Effects of Myrrh extracts on the anti-wrinkle activity and anti tyrosinase activity in Hs68 human fibroblasts

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Abstract. This study was designed to investigate the collagen metabolism and tyrosinase activity of Myrrh Extracts (ME). The currently known effects of ME are to move blood, calm pain, solve oedema, and promote tissue regeneration. The effect of ME 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 the ELISA method. The tyrosinase activity after treatment with ME was measured as well. There was no cytotoxicity at all tested concentrations. ME significantly inhibited the increased collagenase activity after UVB damage, whereas it did not recover the reduced type I procollagen production in UVB damaged Hs68 cells. It did not reduce the L-DOPA oxidation. However, it significantly reduced the tyrosinase activity. In conclusion, ME showed the anti-wrinkle effects via the collagenase inhibitory mechanism and whitening effects via the tyrosinase inhibitory mechanism. Although further research is needed to validate its efficacy, these results suggest that ME may have potential as an anti-aging ingredient in cosmetic herb markets.

Keywords: Myrrh, type I procollagen, collagenase, tyrosinase

1 Introduction

Myrrh is a resin obtained from Commiphora myrrha Engl. Its effects are to activate blood circulation and relieve pain. It reduces swelling and promotes generation of flesh in the cases of skin lesions and ulcerations. It has an excellent ability to reduce swelling, relieve pain, promote generation of flesh, and enhance healing of skin lesions and ulcerations. Heat-clearing and blood-cooling herbs are usually added to reduce inflammation. It has been applied with Ru Xiang (olibanum) as powder topically for the treatment of skin lesions and ulcerations [1,2].

It is the latest trend to look younger than one’s age. In these days, aging seems to be treated not as the inescapable destiny to accept but as a disease or a disorder to overcome. There are two major theories of aging: the programmatic theory that aging is an inherent genetic process, and the stochastic theory 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 for growth factors, and increased responsiveness to growth inhibitors. All these findings are more pronounced in cells derived from photodamaged skin [3].

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 vivo [4,5]. MMPs including collag enase are considered key factors in the photoaging process.

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

2 Methods

2.1 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.

2.2 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.

2.3 Assays of collagen type I synthesis and collagenase inhibition

Hs68 human fibroblasts were inoculated into 24-well plate (2×10⁵ 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 biotrad ELISA system (Amersham life science, USA).

2.4 Tyrosinase inhibition assay

Tyrosinase activity was determined essentially as previously described [6]. The reaction mixtures were prepared by adding 40U of mushroom tyrosinase to 20 μl of ME 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 ME extract, was used as a control.

2.5 Statistical analysis

The results were expressed as means ± standard error of the mean (SEM). Significances of changes were evaluated using the one-way ANOVA with Dunnett's post-hoc test. Values of p < 0.05 were considered significant.

3 Results

3.1 Assay of collagen type I synthesis

To evaluate the amount of collagen type I synthesis that occurred upon exposure to the sample, collagen type I was quantitatively detected by using the procollagen type I C-peptide assay kit previously described in methods section. Collagens are synthesized as precursor molecules, called procollagens. These molecules contain additional peptide sequences, usually referred to as 'propeptides', at both the amino-terminal end and the carboxy-terminal end. These propeptides are cleaved from the collagen triple-helix molecule during its secretion, after which the triple-helix collagens are polymerized into extracellular collagen fibrils. Thus, the amount of free propeptide stoichiometrically reflects the amount of collagen molecules synthesized [8]. ME did not increase the expression of type I collagen at all concentrations of 10, 30, and 100 μg/ml (7.9 ± 1.3 ng/ml, 5.4 ± 2.0 ng/ml, and 5.4 ± 2.1 ng/ml) compared with control group (14.0 ± 0.5 ng/ml).

3.2 Assay of collagenase activity

To evaluate the collagenase activity, MMP-1 activity was quantitatively measured by using the previously described matrix metalloproteinase-1 assay kit. The activities of MMP-1 of ME treatment were recalculated into 100% of control group. ME reduced
the MMP-1 activity at concentrations of 10 μg/ml, 30 μg/ml, and 100 μg/ml in a dose dependent manner (32.4 ± 8.4%, 20.7 ± 5.8%, and 13.0 ± 3.4%). All ME concentrations showed the statistical significances (p < 0.05).

3.3 Inhibitory effects on tyrosinase activity

The activities of ME on tyrosinase activity were recalculated into 100% of control group. The tyrosinase activity of ME 0.1, 1, and 10 mg/ml treated groups showed the dose-dependant reductions (85.7 ± 3.2%, 76.2 ± 5.8%, and 10.3 ± 4.7%). 10 mg/ml treated group showed the statistical difference (p < 0.05).

4 Conclusions

In conclusion, ME showed the inhibitory activity on the collagenase (MMP-1) and tyrosinase. The reduced collagenase activity might delay the process of skin-aging. Accordingly, these results suggest that ME may have potential as an anti-aging ingredient in cosmetic herbal drugs. It is to be hoped that further studies will be needed to unravel the underlying molecular mechanisms.

References