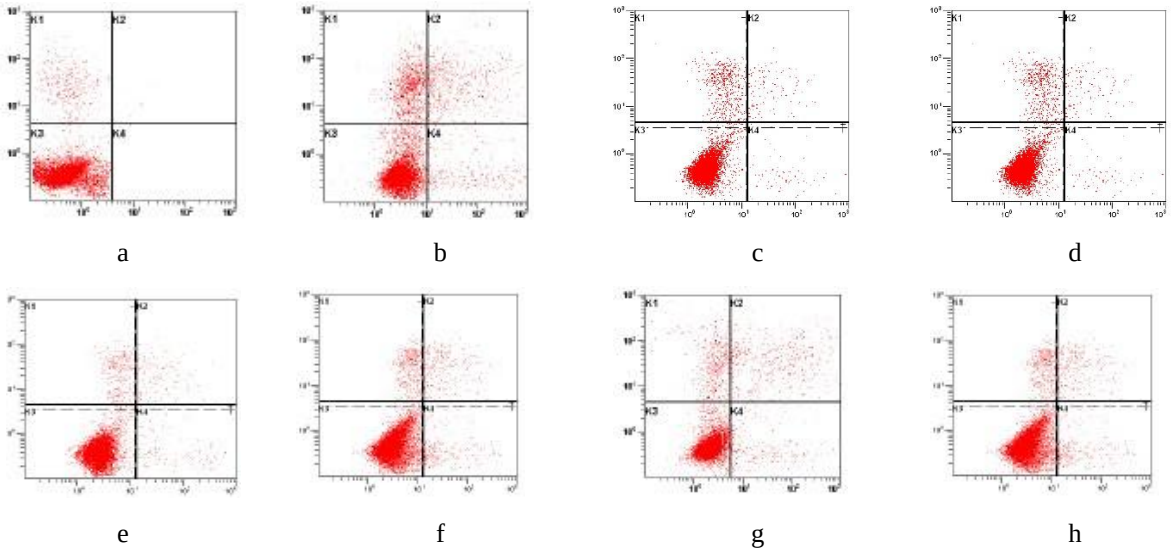
2.2. Methods

2.2.1. Grouping

HaCat cells were divided into eight groups, including group A: control group; group B: UVB irradiation group; Group C: 0.25mg/L Paeoniflorin experimental group; group D: 0.5 mg/L Paeoniflorin experimental group; group E: 2.5 mg/L paeoniflorin experimental group; group F: 5 mg /L paeoniflorin experimental group; group G: 10 mg/L paeoniflorin experimental group and group H: 12.5mg/L paeoniflorin experimental group. In addition to the group A, the other groups were irradiated by UVB.

2.2.2. Cell culture

The HaCaT cells were cultured in Dulbecco's modified eagle's media supplemented with 10% fetal bovine serum, 4.5 g/L glucose and L-glutamine, 100 U/mL penicillin and100 mg/(mL) streptomycin and maintained at 37℃ in an atmosphere containing 5% CO_2.



a b c d e f g h

Figure 1. The results of apoptosis of HaCat cells in A-H group

Table 1 GAPDH used as a reference gene P21 used as purpose primer sequences.

	primer sequence	Tm(℃)	product length（bp）
P21	Upstream 5'-GCGGAACAAGGAGTCAGA-3'	58	233
	Downstream 5'-GGAGAAACGGGAACCAG-3'	58	233
GAPDH	Upstream 5'-TCATGGGTGTGAACCATGAGAA-3'	58	146
	Downstream 5'-GGCATGGACTGTGGTCATGAG-3'	58	146

2.2.3. UVB treatment

Reaching 70–80% confluence the cells were sub-cultured and were used for further experiments. The control group and UVB irradiation group was consisted of normal cells to which Paeoniflorin were not administrated. The Paeoniflorin experimental groups consisted of normal cells to which different concentration of Paeoniflorin were administrated. The HaCaT cells were cultured at 37℃, atmosphere containing 5% CO2 for 24h. Then in addition to the control group, the other groups were irradiated under a UVB lamp. The dosage irradiated in these cells was 20mJ/cm^2. After irradiation these cells were then cultured at 37℃ in an atmosphere containing 5% CO_2 for 6h.

2.2.4. Flow cytometry

A quantitative assessment of apoptosis was made 24 h after UVB exposure using the annexin V-FITC apoptosis detection kit as described by the manufacturer. Briefly, for apoptosis analysis, (0.5–1) ×10^6 cells were resuspended at a concentration of 1×10^6 cells/ml in ice-cold PBS for three times and suspended in 100 μl of binding buffer solution. Cells were then treated with 5 μl of annexin V-FITC and 5 μl of propidium iodide (PI) and placed in the dark at room temperature for 15 min. Fluorescence activated cell sorting (FACS) analysis was done on a cytometer. Using Quest Cell software to collect, store and analyze the apoptosis rate of HaCat cells in each group.

Table 2 The effect of Paeoniflorin on UVB-induced apoptosis and P21mRNA expression in HaCat cells ($\bar{x} \pm s$).

groups	Apoptosis rate(%）	P21mRNA expression
A group	$0.047 \pm 0.015^{*\#\blacklozenge+\star\bullet}$	$0.584 \pm 0.023^{*\circ\blacklozenge+\star\bullet}$
B group	$4.840 \pm 0.236^{\blacktriangle\circ\#\blacklozenge+}$	$1.040 \pm 0.007^{\blacktriangle\circ\#\blacklozenge+\star\bullet}$
C group	$0.423 \pm 0.179^{*\blacklozenge+\star\bullet}$	$0.440 \pm 0.015^{\blacktriangle*\#\blacklozenge+\star\bullet}$
D group	$1.127 \pm 0.235^{\blacktriangle*\blacklozenge+\star\bullet}$	$0.551 \pm 0.013^{*\circ\blacklozenge+\star\bullet}$
E group	$2.570 \pm 0.239^{\blacktriangle*\circ\#\star\bullet}$	$0.848 \pm 0.027^{\blacktriangle*\circ\#\star\bullet}$
F group	$3.137 \pm 0.347^{\blacktriangle*\circ\#\star\bullet}$	$0.850 \pm 0.052^{\blacktriangle*\circ\#\star\bullet}$
G group	$4.203 \pm 0.255^{\blacktriangle\circ\#\blacklozenge+}$	$0.923 \pm 0.035^{\blacktriangle*\circ\#\blacklozenge+}$
H group	$4.323 \pm 0.205^{\blacktriangle\circ\#\blacklozenge+}$	$0.936 \pm 0.041^{\blacktriangle*\circ\#\blacklozenge+}$

▲The other groups were compared with A group: P<0.05； *The other groups were compared with B group: P<0.05；○The other groups were compared with

C group: P<0.05；#The other groups were compared with D group: P<0.05；◆The other groups were compared with E group: P<0.05；+The other groups were compared with F group: P<0.05；☆The other groups were compared with G group: P<0.05；●The other groups were compared with H group: P<0.05.

2.2.5. P21mRNA expression

After treatment with different concentrations of 18h, the cells were treated with UVB. Then the expression of P21mRNA was detected. P21mRNA expression was detected by RT-PCR method, and the gray ratio of P21/GAPDH was analyzed by gel imaging system. P21 and GAPDH primer sequence, reaction conditions, amplification products molecular weight were shown in Table 1.

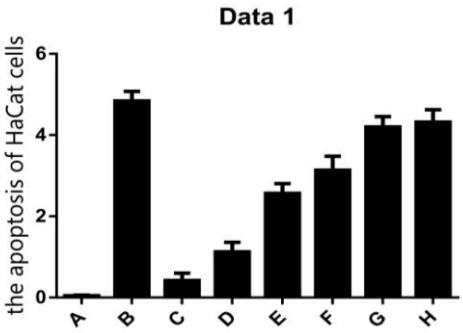


Figure 2. The results of apoptosis of HaCat cells in A-H groups.

3. Result

3.1. The effect of paeoniflorin on UVB-induced apoptosis of HaCat cells

The effect of paeoniflorin on UVB-induced apoptosis of HaCat cells was shown in Figure 1, Figure 2, Table 2. Compared with the group A, the apoptosis rate of HaCat cells was significantly increasing (P<0.05).

The apoptosis rate of group C~group F was significantly lower than that in group B (P<0.05). Among the experimental groups, the apoptosis rate of group C~group D was lower than those in groups E~H (P <0.05).

2.3. Statistical treatment

Statistical analysis using SPSS 17.0 software, the experimental results are expressed as $\bar{x} \pm s$. Among multiple groups were compared using ANOVA and between the two groups were compared using LSD test a small sample. P <0.05 indicates significant difference.

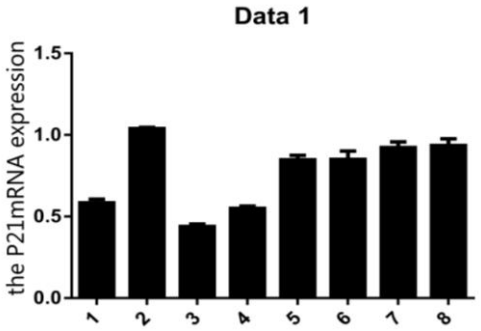


Figure 3. A-H were the results of P21mRNA expression of HaCat cells in A-H group.

3.2. The effect of Paeoniflorin on UVB-induced P21mRNA expression of HaCat cells

The expressions of P21mRNA and GAPDH in the group were significantly amplified, which indicated that the quality of RNA and the process of RT-PCR in the cells were all good. The relative expression of P21/GAPDH in each group was shown in Figure 3 Figure 4 Table 2. Comparing with the group A, the expression of P21mRNA in HaCat cells was significantly increasing (P<0.05).

The expression of P21mRNA in the group B was the highest, and the expression of P21mRNA in other experimental groups was significantly decreased, the difference was statistically significant (P<0.05).

4. Discussion

In recent years, due to the destruction of the ozone layer in the atmosphere, radiation to the surface of the earth's UVB increased year by year. UVB can cause DNA damage, oxidative stress, immune suppression, apoptosis, etc. [7-8]. So the protection of UVB is a meaningful and important measure.

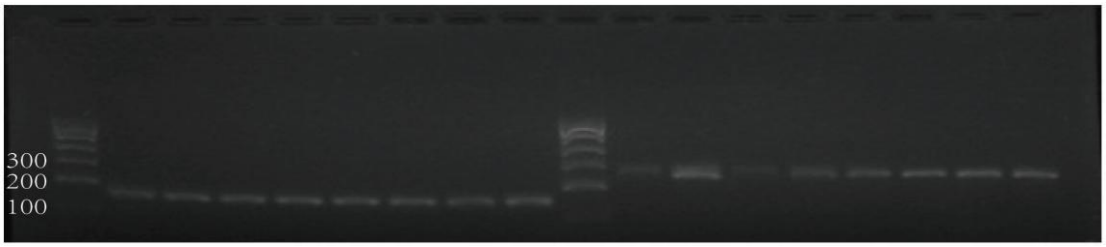


Figure 4. Gel image expression of P21/GAPDH.

Cell cycle is the process of the strict and orderly regulation of the periodic regulation factors (P21、P53), including the S phase, M phase, G1 phase, and G2 phase. P53 plays a crucial role in the orchestration of a cell's response to UV-induced damage. In response to UV-induced damage, it is known that induction of p21 via transcriptional activation is critical for G1 checkpoint control [9]. Some scholars believe that cell cycle arrest is a mechanism of cell self-protection [10]. The experiments show that G1/G0 phase arrest in HaCat cells was found after UVB irradiation. Therefore, flow cytometry was used to quantitatively analyze cell cycle phase of different cell cycle, measuring the apoptosis rate in this study.

This experiment was compared with the blank control group, the apoptosis rate of HaCat cells increased significantly ($P<0.05$). The expression of P21mRNA in HaCat cells increased significantly ($P<0.05$). This indicates that the apoptosis rate and the expression of P21mRNA can be increased after UVB irradiation. The apoptosis rate was significantly decreased in the group C ~ group F compared with the control group ($P<0.05$). The expression of P21mRNA in the group C~ group H was significantly decreased ($P<0.05$). This experiment show that Paeoniflorin can reduce the expression of P21mRNA in the 0.25 mg/L ~ 5 mg/L concentration, reducing UVB-induced apoptosis rate HaCat cells. We speculate that the anti-apoptotic effects of paeoniflorin may be related to the decrease of UVB-induced P21mRNA expression. Previous studies have indicated that paeoniflorin has a protective effect against oxidative damage. By study, Zhang Hongying[11] etc. approved that total glucosides of paeony (in the main active ingredients of total glucosides of paeony) provides a boost for HaCat cell proliferation at low concentrations（0.5 mg/L、2.5 mg/L）. This conclusion was tested by us that is in accordance with this view. According this, we speculated that paeoniflorin in low concentration promoted cell proliferation and inhibited apoptosis, so as to exert its light protection.

In conclusion, we validate the paeoniflorin for all the UVB HaCat apoptosis has protective effect on HaCat apoptosis which is caused by UVB in certain concentration range (0.25 mg/L~5 mg/L). Its mechanism may be associated with lower HaCat cells P21mRNA expression level

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