Fucoidan Inhibits UVB-Induced MMP-1 Expression in Human Skin Fibroblasts

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Ultraviolet (UV)B irradiation induces the production of matrix metalloproteinases (MMPs) by activating cellular signaling transduction pathways, which are responsible for the degradation or synthesis inhibition of collagenous extracellular matrix in connective tissues, causing skin photoaging. Using the human skin fibroblast (HS68) cell line in the present study, we investigated the inhibitory effects of fucoidan on MMP-1 expression by various *in vitro* experiments and elucidated the pathways of inhibition. Pretreatment with fucoidan inhibited UVB-induced MMP-1 expression in a dose-dependent manner. Extracellular signal regulated kinase (ERK) activation was markedly inhibited by treatment with fucoidan, though JNK activation was very slightly affected by fucoidan. We also found that fucoidan pretreatment significantly reduced MMP-1 mRNA expression in comparison with UVB irradiation only. In conclusion, our results demonstrate that fucoidan can mainly inhibit UVB-induced MMP-1 expression by inhibiting the ERK pathways. Therefore, fucoidan might be used as a potential agent for the prevention and treatment of skin photoaging.

Key words matrix metalloproteinase-1; fucoidan; ultraviolet B

Skin can age in two ways: (i) intrinsic or chronologic aging, which is the process of senescence that affects all body organs; and (ii) extrinsic aging which occurs as a consequence of exposure to environmental factors including sunlight and cigarette smoke.[1)]

The most important of these factors is sunlight, particularly exposure to ultraviolet (UV)B irradiation, which causes photoaging. The photoaging process increases skin fragility, laxity, blister formation, leathery appearance and formation of wrinkles.[2)]

UVB induces the production of matrix metalloproteinases (MMPs) by activating cellular signaling transduction pathways[3)]; MMPs are responsible for the degradation or synthesis inhibition of collagenous extracellular matrix in connective tissues.[3)] Collagen represents the main component of the extracellular matrix of dermal connective tissue, and its concentration decreases in chronoaging and photoaging.

All matrix macromolecules in the dermis can be digested by MMPs acting alone or in combination. The MMPs form a family of structurally and functionally related zinc endopeptidases that exhibit various substrate specificities.[4)] Once collagen is initially cleaved by MMP-1, MMP-3 and other MMPs, collagen breakdown is further promoted. The enzyme mainly responsible for collagen breakdown in skin is, MMP-1 (fibroblast collagenase), which cleaves type I, III, VII, VIII and X collagen.

Human fibroblast collagenase was the first vertebrate collagenase both purified to homogeneity as a protein, and cloned as a cDNA. This enzyme has been designated as matrix metalloproteinase-1 (MMP-1) and has served as the prototype for all the interstitial collagenases. MMP-1 is also known as collagenase-1. Starting from the N terminus the following features of domain organization are observed.[5)]

The pre-domain specifies a signal rich in hydrophobic amino acids that destines the synthesized polypeptide to the endoplasmic reticulum where it is removed during the transportation of the molecule from the cell to the outside. The propeptide domain indicates a sequence that is responsible of keeping the pro-form inactive. It presents a cysteine residue 73 that is located in a conserved sequence PRCGVPD opposite to a zinc atom at the active-form site and coordinated to it through an –SH group. The enzymatic activity of the pro-form enzyme is turned on by the displacement of this cysteine residue ("cysteine switch") and occurs by proteolytic cleavage, or by chemical disruption as in the case of oxidation or treatment with mercurial compounds.[6)]

Recent studies have shown that hairless mice exposed to UVB developed wrinkled skin and significantly enhanced MMP-1 mRNA expression. However, they have shown that the inihibition of MMP-1 activities by a specific MMP inhibitor suppresses UVB-induced wrinkle formation.[7)] This evidence suggests that MMP-1 plays a major role in the process of photoaging.

Brown seaweed has been a staple of both Korean and Japanese diets and has also been documented as being used in traditional Chinese medicine for over 1000 years.[8)] Sulphated polysaccharides extracted from marine algae represent a good source of marine compounds with potential applications in medicine. Fucoidans, the sulphated polysaccharides extracted from brown algae, were first isolated by Kylin almost a century ago,[9)] and were found to contain a significant amount of L-fucose and sulphate ester groups. Before this, fucoidan was considered as an alternative to heparin and the focus was mainly on its anticoagulant activity.

Recently, fucoidan is being studied extensively due to potential antitumor, antiviral, anticomplement and anti-inflammatory activities.[10)] Also, other recent studies have shown that fucoidan was able to increase the antioxidant enzymes in rats treated with ethylene glycol (EG).[11)] Senni *et al.*[12)] reported that polysaccharides were able to stimulate dermal fibroblast proliferation and extracellular matrix deposition *in vitro*, to control important parameters involved in connective

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tissue breakdown in human skin. Yet, the effects of fucoidan on skin photoaging have not been investigated. In this study, we investigated the inhibitory effects of fucoidan on MMP-1 expression by various *in vitro* experiments and elucidated the pathways of inhibition.

MATERIALS AND METHODS

Cell Culture The normal human newborn foreskin fibroblasts cell lines, HS68 cell (ATCC CRL 1635), were obtained from American Type Culture Collection (Rockville, MD, U.S.A). HS68 cells were 10 passage. HS68 cells were plated in 100 mm tissue culture dishes and grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (all from GIBCO, Grand Island, NY, U.S.A.). Fucoidan (Sigma, St. Louis, Mo, U.S.A.) dissolved in distilled water. For treatment, cells were maintained in culture media without FBS overnight and followed by pretreatment with fucoidan for 24 h. The cells were rinsed twice with phosphate-buffered saline (PBS), and all UVB irradiations were performed under a thin layer of PBS (GIBCO, Grand Island, NY, U.S.A.). Immediately after irradiation, cells were further incubated in fresh culture media without serum and in the presence of fucoidan.

Ultraviolet Irradiation The UV light source was a Philips TL 20W/12RS fluorescent sun lamp (Amsterdam, Holland), with an emission spectrum between 285—350 nm (peak at 310—315 nm). Following this, the cells were exposed to a 100 mJ/cm^2 dose of UVB light.

Cytotoxicity Cell viability was assessed using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Sigma, St. Louis, MO, U.S.A.). Cells were pretreated with fucoidan at concentrations ranging from 0, 10, 25, 50, and 100 μg/ml prior to UVB irradiation. After incubation periods ranging from 24, 48 and 72 h, MTT solution (final concentration: 0.5 μg/μl) was added and cells were incubated at 37 °C for 3 h. The supernatant was then removed, and 100 μl of dimethyl sulfoxide (DMSO) was added. Finally, absorbance was measured on a microplate reader at 570 nm to obtain the percentage of viable cells.

Western Blotting Cells were lysed with lysis buffer (50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 10 μg/μl aprotinin, 10 μg/μl leupeptin, 5 mM phenylme-thanesulfonyluoride (PMSF), 1 mM dithiolthreitol (DTT) containing 1% Triton X-100). Insoluble debris was removed by centrifugation at 12000 rpm for 10 min, and protein content was determined using Bradford reagent (Bio-Rad, Hercules, CA, U.S.A.). Equal amounts of protein were resolved in gradient (10%) SDS PAGE gels (Invitrogen, Carlsbad, CA, U.S.A.) and electrophoretically transferred to nitrocellulose membranes. Membranes were subsequently blocked with 5% skimmed milk in TBST (20 mM Tris–HCl (pH 7.6), 137 mM NaCl, 0.05% Tween 20) and incubated with the indicated antibodies. Western blotting proteins were visualized by enhanced chemiluminescence. Western blotting was performed using the anti-human MMP-1 antibody (1 : 250) (Calbiochem, San Diego, CA, U.S.A.), anti-phospho-Jun N terminal kinase (JNK) (1 : 250), anti-phospho-extracellular signal related kinase (ERK) (1 : 500), anti-total-JNK (1 : 500), and anti-total-ERK (1 : 500) (Cell signaling, Boston, MA, U.S.A). In some experiments, cells were treated with chemical MAPK inhibitors. The specific MEK inhibitors PD98059 (Calbiochem, San Diego, CA, U.S.A.) was added to final 10 μM/l, while the JNK inhibitor SP600125 (Calbiochem, San Diego, CA, U.S.A.) was added to final 25 μM/l for 30 min.

RNA Extraction and Reverse Transcriptase (RT)-Polymerase Chain Reaction (PCR) To assay for the MMP-1 mRNA, total RNA was first isolated using the procedure of Chomczynski and Sacchi.[13] RNA concentration was quantified by UV spectrophotometer at 260 nm and the purity was determined using the A_{260}/A_{280} ratio. All samples were reverse-transcribed using moloneymurine leukemia virus reverse transcriptase (Bioneer, Daejeon, Korea) and 30 pM oligo dT19 in 20 μl of total reaction volume containing 5× RT buffer (250 mM Tris–HCl pH 8.3, 375 mM KCl, 15 mM $MgCl_2$, 50 mM DTT), and 1 mM dNTPs. RT-PCR assay was performed to specifically quantify mRNA level. In all assays, cDNA was amplified using a standardized program (10 min denaturing step; 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C: melting point analysis in 1 °C step: final cooling step) using a Gene Amp PCR 2400 (Applied Biosystem, CA, U.S.A). The primers used for β-actin were forward; 5′-GGA CCT GAC AGA CTA CCT CA-3′, reverse; 5′-GTT GCC AAT AGT GAT GAC CT-3′, for MMP-1 were forward; 5′-GGT GAT GAA GCA GCC CAG-3′ reverse; 5′-CAG TAG AAT GGG AGA GTC-3′.

Statistical Analysis Data were expressed as the mean±S.E.M. and were analyzed by analysis of variance (ANOVA) and Kruskal–Wallis test. Statistical significance was set a *priori* at $p<0.05$.

RESULTS

Changes in Cell Viability by Fucoidan under UVB Irradiation Cytotoxicity induced by UVB irradiation was measured after 24, 48, and 72 h at doses of 0, 50, 75, and 100 mJ/cm^2 (Table 1). Cell viability was significantly higher 24 h after UVB irradiation in the groups pretreated with fucoidan at doses of 10, 25, and 50 μg/ml in comparison to the group with UVB irradiation only, while at doses of 100 μg/ml was not significantly affected. These results suggest that fucoidan has a slight toxicity at dose of 100 μg/ml.

Inhibitory Effect of Fucoidan on MMP-1 Expression To examine UVB-induced MMP-1 expression in cultured cells, cells were irradiated with UVB (0—100 mJ/cm^2). As expected, UVB radiation induced MMP-1 expression in a dose-dependent manner (Fig. 1). Cells were pretreated with various treatment concentrations of fucoidan for 24 h followed by irradiation with UVB (100 mJ/cm^2). Cells were further incubated for an additional 24 h (Fig. 2A), 48 h (Fig. 2B), and 72 h (Fig. 2C). UVB-induced MMP-1 expression was inhibited as a function of fucoidan dose; after 24 h of fucoidan incubation, MMP-1 expression was reduced by 13, 49, and 66% with 10, 25, and 50 μg/ml of fucoidan, respectively, compared to UVB irradiation only (Fig. 2A). After a 48 h incubation period, MMP-1 expression was inhibited by 27, 55, and 77% with 10, 25, and 50 μg/ml of fucoidan, respectively, compared to UVB irradiation only (Fig. 2B). After a 72 h incubation period, similar results were observed (Fig. 2C).

Table 1. Effect of Fucoidan on Cell Viabilities under UVB Irradiation

Source[a]	Harvested time	Dose	Fucoidan (μg/ml)[b]				
			0	10	25	50	100
UVB (mJ/cm^2)	24 h	0	100±0.00	98.2±0.13	100.38±0.90	99.15±1.52	94.68±3.53
	24 h	50[c]	78.31±1.55	85.96±6.02	89.78±1.15	91.45±0.68	89.48±2.07
	24 h	75[d]	70.97±2.14	75.71±0.08	78.09±1.93	85.57±6.58	74.75±4.04
	24 h	100[e]	66.38±1.86	74.84±0.19	76.99±1.18	81.06±0.16	70.98±0.34
UVB (mJ/cm^2)	48 h	0	100±0.00	100±3.46	97.5±7.26	96.73±5.33	100.98±4.33
	48 h	50[f]	6.56±1.60	70.30±1.18	69.14±0.06	76.25±3.62	75.44±2.77
	48 h	75	59.11±2.50	63.13±1.57	64.35±0.74	65.54±0.37	61.88±2.31
	48 h	100	50.27±3.03	52.71±4.64	54.40±5.51	56.56±5.27	48.37±2.91
UVB (mJ/cm^2)	72 h	0	100±0.00	99.57±5.16	102.84±3.59	98.52±4.95	100.58±3.07
	72 h	50	42.05±4.52	42.88±0.62	45.86±0.62	45.09±5.61	41.28±2.23
	72 h	75	31.28±0.04	35.07±0.02	36.20±0.02	42.67±0.01	30.25±0.02
	72 h	100	25.82±3.65	29.75±2.94	8.61±0.59	32.05±1.45	31.43±7.38

a) The cells were treated with UVB (0, 50, 75, 100 mJ/cm^2). *b*) The cells were pretreated with fucoidan (0, 10, 25, 50, 100 μg/ml) prior to UVB irradiation and incubated 24, 48 and 72 h later. *c*) Cell viability was significantly higher in doses of 25, 50 and 100 μg/ml than 0 μg/ml ($p<0.05$). *d*) Cell viability was significantly higher in dose of 50 μg/ml than 0, 10, 25 and 100 μg/ml ($p<0.05$) and significantly higher in dose of 25 μg/ml than 0 μg/ml ($p<0.05$). *e*) Cell viability was significantly higher in dose of 50 μg/ml than 0 and 100 μg/ml ($p<0.05$) and significantly higher in doses of 10 and 25 μg/ml than 0 μg/ml ($p<0.05$). *f*) Cell viability was significantly higher in doses of 50 and 100 μg/ml than 0 μg/ml ($p<0.05$). Cell viability was measured by MTT assay, as described in Materials and Methods and was calculated in terms of relative values. Each value represents mean±S.D. ($n=6$).

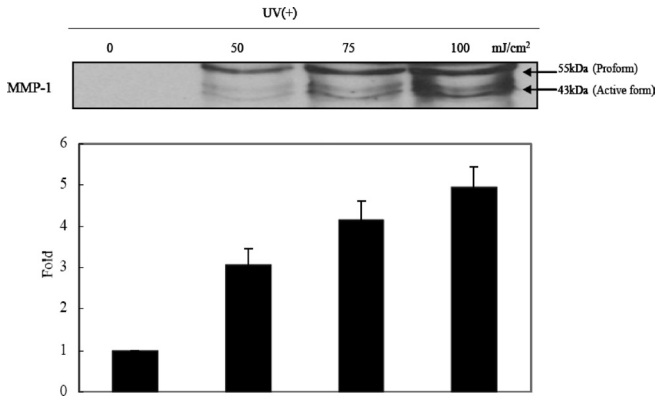

Fig. 1. MMP-1 Protein Expression after Various Doses of UVB Irradition in Human Foreskin Fibroblasts (HS68)

PBS was added to the quiescent cells before UVB exposure (0—100 mJ/cm^2). After UVB irradiation, the cells were washed with PBS incubated for an additional 72 h. MMP-1 expression was determined in the culture media by Western blotting. Each bar of the lower figures shows combined data of proform and active form of MMP-1 quantified by densitometry.

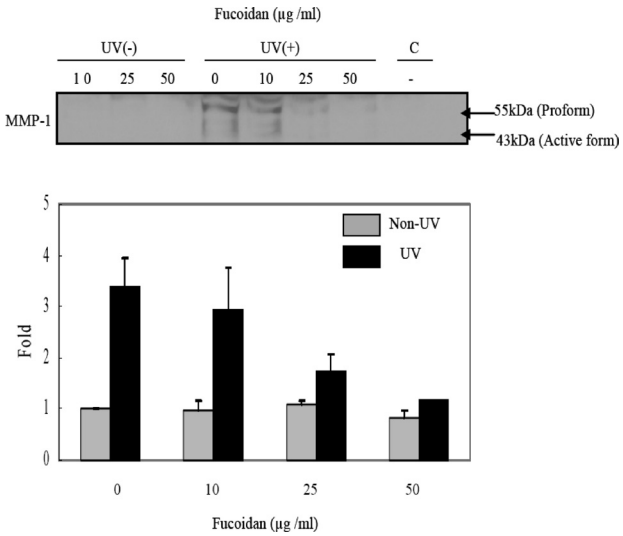

Fig. 2A. Inhibition of UVB-Induced MMP-1 Expression by Fucoidan in Human Foreskin Fibroblasts (HS68)

After pretreated for 24 h with fucoidan, cells were mock-treated or irradiated with UVB (100 mJ/cm^2). The cells were washed PBS and cells were further incubated for 24 h. MMP-1 expression was determined in culture media by Western blotting. Each bar of the lower figures shows combined data of proform and active form of MMP-1 quantified by densitometry.

Effects of Fucoidan on UVB-Induced ERK and JNK Activation To examine the roles of ERK and JNK protein expression in UVB-induced MMP-1 expression, a western blot analysis was performed at 0, 5, 15, 30, 60, and 480 min after UVB (100 mJ/cm^2) exposure. ERK and JNK expression was maximally induced at 30 min after UVB irradiation and subsequently declined to basal levels in cells (Fig. 3A). Cells were pretreated with PD98059 (10 μM: mitogen activated ERK activating kinases (MEK) inhibitor), and SP600125 (25 μM: JNK inhibitor) for 30 min and followed with UVB irradiation (100 mJ/cm^2). UVB-induced MMP-1 expression pretreated with PD98059 and SP600125, was inhibited by 59% and 76%, respectively (Fig. 3B). Given that ERK and JNK activation is required for UVB-induced MMP-1 expression in cells, we investigated the effects of fucoidan on UVB-induced ERK and JNK activation. ERK activation was markedly inhibited by treatment with fucoidan, but JNK activation was very slightly affected. However, no change was observed in total ERK and JNK levels (Fig. 3C).

Effect of Fucoidan on UVB Induced MMP-1 mRNA Levels In order to study the inhibitory effects of fucoidan on UVB-induced MMP-1 expression at the transcription level, RT-PCR analysis was performed using total RNA isolated from the cells. As expected, the level of UVB-induced MMP-1 mRNA expression showed positive dependence with fucoidan dose (Fig. 4A). Pretreatment with fucoidan was reduced MMP-1 mRNA expression compared to UVB irradiation alone (Fig. 4B).

DISCUSSION

It is well established that UV irradiation of cultured human skin fibroblasts *in vitro* or human skin *in vivo* induces the expression of MMPs which play important roles in the degradation of extracelluar matrix components during pre-

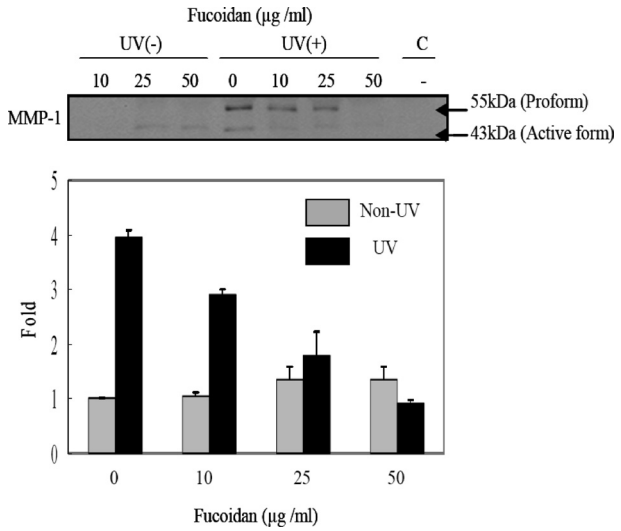

Fig. 2B. Inhibition of UVB-Induced MMP-1 Expression by Fucoidan in Human Skin Fibroblasts (HS68)

After pretreated for 24 h with fucoidan, cells were mock-treated or irradiated with UVB (100 mJ/cm^2). The cells were washed PBS and cells were further incubated for 48 h. MMP-1 expression was determined in culture media by Western blotting. Each bar of the lower figures shows combined data of proform and active form of MMP-1 quantified by densitometry.

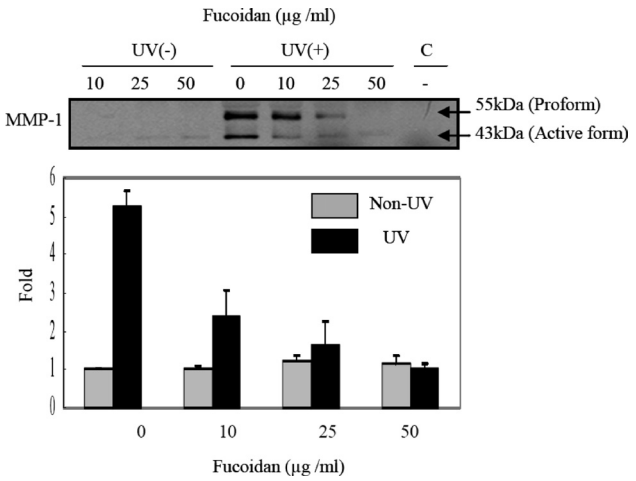

Fig. 2C. Inhibition of UVB-Induced MMP-1 Expression by Fucoidan in Human Skin Fibroblasts (HS68)

After pretreatment for 24 h with fucoidan, cells were mock-treated or irradiated with UVB (100 mJ/cm^2). The cell were washed PBS and cells were further incubated for 72 h. MMP-1 expression was determined in culture media by Western blotting. Each bar of the lower figures shows combined data of proform and active form of MMP-1 quantified by densitometry.

mature skin aging (photoaging).[14—16] Varani *et al.*[17] reported that with increasing age, MMP levels rise and collagen synthesis decline for sun-protected human skin *in vivo*. Hence, the development of MMP inhibitors is considered to be a promising strategy for skin cancer therapy and photoaging. In recent years, the development of compounds with MMP inhibition activities from natural plants has received a great deal of attention.[3]

We investigated the inhibitory effect of fucoidan on MMP-1 as a new anti-photoaging substance. Senni *et al.*[12] have already reported that fucoidan was able to stimulate dermal fibroblast proliferation and extracellular matrix deposition *in vitro*. However, no *in vitro* investigations of fucoidan on the MMP-1 expression have been reported to date. Hence, we in-

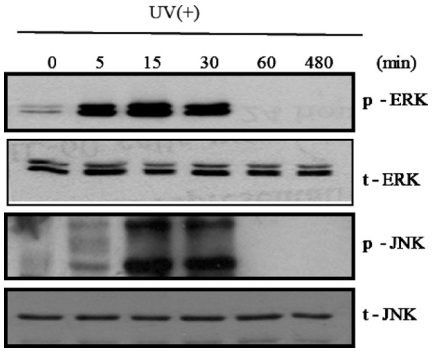

Fig. 3A. The Role of MAP Kinases (ERK, JNK) in UVB-Induced MMP-1 Expression

Cells were irradiated with UVB (100 mJ/cm^2) and incubated. Total cell lysates were prepared as described in the Materials and Methods section and subjected to Western blotting using phosphor-specific (-p) ERK, JNK; Levels of total (-t) ERK, JNK were used as loading controls.

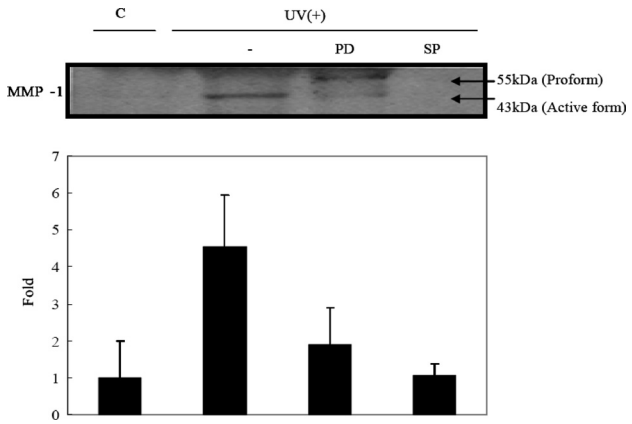

Fig. 3B. The Roles MAP Kinases (ERK, JNK) in UVB-Induced MMP-1 Expression

Cells were pretreated with PD98059 [(PD) a MEK kinase inhibitor; 10 μM], SP600125 [(SP) a JNK kinase inhibitor; 25 μM] for 30 min. Cells were washed with PBS and irradiated with UVB (100 mJ/cm^2). The cells were then washed again with PBS and further incubated for 72 h. MMP-1 expression was assessed in culture media by Western blotting. Each bar of the lower figures shows combined data of proform and active form of MMP-1 quantified by densitometry.

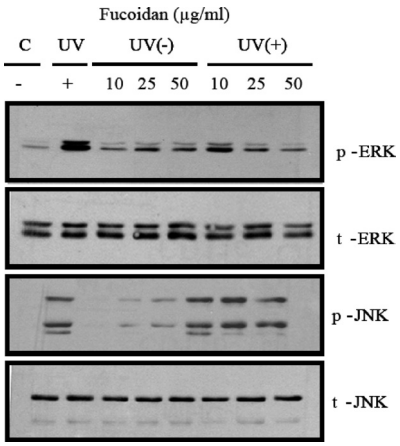

Fig. 3C. Fucoidan Inhibits UVB-Induced MMP-1 Expression by Inhibiting ERK and JNK Activation

Cells were pretreated fucoidan for 24 h and irradiated with UVB (100 mJ/cm^2). After a 30 min incubation for period, total cell lysates were prepared as described in Materials and Methods. Western blotting was performed using phosphor-specific (-p) ERK and JNK antibodies; Levels of total (-t) ERK and JNK were used as loading controls.

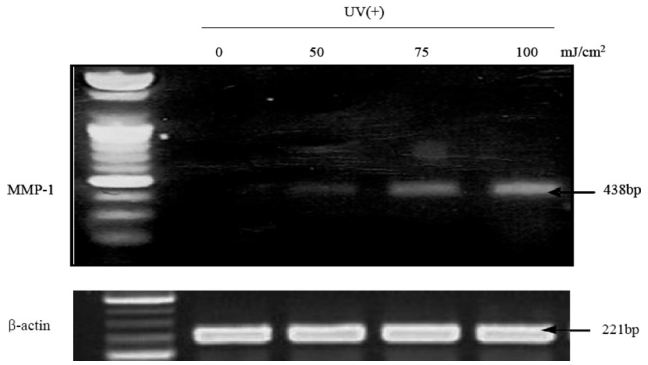

Fig. 4A. MMP-1 mRNA Expression after Dose-Dependent Irradiation of UVB in Human Foreskin Fibroblasts (HS68)

PBS was added to quiescent cells prior to UVB (0—100 mJ/cm^2). After UVB irradiation, the cells were washed with PBS, and further incubated for 24 h. MMP-1 mRNA was determined by RT-PCR.

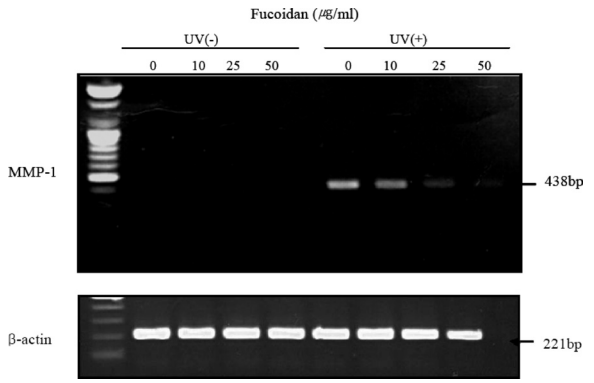

Fig. 4B. Effect of UVB-Induced MMP-mRNA Expression by Fucoidan in Human Skin Fibroblasts

PBS was added to quiescent cells prior to UVB exposure (100 mJ/cm^2). After UVB irradiation, cells were washed with PBS, and were further incubated for 24 h. MMP-1 mRNA was determined by RT-PCR.

vestigated the inhibitory effect of fucoidan on the MMP-1 expression by various *in vitro* experiments and elucidated the pathways of inhibition.

To examine the effect of fucoidan in cell viability after UVB irradiation, fucoidan was pretreated to the cells. But it didn't suppress the cell death at 48 and 72 after UVB, fucoidan delayed only the cell death.

UVB can induce MMP-1 expression as a function of dose. Südel *et al.*[2] suggested that time after UV exposure *in vitro* directly determines the extent of damage that is inflicted on MMP-1 expression. Di Girolamo *et al.*[18] reported that MMP-1 expression increases both as a function of time and dose in cultured cells in response to UV exposure. To characterize the time course of MMP-1 activation after UVB exposure *in vitro*, cells were irradiated with 100 mJ/cm^2 and incubated for 24 h, 48 h and 72 h. We found that pretreatment with fucoidan, followed by a 24 h incubation period, inhibited UVB-induced MMP-1 expression as a function of fucoidan dose by 13% at 10 μg/μl, 49% at 25 μg/ml and 66% at 50 μg/ml, compared to the UVB-only irradiated group. After a 48 h and 72 h incubation period, inhibitory effects of fucoidan on MMP-1 expression were observed.

Fucoidan is sulfated polysaccharides from brown algae cell wall which are described after fragmentation to mimic some heparin properties.[19,20] Gaultier *et al.*[21] reported that heparin and over sulfated chondroitin sulfate had the capability to inhibit MMP-1 protein and mRNA expression. Wang *et al.*[22] reported that intracellular MMP-1 was down-regulated by polysulphated polysaccharide which is consistent with our results. Collagen is the most abundant protein in skin connective tissue, and is initially cleaved by MMP-1, promotes further collagen breakdowns followed by photoaging processes. Our results suggest that fucoidan may attenuate connective tissue damage by inhibition of UVB-induced MMP-1 expression.

Earlier investigations demonstrated that MMP-1 was up-regulated *via* UVB-induced ERK and JNK signaling pathway. Kim *et al.*[23] reported that ERK and JNK pathways were involved in UVB-induced MMP-1 expression. Similar to their results, we also observed that ERK and JNK kinases were activated within 30 min of UVB irradiation, followed by a decline to a baseline level of cells. To investigate the role of ERK and JNK in UVB-induced MMP-1 expression, cells were pretreated with PD98059 (10 μM, MEK-specific inhibitor), SP600125 (25 μM, JNK-specific inhibitor). We found that UVB-induced MMP-1 expression was inhibited by these two substances. Therefore, we hypothesized that fucoidan may inhibit MMP-1 expression by prevention of the ERK and JNK signaling pathway. We also found that ERK activation was markedly inhibited by treatment with fucoidan. However, JNK activation was very slightly affected by fucoidan. Our results demonstrates that fucoidan mainly affects inhibition of ERK activation. In this study, fucoidan enhanced the expression of p-ERK and p-JNK in non-UVB-induced cells. Wu *et al.*[24] also observed that treatments with catechin in non-UVB cell had a slightly activated the ERK and JNK phosphorylation. Shin *et al.*[25] reported that pretreatment with DHEA inhibited also c-fos and c-jun (downstream of ERK and JNK) expression. Further studies will be needed to evaluate the effects of fucoidan.

Gene transcription has been demonstrated to be an important regulatory mechanism for MMP-1.[2] To investigate the extent to which the increase in MMP-1 mRNA is reflected by the activity level, cells were irradiated with UVB, and a MMP-1 mRNA expression assay was performed. We observed that MMP-1 mRNA expression was activated within 24 h after UVB irradiation followed by a decline to the level in cells (not shown data). Südel *et al.*[2] reported that after UV irradiation, MMP-1 was time-dependently induced on the mRNA level in dermal fibroblasts *in vitro*/*in vivo* in human skin and then a strong induction of MMP-1 mRNA at 24 h after UV irradiation as similar as our results. We also found that UVB-induced MMP-1 mRNA expression showed a negative relationship with fucoidan levels followed by a 24 h incubation period. In summary, we found that the inhibitory effect of fucoidan on MMP-1 expression at the level of transcription.

This study demonstrates the inhibitory effect of fucoidan on the MMP-1 expression *via* protein and mRNA assay. Also, our data indicate that fucoidan may prevent UVB-induced MMP-1 expression by inhibiting the UVB-induced activation of the ERK signaling pathway. Therefore, we suggest that fucoidan should be viewed as a potential therapeutic agent for preventing and treating skin photoaging.

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