

Effects of Ultraviolet A and B Irradiation on the Transcriptional Regulation of Stromelysin-1 Gene in Human Fibroblast Cultures

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Background : Sun exposure and therapeutic irradiation have been shown to induce alterations in extracellular matrix (ECM) proteins, including elastin, glycosaminoglycan and collagens. The integrity of the connective tissue mainly depends on balanced rates of matrix synthesis and degradation of the extracellular matrix. Therefore, matrix metalloproteinases (MMPs) may be involved in ultraviolet irradiation (UVR)-induced alterations in ECM proteins.

Objective : To evaluate the effects of UVA as well as UVB irradiations on ST-1 gene expression in cultured human skin fibroblasts.

Methods : After exposure of different doses of UVA and UVB on cultured human skin fibroblasts, we examined the expression of ST-1 gene by Northern blot analysis, chloramphenicol acetyltransferase (CAT) assay with CAT construct containing AP-1 binding site. Additionally, we carried out the gel mobility shift assay to investigate the effects of UVR on the DNA-binding activity of AP-1

Results : After UVR on fibroblasts, the steady-state levels of ST-1 mRNA were increased in response to UVA and UVB by 2.5-fold and 4.2-fold, respectively, as compared with controls. Similar results were obtained by CAT assay showing that CAT activity increased as the UVA and UVB doses increased. Furthermore, gel mobility shift assay demonstrated that both UVA and UVB increased AP-1 DNA binding complexes.

Conclusion : UVB as well as UVA up-regulated ST-1 gene expression at transcriptional levels in vitro. We speculate that modulation of MMPs, including ST-1, gene expression by UVR may contribute to the connective tissue damage related to photoaging and other photocutaneous disorders. (*Ann Dermatol* 11(4) 225~231, 1999).

Key Words : UVA, UVB, ST-1, In vitro.

Photoaging by chronic sun exposure is characterized by dermal connective tissue changes, which gives the skin a yellowish hue and leathery consistency. These clinical manifestations indicate ultraviolet irradiation (UVR) induces alterations in extracellular matrix (ECM) proteins, including elastin, glycosaminoglycan and collagens¹⁻⁵.

The integrity of the connective tissue mainly depends on balanced rates of matrix synthesis and degradation of the extracellular matrix. Therefore, matrix metalloproteinases (MMPs) play a central role in chronic ultraviolet radiation-induced alterations in ECM proteins^{6,7}. Stromelysin-1 (ST-1), MMP 3, is a key member of the MMP family with a broad substrate specificity. It can degrade several structural matrix glycoproteins, including fibronectin, laminin, gelatin and collagens types III, IV, V, and IX. In addition, ST-1 can also activate other MMPs such as 92-kDa type IV collagenase, matrilysin, and gelatinase B, rendering ST-1 crucial in remodeling of connective tissue during development, growth, aging, and wound healing^{8,9}.

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Fig. 1. A, Dose-dependent enhancement of stromelysin-1 mRNA levels by UVA irradiation. Cultured Fibroblasts were exposed to varying doses of UVA (0-30 J/cm²). Twenty-four hrs after UVA irradiation, total RNA was isolated and the stromelysin-1 and GAPDH mRNAs were detected by Northern hybridizations with corresponding cDNAs (Upper panel). The relative levels of stromelysin-1 mRNAs were presented as fold induction of unirradiated control group (Lower panel). B, Dose-related induction of stromelysin-1 mRNA levels by UVB irradiation. Cultured Fibroblasts were exposed to varying doses of UVB (0-30 mJ/cm²).

The production of ST-1 is under tight control and primarily regulated at the level of transcription. Enzyme synthesis and secretion are induced by a variety of treatments and extracellular signals such as cytokines, growth factors and oncogenic transformation¹⁰. Induction of human ST-1 gene expression has been specifically linked to exposure of cultured fibroblast cells to the tumor-promoting phorbol 12-myristate 13-acetate (PMA) and interleukin-1^{11,12}. Conversely, ST-1 gene expression is inhibited by retinoic acid, dexamethasone and transforming growth factor- β ^{13,14}. Recently, it was reported that UVA irradiation stimulates ST-1 gene expression, suggesting that MMPs contribute to UVR-induced ECM damage¹⁵. Additionally, Sawamura *et al.*¹⁶ have suggested that activation of ST-1 gene transcription by UVA irradiation is mediated, in part, through the AP-1 site within the 5'-flanking region from -70 to -64, which is also involved in induction of ST-1 by PMA and interleukin-1¹⁷. But the UVB effect on MMPs is still not resolved. The question whether UVB up-regulates interstitial collagenase (MMP-1) gene expression is in dispute in the literature. While

many investigations^{13, 18, 19} reported an induction of MMP-1 and ST-1 mRNA after UVB irradiation in vivo or in vitro, Petersen *et al.*⁷ did not demonstrate an increase in MMP-1 mRNA both in fibroblasts and keratinocytes after irradiation with UVB (0-100 mJ/cm²). Due to promoter similarities sharing AP-1, PEA-3 and TATA, MMP-1 and ST-1 have been shown to be similarly regulated in different experimental settings²⁰.

Consequently, we examined the effects of UVA and UVB irradiations on ST-1 gene expression by Northern blot analyses, chloramphenicol acetyltransferase (CAT) assay, and gel mobility shift assay in cultured human skin fibroblasts.

MATERIALS AND METHODS

Fibroblast Culture

Primary cultures of dermal fibroblasts were established from a child's skin (n=3, mean age:3-year-old) left over from surgery and subcultivated on plastic culture dishes in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, penicillin (100U/ml), streptomycin

Fig. 2. A, Dose dependent increase of stromelysin-1 promoter activity in transient transfections of cultured skin fibroblasts by UVA irradiation. The cells transfected with the stromelysin-1 promoter/CAT gene construct were exposed to varying doses of UVA (0-20 J/cm²). Twenty-four hrs following UVA irradiation, the CAT activity was determined by the radioactivity in the acetylated (AC) and nonacetylated (C) forms of ¹⁴C chloramphenicol. B, Dose dependent enhancement of stromelysin-1 promoter activity by UVB irradiation. in transient transfections. The transfected cells were exposed to varying doses of UVB (0-20 mJ/cm²).

(100µg/ml), and amphotericin B (1µg/ml). The cells were maintained in a humidified 5% CO₂ - 95% air incubator at 37 °C. Analyses of confluent fibroblast cultures were carried out at 3 - 6 passages of subcultivation.

UV Source and Irradiation

UVA was supplied by UVASUN 3000® emitting wavelengths in the 340-450 nm range (Mutzhas, Munich, Germany) and UVB was supplied by FS-40 sunlamps emitting radiation between 280 and 315 nm (National biological corp, Twinsberg, OH, USA). Both delivered uniform irradiation at a dis-

tance of 38cm. The energy output of UV at 38cm was measured with a UVA/UVB photometer (IL 1350 photometer, International Light Inc, MA, USA). The output of UVA and UVB was 72mW/cm²/sec and 0.1mW/cm²/sec at 38cm, respectively.

Fibroblast cultures (passage 3-6) were exposed to UVA or UVB at doses of 10, 20 and 30 J/cm² or 10, 20 and 30 mJ/cm², respectively. After 24 h of incubation, total RNA was isolated and CAT activity was determined. To prevent light absorption by tissue-culture medium, culture medium was removed just prior to irradiation and replaced with a

Fig. 3. Both UVA and UVB irradiations upregulate AP-1 DNA binding activity. A radioactively labeled double-stranded oligomer containing the AP-1 consensus sequence was incubated with nuclear extracts from cultured fibroblasts irradiated with or without UVA or UVB for 1 hr. The reaction mixture was subsequently analyzed by electrophoresis on 4% non-denaturing polyacrylamide gels.

A) Effects of UVA and UVB irradiations

B) 10 and 50 molar excesses of consensus AP-1 site oligo abolished retarded complexes induced by UVA (10 J/cm²). C:competitor

thin layer of phosphate-buffered saline (PBS) sufficient to cover the cells. Unirradiated control cells were also replaced with PBS. Tissue-culture medium was replaced in all dishes immediately after the last UV dose was administered. Cell viability was determined by trypan blue exclusion 16h after irradiation. Survival rates were more than 90% in all experiments presented. All experiments were performed in triplicate.

Northern Analyses

Total RNA was isolated by the method of Chomczynski and Sacchi²¹ from cultured normal skin fibroblasts. Extracted RNA was analyzed by Northern hybridization with ³²P labeled 1.5-kilobase pair (kb) human ST-1 cDNA probe²². The ³²P cDNA-mRNA hybrids were visualized by autoradiography, and the steady-state levels of mRNA were quantitated by laser densitometer (LKB Instrument, Inc., Bromma, Sweden). ST-1 mRNA levels were standardized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA levels in the same samples²³.

CAT Assay

The human ST-1 promoter/CAT reporter gene construct, ST56/CAT was a gift from D. Sawamura (Hirosaki University, Hirosaki, Japan). The plasmid contained 0.5 kb of the 5'-flanking DNA region of the human ST-1 gene linked to the CAT reporter gene for transient transfections of cultured fibroblasts¹⁶. The transfections were performed with the calcium phosphate/DNA coprecipitation method²⁴, followed by a 1 min (15%) glycerol shock. Four hrs after transfection, cells were irradiated with varying doses of UVA or UVB. After a total of 24hrs incubation, the cells were harvested, as described previously¹⁷. CAT activity was determined by incubating cell extracts with ¹⁴C chloramphenicol, followed by separation of its acetylated and non-acetylated forms by thin-layer chromatography as described elsewhere²⁵. CAT activity in the cell extracts was calculated by the radioactivity in the acetylated forms as a percent of the total radioactivity in each sample.

Gel Mobility Shift Assay

Nuclear proteins were isolated from cultured skin fibroblasts 1 h after irradiation of varying doses of UVA or UVB. For binding assay, a 21 bp, 5'-CGCTTGATGAGTCAGCCGAA-3' (the AP-1 site is indicated by the underline), double-stranded oligomer containing the AP-1 consensus sequence was labeled by [³²P]-dATP and used as a probe for protein binding. The oligomer containing approximately 5 × 10⁴ cpm of radioactivity was incubated with 8μg of the nuclear protein extract, and the DNA-protein complexes were fractionated on 4% polyacrylamide gel containing 0.4 × TBE under non-denaturing conditions, as described elsewhere²⁶. The radioactivity of oligomer-protein complexes were visualized by exposure of the gels to X-ray films with intensifying screens at -70°C.

RESULTS

Effect of Single Exposure of UVA or UVB Irradiations on the Steady State Level of ST-1 mRNA

To examine the effect of UVA or UVB irradiations on ST-1 gene expression at the mRNA level, cultured human fibroblasts were exposed to varying doses of UVA or UVB, and the corresponding mRNA levels were determined by Northern hy-

bridizations (Fig 1). In UVA or UVB treated and unirradiated cell cultures, characteristic 1.8kb mRNA transcripts for human ST-1 were detected. A dose-related induction of ST-1 was observed with an increase of ST-1 steady-state mRNA levels. Its range was from 1.3-fold to 2.5-fold and from 2.5- fold to 4.2-fold at 24 h following UVA and UVB irradiations, respectively, as compared with controls which were set as 1.0. Induction of ST-1 was maximal at dose of 30 J/cm² (mJ/cm²) UVA (UVB) irradiations.

Transcriptional Regulation of the Stromelysin-1 Gene by UVR

To examine the effect of UVA or UVB irradiation on ST-1 promoter activity, transient transfections were performed. Human ST56/CAT construct which contained segment -560 to +6 with AP-1 site located between -70 and -64 were transfected into the cultured skin fibroblasts and CAT activities were measured. Both single exposure of UVA and UVB resulted in an elevation of promoter activity in a dose dependent manner. Maximal promoter activation was occurred after single dose of 20 J/cm² (mJ/cm²) of UVA (UVB) irradiations, respectively. Relative CAT activity was 1.0 in the nonirradiated control, 6.25 (13.4) after a single dose of 10 J/cm² (mJ/cm²), 14.5 (20.1) after 20 J/cm² (mJ/cm²) of UVA (UVB) irradiations, respectively (Fig 2).

Effect of UVA or UVB Irradiations on AP-1 DNA binding

Both UVA and UVB irradiation increased AP-1 DNA binding. Additionally, increases of AP-1 DNA binding by UVA and UVB were dose-related (Fig. 3A). Binding of AP-1 to the double-stranded DNA probe was specific, as demonstrated by loss of retarded complexes with both 10 and 50 molar excesses of consensus AP-1 site competitor (Fig. 3B).

DISCUSSION

We found that both UVA and UVB irradiations up-regulated ST-1 gene expression at the transcriptional level. The induction of ST-1 gene expression was dose-dependent. The results of this study demonstrated transcriptional regulation of ST-1 by both UVA and UVB irradiations in cultured human skin fibroblasts. Our results are well in accordance with previous results^{6,13,16,19}. It is well

recognized that UVA irradiation induces mRNA and promoter activity of ST-1. However, the question whether UVB up-regulates MMP-1 gene expression is not settled. While several scientists^{13,18,19} reported an induction of MMP-1 and ST-1 mRNA after UVB irradiation in vivo and in vitro, respectively, Petersen et al.¹⁷ did not demonstrate an increase of MMP-1 mRNA both in fibroblasts and keratinocytes after irradiation with UVB (0-100 mJ/cm²). Brenneisen et al.¹⁹ suggested that the discrepancy may be due to the differences in the emission spectrum. In their studies, the wavelength dependence for the induction of MMP-1 is longer (300-320 nm) than that (280nm) in previous results obtained by Stein et al.¹⁸. MMP-1 and ST-1 have been shown to be similarly regulated in different experimental settings because of promoter similarities sharing AP-1, PEA-3 and TATA²⁰. In addition, the gene expression of ST-1 by UVB irradiation has not been widely known. Only Brenneisen et al.^{19,27} reported in vitro data. In these regards, our result of increased steady-state ST-1 mRNA levels by UVB supports previous data by Brenneisen et al.^{19,27}. Also, we found that not only UVA but also UVB irradiation stimulates ST-1 gene expression at transcriptional level. The promoter of ST-1 has a single AP-1 site, TGAGTCA, located in the region from -70 to -64¹⁶. The site is transactivated by the binding of newly synthesized and heterodimerized Fos and Jun, which constitute the AP-1 transcription factor¹³. Therefore, we examined whether the level of nuclear protein which bound to the AP-1 site changes after UVR. The gel mobility shift assay showed that UVB as well as UVA irradiation increased AP-1 DNA binding complexes. Increases of AP-1 binding by UVR were dose-related. Loss of intensity of retarded complexes with both 10 and 50-fold molar excesses of consensus AP-1 site competitor indicated that binding of AP-1 to the double-stranded DNA probe was specific. Our in vitro data was consistent with previous results^{13,16}. It has been reported some cis elements including PEA-3 associated with PMA response, zeta PKC related to platelet-derived growth factor response and another UVR-responsive element (TGACAACA) other than AP-1 binding site are important to the regulation of ST-1 gene expression. Although we did not perform the deletion analyses of the 5'-flanking region of ST-1 gene, our results suggest a possible involvement of

both UVA and UVB irradiations in the activation of ST-1 gene transcription through the AP-1 site. Little is known about the mechanisms of upregulation and induction of AP-1 and AP-1 regulated MMP genes. Currently, Some have reported that UVB activates AP-1 and thus induces MMP genes through activation of ERK and JNK/p38 pathways^{27,28}. Interestingly, Brenneisen *et al.*²⁷ demonstrated that the iron-derived generation of lipid peroxides and hydroxyl radicals after UVB irradiation in human dermal fibroblasts induce JNK2 activity, not ERK activity. However, the upstream signaling steps preceding the induction of the JNK activity remain to be elucidated.

In conclusion, UVB as well as UVA up-regulated ST-1 gene expression at transcriptional levels *in vitro*. We speculate that modulation of MMPs, including ST-1, gene expression by UVR may contribute to the connective tissue damage related to photoaging and other photocutaneous disorders.

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