Action Spectrum for Cytotoxicity in the UVA- and UVB-Wavelength Region in Cultured Lens Epithelial Cells

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Purpose. This study was done to quantitate the biologic effects of different wavelengths of radiation in the UVA- and UVB-wavelength region on cultured rabbit lens epithelial cells.

Methods. An action spectrum for UV-induced cytotoxicity as measured by colony-forming ability was determined using six different monochromatic wavelengths from 297 to 405 nm in rabbit lens epithelial cell line N/N1003A. Cell survival was determined by clonogenic assay. Fluence rates were monitored with a calibrated radiometer.

Results. Survival curves show that cell killing was most efficient at 297 nm. After quantum correction, the efficiency of 297-nm radiation in cell killing was 7 times greater than was 302-nm radiation. Radiation at 297 nm was more than 170, 340, 560, and 2000 times as effective in cell killing as 313-, 325-, 334-, and 365-nm radiation, respectively. The action spectrum had a shape similar to the DNA absorption spectrum in the UVB region, suggesting that DNA may be one of the critical targets for damage to the cells. At wavelengths longer than 313 nm, the shape of the action spectrum deviated from the DNA absorption spectrum.

Conclusions. Cytotoxicity of UV radiation in cultured lens epithelial cells varies greatly with wavelength within the UVA and UVB regions. Different mechanisms may predominate in the two wavelength regions. Cultured cells may provide a suitable system for investigating the mechanisms by which UV radiation damages lens epithelial cells and leads to cataract formation. Invest Ophthalmol Vis Sci. 1994;35:367-373.

Epidemiologic studies suggest an association between solar UV radiation and the increased risk of human cortical and posterior subcapsular cataract formation.1² Experimental evidence in animals also indicates that long-term exposure to either predominantly UVB (290- to 315-nm) radiation or to higher levels of UVA (315 to 400 nm) or possibly a combination of UVA and UVB, causes detrimental changes in the lens cortex including posterior subcapsular regions.5-8

The cornea absorbs all wavelengths of radiation below 297 nm and transmits increasing amounts of longer wavelengths of UV radiation to the lens.6-7 Solar UV radiation reaching the earth's surface contains only 3% of UVB; the fluence rates vary with geographic, physical and meteorologic factors.9-10 Only 2% to 17% of the ambient UVB radiation is estimated to impinge on the cornea.10 Transmission characteristics of the human cornea allow approximately 3% to 8% of UVB radiation and 40% to 60% of UVA to reach the lens epithelium.11 It is estimated that 0.006 to 0.05 W/m² of UVB radiation and 10 to 100 W/m² of UVA is transmitted to the human lens epithelium. Even these low levels of UV radiation can have an adverse effect on the lens after many exposures over decades.

Lens fiber cells derive from the lens epithelium, and cortical or posterior subcapsular cataracts may both result from initial damage to the epithelial cell layer. Age-related cortical cataracts involve opacification of lens fibers acquired in adulthood. Human posterior subcapsular cataracts involve posterior migration of lens epithelial cells.12 The lens epithelium is thought to play a pivotal role in the development and progression of human cataracts caused by exogenous mutagens13 such as solar UV radiation. The epithelial...
cells are likely targets for UV damage because they are the first cells in the lens to be exposed to UV in vivo. Indeed, exposure of the eye in vivo to predominantly UVB radiation induces unscheduled DNA synthesis in the lens epithelium. Exposure of cultured lens epithelial cells to broadband UVB radiation induces DNA damage and repair, and triggers an alteration in the synthesis of specific proteins. In normal course of events, lens epithelial cells undergo mitosis and differentiate into lens fibers throughout life. It has been hypothesized that damage to lens epithelial DNA leads to aberrant fibrogenesis, which is thought to initiate cataract formation. The lens continues to grow throughout life by the deposition of outer cortical fibers over the older nuclear fibers. Thus damage to the epithelium by agents such as UV radiation can be expressed in fibers later in life.

The epithelial cells serve key transport functions for the entire lens and are the primary site of the enzymatic systems that protect the lens from oxidative stress. Exposure of the anterior surface of the cultured rabbit lens to UV radiation causes alterations in Na⁺ and Ca²⁺ concentrations, a lowering of reduced glutathione, a reduction in Na⁺-K⁺ adenosine triphosphatase activity, and the induction of lens opacification. In marked contrast, UV exposure of the posterior side of the lens, the side that lacks an epithelial layer, causes neither ionic imbalances nor opacification. These findings strongly suggest that the epithelium is of vital importance in the maintenance of lens homeostasis after UV insult. The exact targets and the detailed mechanisms that are responsible for UV-induced cataracts remain to be determined.

Because the cellular targets and mechanisms of action of UV radiation vary as a function of wavelength, we investigated the effect of a range of specific wavelengths of UV radiation on cultured lens epithelial cells. This information is currently unavailable. Quantitation of the biologic effect of UV on the lens is important for (1) information regarding the chromophores and mechanism(s) involved in cataract formation; (2) estimation of the response to changing ocular dose of UVB, to assess the effect on human cataract caused by an increase in UVB reaching the earth due to ozone depletion; (3) interspecies comparison; and (4) determination of safe ocular exposure durations of in vivo optical devices. In the current study, we investigated the action spectrum for killing cultured lens epithelial cells at six different wavelengths in the solar UV region known to be transmitted to the lens.

METHODS

This investigation adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Cell Culture

Rabbit lens epithelial cells (cell line N/N1003A) were cultured in minimum essential medium (MEM, Sigma, St. Louis, MO) containing 8% rabbit serum and 50 μg/ml gentamicin (Sigma) at 37°C in a water-saturated air atmosphere as described previously. Cells from this line have a stable epithelial morphology, are non-tumorigenic in vivo and have a normal chromosomal banding pattern. Transfection of these cells by a plasmid containing αA or γ-crystallin gene promoters results in efficient expression of a reporter gene, indicating that they retain functions typical of lens epithelial cells in vivo. All experiments were performed on cells between population-doubling levels 30 and 50.

Irradiation and Dosimetry

The UV source was a 1-kW Hg-Xe ozone-free lamp (Oriel, Stratford, CT). Infrared radiation was filtered using a water filter (model 61940, Oriel). Monochromatic radiation at defined wavelengths was obtained using an Oriel (model 77320) 0.25-m monochromator with a 1200-line/mm grating blazed at 280 nm. A beam turner (model 66215, Oriel) was used to turn the beam by 90° for irradiation of the cell monolayer at a distance of 12 cm. Because shorter wavelengths are more effective in producing cytotoxicity, additional filtration was used to provide improved spectral purity. Details of additional light filters, slit widths and approximate fluence rates used at different wavelengths are given in Table 1. Fluence rates were measured with a research radiometer (IL1700, International Light, Newburyport, MA) using a SED 240 probe calibrated at 297, 302, and 313 nm and a SED 038 probe calibrated at 325, 334, 345, and 405 nm.

In initial experiments, a 5-kW Hg–Xe lamp and a Jobin-Yvon monochromator were used to irradiate cells at 302 and 365 nm. Cell monolayers were irradiated at a distance of 11 cm from the beam-turner using fluence rates of 10 W/m² at 302 nm and 102 W/m² at 365 nm. Fluences were between 0 and 1500 J/m² at 302 nm and were between 0 and 3.1 × 10⁵ J/m² at 365 nm. Bandwidths were 5 nm at each wavelength, and Schott filters (WG 320 at 302 and WG 345 at 365 nm) were used to filter shorter wavelengths. For irradiation, cells from a confluent 100-mm Petri dish were trypsinized and plated in 96-well plates (10⁵ cells/well) in MEM containing 1% rabbit serum overnight. Confluent cells exhibit contact inhibition of cell division and exhibit little growth when cultured in

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MEM containing 1% rabbit serum. This approach allowed us to work with a fairly synchronous population of cells relative to the cell cycle. Immediately before UV exposure, the monolayer was rinsed twice with 0.2 ml phosphate-buffered saline containing Ca²⁺ and Mg²⁺, and 0.1 ml phosphate-buffered saline was added to the well. Cells were exposed to different fluences of UV radiation by placing the culture plate without cover on ice. One control nonirradiated well was used for measurement of viability at each time of irradiation. No loss of viability was observed when cells were kept on ice in phosphate-buffered saline with no irradiation for as long as 5 hours, the longest time of irradiation. Fluence rates were measured at the same position that the epithelial cells occupied during the exposures.

Assessment of Cell Survival
Survival was determined by the relative colony-forming ability of the cells.25 Immediately after irradiation, cells were trypsinized and approximately 8 × 10⁶ cells/ml were suspended in MEM containing 8% rabbit serum. An aliquot of the suspension was diluted to 20 ml using Isoton II and the cells were counted in a Coulter Counter. The cells were diluted to 200 cell/ml and colony-forming ability at different fluences of radiation was determined by plating the cells in a 24 well plate at a density of 100, 50, and 20 cells/well containing 0.5 ml of MEM containing 8% rabbit serum. Four wells were plated at each concentration (a total of 12 wells per treatment) and incubated at 37°C for 1 to 2 weeks. At this time, the growth medium was discarded, and colonies were stained using 0.5 ml of staining solution (0.5% methylene blue in 50% methanol). After incubation for 15 minutes, the staining solution was discarded and cells were rinsed with deionized water, dried and colonies were counted. Only colonies containing more than 32 cells were scored. The colony-forming ability of the nonirradiated controls was estimated to be 10%. The surviving fraction was calculated at each cell density and the experiments were repeated at least twice. At two wavelengths (302 and 365 nm), a microtiter well assay was also used to confirm the results.24,26 In the latter, cells were plated in 96-well plates at densities of 2 to 20 cells/well. The ability of cells to initiate a microculture was determined after 2 weeks of incubation at 37°C. Both methods gave nearly identical survival curves at 302 and 365 nm.

Data Analysis
The linear portion of the survival curves was analyzed by least squares regression to give a slope and a y intercept. At each wavelength, the fluence producing a surviving fraction of 1/e (37%, F₃₇) was determined. An action spectrum was plotted using 297 nm as reference wavelength, with necessary quantum correction at each wavelength.

RESULTS
Cytotoxicity of UV radiation in lens epithelial cells was determined using monochromatic radiation at 297, 302, 313, 325, 334, 365, and 405 nm. Survival curves (Fig. 1) show that cell killing, as measured by colony-forming ability was most efficient at 297 nm. As the wavelength increased, the extent of killing decreased. Significant shoulders were observed in the survival curves at 302, 313, and 365 nm. These shoulders represent the nonresponsive regions of the survival curves, indicative of the extent of repair during the assay period. The absence of shoulders from the survival curves at 325 and 334 nm may possibly reflect differences in repair after irradiation at these wavelengths. The final slopes of the curves differed significantly at the monochromatic wavelengths studied (Table 2). The dose giving 1/e survival (37%, F₃₇) was determined from survival curves at each wavelength, except at 325 nm, where extrapolation of the curve was necessary. At 405 nm, the highest dose that could practically be given produced no adverse effect on survival.

After quantum correction, the F₃₇ for 297-nm radiation was 96 J/m², whereas for 302-nm radiation F₃₇ was 680 J/m². The F₃₇ at 297 and 302 nm may actually be higher, because the bandpass was 3 and 5 nm, respectively, and some shorter wavelength radiation may be present. The efficiency of 297-nm radiation in cell killing was 7 times greater than 313-nm radiation. Radiation at 297 nm was more than 170, 340, 560, and 2000 times as effective in cell killing as 313-, 325-, 334-, and 365-nm radiation, respectively. At 365 nm, a fluence rate of either 22 or 102 W/m² gave the same response with respect to 297-nm radiation. Cytotoxicity was also measured by a microtiter well assay.26 Results from this assay at 302 and 365 nm were identical.

### TABLE 1. Bandwidths, Corning Cut-off Filters, and Approximate Fluence Rates Used in Action Spectrum Experiments

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Bandwidth (nm)</th>
<th>Filter</th>
<th>Fluence Rate (Wm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>297</td>
<td>3.6</td>
<td>CS 0-53, 1 mm</td>
<td>2.0</td>
</tr>
<tr>
<td>302</td>
<td>5.1</td>
<td>CS 0-53, 1 mm</td>
<td>8.0</td>
</tr>
<tr>
<td>313</td>
<td>6.3</td>
<td>CS 0-54, 0.5 mm</td>
<td>12.0</td>
</tr>
<tr>
<td>325</td>
<td>9.3</td>
<td>CS 0-54, 2 mm</td>
<td>2.2</td>
</tr>
<tr>
<td>334</td>
<td>9.3</td>
<td>CS 0-54, 2 mm</td>
<td>4.5</td>
</tr>
<tr>
<td>365</td>
<td>10.0</td>
<td>CS 0-52, 1 mm</td>
<td>22.0</td>
</tr>
<tr>
<td>405</td>
<td>10.0</td>
<td>CS 0-52, 1 mm</td>
<td>6.6</td>
</tr>
</tbody>
</table>

* Bandwidth at 100% transmission.
DISCUSSION

The action spectrum for cytotoxicity of UV radiation to lens epithelial cells gives the relative effectiveness per incident photon at each wavelength for a single UV exposure. Biologic effectiveness of solar radiation on the lens epithelium would result from many exposures to both UVB and UVA radiation over decades. The current action spectrum may be useful to investigate the cataractogenic potential of different UV wavelengths. In this regard, Pitts reported that the action spectrum for UV-induced lens opacities in rabbits in vivo begins at 295 nm and extends to 320 nm. Radiation at 300 nm (using a 6.6-nm full bandpass) was 30
TABLE 2. Parameters Obtained by Statistical Analyses of Survival Curves at Monochromatic Wavelengths

<table>
<thead>
<tr>
<th>Wavelength (nm)</th>
<th>Slope ± SD \times 10^6</th>
<th>y Intercept ± SD</th>
<th>Corrected F_{37} (Jm⁻²)*</th>
<th>Relative Response†</th>
</tr>
</thead>
<tbody>
<tr>
<td>297</td>
<td>-5380 ± 500</td>
<td>0.89 ± 0.15</td>
<td>96</td>
<td>1.0</td>
</tr>
<tr>
<td>302</td>
<td>-932 ± 99</td>
<td>1.01 ± 0.03</td>
<td>680</td>
<td>1.41 \times 10⁻¹</td>
</tr>
<tr>
<td>313</td>
<td>-22.6 ± 5</td>
<td>0.79 ± 0.09</td>
<td>16,400</td>
<td>5.84 \times 10⁻³</td>
</tr>
<tr>
<td>325</td>
<td>-14.0 ± 0.9</td>
<td>0.89 ± 0.08</td>
<td>33,800</td>
<td>2.87 \times 10⁻³</td>
</tr>
<tr>
<td>334</td>
<td>-11.4 ± 2.0</td>
<td>1.06 ± 0.016</td>
<td>53,800</td>
<td>1.78 \times 10⁻³</td>
</tr>
<tr>
<td>365</td>
<td>-2.5 ± 0.4</td>
<td>0.96 ± 0.08</td>
<td>194,000</td>
<td>4.93 \times 10⁻⁴</td>
</tr>
<tr>
<td>405</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Values are average of two to four experiments at each monochromatic wavelength. Final slopes of the survival curves were determined using linear regression analysis. ND = not determined.

* Quantum corrected F_{37} = F_{37\lambda} \times 297/\lambda.
† Relative response = F_{37\lambda}/F_{37\lambda}.

Wavelengths of light that are most effective at inducing cataracts in the lens in vivo. Sublethal doses of UVB radiation alter or interfere with the synthesis of specific intracellular and extracellular proteins in cultured lens epithelial cells. The mechanism(s) of formation of cataracts by UV radiation is likely to involve the lens epithelium. UVB and UVA radiation may result in DNA damage that could be cumulative throughout life, because not all damage is repaired successfully. If epithelial cell DNA is irreparably damaged the cells may differentiate into abnormal lens fibers. The presence of micro and macronuclei (damaged pieces of chromosomes) in human lens epithelial "tags" supports the occurrence of DNA damage in cortical cataracts. Whether UV is the causative agent of this damage is unknown. However it is known that UVB radiation produces posterior cortical cataracts in mice that resemble those observed in human senile lens.

The action spectrum for cytotoxicity of UV radiation reported here resembles that of human epithelial and other cell lines and is similar to the DNA absorption spectrum over the range of 297 to 313 nm (Fig. 2). The formation of pyrimidine dimers and DNA repair after UVB exposure occurs in cultured bovine lens epithelial cells. The data in the literature do not preclude the possibility that UV-induced changes in membrane transport and permeability play a role in cell killing. In this regard, our recent findings indicate that UVB radiation brings about increased prostaglandin synthesis in cultured lens epithelial cells.

At wavelengths above 313 nm, the importance of pyrimidine dimers in DNA as the lesion responsible for cell killing decreases and it is likely that a different mechanism of cell killing operates at longer wavelengths. The nature of the different lethal mechanisms in UVA region is not understood, but is thought to involve molecules other than DNA as the primary chromophore, and DNA the ultimate target. UVA radiation may be absorbed by chromophores such as NADPH, NADH, and riboflavin. In terms of mecha-
nisms, UVA radiation generates damaging reactive oxygen species in cultured mammalian cells (V79). 55 UV radiation also induces a decrease in reduced glutathione in cultured skin cells 56 and in the epithelium of cultured lenses. 20 Whether glutathione protects lens epithelial cells from UV in vivo or in vitro remains to be determined. It is of interest that the lens epithelium in vivo has one of the highest concentrations of reduced glutathione of any cell type. 57

Photochemical damage to the lens includes the effects of UV radiation on the lens epithelial and fiber cells. Posttranslational changes in fiber cell proteins induced by UV radiation have been extensively studied. 58 The current study is in accord with the results of Zigman et al, 59 on squirrel lenses in vivo, where broadband UVA radiation centered at 365 nm damaged the lens. Further studies to determine the complete action spectra (297 to 405 nm) for different biologic endpoints that alter the lens epithelium (eg, cytotoxicity and mutation, protein synthesis, membrane effects) and lens fiber cells (aggregation of proteins, formation of tryptophan photoproducts, loss of visible light transmission) are needed to elucidate the overall UV action spectrum for cataract formation. Cultured lens epithelial cells appear to be a suitable model system for addressing some of these questions and for investigating compounds that prevent UV damage.

Key Words
action spectrum, lens epithelial cells, UV radiation, cell killing, cataract

References


