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UV-B Induced Production of MMP-2 and MMP-9 in Human Corneal Cells

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Summary

The purpose of this study was to determine the production of metalloproteinases (MMP) 2 and 9 following UV-B irradiation in human corneal epithelial cells and fibroblasts. Epithelial cells and fibroblasts were separated from human donor corneas and exposed to UV-B lamp irradiation for 20, 40, 80 and 120 s. Media samples were collected at 8, 24, 48 and 72 h and gelatinase A and B production was assayed by the ELISA test. Statistical significance of production was assessed by the paired t-test. Increased production of MMP-2 was found in human corneal fibroblasts in response to UV-B irradiation. A statistically significant production of MMP-2 was not observed in human corneal epithelial cells following UV-B exposure. We did not detect any increase in MMP-9 after irradiation in either epithelial cells or fibroblasts. MMP-2 is produced by the corneal fibroblasts in the acute phase after UV-B irradiation. MMP-9 is not released *in vitro* following UV-B irradiation damage and therefore does not directly participate in the pathophysiology of acute photokeratitis.

Key words
UV-B irradiation • Human corneal epithelial cells • Human corneal fibroblasts • MMP-2 production • MMP-9 production • Acute photokeratitis

Introduction

The acute response of the cornea to excessive ultraviolet irradiation is referred to as photokeratitis. Photokeratitis occurs after accidental exposure to UV-irradiation during welding (ophthalmia photoelectrica), from sun at high altitudes (solar/actinic keratitis), snow (mountain keratitis) or sand.

Various proinflammatory molecules including interleukins, cytokines and matrix metalloproteinases (MMPs) have been implicated in the pathophysiology of photokeratitis (Kennedy *et al.* 1997). MMPs are zinc-binding proteolytic enzymes that participate in degrading and remodeling of the extracellular matrix (ECM) in various physiological and pathological conditions (Fini *et al.* 1992). Metalloproteinases are produced by corneal cells *de novo* and have the ability to degrade basement membrane components (Martisian 1990). They are ordinarily secreted as proenzymes and are activated in the extracellular compartment (Stetler-Stevenson *et al.* 1989). All MMPs have similar structures but differ in the ECM substrates they cleave. The activity of metalloproteinases on ECM substrates depends on the balance between the enzymes and their specific tissue inhibitors of metalloproteinases (TIMPs) (Azar *et al.* 1996).

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Gelatinases belong to one of three groups of metalloproteinases. They include the 72-kD (MMP-2) and 92-kD (MMP-9) forms called gelatinase A and B, respectively (Stetler-Stevenson *et al.* 1989). They are often referred to as gelatinases as they readily digest heat-denaturated collagens. Gelatinase A is widely expressed in various normal tissues and is upregulated in several diseases and pathological conditions including ECM remodeling during wound healing (Fini and Girard 1992). Gelatinase B is produced in many tumor tissues and is thought to play an important role in tumor progression and metastasis (Stetler 1990). More recently gelatinase B has been found to be released in excimer laser-wounded rat and rabbit corneas (Azar *et al.* 1996).

Previous studies by Herrmann *et al.* (1993) and Koivukangas *et al.* (1994) demonstrated that both UV-A and UV-B irradiation stimulates the production of gelatinases in human skin fibroblasts. We have, therefore, conducted experiments to detect the same phenomenon after UV-B exposure in human corneal epithelial cells and fibroblasts.

Methods

Establishment of human corneal epithelial and keratocyte cell cultures

Human corneas were obtained from the National Disease Research Institute Interchange (Philadelphia, PA) and placed into culture within 4 days of enucleation. After trimming off the corneoscleral rims and peeling off the endothelial layer, the corneas were washed repeatedly in RPMI-1640 medium (GIBCO, Grand Island, NY). To dissociate the epithelial layer from the stroma, the concave side of the cornea was placed in a 60-mm Petri dish on a drop of Dispase, grade II (Boehringer Mannheim, Indianapolis, IN) containing 25 caseinolytic units/ml Dispase and 20 μg/ml gentamycin in Hanks balanced salt solution. Care was taken to prevent direct contact of the epithelial layer with Dispase. After incubation with Dispase at 4 °C for 24 h in a humidified environment, the epithelial cell layer was lifted from the stroma with a pair of surgical forceps.

A single cell suspension was prepared by trypsinizing the epithelial cell layer in 1.5 ml of 0.05 % trypsin in 0.53 mmol/l ethylendiaminetetraacetic acid for 10 to 15 min at 37 °C, and then passing the preparation through a 20 G hypodermic needle 4 to 5 times with moderate pressure. Trypsinization was halted by addition of 10 mg/ml soybean trypsin inhibitor (GIBCO, Grand Island, NY) in phosphate-buffered saline without Ca^{2+} and Mg^{2+}. The cell suspension was centrifuged at 1000x*g* for 10 min and resuspended in Keratinocyte Serum Free Medium (GIBCO) containing 5 μg/ml gentamycin. The cells from each cornea were seeded into two Falcon Primaria positively charged 25 cm^2 tissue culture flasks (Becton Dickinson, Lincoln Park, NJ) with 5 ml medium per flask, and incubated at 37 °C in a 5 % CO_2 atmosphere. After 24 h, unattached cells and the medium were discarded and 5 ml fresh medium was added. The epithelial cells were subcultured when 75 to 90 % confluence was reached (usually within 7 days). Cells were dislodged from the flasks with 0.05 % trypsin in 0.53 mmol/l ethylene-diaminetetraacetic acid at 37 °C, washed with soybean trypsin inhibitor solution and reseeded to flasks with a fresh medium at a passage ratio of 1:2 or 1:3.

The stroma, now lacking an epithelial layer, was cut into approximately 2 mm^3 portions and partially digested with 300 U/ml collagenase type I (SIGMA, St. Louis, MO) in RPMI-1640. Digestion was carried out at 37 °C for 1 to 2 h after which the cells were washed with Dulbecco's modified Eagle's medium containing 20 % fetal calf serum (FCS). The particulate material from each cornea was seeded into one 25 cm^2 flask containing 1 ml of medium with 20 % FCS and 1 x antibiotic-antimycotic (GIBCO). The cultures were incubated at 37 °C in a 5 % CO_2 atmosphere. After confluence was reached, the cultures were split 1:2 or 1:4 in Dulbecco's modified Eagle's medium containing 10 % FCS and 1 x antibiotic-antimycotic.

Irradiation

Subconfluent cells in small Petri dishes were washed with phosphate-buffered saline (PBS) and exposed to UV light through a colorless medium. For UV irradiation we used the Rayonet Photochemical Chamber Reactor (Dayton Electric MFG. Co., Chicago, IL) with four pre-installed RPR-3000 photochemical lamps (Southern N.E. Ultraviolet Co., Branford, CT). The emission peak reached 313 nm at the reactor temperature of 44 °C. The samples were irradiated for 20, 40, 80 and 120 s. At a distance of 5 cm from the lamps, the intensity readings were 4 mW/cm^2. PBS was changed for the Keratinocyte Serum Free Medium and the dishes were stored in an incubator at 37 °C. The samples were collected 8, 24, 48 and 72 h after the treatment.

Metalloproteinase assays

MMP-2 and MMP-9 in collected samples were quantified using enzyme-linked immunosorbent assay

(ELISA) kits obtained from R&D Systems (Minneapolis, MN). Parallel measurements of standards and samples were performed by applying them to an antibody-precoated microplate, and the procedure was followed as indicated by the commercial kit. For total MMP-2, 9 measurement, the samples were acidified before assay. Colorimetric results were read at 450 nm using an EL 308 microplate reader (BIO-TEK Intruments, Winooski, VT). MMP-2, 9 concentrations were determined from the best linear curve drawn with the absorbance of standards versus their concentrations. The concentration was expressed in pg/mg protein. Significant differences between MMP-2 and MMP-9 levels were evaluated by small paired t-statistics.

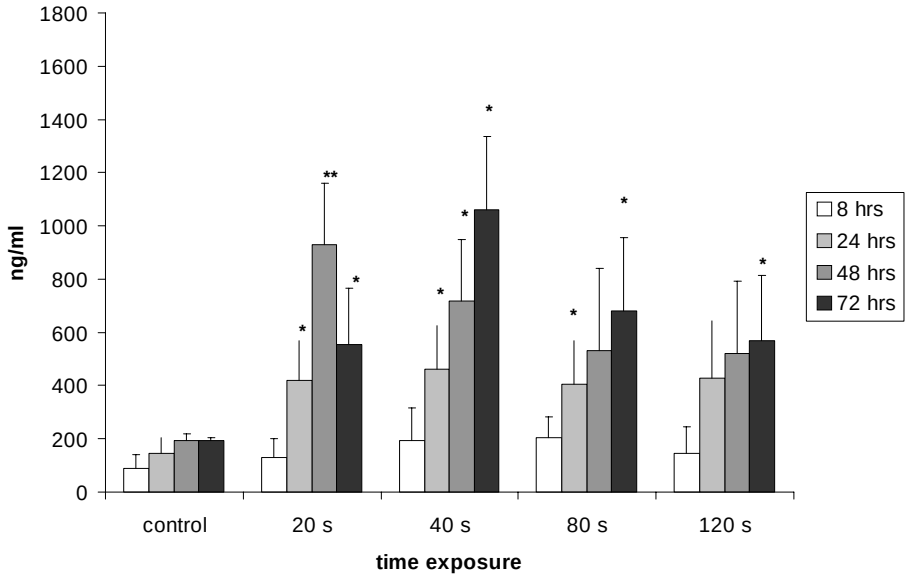

Fig. 1. *Expression of MMP-2 after UV-B irradiation in human corneal fibroblasts. Statistical significance p<0.05 (*) or p<0.01 (**) compared to untreated fibroblasts.*

Results

We have used human corneas from 6 different donors for UV-B irradiation and subsequent detection of MMP-2 and MMP-9 expression by human corneal fibroblasts and epithelial cells. Even though the detectable level of MMP-2 was present in untreated corneal fibroblasts, the UV-B light exposure resulted in a significant increase in the MMP-2 levels at the following time points: the values in samples irradiated for 20 s were 421 ng/ml after 24 h ($p<0.01$), 930 ng/ml after 48 h ($p<0.01$) and 552 ng/ml after 72 h ($p<0.05$). The values in samples irradiated for 40 s were 461 ng/ml after 24 h ($p<0.05$), 719 ng/ml after 48 h ($p<0.05$) and 1060 ng/ml after 72 h ($p<0.05$). The corresponding values in samples irradiated for 80 s were 404 ng/ml after 24 h ($p<0.05$), 679 ng/ml after 72 h ($p<0.05$) and in samples irradiated for 120 s were 569 ng/ml after 72 h ($p<0.05$) (Fig. 1). However, the increase in MMP-2 expression was not dose-dependent.

We observed that UV-B irradiation did not increase the production of MMP-2 in human corneal epithelial cells in any of the measured samples (Fig. 2). Although we detected trace amounts of MMP-9 in untreated human corneal epithelial cells, the UV-B light exposure did not stimulate the cells to further production of MMP-9 (Fig. 3). Neither did we detect any increase in MMP-9 production following UV-B irradiation in any of the samples of the human corneal fibroblasts (Fig. 4).

Discussion

Ultraviolet light is an environmental agent that can cause significant ocular inflammation and pathological changes. The ability of UV irradiation to induce metalloproteinases production in the skin is well established (Herrmann *et al.* 1993, Koivukangas *et al.* 1994). In this study we investigated the production of MMP-2 and -9 in human corneal epithelial cells and fibroblasts in response to various time exposures to UV-B (290-320 nm) irradiation.

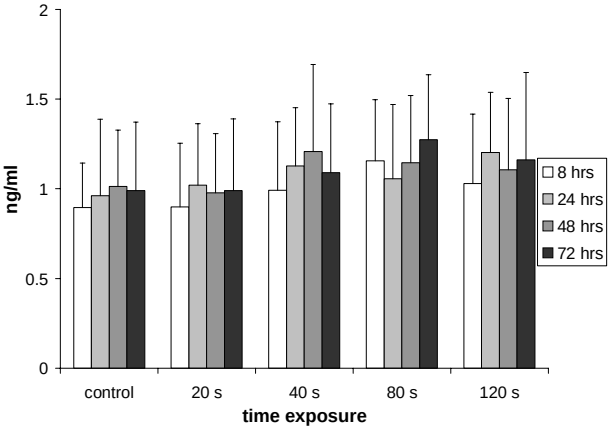

Fig. 2. *Expression of MMP-2 after UV-B irradiation in human corneal epithelial cells.*

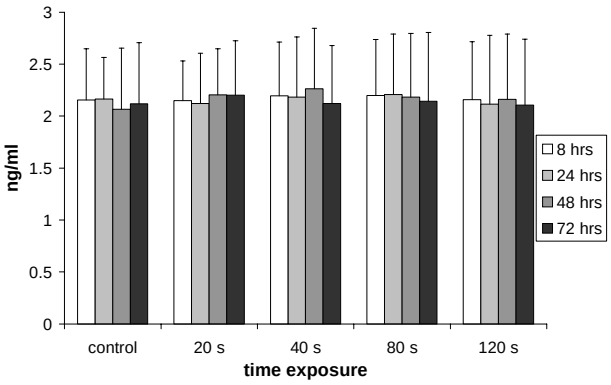

Fig. 3. *Expression of MMP-9 after UV-B irradiation in human corneal epithelial cells.*

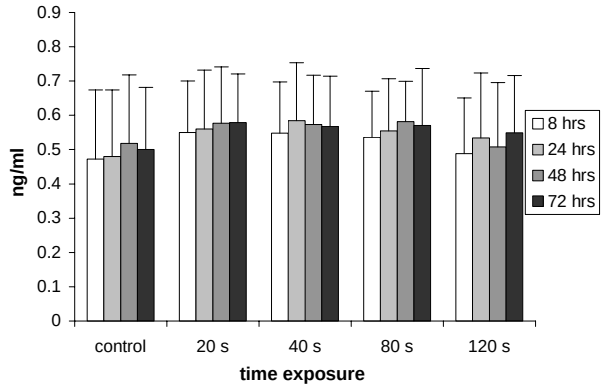

Fig. 4. *Expression of MMP-9 after UV-B irradiation in human corneal fibroblasts.*

The samples were collected to measure the MMP-2 and MMP-9 production in the acute phase. We observed that UV-B irradiation induces a significant increase in MMP-2 production in corneal stromal fibroblasts but not in epithelial cells. We have detected some basal MMP-2 production in the corneal fibroblasts which had also been reported by other authors (Azar *et al.* 1996, Fini and Girard 1990, Matsubara *et al.* 1991a). However, UV-B irradiation further increased MMP-2 levels. Although we detected some baseline MMP-9 levels in the corneal epithelial cells prior to treatment, the UV-B irradiation did not increase MMP-9 production in neither epithelial or stromal fibroblasts.

The results of previous studies indicate that exposure of the cornea to excessive UV irradiation leads to damage at both cellular and molecular levels (Kennedy *et al* 1997). Acute UV-B exposure causes corneal edema and photokeratitis and is associated with an inflammatory reaction in the cornea (Schein 1992, Čejková and Lojda 1995). Our findings indicate that MMP-2 could be a part of this inflammatory cascade. The source of MMP-2 are stromal fibroblasts, while corneal epithelial cells produce very little of this enzyme (Fini and Girard 1990).

MMP-2, apart from its elastinolytic properties in actinically damaged skin, is able to degrade basement membrane compounds (Woessner 1991). Even though most of the UV-B light is absorbed by epithelial cells, pronounced expression of MMP-2 could pose a danger for basement membrane structures.

As mentioned above, the production of MMP-9 was not increased following UV-B exposure. MMP-9 is produced by cells in the basal layer of the epithelium directly adjacent to the basement membrane in human corneas with repair failures and basement membrane defects (Matsubara *et al.* 1991b). Previous studies indicate that MMP-9 did not appear in the corneal tissue after epithelial scraping, injury that heals without loss of the original basement membrane (Fujikawa *et al.* 1984). It is therefore evident that the production of MMP-9 is only associated with the damage producing basement membrane defects.

The metalloproteinases are secreted as proenzymes. The mechanism of activation of the MMP proenzymes includes removal of the propeptide region by proteolytic cleavage of the N-terminal with a corresponding reduction in the molecular size of about 8-10 kD (Matsubara *et al.* 1991a). In MMP-9 this can be achieved by the action of various proteinases whereas MMP-2 seems to be insensitive to the activation by many proteinases probably due to a different secondary structure in the propeptide area (Okada *et al.* 1990).

Previous studies have shown that human corneal fibroblasts and epithelial cells can produce a number of proinflammatory cytokines either spontaneously or in

diseased states (Ansel *et al.* 1991, Cubitt *et al.* 1993, Rosenbaum *et al.* 1995). Some of these substances can be identified in human corneal tissue after UV-B irradiation (Kennedy et *al.* 1997). Moreover, after UV-B irradiation human corneal fibroblasts also produce MMP-2. All these as well as their inhibitors can participate in the pathophysiology of photokeratitis and chronic radiation damage to the cornea. Their exact role needs to be elucidated by further experiments in order to enable successful treatment of this fairly common event in ophthalmologic practice.

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