

Ultraviolet-A Irradiation Upregulated Urokinase-Type Plasminogen Activator in Pterygium Fibroblasts through ERK and JNK Pathways

Shih-Chun Chao,¹⁻³ Dan-Ning Hu,³⁻⁵ Pei-Yu Yang,⁴ Ching-Yang Lin,¹ Chan-Wei Nien,¹ Shun-Fa Yang,^{6,7} and Joan E. Roberts⁸

PURPOSE. Effects of ultraviolet (UV) B on the pterygium and its cells have been studied previously, whereas little is known on the effects of UVA. Urokinase-type plasminogen activator (uPA) is a protease involved in tissue remodeling and cell migration, and its levels are increased in pterygium. The purpose of our study was to investigate the effects of UVA on the expression of uPA in cultured pterygium fibroblasts.

METHODS. Cultured fibroblasts from early-stage pterygia and normal conjunctiva were irradiated with different dosages of UVA and compared to nonirradiated cells. uPA activities in the medium and uPA mRNA in the cells were measured by casein zymography and RT-PCR, respectively. Total and phosphorylated p38 mitogen-activated protein kinase (MAPK), extracellular signal-related kinase (ERK), and c-Jun N-terminal kinase (JNK) levels of cells treated with and without UVA were measured by Western blotting. Inhibitors of p38 (SB203580), ERK (UO1026), and JNK (SP600125) were added before the irradiation of UVA to test their effects.

RESULTS. UVA irradiation increased the uPA mRNA levels in pterygium fibroblasts and the uPA activities in cultured medium, which was accompanied with an increase in phosphorylated ERK and JNK. The ERK and JNK inhibitor, but not p38 MAPK inhibitors, significantly decreased the UVA-induced expression of uPA by pterygium fibroblasts. Normal conjunctival fibroblasts were less sensitive to UVA irradiation compared to the pterygium fibroblasts.

CONCLUSIONS. UVA stimulated the production of uPA, a key factor in the modulation of extracellular matrixes, inflammatory processes, and angiogenesis. This may have a role in the development and progression of pterygium. (*Invest Ophthalmol Vis Sci.* 2013;54:999-1007) DOI:10.1167/iovs.12-10469

Pterygium is a common ocular surface disease associated with chronic ultraviolet (UV) exposure, and is characterized by proliferation, inflammatory infiltrates, angiogenesis, and extracellular matrix (ECM) breakdown.^{1,2} Compared to normal fibroblasts, pterygium fibroblasts grow much faster in a medium containing a low concentration of serum and can grow in a semisolid agar, indicating that these cells represent tumor-like transformed cells.³

UV radiation in sunlight (UVA and UVB) has an important role in the occurrence of pterygium, cataracts, and skin tumors. Early work on the mechanism of UV radiation-induced disease had focused on UVB, whereas the contribution of UVA has been ignored. Only in recent years has UVA been shown to cause damage to various cellular biomolecules, which can lead to the development of skin cancers and melanoma.⁴⁻¹²

Whereas UVB radiation does direct damage to cellular DNA, UVA radiation generates significant oxidative stress in cells, which in turn oxidizes cellular biomolecules, including DNA and lipids; initiates gene mutation and ECM degradation; activates protein kinases and phosphatases; modulates transcription factors; and induces inflammation. All these effects can lead to the development of skin tumors.⁷⁻¹²

In the past decades, *in vitro* studies have indicated that irradiation of pterygium tissues and cells with UVB induced expression of various cytokines, growth factors, and metalloproteinases (MMPs). These effects have an important role in the pathogenesis of pterygium.¹³⁻¹⁵ Epidemiologic studies have shown that the occurrence of pterygium was associated significantly with UVA and UVB.^{16,17} However, the effect of UVA on pterygium cells and its mechanism has yet to be defined.

Urokinase-type plasminogen activator (uPA) is a serine protease that converts plasminogen to plasmin, and then activates pro-MMPs into MMPs; degrades various ECM; stimulates cell migration, proliferation, and chemotaxis; inhibits apoptosis; and induces angiogenesis. uPA has an important role in tissue remodeling; angiogenesis; and the progression, invasion, and metastasis of tumors.¹⁸⁻²²

Recently, we found that the expression and secretion of uPA were increased in pterygium and its fibroblast. The expression of uPA by pterygium increased significantly following the progression of the pterygium. The increased expression of uPA may have an important role in the development and progression of pterygium.²³ It has been reported that UVB irradiation stimulated the expression of uPA in corneal cells.²⁴

From the ¹Department of Ophthalmology, Show Chwan Memorial Hospital, Changhua, Taiwan; the ²Institute of Electrical and Computer Engineering, National Chiao Tung University, Hsinchu, Taiwan; the ³Cell Culture Laboratory of Department of Medical Research, Show Chwan Memorial Hospital, Changhua, Taiwan; the ⁴Tissue Culture Center, New York Eye and Ear Infirmary, New York, New York; The ⁵Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan; the ⁶Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan; and ⁸Fordham University, New York, New York.

³These authors contributed equally to the work presented here and should therefore be regarded as equivalent authors.

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Corresponding author: Shun-Fa Yang, Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan; ysf@csmu.edu.tw.

However, to our knowledge the effects of UVA on the expression of uPA in pterygium fibroblasts have not been reported previously.

Our study investigated the effects of UVA radiation on the expression and secretion of uPA by fibroblasts isolated from surgical excised specimens of pterygium. The signaling pathways involved in the UVA-induced uPA expression also were studied.

MATERIALS AND METHODS

Subjects

Excised pterygia specimens were obtained from patients after surgery with the patients' informed consent, in accordance with the tenants of the Declaration of Helsinki. Our study was reviewed and approved by the Institutional Review Board of Show Chwan Memorial Hospital on February 15, 2007 (code: 960102).

The external eye of each patient was photographed before the operation. Pterygia were classified into three stages by the surgeon, based on the extent of the pterygium: Stage 1 - The head of pterygia did not reach the midline between the limbus and pupillary margin. Stage 2 - The head of pterygia passed the midline, but did not reach the pupil. Stage 3 - The head of pterygia passed the pupillary margin.

The tissue specimens used in our study have been reported previously.²³ Briefly, surgical specimens were collected from 15 pterygium patients. All of these pterygia were progressive in nature. Normal conjunctival tissues were obtained from 5 individuals.

Isolation and Cultivation of Pterygium and Normal Fibroblasts

Fibroblasts were obtained from pterygia or normal conjunctiva specimens by using the explants methods reported previously.²³ Briefly, the head of pterygium specimen or normal conjunctiva was cut into small pieces, washed, and placed into a culture dish. Dulbecco's modified Eagle's medium (DMEM) with 15% fetal bovine serum (FBS) was added to cover the explants (all from Gibco, Carlsbad, CA). The culture dish was put in a CO₂-regulated incubator. The culture medium was replaced 3 times a week after the appearance of the outgrowth of cells from the explants. After the primary cultures became confluent, cultured fibroblasts were detached from the dish with a 0.05% trypsin/0.01% EDTA solution (Gibco) and passaged for subcultures with a 1:3 split.

An immunocytochemical study was performed to identify the cell types. The epithelial cells contained cytokeratin antigens, but the fibroblasts did not.²³

Effects of UVA on the Viability of Pterygium Fibroblasts

Stage 1 pterygium fibroblasts in the second or third subcultures were seeded into 96 well plates (cell density at 5×10^3 per well) and cultured with DMEM with 10% FBS. When cells were nearly confluent (cells covered approximately 90% of the surface of the culture dish bottom), culture medium was aspirated and the culture was washed with PBS (with CaCl⁺⁺ and MgCl⁺⁺). Cells were cultured with PBS and irradiated with UVA by using a Daavlin 3 series 311/350 UV irradiator (Daavlin Company, Bryan, OH).²⁵ This UV irradiator emits a broad band of UVA from 320 to 400 nm at 0.05 W/cm². The UVA irradiance was measured with a spectroradiometer (LuzChem Research, Inc., Ottawa, ON, Canada). Cells were irradiated (covered with the plate lid) for 90 seconds, and 3.75, 7.0, and 15 minutes to obtain the UVA dosage at 2, 5, 10, and 20 J/cm², respectively. After UV irradiation, PBS was replaced by DMEM without serum. After 24 hours of culture, 50 μ L of tetrazolium bromide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 1 mg/mL; Sigma, St. Louis, MO) were added to

each well and incubated for 4 hours. The medium was withdrawn and 100 μ L of dimethyl sulfoxide (DMSO; Sigma) were added to each well. The optical density was read at 540 nm using a microplate reader (Multiskan EX; Thermo, Vantaa, Finland). Cells cultured with PBS, but without UVA radiation were used as the controls. All groups were tested in triplicate.

Effects of UVA on uPA Activities Secreted by Pterygium and Normal Fibroblasts

Stage 1 pterygium fibroblasts or normal conjunctival fibroblasts (Fig. 1) in the second or third subculture were seeded into a 35-mm dish (cell density at 2×10^5 per well) and cultured with DMEM with 10% FBS. Culture medium was aspirated and the culture was washed with PBS after near confluence. Cells were irradiated by UVA at 0, 0.5, 1.0, and 2.0 J/cm² as described above. After UV irradiation, PBS was replaced by DMEM without serum. After 48 hours of culture, the conditioned medium was collected and centrifuged. The supernatant was stored at -70°C until zymography analysis was conducted on the uPA activity. All tests were performed in triplicate.

Effects of UVA on mRNA Levels of uPA in Fibroblasts

Pterygium fibroblasts or normal conjunctival fibroblasts were irradiated with UVA at 0, 0.5, 1.0, and 2 J/cm² as described above. After 48 hours of culture, the culture medium was withdrawn. The fibroblasts were trypsinized and stored at -70°C until the mRNA of uPA was measured. All tests were performed in triplicate.

Determination of uPA Activities by Casein Zymography

The activities of uPA in the conditioned culture medium were measured by casein zymography protease assays, as we reported previously.²³ Briefly, the cultured media were prepared with an SDS sample buffer without boiling or reduction and then subjected to 2% casein and 20 μ g/mL plasminogen in 8% SDS-PAGE electrophoresis. After electrophoresis, the gels were washed with 2.5% Triton X-100 and then incubated in a reaction buffer (40 mM Tris-HCl, pH 8.0; 10 mM CaCl₂; and 0.01% NaN₃) at 37°C for 12 hours. Then the gels were stained with Coomassie brilliant blue R-250 (all from Sigma). The uPA standard from ELISA kits by American Diagnostica (Stamford, CT) was used as the positive control and buffer was loaded as the negative control. μ -Actin was used as an internal loading control. The relative photographic densities of uPA were quantified by scanning the photographic negatives on a gel documentation and analysis system (AlphaImager 2000; Alpha Innotech Corporation, San Leandro, CA).

RNA Preparation and Quantitative Real-Time PCR

Methods for RNA extraction and measurement of uPA mRNA have been described previously.²³ Briefly, total RNA was isolated from cultured fibroblast cells using Trizol (Life Technologies, Grand Island, NY) according to the manufacturer's instructions. For reverse transcription, first-strand cDNA synthesis was performed with random primers (hexamers; Promega, Madison, WI) and 100 U of Moloney murine leukemia virus reverse transcriptase (Promega), and carried out at 42°C for 60 minutes and terminated at 90°C for 10 minutes. The quantitative real-time PCR analysis was done using Taqman one-step PCR Master Mix (Applied Biosystems, Carlsbad, CA). A total of 100 ng cDNA was added per 25 μ L reaction with uPA primers and Taqman probes. The uPA (Hs01547054_m1) and GAPDH (Hs99999905_m1) primers and probe were designed using commercial software (ABI PRISM Sequence Detection System; Applied Biosystems).²³ Quantitative real-time PCR assays were done in triplicate on a StepOnePlus sequence detection system (Applied Biosystems). The cycling conditions were 10 minutes of polymerase activation at 95°C followed by 40 cycles at 95°C for 15

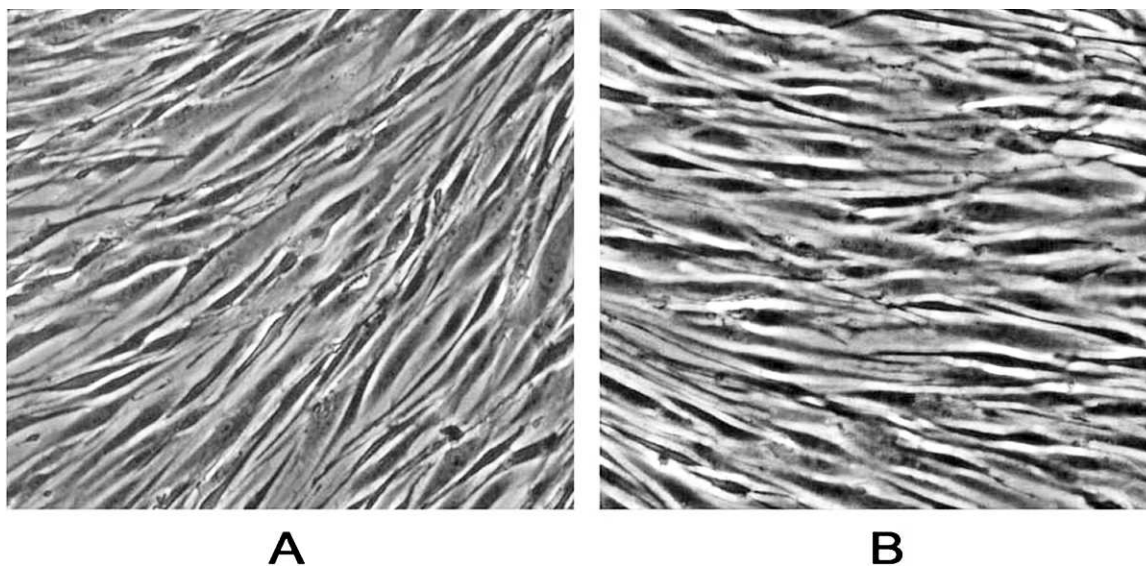


FIGURE 1. Morphology of pterygium fibroblasts and normal conjunctival fibroblasts (phase-contrast microscopy). (A) Pterygium fibroblasts. (B) Normal conjunctival fibroblasts.

seconds and 60°C for 60 seconds. The threshold was set above the nontemplate control background and within the linear phase of the target gene amplification to calculate the cycle number at which the transcript was detected.

UVA Irradiation, and Measurement of Total and Phosphorylated Extracellular Signal-Related Kinase (ERK), c-Jun N-Terminal Kinase (JNK), and p38 Mitogen-Activated Protein Kinase (MAPK)

Pterygium or normal conjunctival fibroblasts were plated into 10 cm culture dishes at a density of 1×10^6 cells per well. When cells were nearly confluent, the medium was changed to PBS and irradiated with UVA at 2.0 J/cm² as described above. After 0.5, 6, and 24 hours, the culture medium was withdrawn. Cells were washed with cold PBS three times and then scraped from the well. After cell counting and centrifugation at 1500 revolutions per minute (rpm) for 5 minutes at 4°C, the cell pellets were collected. Cells were lysed using Cell Extraction Buffer (BioSource, Camarillo, CA) with Protease Inhibitor Cocktail (Sigma) and phenylmethanesulfonylfluoride (PMSF; BioSource), incubated on ice for 30 minutes, and vortexed for 30 seconds. The lysates were centrifuged at 10,000 rpm for 10 minutes at 4°C. The supernatants were stored at -70°C until analysis.

The amount of phosphorylated, and total p38 MAPK, ERK, and JNK in cell lysates was measured by Western blotting. Briefly, the cell lysates were separated in a 10% polyacrylamide gel and transferred onto a nitrocellulose membrane. The blot subsequently was incubated with 5% nonfat milk in Tris-buffered saline (20 mM Tris, 137 mM NaCl, pH 7.6) for 1 hour to block nonspecific binding and then overnight with polyclonal antibodies against three MAPKs pathway (ERK 1/2, JNK 1/2, and p38) with the specific antibodies for unphosphorylated or phosphorylated activated forms of the corresponding ERK 1/2, JNK 1/2, and p38. Blots then were incubated with a horseradish peroxidase goat anti-rabbit or anti-mouse IgG for 1 hour. Afterwards, signal was detected by using enhanced chemiluminescence (ECL) commercial kit (Amersham Biosciences, Little Chalfont, UK), and relative photographic density was quantitated by scanning the photographic negatives on a gel documentation and analysis system (AlphaImager 2000; Alpha Innotech Corporation). All tests were performed in triplicate.

Effects of MAPK Inhibitors on UVA-Induced Release of uPA by Pterygium Fibroblasts

Pterygium fibroblasts cells were plated into 35 cm culture dishes at a density of 2×10^5 cells per well. When cells were nearly confluent, various MAPK inhibitors were added to the medium separately, including UO1026 (ERK inhibitor), SP600125 (JNK inhibitor), and SB203580 (p38 MAPK inhibitor) at 5 and 10 μM (all from Calbiochem, San Diego, CA). One hour later, the medium was changed to PBS, cells were irradiated with UVA at 2.0 J/cm² as described above. After UVA irradiation, PBS was replaced by DMEM without serum and cultured for 48 hours, the conditioned medium was collected and centrifuged. The supernatant was stored at -70°C until zymography analysis was conducted on the uPA activity.

Statistical Analysis

Statistical significances of differences throughout our study were calculated by an ANOVA one-way test in comparing data from more than two groups and using a Student's *t*-test for comparing data between two groups. The data were analyzed using SPSS statistical software (SPSS Inc., Chicago, IL). A difference at $P < 0.05$ was considered statistically significant.

RESULTS

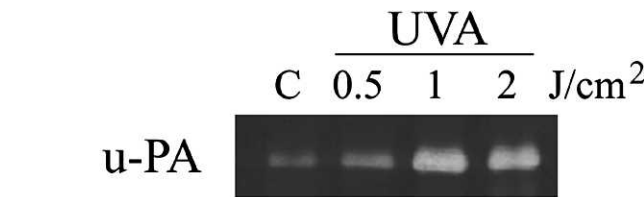
Effects of UVA on the Viability of Pterygium Fibroblasts

Cell viability in pterygium fibroblasts irradiated with UVA at 1, 2, 5, and 10 J/cm² was $100.7 \pm 5.1\%$, $103.0 \pm 4.4\%$, $81.6 \pm 5.4\%$, and $58.7 \pm 4.0\%$ of the controls (cells without irradiation of UVA), respectively. The difference between cells irradiated with and without UVA was significant at cells irradiated with 5 and 10 J/cm² ($P < 0.05$), but not in cells irradiated with 1.0 and 2.0 J/cm². Therefore, 2.0 J/cm² was used as the maximum dosage for the studies of effects of UVA on the expression and secretion of uPA from pterygium fibroblasts.

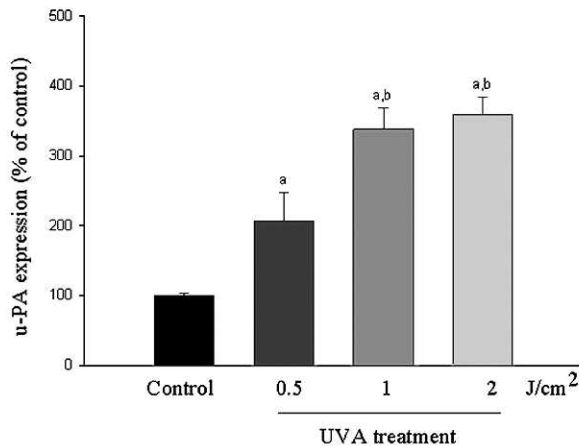
Effects of UVA Irradiation on uPA Activities in Cultured Medium from Pterygium and Normal Fibroblasts

uPA activities in the conditioned culture medium were measured by casein zymography. Pterygium fibroblasts showed constitutive secretion of activated uPA. Upon irradiation of cells with UVA, the secretion of uPA increased (Figs. 2A, 2B). uPA activities in medium from cells irradiated with 0.5, 1.0, and 2.0 J/cm² UVA were 2.09-, 3.42-, and 3.68-fold of that in nonirradiated fibroblasts, respectively (Figs. 2A, 2B). The differences in uPA activities in the cultured medium between fibroblasts irradiated with 0.5, 1.0, and 2.0 J/cm² UVA, and nonirradiated fibroblasts all were statistically significant (*P* < 0.05).

UVA irradiation also caused increase of uPA activities in the culture medium from normal fibroblasts. uPA activities in medium from cells irradiated with 0.5, 1.0, and 2.0 J/cm² UVA were 1.05-, 2.11-, and 1.78-fold of that in nonirradiated fibroblasts, respectively (Figs. 3A, 3B). The difference in uPA activities in the cultured medium between fibroblasts with or without UVA irradiation was statistically significant only in cells

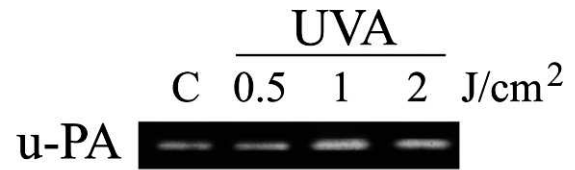


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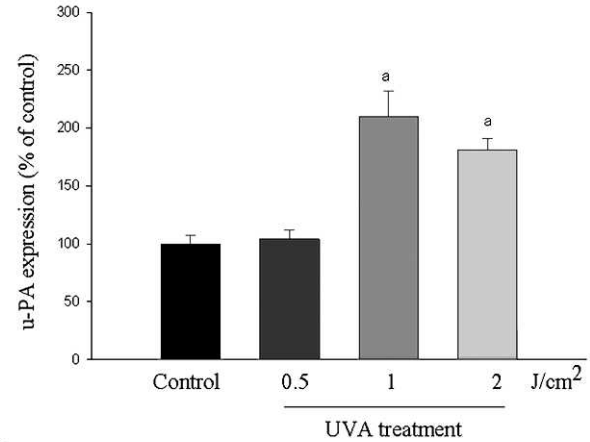


B

FIGURE 2. Effects of UVA irradiation on uPA activities in cultured medium from pterygium fibroblasts as measured by casein zymography. Cells were plated into 24-well plates and treated with or without UVA. After 48 hours, conditioned culture media were collected and uPA activities were measured using casein zymography as described in Materials and Methods. (A) Casein zymography. (B) Levels of uPA activity in fibroblasts irradiated with 0.5, 1, and 2 J/cm² of UVA. Data are given as mean ± SD (*n* = 3). (a) Significant difference (*P* < 0.05) compared to nonirradiated cells. (b) Significant difference (*P* < 0.05) compared to cells irradiated with 0.5 J/cm² UVA.



A



B

FIGURE 3. Effects of UVA irradiation on uPA activities in cultured medium from normal conjunctival fibroblasts as measured by casein zymography. Cells were plated into 24-well plates and treated with or without UVA. After 48 hours, conditioned culture media were collected and uPA activities were measured using casein zymography as described in Materials and Methods. (A) Casein zymography. (B) Levels of uPA activity in fibroblasts irradiated with 0.5, 1, and 2 J/cm² of UVA. Data are given as mean ± SD (*n* = 3). (a) Significant difference (*P* < 0.05) compared to nonirradiated cells.

irradiated with relatively high dosages of UVA (1.0 and 2.0 J/cm², *P* < 0.05), but not in 0.5 J/cm² UVA, indicating that normal fibroblasts were less sensitive to UVA irradiation compared to pterygium fibroblasts.

Effects of UVA Irradiation on uPA mRNA of Cultured Pterygium Fibroblasts

Real time RT-PCR study showed that uPA was expressed constitutively in pterygium fibroblasts. UVA induced the expression of uPA in a dose-dependent manner in cells irradiated with 0.5, 1.0, and 2.0 J/cm² UVA (Fig. 4A). The difference in uPA mRNA levels between the controls versus UVA-irradiated fibroblasts was statistically significant at all dosages of UVA (*P* < 0.05). UVA irradiation caused a slight increase of expression of uPA mRNA in normal fibroblasts, which was less sensitive to UVA irradiation compared to that of pterygium fibroblasts (Fig. 4B).

Effects of UVA Irradiation on ERK, JNK, and p38 MAPK Levels in Pterygium Fibroblasts

Irradiation of cultured pterygium fibroblasts did not affect the levels of total ERK, JNK, and p38 MAPK in these cells. Phosphorylated ERK and JNK levels in UVA-irradiated cells

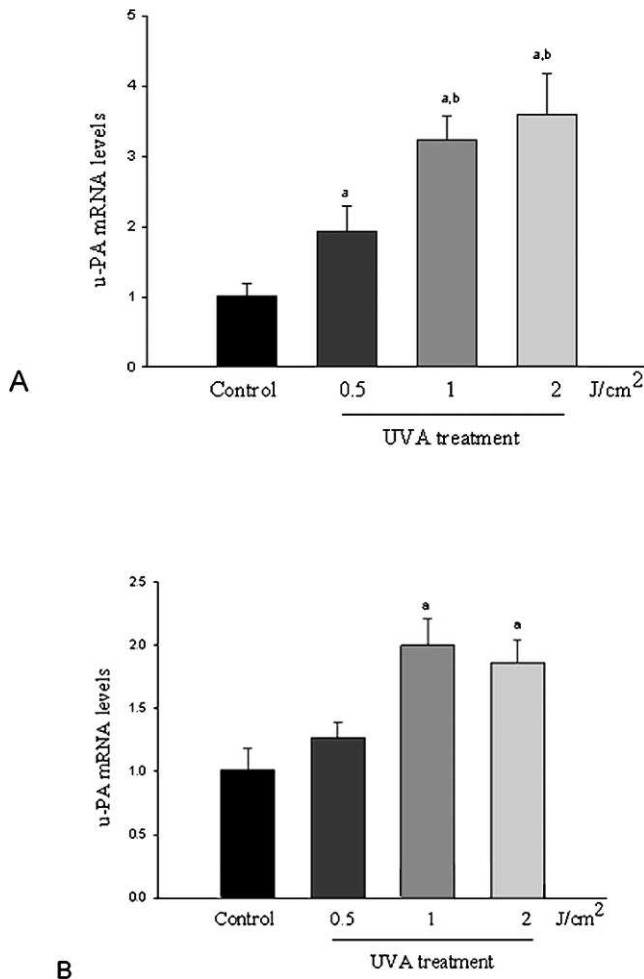


FIGURE 4. Effects of UVA irradiation on uPA mRNA expression in pterygium and normal fibroblasts measured by quantitative real-time PCR. Comparison of uPA mRNA levels between cells treated with 0.5, 1.0, and 2.0 J/cm² UVA, or without UVA. Data are given as mean ± SD (*n* = 3). **(A)** Levels of uPA activity in pterygium fibroblasts irradiated with 0.5, 1, and 2 J/cm² of UVA. **(a)** Significant difference (*P* < 0.05) compared to nonirradiated cells. **(b)** Significant difference (*P* < 0.05) compared to cells irradiated with 0.5 J/cm² UVA. **(B)** Levels of uPA activity in normal fibroblasts irradiated with 0.5, 1, and 2 J/cm² of UVA. **(a)** Significant difference (*P* < 0.05) compared to nonirradiated cells.

were significantly increased, whereas phosphorylated p38 MAPK levels were not affected (Figs. 5A, 5B). The difference of phosphorylated ERK levels between UVA-irradiated and nonirradiated cells was statistically significant 6 and 24 hours after irradiation (*P* < 0.05). The difference of phosphorylated JNK levels between UVA-irradiated and nonirradiated cells was statistically significant 0.5, 6, and 24 hours after irradiation (*P* < 0.05, Fig. 5B).

Effects of MAPK Inhibitors on UVA-Induced Increase of uPA Activities in Cultured Medium from Pterygium Fibroblasts

Treatment of SB203580 (p38 MAPK inhibitor) 30 minutes before UVA irradiation did not decrease significantly uPA activities in cultured medium from UVA-irradiated cells (*P* > 0.05; Figs. 6A, 6B). U0126 (ERK inhibitor) treatment (10 μM) decreased significantly UVA-induced uPA activities (*P* < 0.05). SP600125 (JNK inhibitor) treatment (5 and 10 μM) also

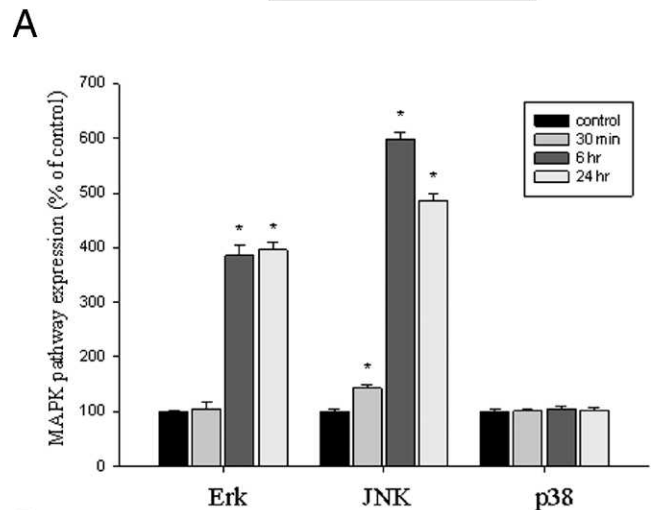
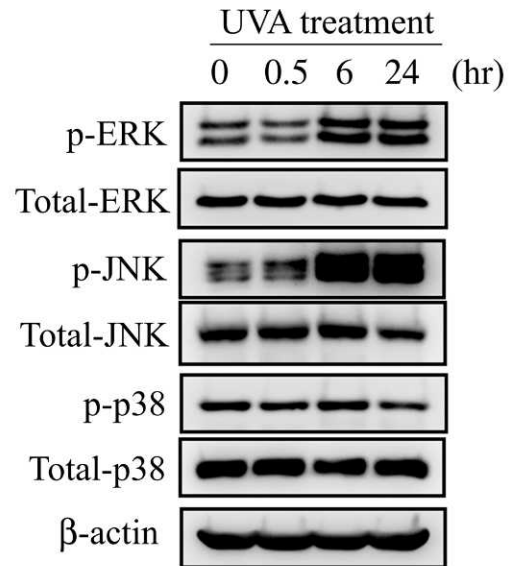


FIGURE 5. Effects of UVA irradiation on ERK, JNK, and p38 MAPK levels in pterygium fibroblasts. Cells were plated into 10 cm culture dishes and treated with or without UVA (2.0 J/cm²). After 0.5, 6, and 24 hours, cells were collected, and the amount of phosphorylated and total p38 MAPK, ERK, and JNK in cell lysates were measured by Western blotting. All tests were performed in triplicate. **(A)** Western blot analysis: Total and phosphorylated levels of ERK, JNK, and p38 were measured. β-actin was used as an internal loading control. **(B)** Levels of phosphorylated ERK, JNK, and p38 MAPK in cells treated with and without UVA (2.0 J/cm²) at 0.5, 6, and 24 hours. *Significant difference (*P* < 0.05) compared to nonirradiated cells.

decreased significantly UVA-induced uPA activities at (*P* < 0.05; Figs. 6A, 6B), indicating that ERK and JNK signal pathways are involved in UVA-induced expression of uPA by fibroblasts isolated from early stage pterygia.

DISCUSSION

The sun emits UV radiation in the UVA (320–400 nm), UVB (290–320 nm), and UVC (100–290 nm) regions. The Earth's ozone layer blocks all of the UVC and all but 10% of the solar radiation from UVB from penetrating through the atmosphere. However, most of the solar UVA radiation (90%–99%) is transmitted to the Earth's surface.^{11,16}

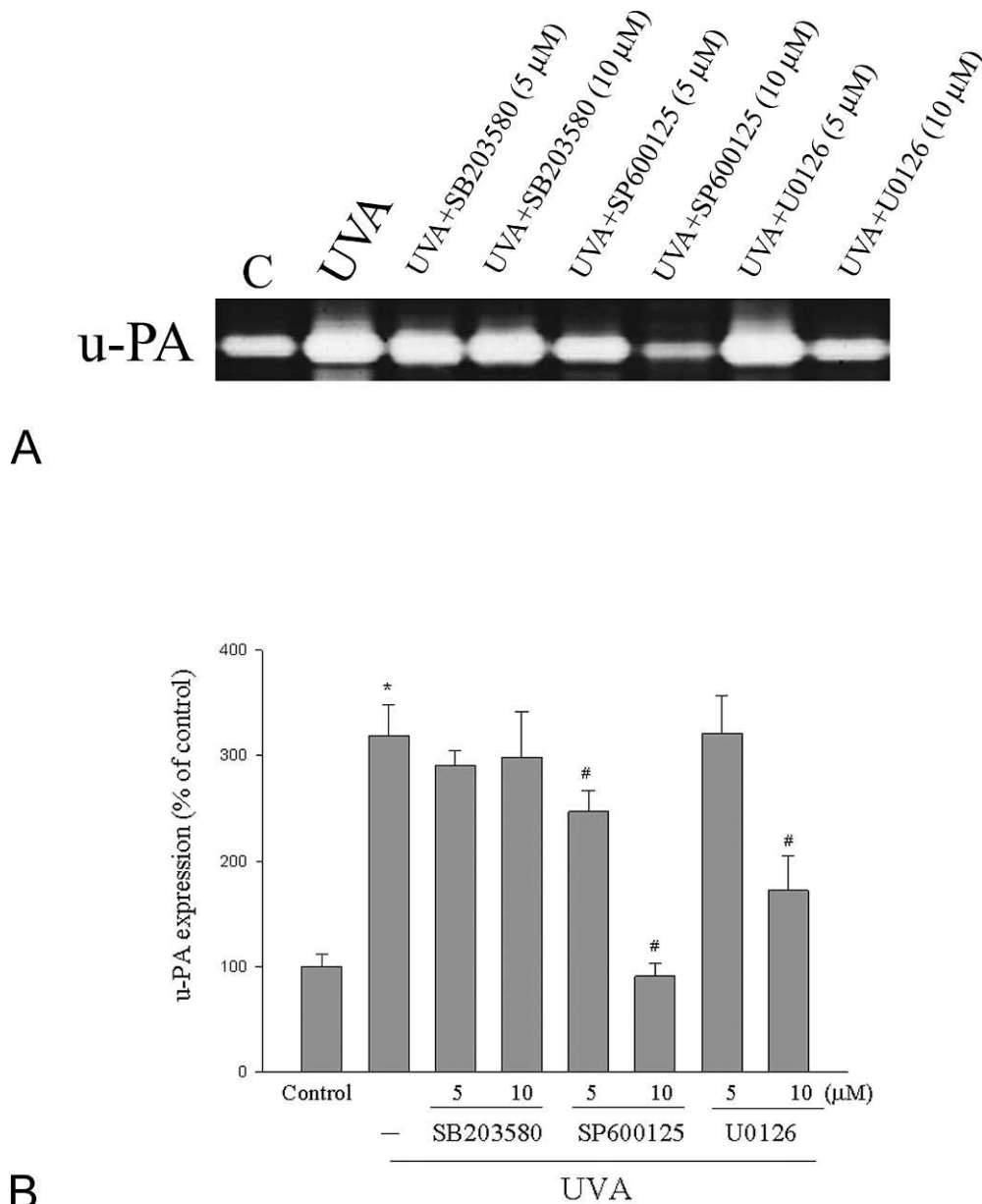


FIGURE 6. Effects of MAPK inhibitors on UVA-induced increase of uPA activities in cultured medium from pterygium fibroblast. Cells were plated into 24-well plates. Inhibitors of ERK (U0126), JNK (SP600125), and p38 MAPK (SB203580) were added at concentrations of 5 and 10 μM ; 1 hour later cells were irradiated with UVA (2.0 J/cm²). After 48 hours, cells were collected and the amount of uPA in cell lysates was measured by casein zymography. Cells not irradiated were used as the negative controls and irradiated with UVA, but no MAPK inhibitors were used as the positive controls. All tests were performed in triplicate. **(A)** Zymography. **(B)** Levels of uPA in cells treated with and without UVA, and various MAPK inhibitors. *Significant difference ($P < 0.05$) compared to negative controls. #Significant difference ($P < 0.05$) compared to cells treated with UVA alone.

In our study, UVA irradiation significantly increases the expression and secretion of uPA in fibroblasts from early stage of pterygia specimens in a dose-dependent manner.

It is well known that overexposure of UVB causes skin sunburn, and UVA causes skin tanning and aging. Epidemiologic and experimental studies have indicated that UV radiation is associated closely with the development of various diseases, including skin cancer, cutaneous melanoma, pterygium, and cataracts.^{5,6,26-28} In the past, most work on the mechanisms that underlie UV-induced skin carcinogenesis focused on UVB, which causes direct DNA damage and immunosuppression, which often results in the development of various skin tumors.^{25-27,29}

However, in recent years, numerous studies determined that UVA induces damage to skin and eye cells through mechanisms that differed from UVB damage.⁴⁻¹² UVA causes oxidative stress, oxidative damage to DNA and lipids, gene mutation, ECM degradation, and inflammation, which leads to the development of benign skin tumors, skin cancer, and cutaneous melanoma.⁷⁻¹² In vitro and experimental studies have shown that UVB is absorbed mainly by epidermal epithelial cells, whereas UVA penetrates deeply into the skin, reaching the dermal fibroblasts.⁸ UVA induces expression of various MMPs and pro-inflammation cytokines by dermal fibroblasts and keratinocytes.^{7,30-37}

Epidemiologic studies indicated that exposure to UV radiation was associated significantly with the occurrence of pterygium.^{1,2,16,17,28,38-40} In the past decades, most of the pterygium studies focused on the effects of UVB. In vitro studies indicated that UVB stimulated the expression of MMP-1, pro-inflammatory cytokines (IL-6 and -8), and VEGF by pterygium epithelial cells. These studies provide a better understanding of the role of UVB in the pathogenesis of pterygium.¹³⁻¹⁵ However, very little is known on the effects of UVA on pterygium tissues or cells. An epidemiologic study on the association between exposure to UV radiation and the development of pterygium in 838 watermen showed that the occurrence of pterygium was associated with UVB and UVA exposure. Logistic analysis showed that the regression coefficient (95% confidence interval) between UVB (290-320 nm), UVA1 (320-340 nm), and UVA2 (340-400 nm), and pterygium was 0.65 (0.33-0.98), 0.82 (0.45-1.19), and 0.86 (0.48-1.25), respectively.^{16,17} A computer-assisted optical ray tracing analysis showed that the peak light intensity at the distal (nasal) limbus is approximately 20 times that of the incident light intensity (from the temporal side). This phenomenon is seen in the visible light and also in the UV radiation, especially UVA. The cornea transmits more UVA than UVB (80% at 400 nm and 60% at 320 nm). Therefore, the association of UVA with pterygium is not surprising.³⁸

uPA is a protease that converts plasminogen to plasmin, which is capable of degrading fibrin. Plasmin also degrades basement membrane and a broad spectrum of ECM, including fibronectin, vitronectin, and laminin.¹⁸⁻²² Plasmin activates promatrix MMP into MMP, including MMPs-1, -2, -3, -9, -10, and -13.²⁰⁻²² Many growth factors, including VEGF, basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF), and so forth, are activated or released from ECM by plasmin or MMPs.²⁰⁻²² The binding of uPA with its receptor uPAR can activate downstream signaling molecules and various transcription factors, which in turn lead to cell proliferation, migration, and invasion.¹⁹

uPA has an important role in various physiologic and pathologic processes, including wound healing, tissue remodeling and regeneration, angiogenesis, inflammation, and tumor progression.^{18,21,22} uPA content in various tumor specimens is increased significantly.^{18-22,41} High uPA levels in primary malignant tumor specimens correlate with a high incidence of relapse, poor prognosis, and high mortality in various tumors.^{18-22,41} It has been reported that UVA induced uPA expression in fetal fibroblasts.⁴² The effects of UVA on the expression of uPA in pterygium fibroblast have not been reported to our knowledge.

The expression of several types of MMPs is increased in pterygium and related cell types (fibroblasts and epithelial cells).^{2,43-49} MMPs are secreted in a latent precursor and can be activated by plasmin.²⁰⁻²² uPA is the most important serine proteinase that activates plasminogen into plasmin, and is responsible for the tissue degradation and tumor cell invasion.^{18-22,41} Our previous studies found that overexpression of uPA mRNA and activities was present in pterygium and its fibroblasts. The expression of uPA by pterygium and its fibroblasts is increased significantly with the progression of pterygium.²³

We compared the UVA dosages used in our study to the dosages of ocular UVA exposure in vivo. The UVA fluences in Paris between 11 AM and 1 PM in summer are 54 W/m², which equals to 19 J/cm² per hour.⁵⁰ The ratio of ocular exposure to ambient exposure of UV radiation is 4.6% in outside workers (grounds man).⁵¹ Therefore, the UVA dosages used in our study (0.5-2.0 J/cm²) approximate to 0.5 to 2 hours of exposure in summer Paris at noon.

UVA irradiation also induces the expression of uPA in normal conjunctival fibroblasts, but only at relatively high dosages (1.0-2.0 J/cm²), and the increase of uPA expression is less than that in pterygium fibroblasts, indicating that pterygium fibroblasts are more sensitive to UVA radiation compared to normal fibroblasts.

Our study has shown that UVA-induced expression was associated with increase of phosphorylated ERK and JNK. This is consistent with the previous studies, which have shown that the MAPK signal pathways, especially ERK and JNK pathways, are involved in the UVA regulation of many cell functions in several cell types.^{49,52-62}

Our study indicated UVA may stimulate the progression of pterygium via the expression of uPA by fibroblasts. To our knowledge, this is the first in vitro study to define the association between UVA and pterygium. If further studies document the role of UVA in the pathogenesis of pterygium, then filtering ocular exposure from UVA and UVB radiation is required to provide adequate protection against pterygium. Sunglasses used for filtering UVB do not filter UVA. With incorrect sunglasses, the eye would not be protected completely from potential sunlight-induced pterygium. UVA radiation is 95% of the UV transmitted to earth from the sun, while UVB is approximately 5%. Also, because of the geometry of the eye, sunlight reflected off of sand, snow, and water is the most hazardous to the eye, and requires maximum ocular protection from UVA and UVB.⁶³ As suggested by Taylor nearly 20 years ago, for maximum protection, people should wear a hat with a brim and close-fitting sunglasses with lenses that absorb UVA and UVB when exposed to sufficient solar radiation to cause sunburn.¹⁶

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