Expression of c-Fos and c-Jun in the cornea, lens, and retina after ultraviolet irradiation of the rat eye and effects of topical antisense oligodeoxynucleotides

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Abstract
Aims—Immunohistochemical techniques were used to investigate c-Fos and c-Jun proto-oncogene expression in the cornea, lens, and retina after ultraviolet irradiation of the rat eye.

Methods—Eyes of anaesthetised rats were exposed to 1.5 J/cm² of ultraviolet radiation (280–380 nm). Animals were perfused, or 6 hours after irradiation and tissue sections were incubated with specific antisera to c-Fos and c-Jun, respectively.

Results—Non-irradiated contralateral eyes displayed no c-Fos and c-Jun immunoreactivity. One and 6 hours after ultraviolet exposure numerous c-Fos and c-Jun immunopositive nuclei were observed mainly in the epithelial cell layers of the cornea and the lens epithelium. Scattered labelled nuclei were detectable in the retinal ganglion cell layer and the inner nuclear layer. Twenty-four hours after irradiation c-Fos and c-Jun protein expression returned to near control levels. Histological signs of ultraviolet damage (for example, chromatin condensation, nuclear fragmentation) were first recognisable in the corneal epithelium 6 hours after irradiation and became more apparent at later times.

Conclusion—Thus, the rapid and sustained activation of c-Fos and c-Jun expression in the eye after single ultraviolet exposure may represent the molecular mechanism underlying ultraviolet induced photodamage and initiation of cell death. Furthermore, topical application of a c-fos antisense oligodeoxynucleotide to the ultraviolet exposed rat eye inhibited the increase in c-Fos expression in the cornea, suggesting therapeutic activity of antisense drugs in corneal malignant and infectious diseases.


Ultraviolet (UV) radiation induced photodamage of the eye has been demonstrated in both humans and experimental animals. Short wavelength UV C radiation (< 280 nm) is cut off by the cornea. Excessive exposure to UV light results in inflammatory damage of the cornea and even neoplastic alterations. Action spectrum measurements for UV keratitis revealed a maximum sensitivity at about 270 nm, which corresponds to the absorption of UV light by nucleic acids. In the corneal epithelium, UV radiation inhibits mitosis, produces nuclear fragmentation, and desquamation. Neoplasms occur most frequently in those portions of the eye exposed to UV radiation (conjunctiva, cornea, and eyelids). Although primary corneal tumours are exceedingly rare suggesting a relative resistance to malignant transformation, an enhanced susceptibility has been demonstrated in humans who suffer from xeroderma pigmentosum. Longer wavelength UV radiation (UV-B range: 280–320 nm) is strongly absorbed by the lens. In cultured rabbit lens epithelial cells, cytotoxicity of UV radiation was most efficient at 297 nm. Numerous epidemiological studies have shown that long term exposure to UV light correlates with an irreversible loss of human lens transparency, and cataract induction by UV-B radiation has also been demonstrated in albino mice. In vitro studies have identified amino acids (for example, tryptophan, tyrosine) and DNA in lens epithelial cells as targets of UV-B radiation, and membrane alterations and DNA strand breaks have been suggested as possible molecular mechanisms involved in acute cataract development.

UV-A wavelengths (320–440 nm) can pass through all optic media and cause photochemical damage to the retina. At high intensities or in aphakic and pseudophakic patients cellular damage occurs in the pigment epithelium, the photoreceptors, and occasionally other retinal neurons.

In mammalian skin, UV radiation promotes similar cellular effects, ranging from growth inhibition and cell death to hyperproliferation and carcinogenesis. The underlying molecular mechanisms are as yet incompletely understood. Single UV exposure of mammalian cells in culture leads to the induction of various genes, among them members of the Fos/Jun family of transcription factors, which are known to be associated with cell growth and differentiation. Recently, the activation of c-Fos protein expression has been demonstrated to precede both naturally occurring and UV induced programmed cell death in mammalian skin. However, little is known about fos/jun proto-oncogene expression in the mammalian eye following UV exposure. Therefore, we investigated whether single UV
irradiation modulates c-Fos and c-Jun protein expression in the cornea, lens, and retina of the rat. Furthermore, we tested the in vivo efficacy of topically administered antisense oligodeoxynucleotides (ODNs) in blocking corneal proto-oncogene expression.

Materials and methods
Male albino Sprague-Dawley rats (250–300 g) were purchased from the Zentralinstitut für Versuchstierzucht (Hannover, Germany). Animals were kept under a 12 hour light–12 hour dark cycle and given food and water ad libitum.

All animals were irradiated 4 hours after the onset of the light period. The UV radiation source consisted of a high pressure mercury arc lamp (Osram HBO 100W/2), two concave quartz lenses, and a UV filter (Spindler and Hoyer UG 11). The eyes were irradiated at an intensity of 14 mW/cm² in the focus of the condenser system.¹⁶ Irradiance was measured with a UV radiometer (Laser Instrumentation). Sixty five per cent of the energy was emitted within the UV-B wavelength range (280–320 nm), the remainder within the UV-A region (320–380 nm). Animals were anaesthetised (Nembutal, 60 mg/kg, intraperitoneally) and the right eye (with lids clamped open) was exposed once to 1.5 J/cm², which is just below the amount to produce threshold keratitis.³ The left eye was shielded from the radiation by aluminium foil and used as a control.

After different survival times (1, 6, and 24 hours, respectively) reanaesthetised rats (n=3 per time point) were perfused transcardially with 4% paraformaldehyde in phosphate buffered saline solution. Eyes were excised and stored in fixative overnight. Tissue was equilibrated in 30% sucrose and sectioned on a freezing microtome (20 µm). Sections were then thaw mounted onto gelatine coated slides and incubated for 48 hours at room temperature in rabbit antiserum to c-Fos (689/5) (1:10 000) or c-Jun (607/3) (1:1000). Antibody binding was localised by the avidin-biotin-peroxidase method (Vector, Burlingame, VA, USA) using diaminobenzidine as chromogen. For histological examination sections were stained with haematoxylin and eosin using standard protocols.

Generation of antisera and their specificity has been described in detail elsewhere.¹⁷ ¹⁸ In short, all antibodies were raised against bacteria expressed fusion proteins. As determined by immunoprecipitation analysis of in vitro translated proteins, the rabbit anti-c-Fos antibody showed a strong reaction with the c-Fos protein, no cross reactivity with Fos B and a weak reaction with the Fos related antigen, Fra-1. The anti-c-Jun antiserum showed a very strong reaction with the c-Jun protein and no cross reactivity with both the Jun B and Jun D proteins. Immunoreactivity was absent in sections after preabsorption of the primary antisera with the respective antigen, whereas incubation with related proteins did not affect immunoreactivity.

Oligodeoxynucleotides with 3’-,5’-terminal phosphoroethioate internucleoside backbones were synthesised and purified as described elsewhere.¹⁵ ¹² A 20-mer c-fos antisense ODN (5’-C*G*AGAACATCAGGCTGTG*G*-3’) was topically applied to the cornea (1 nmol in 10 µl sterile phosphate buffered saline) immediately after UV exposure. Control eyes were exposed and treated with a random sequence ODN (5’-C*C*TTATTACTTTT*G*-3’) (asterisks indicate sites of phosphoroethioate modification). ODN solution was applied at 1 hour intervals, and 6 hour post-irradiation tissue was processed as described above.

Results
One and 6 hours after UV exposure numerous c-Fos and c-Jun positive nuclei were visible mainly in the basal cell layer of the corneal epithelium, whereas less immunoreactive cells were observed in the corneal stroma and endothelium (Fig 1). At the same timepoints the lens epithelial cell layer exhibited c-Fos and c-Jun positive nuclei in the central zone (Fig 1B) and to a significant higher level in the germinative zone (Fig 2A). Cornea, lens, and retina of non-irradiated control eyes displayed no detectable nuclear c-Fos and c-Jun immunostaining (Figs 2B and 3B). Scattered c-Fos and c-Jun immunostained nuclei were observed in the retinal ganglion cell layer and in the inner nuclear layer after irradiation; however, no staining of photoreceptor nuclei

Figure 1 Cross section of the albino rat eye 6 hours after UV irradiation in vivo. (A) c-Jun immunopositive nuclei are concentrated in the basal layer of the corneal epithelium. Magnification ×320. (B) At lower magnification nuclear c-Jun immunostaining is visible both in the cornea and in the central zone of the lens epithelium (arrowhead). The separation of the corneal endothelium from the stroma represents a fixation artefact. Magnification ×240.
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could be detected (Fig 3A). Similar temporal and spatial expression patterns were obtained for both proto-oncogenes. Twenty hours after UV irradiation c-Fos and c-Jun immunoreactivity returned to control levels.

The earliest histological alterations (chromatin condensation, pyknotic nuclei) were observed in the corneal epithelium 6 hours after exposure (Fig 4). Twenty-four hours after irradiation nuclear fragmentation, vacuolisation and desquamation became visible in all epithelial cell layers of the cornea. Pyknotic nuclei were less frequent in the UV irradiated lens epithelium and not detectable in the corneal stroma and endothelium and the retina (not shown).

Topical administration of a terminal phosphorothioated c-Fos antisense ODN inhibited the UV induced increase in c-Fos immunoreactive cells in the cornea but not in the underlying lens epithelium, whereas a random sequence control ODN did not show significant inhibitory activity (Fig 5).

Discussion

In the present study, a rapid and sustained activation of both c-Fos and c-Jun expression has been demonstrated in different cell populations of the rat eye following single UV exposure in vivo. The time course of induction of Fos/Jun transcription factors in the UV irradiated cornea, lens, and retina is very similar to that observed both in the rat epidermis and in epidermal cells in culture, and seems to be a general response of mammalian cells when exposed to environmental stress. The molecular mechanisms eliciting the activation of c-Fos and c-Jun in the UV exposed eye remain unknown. In keratinocytes in culture, a role for DNA damage and oxidative stress in mediating the UV response has been described, and DNA damage and hydrogen peroxide formation have already been detected in cultured lenses after UV irradiation. Furthermore, platelet activating factor, an inflammatory mediator rapidly appearing in the cornea after alkali injury, induces c-Fos and c-Jun expression in epithelial cells of cultured rabbit corneas within 1 hour, and mechanical wounding of rabbit corneal epithelium in vivo activates c-Fos expression within 15 minutes. In these studies, however, proto-oncogene expression was induced only transiently in non-injured epithelial cells of the cornea, whereas in the present study c-Fos and c-Jun activation in putative photodamaged cells lasted at least 6 hours after irradiation.
Figure 4  Haematoxylin and eosin stained sections of non-irradiated control cornea (left), UV irradiated cornea 6 hours after exposure (middle), demonstrating chromatin condensation (arrow), and cornea 24 hours after irradiation (right), showing extensive nuclear fragmentation and vacuolisation. Magnification ×5100.

The physiological significance of the UV induced activation of proto-oncogene expression in the eye remains speculative. The rapid induction of fos/jun expression after single UV exposure is not related to corneal cell proliferation, since mitotic activity in UV irradiated rat corneal epithelium is strongly inhibited as early as 1 hour for up to 24 hours after high dose UV exposure.4 Furthermore, our histochemical analysis revealed a UV induced increase in fos/jun immunoreactivity throughout all corneal epithelial cell layers and no restriction to proliferating basal cells. The rat lens displayed positive nuclei both in the UV exposed non-mitotic central zone of the epithelium, and in the mitotic germinative zone, which is barely protected from UV radiation by the non-pigmented iris of the albino rat. Nevertheless, a higher degree of nuclear staining was observed in mitotic areas, which may be explained by the higher susceptibility of proliferating cells to UV induced cell death.21 UV light is mostly absorbed by the cornea and the lens,1,2 and transmittance of the isolated rat lens decreases to <2% at 310–330 nm.22a In cell culture, the action spectrum for UV induced c-Fos expression peaks at 275 nm and falls off steeply at longer wavelengths.22b Consistently, few nuclei were immunostained in the retinal ganglion cell layer and the inner nuclear layer, where c-Fos expression is usually not detectable during the light period.22c 23 Additionally, the retina did not show significant histopathological alterations, suggesting the photochemical damage may occur after quite a long latency or after longer exposure times.2 10

UV radiation is a potent agent for the induction of programmed cell death/apoptosis in human skin, and different cell lines undergo apoptosis en masse within 2–3 hours after UV-B irradiation.24 The induction of c-Fos and c-Jun expression in response to stimuli evoking apoptosis has been demonstrated in various systems,25 and c-Fos expression has recently been localised in apoptotic fibroblasts.15b There is evidence that apoptosis is
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the molecular mechanism of cell death in different types of retinal degeneration,26 and following UV exposure histological signs of apoptosis (for example, chromatin condensation, nuclear fragmentation) are visible in the rat cornea, the lens, and retina of albino mice, and in the aphakic monkey retina.4 10 27 Consistent with our histological data, studies in rodents have revealed major cellular damage in the corneal epithelium following UV irradiation with a latent period of several hours and have suggested that stromal and endothelial cells may be protected by a higher unscheduled DNA repair activity.4 28 Thus, the rapid induction of c-Fos and c-Jun expression precedes morphological signs of UV phototoxicity and may reflect the initiation of programmed cell death at least in some affected cells of the UV irradiated eye.

Finally, topographical administration of a terminal phosphorothioated c-fos antisense ODN to the UV exposed cornea blocked the activation of c-Fos expression. These findings demonstrate that ionizing antisense ODNs when instilled as eyedrops are able to penetrate into the cornea and to sequence specifically modulate corneal gene expression. This may be of clinical relevance.

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