Regulation Effect of 2',4',7-Trihydroxyisoflavone on the Expression of Matrix Metalloproteinase-1, 2 in Ultraviolet-B Irradiated Primary Cultured Old Aged Human Skin Fibroblasts

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Long term and repeated exposure of UV light on the skin often induces chronic skin diseases such as skin cancer as well as photoaging, and the mechanisms of these skin damages are closely associated with up-regulation of matrix metalloproteinase's (MMPs) activities. We investigated the effect of 2',4',7-trihydroxyisoflavone purified from the whole plants of *Viola hondoensis* W. BECKER *et* H BOISSIEU (Violaceae) on the expression of MMPs in UV-B irradiated old aged human skin fibroblasts. 2',4',7-trihydroxyisoflavone markedly reduced UV-induced MMP-1 expression, but not MMP-2, at the both mRNA and protein levels in a dose-dependent manner. Our report is the first description for the ability of 2',4',7-trihydroxyisoflavone to regulate MMP-1 expression from ultraviolet-B irradiated primary cultured old aged human skin fibroblasts.

Key words *Viola hondoensis*; 2',4',7-trihydroxyisoflavone; matrix metalloproteinase (MMP)-1; MMP-2; aged human skin fibroblast

The skin aging process can be divided into intrinsic aging and photoaging. Damage to human skin due to repeated exposure to ultraviolet (UV) radiation (photoaging) and damage occurring as a result of the passage of time (intrinsic aging) are considered to be distinct entities rather than similar skin aging processes. Photoaging of skin is caused by genetically determined or intrinsic processes superimposed upon the degenerative changes due to solar radiation.¹⁾ Alterations and deficiencies of collagen, the major structural component of skin, have been suggested to be a cause of the skin wrinkling observed in photoaged and naturally aged skin.^{2,3)} The dermis contains predominantly type I and type III collagen, elastin, proteoglycans, and fibronectin. Since collagen fibrils and elastin are responsible for the strength and resiliency of skin, their disarrangement during photoaging causes the skin to appear aged. Recently, it was suggested that excessive matrix degradation by UV-induced MMP-1 secreted by various cells, including, keratinocytes, fibroblasts, and inflammatory cells, contributes substantially to the connective tissue damage that occurs during photoaging.^{4–6)} The expression of MMPs proteins in the dermis of sun-exposed and sun-protected skin of elderly subjects is known to differ.⁵⁾ Photodamaged skin of the elderly shows higher MMP-1 protein expression, than the intrinsically aged skin in the same individual.⁵⁾ Thus, this resulting higher expression of MMP-1 in photoaged skin may cause severe collagen deficiency and wrinkling. Matrix metalloproteinases (MMPs) are

a family of zinc-dependent endoproteases that play pivotal roles in the dynamic remodeling of extracellular matrix. Based on substrate preference and structural homology, MMPs are sub-classified into functional groups: collagenases, gelatinases, stromelysins, matrilysins, membrane type-MMPs (MT-MMPs) and other non-classified MMPs.⁷⁾ MMPs are frequently overexpressed by the various extracellular stimuli including growth factors, cytokines, tumor promoters, and UV, and this increase in MMP-related activities may be involved in the pathogenesis of diseases such as cancer and inflammation as well as in physiologic processes.^{7,8)} It has been also reported that up-regulation of some MMPs is responsible for the enhanced degradation of dermal collagen during chronological and UV-induced skin aging.⁴⁾ With increasing age, collagen synthesis becomes lower and MMP-1 levels become higher in sun-protected human skin *in vivo*.³⁾ Therefore, the regulation of MMP activity might be a potential strategy for prevention and/or treatment of UV-induced skin damage. During our screening program for MMP-1 inhibitors from *Viola* species. Very recently, we reported the isolation of the MMP-1 inhibitor from the whole plants of *V. hondoensis*.⁹⁾ In this study, we investigated the rare occurrence effect of 2',4',7-trihydroxyisoflavone (2',4',7-THIF), isoflavonoid from *V. hondoensis* W. BECKER *et* H BOISSIEU, on the expression of UV-B induced MMP-1 and MMP-2 mRNA and protein in aged human skin fibroblasts.

MATERIALS AND METHODS

2',4',7-Trihydroxyisoflavone (2',4',7-THIF) was isolated as pure compound (purity >99%) from the whole plants of *Viola hondoensis* W. BECKER *et* H BOISSIEU as described previously.⁹⁾ Genistein (2',5,7-trihydroxyisoflavone) was obtained from one of the authors (Dr. Jong Hwan Kwak). Primary aged human skin fibroblasts were established from biopsies of good donors of 65–80 years and cultured in Dulbecco's modified Eagle's media (DMEM) supplemented with glutamine, penicillin, streptomycin, and 10% FCS in a humidified 5% CO₂ atmosphere at 37 °C. The UV-B light source was F75/85W/UV21 fluorescent sun lamps, having an emission spectrum between 285–350 nm (peak at 310–315 nm) as previously described.¹⁰⁾ Subconfluent cells were serum-starved for 24 h to synchronize cells in G1/G0 phase of the cell cycle. Fresh phosphate-buffered saline was added to the quiescent cells prior to UV-B light exposure (0–100 mJ/cm²). After UV-B irradiation, the cells were washed with PBS, and incubated for the indicated time in DMEM with or without compound. Total RNA was extracted using the TRIZOL reagent. The extract of total RNA was reverse transcribed using a first strand cDNA synthesis kit for

Fig. 1. Chemical Structure of Isoflavonoid Compounds

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reverse transcription-polymerase chain reaction (RT-PCR). Semiquantitative PCR was performed using primers for human MMP-1 (forward, 5'-ATTCTACTGATATCGGGGCTTTGA-3'; reverse, 5'-ATGTCCTTGGGGTATCCGTG TAG-3'). MMP-2 (forward, 5'-GGCCAAGTGGTCCGTGTG-3'; reverse, 5'-GAGGCCCCATAGAGCTCC-3'). The PCR con-

dition used was as follows: initial denaturation (for 5 min at 94 °C), 28 cycles of amplification (for 1 min at 94 °C, for 1 min at 60 °C, and for 1 min at 72 °C), and final extension (for 10 min at 72 °C). Primer sequences and PCR conditions for GAPDH were described previously.¹⁰ Reaction products were electrophoresed in 2.0% agarose gels and visualized

Fig. 2. Effects of 2',4',7-Trihydroxyisoflavone (2',4',7-THIF) and Genistein on the Expression of MMP-1 and MMP-2 in Aged Human Skin Fibroblasts

Quiescent primary aged human skin fibroblasts were exposed to UV 100 mJ/cm² and collected at indicated time points. RT-PCR analysis of MMP-1(A) and MMP-2(B). Compounds suppressed UV-B induced MMP-1 mRNA expression at 24 h. Expression levels of MMP-1 were normalized to GAPDH control. Values represent the means ± S.E. of data from three independent experiments (C) (**p* < 0.05, *n* = 5). Conditioned media of fibroblasts at 72 h time point following exposure to UV-B light were collected and Western blotted with anti-MMP-1 antibody, and also subjected to gelatin zymography for MMP-2 expression. Quantitative analysis of gelatinase activity by zymography was used to measure the relative amounts of MMP-2 present. To determine the amount of MMP-2 secreted into culture media by aged human skin fibroblasts, equal aliquots of conditioned culture media containing equal cell densities were fractionated using precast zymogram gels containing gelatin, according to the manufacturer's protocol (NOVEX, San Diego, CA, U.S.A.). Data shown are the representative of four independent experiments.

with ethidium bromide. Following normalization by GAPDH intensity the results are presented as the percentage of basal MMP-1, 2 mRNA expression. The signal strengths were quantified using a densitometric program. Western blot, Zymography and Statistical analysis were previously described.¹⁰⁾

RESULTS AND DISCUSSION

2',4',7-Trihydroxyisoflavone (2',4',7-THIF), isoflavonoid from the whole plants of *V. hondoensis* W. BECKER *et* H BOISSIEU, was verified by comparing NMR spectral data with those of previous reports⁹⁾ (Fig. 1). However, specificity of those compounds toward other MMPs remains to be determined. These observations have led us to investigate the ability of 2',4',7-THIF (Fig. 1), to regulate a specific MMP activity in aged human skin fibroblasts. 2',4',7-THIF treatment completely inhibited UV-B induced MMP-1 mRNA levels to the control levels in a dose-dependent manner (Fig. 2A). In contrast, changes in MMP-2 mRNA levels by UV-B irradiation were not observed and these findings are consistent with previous report.⁴⁾ 2',4',7-THIF also failed to change the expression of MMP-2 mRNA (Fig. 2B). In contrast, Genistein treatment completely inhibited UV-B induced MMP-1, 2 mRNA levels to the control levels at 1 μ M, and these findings are consistent with previous report¹¹⁾ (Figs. 2A, B). *In vitro* cytotoxicity assay was performed to determine whether the inhibitory effect of compounds on MMP-1 mRNA expression were attributable to nonspecific cytotoxicity, as previously reported.¹²⁾ In the presence of up to 1 μ M, compounds did not show cytotoxicity in these aged human skin fibroblasts.

To further ascertain whether compounds regulate the expression of proMMP-1 and MMP-2 (pro and active form) at the protein levels. As expected, 2',4',7-THIF markedly inhibited UV-B induced MMP-1 expression to that of control, and did not alter MMP-2 protein levels (Fig. 2C). Genistein markedly inhibited UV-induced MMP-1, 2 protein level expression to that of control (Fig. 2C). These results demonstrate that 2',4',7-THIF specifically reduces the expression of MMP-1, but not of MMP-2, at both the mRNA and protein levels. It is well established that the UV irradiation of cultured aged human skin *in vitro* or human skin *in vivo* induces the expression of MMPs which play important roles in the

degradation of extracellular matrix components during premature skin aging (photoaging).⁴⁾ Therefore, the development of MMP inhibitors has been considered to be promising strategy for therapy of skin cancer and photoaging. However, most synthetic MMP inhibitors have broad-spectrum activity against MMPs. This lack of specificity toward MMPs family restricts their use in clinical trials.¹³⁾ We investigated the effects of 2',4',7-THIF on the expressions of MMP-1 and MMP-2 protein in cultured aged human skin fibroblasts. In conclusion, this report suggests the possibility of 2',4',7-trihydroxyisoflavone as a therapeutic agent for the chronological and UV-induced aged human skin aging.

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