Action Spectrum and Recovery for In Vitro UV-Induced Cataract Using Whole Lenses

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PURPOSE. To establish the in vitro action spectrum for acute UV cataractogenesis using whole cultured lenses. The recovery pattern of the induced cataract was also investigated.

METHODS. Aseptically dissected porcine lenses were cultured in glass chambers. At 1 week, lenses were exposed to a predetermined UV energy (J/cm²) at specific wavebands ranging from 270 to 370 nm at 5- and 10-nm intervals. The UV energy was generated by a PRA integrated arc lamp system using a water-cooled 1000 W, high-pressure xenon lamp. The lamp output was limited using a deionized water filter, a monochromator, and secondary optics. An electronic shutter was used to control the exposure time. The median effective dose, ED₅₀ (i.e., UV energy threshold) for each waveband was statistically determined using probit analysis. Irradiated spots (3.06 mm²) on the lenses were monitored every 6 to 12 hours up to 48 hours postirradiation for any UV-induced opacity with a dissecting microscope and photomicrography. The ED₅₀,₈ were plotted against wavelengths to obtain the action spectrum.

RESULTS. The threshold values for 270, 300, and 365 nm were 0.057, 0.069, and 137.19 J/cm², respectively. Permanent UV-induced cataract was obtained at twice the threshold values for UVB and UVA.

CONCLUSIONS. An action spectrum for in vitro UV-induced cataract using whole cultured lens is established. These data are comparable to published in vitro (with isolated lens epithelial cells) and in vivo action spectra. The recovery pattern appears to be similar to the in vivo situation. (Invest Ophthalmol Vis Sci. 2001;42:2596–2602)

It is generally agreed that high solar UV irradiance levels in the environment would increase the risk of cataract.¹⁻⁶ Increase in UVB (290–315 nm) insolation would also lead to increase in skin cancer and perhaps immune problems in humans and terrestrial and aquatic animals.⁷ Moreover, elevated UVB levels may perturb marine ecology, killing important algae and bacteria. The gradual thinning of ozone has received considerable attention over the past decade.⁸⁻¹⁴ Atmospheric scientists first detected the ozone hole over the South Pole in 1985, the apparent result of chemical reactions caused by chlorofluorocarbons (CFCs) and other pollutants in the stratosphere.⁷ Ever since, their calculations have predicted that loss of stratospheric ozone (which absorbs much of the harmful UVB radiation) would allow more abiotic UV radiation (particularly UVB) to reach the surface of the Earth.⁷,¹²⁻¹⁵

Recently scientists at the National Institute of Water and Atmospheric Research in Lauder, New Zealand, reported that over the past 10 years, peak levels of dermatogenic and DNA-damaging UV rays have gradually been increasing in New Zealand, just as the concentrations of stratospheric ozone have decreased.¹⁶ According to their report, by the summer of 1998–1999, peak sunburning UV levels were ~12% higher than they were during similar periods earlier in the decade. Their report provides the strongest evidence yet that degradation of the stratospheric ozone layer is ongoing and produces more hazardous UV exposure conditions for life on the Earth’s surface.⁷ It is evident that stratospheric ozone thinning is occurring not just in the sparsely populated polar regions, but also above populous midlatitude regions such as northern Europe, Canada, New Zealand, and Australia.⁷

In the eye, it is believed that UV photochemical damage occurring in the crystalline lens can persist for a long time, causing temporary or permanent impairment of vision. The specific targets and the detailed mechanisms that are responsible for UV-induced cataracts remain to be determined.¹⁷⁻²¹ An important aspect of solar UV radiation investigation is the UV cataractogenic action spectrum in the wavebands to which humans are exposed. In terms of UV-lens research, the in vivo UV action spectrum by Bachem²² and Pitts et al.²³,²⁴ and the in vitro UV action spectrum for lens epithelial cells by Andley et al.²¹ and Andley and Weber²⁵ are available in the literature. However, in vitro UV action spectrum data for the cultured intact whole lens are lacking. Because many experimental models now focus on the intact cultured whole lens and because the cellular targets and mechanisms of action of UV radiation vary as a function of wavelength, it is necessary to establish an in vitro UV action spectrum for the cultured whole crystalline lens. From the action spectrum, the mechanisms of UV effects within each waveband can be effectively studied.

The action spectrum of a biological response is the variation in magnitude of the response with wavelength. The shape of the resulting curve is related to the absorption of the radiation-absorbing molecule initiating the response. The present study determined the action spectrum for in vitro UVR cataract formation (gross superficial opacities) using cultured whole porcine crystalline lens. Pitts et al.²³,²⁴ developed the data on the crystalline lens in vivo UV action spectrum for wavelengths from 295 to 365 nm. These data are still the best existing action spectrum data for in vivo UV cataractogenesis.²⁶ The effort to reduce or eliminate the use of live animals in biomedical research is now shifting experimental approaches from in vivo to in vitro. The data of Pitts et al.²³,²⁴ have been used for some time for most in vivo UV experimental applications. More recently, a series of experiments on in vitro UV action spectra for isolated lens epithelial cells has been reported by Andley et al.²¹ Their data are plausible, but it is uncertain whether or not the data are directly applicable to studies involving the whole intact lens. Thus, the purpose of the present study was to determine the in vitro action spectrum for UV lenticular damage in cultured whole lens and to study the damage recovery pattern. Because the pig’s embryological growth and development are typical of mammals and...
its shape and size are similar to the human lens, some inferences may be made to the human lens.

**Materials and Methods**

**Tissue Preparation and UV Exposure**

Eyeballs from 6- to 8-month-old pigs were collected from a local abattoir. The lenses were aseptically dissected 1 to 3 hours postmortem and placed in 25-ml two-compartment glass chambers for 1 week preincubation in culture medium maintained at 37°C and 4% CO₂. The culture medium consisted of modified M199 (9.8 g/l) without phenol red supplemented with 1% antibiotics (100 units/mL penicillin and 100 µg/mL streptomycin) and 4% sterile-filtered porcine serum, 2.2 g/l sodium bicarbonate and 5.96 g/l HEPES as buffers, and 0.1 g/l L-glutamine (Sigma Chemical Co., St. Louis, MO). The medium was measured periodically and indicated insignificant change over the course of the study. Changes made to the human lens were made every 48 hours. The pH (7.6) of the medium was measured periodically and indicated insignificant change between medium exchanges. At 1 week, the anterior surface of the lens was exposed to a dose of predetermined UV radiant energy (W/cm²) at a specific waveband with the bandwidth centered on 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 330, 340, 350, 360, and 370 nm wavelengths. During exposure, the lens was centered normally to the UV beam. The lens in its culture chamber was placed on a container holder for accurate alignment and distance. Stray UV energy was measured to be <0.1%; and because this amount is negligible, it was ignored in the evaluation of the radiant exposure.

To convert the radiometer measurement to irradiance, the measured value was multiplied by the irradiated area on the lens and the linear multiplication factor from the calibration curve. The irradiated area at the lens surface was 3.06 mm² (0.0306 cm²). The reciprocal of 0.0306 equals 32.68; thus, the unit area multiplication factor for conversion to irradiance was 32.68 throughout this experiment. For example, the linear multiplication factor for 300 nm was 1.08; therefore, a reading of 48 µW was equal to 1694 (i.e., 48 × 32.68) µW/cm² as irradiance. Radiant exposure time was determined using the following radiometric equation: $t = H / E$, where $t$ is exposure duration (seconds), $H$ is radiant exposure (J/cm²), and $E$ is measured irradiance (W/cm²). The duration of exposure was controlled by a preset electronic counter, which automatically closed the shutter after...
A modified staircase (up-and-down) method with doubling, 50%, 25%, and 10% decrement/increment steps, was used to obtain threshold values for each waveband from 270 to 370 nm. On average, five lenses were irradiated for each energy level at each waveband. If none of the five lenses at a given exposure level showed damage, then the dose was doubled. If all five lenses showed damage, the dose was reduced by 50%. If three of five lenses did not show damage, the energy level was arbitrarily increased by 50% or 10% of the immediate previous dose. This was continued until at least three of five lenses were observed to show damage before proceeding to the next energy level or waveband. All lenses were examined under a dissecting light microscope to determine their suitability for experimentation. The pig lenses could be kept viable in culture medium for 5 to 6 weeks. The UV beam was focused at the anterior pole for all the lenses to keep the irradiated area the same.

For the purpose of deciding on the starting point, the threshold data of Pitts et al. were used. They found that the most efficient waveband for lenticular damage was 300 nm, which had a radiant exposure threshold of 0.15 J/cm². An approximate UV energy level of 0.2 J/cm² at 300 nm was chosen as the starting point in the present study. Therefore, the first exposure was made at 300 nm, with 0.2 J/cm² delivered to the anterior lens surface. The sequence involved irradiation for wavelengths from 300 to 270 nm in 5-nm intervals and then 305 to 370 nm in 5- or 10-nm intervals. Observation time was limited to a maximum of 36 to 48 hours after irradiation. Irradiated spots on the lenses were monitored every 6 to 12 hours for any morphologic lesions (i.e., superficial or subcapsular opacities), induction time, and lesion pattern with photomicroscopy (Nikon dissecting microscope, Tokyo, Japan). During photomicroscopy, the eyepiece magnification was set at ×10 and microscope magnification at ×2.5.

Photographs of the lesions patterns (square-shaped area of 3.06 mm²) were randomly taken at appearance or during recovery. UV-irradiated lenses were visually compared with untreated controls. Again, if no lesion was found at 36 to 48 hours, the dose was doubled or increased by 50%, 25%, or 10% for the next exposure. If there was a lesion with the increase, the dose was arbitrarily decreased by 25% or 10% until there was no lesion. For each subsequent set of lenses, the dose was decreased or increased, depending on the response of the previous set of lenses to a lower radiant exposure, until moderate or severe lesions were observed in at least three lenses of five. The ED₅₀ (i.e., 50% probability of damage, with 95% confidence interval) was then calculated by using probit analysis. An SPSS for Windows software (SPSS Inc., Chicago, IL) was used for the probit analysis. As an example, Table 1 shows the results from the probit analysis of the 300-nm waveband data. The induction time and the number of lenses used per radiant exposure were recorded. ED₅₀ was determined for wavebands centered on 270, 275, 280, 285, 290, 295, 300, 305, 310, 315, 320, 330, 340, 350, 360, 365, and 370 nm, and values are presented in Table 2. The 365-nm waveband was included because most studies on UVA effects center on this wavelength. The ED₅₀ values were plotted as a function of wavelength to determine the action spectrum curve (Fig. 2).

The second part of this study investigated the recovery pattern of in vitro UV-induced cataract at 300 and 350 nm, for UVB and UVA exposures, respectively. The threshold values obtained in the first part were used as reference radiant energy levels. Organ cultured porcine lenses (group 1) were irradiated with 300 nm UVB at threshold level (0.07 J/cm²), two times threshold (group 2), and five times threshold (group 3). Group 4 lenses were exposed to 222.6 J/cm² of 350 nm UVA, which was two times threshold. Each irradiated spot on the anterior lens surface (3.06 mm²) was monitored by photomicrography for 4 weeks postirradiation. The lenses were examined every 12 to 24 hours in the first 2 weeks and then once each week postexposure. UVR experimental cataract was defined as a cluster of discrete dots in the lens anterior subcapsular layer. The criterion for recovery was the complete disappearance of the lesions.
Damage Grading

The severity of the lesions in the lenses was graded as follows: −, no damage; +, moderate or threshold damage, lesions half or less of square pattern; ++, severe damage, lesions fill more than half of square pattern; +−, lesions disappearing (sign of recovery); and −−, lesions completely disappeared (full recovery).

Any lens having a + means that the lens showed damage at that particular energy level (dose). For analysis, a lens with a + or ++ grade is counted with the number of lenses damaged. Any lens with a lesion showing recovery was graded +−, and the time that the repair trend was observed was recorded. If a lens lesion showed complete recovery, it was graded −−, and the time of the observation noted. Any lens with a damaged capsule was discarded. The control lenses were also observed for any morphologic changes.

RESULTS

Part 1

Approximately 648 lenses were used in this study. The irradiated spots on the lenses showed anterior subcapsular discrete dot opacities, which appeared initially as small white dots and slowly coalesced into a white patch within the irradiated spot. The pattern of the discrete dot opacities (lesion) was graded using the scheme explained above. Using the 300-nm waveband data as an example, an ED50 value of 0.06952 (95% confidence interval, 0.05746–0.08781) was obtained for 300 nm as indicated in Table 1. The ED50 values (with 95% confidence interval) for all wavebands are shown in Table 2.

Graphically, a threshold can be obtained by connecting the 50% probability on the y-axis to the corresponding radiant exposure value on the x-axis through the regression line intercept. The different ED50 (i.e., 50% probability of damage) values for the wavebands were collated and plotted against wavelengths to obtain the action spectrum (see Fig. 2). The data generally indicate shorter damage latency and slower recovery for higher UV energy levels, whereas the reverse is the case for lower energy levels. It should be noted that in Figures 3, 4, and 5, all the photographs for each figure were of the same lens.

DISCUSSION

The present study demonstrates that probit analysis is useful in determining the threshold for each UV waveband. It enables the conversion of subjective data from the grading method into parametric data (e.g., calculating 50th percentile), thus allowing for the derivation of ED50, which represents 50% probability of a defined UV radiant exposure that could cause photodamage. Simply stated, the ED50 (i.e., median effective dose) is the dose that will produce a response in half the population of the UV exposed lenses at certain radiant exposure levels. The UVR action spectrum obtained by plotting the ED50 versus wavelength (nm) gives the relative effectiveness per incident photon at each waveband for a single UV exposure. Figure 2 illustrates the derived action spectrum for in vitro UV cataract formation for cultured porcine lenses. Pitts et al.23 reported that the in vivo action spectrum for UV-induced lens opacities in rabbits begins at 295 and extends to 320 nm. Radiation at 300 nm, using a 6.6-nm full bandpass, was found to be 30 times as effective as 315-nm radiation in producing lens opacities in vivo.

The in vitro action spectrum obtained in the present study shows a trend similar to the in vivo data of Pitts et al.23 that extended from 295 to 395 nm. Because of the absence of corneal absorption, it was possible to induce lenticular opacity...
with shorter wavelengths down to 270 nm in the present study. This was done in the hope that the obtained threshold (ED$_{50}$) values could be useful for future in vitro UV lens toxicology investigations extending below 290 to 270 nm. Therefore, the data in the present study involved wavelengths from 270 to 370 nm. The limit at the longer UV wavelengths was at 370 nm in the present study because no observable damage could be induced with the highest radiant exposures at the 370-nm waveband. This tends to agree with Andley et al., who reported that the highest dose at the 405-nm waveband in their study produced no adverse effect on lens epithelial cells. Also, using 162 J/cm$^2$ radiant exposure at 365-nm waveband, which was the highest dose in their study, Pitts et al. could not achieve any lenticular opacities in vivo in rabbit.

By comparison, at 300 nm, the present study found 0.069 J/cm$^2$ as threshold, whereas Pitts et al. found 0.15 J/cm$^2$ for in vivo exposure. Andley et al. and Andley and Weber found 0.068 and 0.052 J/cm$^2$ at 302 nm, respectively, using cultured rabbit and human lens epithelial cells. Data in the present study showed radiation at 295-nm waveband to be 25 times more effective than 315-nm radiation in producing UV-induced lens anterior subcapsular lesions (Table 2), whereas the data of Pitts et al. showed that 295-nm radiation was six times more effective than 315-nm radiation. The data of Andley et al. and Andley and Weber show that the radiation at 297 nm is 171 and 261 times more effective than the 313-nm radiation used in their respective studies with rabbit and human lens epithelial cells. The difference in relative effectiveness is not surprising because of the absence of corneal and aqueous absorption in the in vitro conditions. Moreover, in isolated epithelial cells, the influence of overlying capsule, underlying cortex, and adjacent cells are absent or minimal with respect to the impact of the radiation. The in vitro radiant exposure values in the present study are 29 times lower at 295 nm, ~2 times lower at 300 nm, ~3 times at 305 nm, ~3 times at 310 nm.

![Figure 3](image1.png)  
**Figure 3.** A representative illustration of morphologic changes (magnification, ×25) in exposed group 1 lenses at 300 nm UVB threshold (0.07 J/cm$^2$): pre-exposure (A); 36 (B), 48 (C), and 72 hours postexposure (D); and complete recovery at 376 hours (2 weeks) postexposure (E). Bar, 1.75 mm.

![Figure 4](image2.png)  
**Figure 4.** An illustration of morphologic changes (magnification, ×25) in exposed group 2 lenses at 300 nm UVB two times threshold (0.14 J/cm$^2$): pre-exposure (A) and 36 hours (B), 48 hours (C), 72 hours (D), 96 hours (E), 120 hours (F), 216 hours (G), 336 hours (2 weeks; H), 504 hours (3 weeks; I), and 672 hours (4 weeks; J) postexposure, respectively. No complete recovery at 4 weeks in exposed lenses. Bar, 1.75 mm.
The relatively low radiant exposures required to produce damage in the 270- to 315-nm wavelength range suggest that the most effective waveband for producing UV cataract in vitro is from 270 to 315 nm. Looking at the trend of the action spectrum (Fig. 2), it would be expected that wavelengths shorter than 285 nm should be more effective (i.e., have lower thresholds because the shorter the UV wavelength, the higher the photon energy). However, there was a fair rise in effective dose below 285 nm (Fig. 2), which suggests that a different mechanism is responsible for UVR lenticular toxicity below 285 nm. Another possible explanation is that UVR does not affect crystalline lens biomolecules in the same way for each waveband.11 The action spectrum curve shows that 285 nm is the most effective wavelength for producing UV toxicity on porcine lens in vitro. This is relatively close to 280 nm, the shorter absorption maximum of the human crystalline lens that has been reported to exhibit absorption maxima at 280 and 370 nm.31–33 With the advent of the artificial cornea, the maximum effectiveness at the 285-nm wavelength on the lens is important. The present in vitro result support Yamanashi et al.,34 who explained that the wavelength at which UV-induced cataract appears to occur maximally (i.e., 300 nm) due to corneal absorption during an in vivo situation might not be the true maximum of UV-induced cataractogenesis. At the long UV wavelengths, 365 nm appears to be more effective than 360 nm for causing cataract. The present study has established the in vitro UV action spectrum for cultured porcine lens using gross morphologic changes (anterior subcapsular opacities) as damage criteria. However, extrapolations from the data can be applied to the study of in vitro UV-induced cataracts using lenses from other animal species. The UV levels in this study was estimated to be approximately nine times higher than ambient lenticular levels reported by Zigman.35

In terms of recovery from UV damage, the present study confirms the findings by Pitts et al.,17–24 that radiant exposure at two times threshold level results in permanent opacity. At two times UVA threshold exposures, there was no full recovery (Fig. 5), confirming that UVA is cataractogenic. At five times UVB threshold exposure, the photodamage gave rise to prominence of the suture, which might be an indication of permanent opacity with no chance of recovery. Data in the present study support the theory that repair will generally occur for UVR-induced cataract at threshold and subthreshold radiant energy levels. This may tempt one to speculate that the temporary blurring of vision often experienced due to UVR-induced photokeratoconjunctivitis may arise from not only the cornea but also from the lens.

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References


