Protective effects of astragaloside against ultraviolet A-induced photoaging in human fibroblasts

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Objective: In this study, we aim to investigate the protective effects of astragaloside on ultraviolet A (UVA)-induced photoaging in human fibroblasts and its possible mechanisms.

Methods: Subconfluent fibroblasts were cultured and divided into normal control group, astragaloside group, UVA irradiation group, and UVA plus astragaloside group. The cells were sham-irradiated or irradiated with 10 J/cm² of UVA irradiation and treated with 20 μg/mL astragaloside. The aging condition was determined by histochemical staining of senescence-associated β-galactosidase (SA-β-gal). Concentration of transforming growth factor-β1 (TGF-β1) in the supernatant was determined by enzyme-linked immunosorbent assay, and mRNA levels of matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) were measured by real-time polymerase chain reaction.

Results: UVA irradiation raised the proportion of SA-β-gal-positive cells in comparison with the normal control group (P<0.05). Astragaloside treatment was shown to decrease the level of SA-β-gal compared with the UVA group. With UVA irradiation, the concentration of TGF-β1 in the supernatant decreased, and astragaloside treatment recovered the content of TGF-β1 compared with the UVA irradiation alone (P<0.05). UVA irradiation also up-regulated the mRNA levels of MMP-1 and TIMP-1 (P<0.05). Astragaloside decreased the mRNA level of MMP-1 compared with the UVA irradiation alone, while the TIMP-1 expression increased (P<0.05).

Conclusion: Astragaloside can protect the skin from UVA irradiation. The mechanism involved may be related with TGF secretion and decrease of collagen degradation.

Keywords: ultraviolet rays; fibroblast; astragaloside; transforming growth factor-beta 1; matrix metalloproteinase-1; tissue inhibitor of metalloproteinase-1

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Photoaging refers to the effects of long-term ultraviolet exposure and sun damage superimposed on intrinsically aged skin. Ultraviolet A (UVA) irradiation (320 to 400 nm) of sunlight is the most effective wavelength for causing photoaging. The fibroblasts in the dermis are the main target site. Astragaloside is the main effective component of Huangqi (Radix Astragali Mongolici), one kind of Chinese herbal medicine. Astragaloside has many biological effects\(^1\).\(^2\). Earlier studies have shown that astragaloside can reduce oxygen radical generation and strengthen the cellular antioxidative capacity\(^1\).\(^2\). However, whether application of astragaloside could protect skin fibroblasts from UVA-induced photoaging is still rarely investigated.

In the present study, we assessed the effects of astragaloside on UVA-induced photoaging and the correlational mechanisms in human skin fibroblasts.

### 1 Materials and methods

#### 1.1 Instruments and reagents

Astragaloside was bought from National Institutes for Food and Drug Control (Batch number: 107851-200613; standard preparation); Dulbecco’s modified Eagle medium (DMEM) (Gibco-BRL, USA); glutamine (Gibco-BRL, USA); fetal bovine serum (HyClone, USA); SUV-100 solar simulator and radiant emittance monitor (Shanghai Sigma High-tech Co., Ltd., China); β-galactosidase kit (Mirus Bio LLC, USA); enzyme-linked immunosorbent assay (ELISA) kit of human transforming growth factor-β1 (TGF-β1) (R&D Systems Inc., USA); 128C enzyme-labelled meter (ClinicBio, Austria); ABI 7300 real-time polymerase chain reaction (RT-PCR) system (Applied Biotechnology Institute, USA); reverse transcription kit and real-time quantitative PCR kit (TaKaRa Biotechnology Co., Ltd., China).

#### 1.2 Experimental method

**1.2.1 Cell culture and subgroups**

Human fibroblasts deriving from the foreskin of circumcision by surgical excision on young donors (less than 5 years old) were isolated and cultured in DMEM supplemented with 2 mmol/L glutamine and 10% fetal bovine serum at 37°C in 5% CO₂. Cells (1×10⁵/mL) were plated into 6-well plates or 100-mm culture dishes for succeeding experiments. When the fibroblasts growth reached the designed subconfluent state, the culture was then divided into the following subgroups: control group, astragaloside group, UVA irradiation group and UVA plus astragaloside group. The astragaloside group was treated only with tested medicine but no UVA irradiation. The UVA irradiation group was managed by 10 J/cm² UVA irradiation. The UVA plus astragaloside group was treated with 20 μg/mL astragaloside before (2 h) and after UVA irradiation.

**1.2.2 UVA irradiation**

Astragaloside was prepared with DMEM with the stock concentration at 500 μg/mL and stored at −20°C. The fibroblasts of the astragaloside group and the UVA plus astragaloside group were precultured with 20 μg/mL astragaloside solution for 2 h, and then 10 J/cm² UVA irradiation was given to the UVA group and the UVA plus astragaloside group. After UVA irradiation, serum-free medium with 20 μg/mL astragaloside was given to the two groups. Different groups were then used for the experiments of senescence, TGF-β1 secretion assay and real-time quantitative PCR. The accumulation course was designed for 2 weeks and 24 h, respectively. After washing twice with phosphate buffered saline (PBS), cells were irradiated with a thin cover of PBS to avoid drying and in a water bath at room temperature to avoid overheating during irradiation. The emitted intensity of UVA (320 to 400 nm) was 4.4 mW/cm². The irradiation distance of cultured cells to UVA source was 15 cm and the irradiated dosage was controlled by radiometer equipped with UVA sensor. The control group and the astragaloside group were handled identically except that they were shielded with aluminum foil during the irradiation. Each treatment and experiment was conducted in triplicate.

**1.2.3 Histochemical method for β-galactosidase detection**

The semi-quantitative analysis of senescence-associated β-galactosidase (SA-β-gal) positive cells was performed when the confluence of plated fibroblasts reached 50%. Cells were washed in PBS, fixed in 2% formaldehyde/0.2% glutaraldehyde for 5 min (room temperature), washed again, and then incubated at 37°C (no CO₂) with fresh SA-β-gal stain solution. The cells colored blue were considered as indicative of β-galactosidase positive cells; 500 randomly selected cells within a field under the microscope were counted. The percentage of positive cells, which represented the aging rate of the fibroblasts cultures, was calculated. The senescence rate = the number of blue colored cells/the total cell number ×100%.

**1.2.4 ELISA analysis**

Cell-free supernatants were collected and then stored at −80°C for ELISA. Detection of TGF-β1 was carried out by using a human cytokine sandwich ELISA kit. The cytokine levels were measured following the manufacturer’s protocol.

**1.2.5 RT-PCR for matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 gene expressions**

RNA was extracted by using TRIzol according to the supplier’s instructions. Then cDNA was also synthesized according to the manufacturer’s instructions. Reaction system (10 μL): MgCl₂ (25 mmol/L) 2 μL, 10×RT Buffer 1 μL, RNase-free dH₂O 3.75 μL, dNTP mixture (10 mmol/L) 1 μL, PRNase inhibitor (40 U/μL) 0.25 μL, AMV-RT (5 U/μL) 0.5 μL, Oligo dT-adaptor primer (2.5 pmol/μL) 0.5 μL, RNA sample 1 μL. Reaction condition: (42 to 55°C 1 h → 99°C 15 min→5°C 5 min) ×1 cycle. The cDNA
from reverse transcription was stored at $-80^\circ$ C for PCR assay.

Quantitative PCR was performed to amplify the cDNA fragments of matrix metalloproteinase-1 (MMP-1) and tissue inhibitor of metalloproteinase-1 (TIMP-1) genes. The specific primers used were as follows: MMP-1 sense primer: 5'-CTGCT-TACGATTGCGGAC-3' and anti-sense primer: 5'-GCAGCATGATGCCTGAC-3'; TIMP-1 sense primer: 5'-GATGGACTTGTGCACATC-3' and anti-sense primer: 5'-TGGATAAACAGG-GAAACACTG-3'. PCR reaction system (20 $\mu$L): cDNA 2 $\mu$L, 2$\times$QuantiTect SYBR Green I RT-PCR master mix 10 $\mu$L; sense and anti-sense primers (10 mol/L) 0.5 $\mu$L, final concentration 0.15 mol/$\mu$L; QuantiTect RT mix 0.2 $\mu$L; free-DNAase water 6.8 $\mu$L. PCR was conducted through 45 cycles: 95 $^\circ$C 10 min (×1 cycle), 94 $^\circ$C 15 s $\rightarrow$ 58 $^\circ$C 20 s $\rightarrow$ 72 $^\circ$C 30 s (×45 cycles). The relative amount percent is $2^{-\Delta\Delta CT} (\Delta\Delta CT = \Delta CT_{\text{treatment group}} - \Delta CT_{\text{control group}})$.3.3 Statistical analysis Values were expressed as $\bar{x} \pm s$. SPSS 11.0 statistical software was used. Statistical analyses were performed by using analysis of variance followed by the least significant difference-$t$ test. $P<0.05$ was considered significant.

## 2 Results

### 2.1 Effect of astragaloside on UVA-induced expression of β-galactosidase

The morphology of SA-β-gal-stained senescence cells were examined by histochemical staining (Figure 1). The results showed that the rates of senescence cells in the astragaloside group and the control group were both low. There was no statistical significance between the control group and the astragaloside group ($P>0.05$). However, the counted number of SA-β-gal-positive cells of the UVA group was increased by UVA irradiation in comparison with the control group. The senescence rate increased to 71.36%. The UVA plus astragaloside group showed a decrease in the number of SA-β-gal-positive cells compared with the UVA group, which implied some promotive effects of astragaloside on the cell senescence while combined with multiple UVA irradiation ($P<0.05$) (Table 1).

![Figure 1](image1.png)

**Figure 1** Effects of astragaloside on UVA-induced photoaging feature of fibroblasts tested by cytochemical staining of SA-β-gal (Light microscopy, ×200)

Cells were incubated with or without astragaloside (20 $\mu$g/mL), while receiving sham irradiation or irradiated with UVA (10 J/cm$^2$) for 2 weeks. The expression of SA-β-gal, such as blue nuclear staining was observed under a light microscope (×200 magnification). UVA: ultraviolet A; SA-β-gal: senescence-associated β-galactosidase.

### Table 1 Proportion of β-galactosidase-positive cells of different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$\text{SA-}\beta\text{-gal-positive cells}$ ($\bar{x} \pm s$, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>13.16±2.77</td>
</tr>
<tr>
<td>Astragaloside</td>
<td>3</td>
<td>15.42±3.04</td>
</tr>
<tr>
<td>UVA</td>
<td>3</td>
<td>71.36±2.54</td>
</tr>
<tr>
<td>UVA plus astragaloside</td>
<td>3</td>
<td>16.26±3.56</td>
</tr>
</tbody>
</table>

* $P<0.05$, vs control group; $\Delta P<0.05$, vs UVA group. UVA: ultraviolet A; SA-β-gal: senescence-associated β-galactosidase.

### 2.2 TGF-β1 secretion

Astragaloside treatment could significantly promote the secretion of TGF-β1 compared with the control group ($P<0.05$). With UVA irradiation, the content of TGF-β1 in the supernatant decreased. Astragaloside treatment recovered the content of TGF-β1 compared with the UVA group ($P<0.05$) (Table 2).

### 2.3 Expressions of MMP-1 and TIMP-1 mRNAs

Compared with the control group, the expression level of MMP-1 mRNA had no significant change after astragaloside treatment, while the expression level of TIMP-1 mRNA increased slightly.

There was no statistical significance between the control group and the astragaloside group for both the indexes ($P>0.05$). UVA irradiation up-regulated the mRNA levels of MMP-1 and TIMP-1 genes compared with the control group. Astragaloside treatment significantly decreased the expression level of MMP-1 mRNA, though it was still higher than the control group ($P<0.05$); astragaloside had no effect on the TIMP-1 mRNA level compared with the UVA irradiation ($P>0.05$) (Table 3).

### Table 2 Secretion of TGF-β1 in the supernatant of different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>$\text{TGF-\beta}$ ($\bar{x} \pm s$, ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>3343.58±45.58</td>
</tr>
<tr>
<td>Astragaloside</td>
<td>3</td>
<td>2423.54±35.48*</td>
</tr>
<tr>
<td>UVA</td>
<td>3</td>
<td>818.97±37.99*</td>
</tr>
<tr>
<td>UVA+astragaloside</td>
<td>3</td>
<td>1673.15±41.65*</td>
</tr>
</tbody>
</table>

* $P<0.05$, vs control group; $\Delta P<0.05$, vs UVA group. UVA: ultraviolet A; TGF-β: transforming growth factor-β.
### Table 3  Expressions of MMP-1 and TIMP-1 mRNAs in different groups

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MMP-1 (2 SDST)</th>
<th>TIMP-1 (2 SDST)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>1.00±0.03</td>
<td>1.00±0.03</td>
</tr>
<tr>
<td>Astragaloside</td>
<td>3</td>
<td>1.18±0.02</td>
<td>1.40±0.02</td>
</tr>
<tr>
<td>UVA</td>
<td>3</td>
<td>4.43±0.08*</td>
<td>1.85±0.07*</td>
</tr>
<tr>
<td>UVA+astragaloside</td>
<td>3</td>
<td>2.23±0.04*</td>
<td>2.11±0.07</td>
</tr>
</tbody>
</table>

* P<0.05, vs control group; ** P<0.05, vs UVA group.

UVA: ultraviolet A; MMP-1: matrix metalloproteinase-1; TIMP-1: tissue inhibitor of metalloproteinase-1.

### 3 Discussion

The aging process encompasses progressive physiological changes in an organism that lead to senescence; it refers to the decline of biological functions and the organism’s ability to adapt to metabolic stress with time. These changes result from intrinsic as well as extrinsic processes, such as ultraviolet radiation[4]. It is now believed that UVA plays a substantial role in photoaging. Many of the functions of skin that decline with age show an accelerated decline in photoaged skin. The clinical signs associated with photoaging are dyspigmentation, laxity, wrinkles, telangiectasia, a leathery appearance and cutaneous malignancies[5]. More and more, individuals are seeking treatment for reversal of UVA-induced changes in the skin. There has been great interest in chemoprevention of photodamage which means using naturally occurring agents to reduce the risk of photoaging, which are considered as a less toxic and more effective approach[6]. There have been some studies confirming that Huangqi could effectively delay the aging process of body cells in recent years[7]. In the present study, we assessed the protective potency of astragaloside against UVA-induced photoaging in fibroblasts, and discussed its possible mechanisms.

It was confirmed that the positive expression of SA-β-gal increases in aging individuals and SA-β-gal serves as one kind of aging-related biologic markers. Compared with freshly isolated fibroblasts, the fibroblasts cultured for two weeks following UVA treatment presented special aging morphology and SA-β-gal-positive staining. This indicated that UVA-induced senescence shared the common features with intrinsic aging. However, the positive senescence cells decreased by a large degree compared with the UVA group when the fibroblasts were pretreated with astragaloside. It indicated that astragaloside treatment caused a further descent in senescence rate.

TGF-β is a multipotent cytokine that regulates both cell growth and differentiation. Three isoforms of TGF-β (TGF-β1, 2 and 3) have been documented in human skin, and TGF-β1 is the predominant isoform (> 90%). TGF-β1 could activate the fibroblast proliferation and extracellular matrix (ECM) generation, and it could also inhibit the collagenase generation and promote collagen type I synthesis by fibroblasts. There have been reports indicating that the secretion of TGF-β1 and the responses to TGF-β1 by fibroblasts were both reduced in aging skin[8]. Our results confirmed that astragaloside treatment remarkably increased the secretion of TGF-β1 by fibroblasts. After UVA irradiation, TGF-β1 secretion was apparently reduced, while astragaloside treatment recovered the secretion of TGF-β1. In the condition of UVA radiation, the concentration of TGF-β1 in the astragaloside plus UVA group was still higher than that in the control group. These data suggested that the anti-photoaging effect of astragaloside might be associated with increasing the secretion of TGF-β1.

The characteristic histological changes of photoaging skin manifest primarily as the disorganization of collagen fibrils that constitute the bulk of the connective tissue and the accumulation of abnormal, amorphous and elastin-containing material. It was reported that UVA exposure induces the nuclear transcription factor activator protein 1 (AP-1) expression and activation. AP-1 promotes the transcription of MMPs, which are responsible for the degradation of the extracellular matrix. MMP-1 belongs to the MMPs family and is the major collagenase causing collagen type I degradation and is responsible for skin photoaging[9]. TIMPs are one representative of the natural MMP inhibitor family. TIMP-1 expression is decreased with the fibroblast senescence, both ex vivo and in vivo, thus contributing to the increased catabolic activity within the dermis[10].

Therefore, we further investigated whether astragaloside could affect MMP and TIMP-1 expressions induced by UVA. UVA irradiation of 10 J/cm² up-regulated the mRNA levels of MMP-1 and TIMP-1 3.43 times and 1.8 times, respectively, compared with the control group. Astragaloside treatment significantly reduced the expression level of MMP-1, but it did not decrease the mRNA level of TIMP-1. These data showed that astragaloside might inhibit UVA-induced collagen degradation and lessen the skin photoaging through reducing MMP-1 expression and up-regulating TIMP-1 expression. The raise of TIMP-1 expression after UVA irradiation may be one defensive mechanisms of the cells against photodamage.

In summary, the results obtained in the present study indicated that UVA irradiation could induce premature aging in cultured human skin fibroblasts. Astragaloside treatment could protect skin fibroblasts against UVA-induced photoaging, including reduction of the senescence cell rate. The specific mechanisms of the photo-protective effect of astragaloside may be concerned with inhibition of UVA-induced cellular damage and regulation of TGF-β1 and expressions of MMP-1 and TIMP-1. It may provide new clues for developing new strategies to avoid UVA-induced skin aging.
REFERENCES


7 Li WZ, Li WP, Yin YY. Effects of AST and ASI on metabolism of free radical in senescent rats treated by HC. Zhongguo Zhong Yao Za Zhi. 2007; 32(23): 2539-2542. Chinese with abstract in English.


黄芪甲苷对人成纤维细胞光老化的抑制作用

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目的：观察黄芪甲苷对长波紫外线（ultraviolet A, UVA）照射导致的人成纤维细胞衰老的抑制作用，以及对基质金属蛋白酶-1(matrix metalloproteinase-1, MMP-1)和金属蛋白酶组织抑制因子-1(tissue inhibitor of metalloproteinase-1, TIMP-1)等老化相关基因表达的影响。

方法：分离培养人原代成纤维细胞，将亚融合状态的培养细胞分为空白对照组、黄芪甲苷组、UVA组和UVA+黄芪甲苷组，以10 J/cm² UVA进行照射，并加入20 μg/mL黄芪甲苷干预处理。采用组织化学染色法检测衰老相关β半乳糖苷酶表达、以酶联免疫吸附法检测血清中转化生长因子-β1(transforming growth factor-β1, TGF-β1)的含量，实时聚合酶链反应法检测MMP-1和TIMP-1的mRNA表达水平变化。

结果：空白对照组及黄芪甲苷组β半乳糖苷酶阳性细胞均较低，UVA组β半乳糖苷酶阳性细胞数显著升高，加入黄芪甲苷处理可使β 半乳糖苷酶阳性细胞比率明显降低（P<0.05）。黄芪甲苷处理可以促进成纤维细胞分泌TGF-β1；UVA照射成纤维细胞后 TGF-β1分泌量明显降低，UVA照射前加入黄芪甲苷可增加TGF-β1分泌量（P<0.05）。此外，UVA能够诱导MMP-1和TIMP-1的mRNA表达水平升高，而黄芪甲苷可在一定程度上抑制MMP-1 mRNA表达，并诱导TIMP-1 mRNA表达（P<0.05）。

结论：黄芪甲苷可有效延缓人成纤维细胞光老化进程，其机制可能与促进TGF分泌和抑制胶原降解相关。

关键词：紫外线；成纤维细胞；黄芪甲苷；转化生长因子-β1；基质金属蛋白酶-1；金属蛋白酶-1；组织抑制剂