Effects of Polygonati Rhizoma Extracts on the Collagenase Activity and Procollagen Synthesis in Hs68 Human Fibroblasts and Tyrosinase Activity

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ABSTRACT

Objectives: This study was designed to investigate the collagen metabolism and tyrosinase activity of Polygonati Rhizoma extracts (PR). Its effects are to tonify spleen qi and augment the spleen yin. It enrichs the yin and moisten the lung.

Methods: The effect of PR on type I procollagen production and collagenase (matrix metalloproteinase-1, henceforth referred as MMP-1) activity in human normal fibroblasts Hs68 after ultraviolet B (UVB, 312 nm) irradiation was measured by ELISA method. The tyrosinase activity after treatment of PR was measured.

Results: There were no cytotoxicity at concentrations of 10, 30, 100 µg/ml. The reduced type I procollagen production was recovered by PR in UVB damaged Hs68 cells at a concentration of 100 µg/ml (16.2 ± 0.0 ng/ml) from control group (13.9 ± 0.5 ng/ml). However there was no statistical significance. PR reduced the increased MMP-1 activity after UVB damage at concentrations of 10 µg/ml, 30 µg/ml, and 100 µg/ml in a dose dependent manner (42.2 ± 20.5%, 44.8 ± 8.5%, and 22.0 ± 5.8%), PR 100 µg/ml treatment showed the statistical significance (p < 0.05). PR significantly reduced the tyrosinase activity at a concentration of 10 mg/ml (32.0 ± 12.8%, p < 0.05). However, the L-DOPA oxidation was not changed.

Conclusion: PR showed the anti-wrinkle effects and whitening effects in vitro. Although more researches are needed to validate the efficacy, these results suggest that PR may have potential as an anti-aging ingredient in cosmetic herb markets.

Key words: Polygonati Rhizoma, type I procollagen, collagenase, tyrosinase

Introduction

Polygonati Rhizoma (PR) is the root of Polygonatum sibiricum, P. falcatum, or P. kingianum1). Its effects are to tonify spleen qi and augment the spleen yin. It enrichs the yin and moisten the lung1,2). Accordingly, PR has been used to delay the aging process.

There are two major theories of aging: the programmatic theory states that aging is an inherent genetic process, and the stochastic theory states that aging represents random environmental damage, Processes that are associated with cellular damage and aging are the production of free radicals (a process much enhanced after ultraviolet irradiation) and an increasing number of errors during DNA replication, Cellular manifestations of intrinsic aging include decreased life span of cells, decreased responsiveness of cells to growth signals, which may reflect loss of cellular receptors to growth factors, and increased responsiveness to growth inhibitors. All these findings are more pronounced in cells derived from photodamaged skin3).

It has been shown that UV irradiation leads to the formation of reactive oxygen species (ROS) that activate the mitogen–activated protein (MAP) kinase pathway, which subsequently induces the expression and activation of matrix metalloproteinases (MMPs) in human skin in vivo4,5). MMPs including collagenase are considered key factors in the photoaging process.

In the present study, we investigated the effect of PR on type I procollagen production and collagenase activity in human normal fibroblasts Hs68 after UVB...
(312 nm) irradiation, The tyrosinase activity after treatment of PR was measured as well.

Materials and Methods

1. Sample preparation
Polygonati Rhizoma was purchased from Omniherb (Cultivated in Korea). Polygonati Rhizoma extracts (PR) was prepared as follows. 100 g of Polygonati Rhizoma in 2,000 ml distilled water was heated in a heating extractor for 3 hours. The extract was filtered and concentrated by using the rotary evaporator. The extracts were lyophilized by using freeze dryer (15.3 g). The extract was dissolved in water and filtered three times through micro-filter paper and syringe filter (Whatman #2, 0.45 µm to 0.2 µm). Filtered material was placed in the disinfected vial and was sealed for further study.

2. Reagents
All reagents were purchased from Sigma–Aldrich except as mentioned below (St. Louis, MO, USA).

3. Cell culture
Hs68 human fibroblasts (Health Protection Agency Culture Collections, UK) were cultured in Dulbecco's Modified Eagle's medium (Gibco, USA) containing 10% fetal bovine serum, 1% antibiotics at 37°C in a humidified atmosphere of 5% CO2. When cells reached above confluency, subculture was conducted at a split ratio of 1:3.

4. UVB irradiation
A UVB lamp (Vilber Lourmat, France) was used as a UVB source. In brief, Hs68 cells were rinsed twice with phosphate–buffered saline (PBS), and all irradiations were performed under a thin layer of PBS (200 µl/well). Immediately after irradiation, fresh serum–free medium was added to the cells. After 24 hours incubation period, responses were measured. Mock–irradiated blanks followed the same schedule of medium changes without UVB irradiation.

5. Cell viability
General viability of cultured cells was determined by reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan. Hs68 cells were seeded in 24-well plates at a density of 2×10^4/ml per well and cultured at 37°C in 5% CO2. Cells were pretreated with the sample at a concentration of 10, 30, and 100 µg/ml for 24 hours prior to UVB irradiation. After UVB irradiation, cells were retreated with the sample and incubated for additional 24 hours, before being treated with 0.05 mg/ml (final concentration) of MTT. The blank and control group was cultivated without sample treatment. The cells were then incubated at 37°C for additional 4h. The medium containing MTT was discarded, and MTT formazan that had been produced was extracted with 200 µl of DMSO. The absorbance was read at 595 nm with a reference wavelength of 690 nm. The cell viability being calculated as follows:

\[ \text{Cell viability (\%)} = \left( \frac{\text{OD595 of sample}}{\text{OD595 of control}} \right) \times 100 \]

6. Assays of collagen type I synthesis and collagenase inhibition
Hs68 human fibroblasts were inoculated into 24-well plate (2×10^5 cells/well) and cultured at 37°C in 5% CO2. Cells were pretreated with the sample at a concentration of 10, 30, and 100 µg/ml for 24 hours prior to UVB irradiation. After UVB irradiation, cells were retreated with the sample and incubated for additional 24 hours. The blank and control group was cultivated without sample treatment. After culturing, the supernatant was collected from each well, and the amount of pro–collagen type I was measured with a procollagen type I C-peptide assay kit (Takara Bio, Japan). The activity of collagenase was measured with a matrix metalloproteinase-1 (MMP–1) human biotrak ELISA system (Amersham life science, USA).

7. Tyrosinase inhibition assay
Tyrosinase activity was determined essentially as previously described. The reaction mixtures were prepared by adding 40U of mushroom tyrosinase to 20 µl of PR dissolved in distilled water (0.1, 1, and 10 mg/ml), and then adding 40 µl of 1.5 mM L-tyrosine and 220 µl of 0.1 M sodium phosphate buffer (pH 6.5). The resulting mixture (300 µl) was incubated for 10 min at 37°C and then absorbance at 490 nm was measured. The same mixture, but without PR extract, was used as a control.

8. Inhibition of L–DOPA oxidation