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Ultraviolet Action Spectrum for Cell Killing in a Human Lens Epithelial Cell Line

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Abstract: There are many sources of ultraviolet (UV) radiation in the workplace. Action spectrum data are necessary for establishing methods to evaluate UV hazards. We determined a UV action spectrum for cell killing in the human lens epithelial cell line SRA 01/04 by measuring the viability of cells exposed to UV at 8 different wavelengths ranging from 259.5 to 300.5 nm in a carefully designed and controlled experiment. The action spectrum based on LD_{50} is nearly flat or falls slightly with wavelength in the short-wavelength region and then rises increasingly steeply at longer wavelengths. UV at 267.5 nm is most effective in cell killing, with an LD_{50} of 8.86 Jm^{-2}. The action spectrum for killing lens epithelial cells in humans (*in vivo* action spectrum) derived from the present results falls monotonically with wavelength in the range studied, suggesting that UV at wavelengths longer than 300.5 nm may also be highly effective in killing lens epithelial cells in humans and, therefore, in causing cataract. Further studies should be conducted with UV at longer wavelengths.

Key words: Action spectrum, Ultraviolet radiation, Cell killing, Lens epithelial cell, Hazard evaluation

Introduction

Many workers are expected to be exposed to ultraviolet (UV) radiation from the sun, welding arcs, germicidal lamps and other sources, thereby suffering health problems such as photokeratitis, photoconjunctivitis, pterygium, cataract, erythema, skin aging and skin cancer[1, 2]. In fact, photokeratitis[3, 4] and erythema[3] often occur in workplaces where arc welding is performed, and cataract[5, 6] and skin cancer[7–10] have been suggested to be more prevalent in outdoor workers. As a first step toward preventing UV-induced health problems, UV hazards should be evaluated in the workplace.

Action spectrum data are necessary for establishing methods to evaluate UV hazards. An action spectrum is a plot of exposure dose (or sometimes effectiveness) of monochromatic optical radiation to produce a specified photochemical or photobiological effect as a function of wavelength. Action spectra for UV-induced adverse health effects in humans (hazard functions) must be considered in evaluating UV hazards because UV usually consists of different wavelength components which have different effectiveness. In order to evaluate the hazard of a specified health effect, the hazard function for that health effect must be known, because hazard functions generally differ by health effect. A hazard function has been developed from action spectra for photokeratitis in animals and erythema in humans[11] and used by the American Conference of Governmental Industrial Hygienists (ACGIH)[12] and the International Commission on Non-Ionising Radiation Protection (ICNIRP)[13] in their guidelines for the evaluation of hazards of photokeratitis and erythema. For other UV-induced health effects, however, hazard functions remain unknown or unestablished because of the lack of action spectrum data and, therefore, hazards cannot be evaluated.

Action spectra for effects on animals, cultured cells and biological molecules are important in establishing hazard functions, because human action spectra are often difficult or impossible to measure directly. Non-human action spectra are useful also in that they can be accurately determined in carefully designed and controlled experiments. However,

there are still insufficient non-human data to derive reliable hazard functions. More studies are needed on action spectra related to UV hazards.

As part of a project to study action spectra that can be used for UV hazard evaluation, we determined a UV action spectrum for cell killing in a human lens epithelial cell line by measuring the viability of cells exposed to UV at different wavelengths. The experiment was carefully designed and controlled to obtain reliable data. The results will help develop hazard functions, particularly the hazard function for cataract formation, because it has been suggested that UV-induced damage in the lens epithelium in humans leads to cataract formation[14].

Materials and Methods

Cell culture

The human lens epithelial cell line SRA 01/04[15] was used for the experiment. Cells were seeded at 7,000 cells per well in flat-bottomed 96-well plates in complete Dulbecco's Modified Eagle Medium (DMEM, Gibco Life Technologies) supplemented with 20% fetal bovine serum (Gibco Life Technologies), 1% penicillin and streptomycin, 0.19% HEPES and 1% nonessential amino acids (BioWhittaker). They were cultured in monolayer at 37°C in a humidified atmosphere containing 5% CO_2 for 2 d to allow them to enter exponential growth (Fig. 1). Then, the culture medium was replaced with Dulbecco's phosphate buffer saline (PBS) with calcium and magnesium (BioWhittaker), which is transparent to UV in the wavelength range studied, and cells were exposed to UV. After that, cells were cultured in the medium for another 4 d, during which UV-induced cell death occurred (Fig. 1). Then, cells in exponential growth were assessed for viability.

In this procedure, UV exposure and viability assessment were both performed during exponential cell growth to obtain reliable data. If UV exposure or viability assessment is advanced or delayed by a few hours for experimental reasons, basically it does not affect the results.

UV exposure

UV from a xenon lamp system (SXUI500XQ, Ushiospax) was delivered to cells through optical elements (Fig. 2). A bandpass filter was used to isolate a specified wavelength region, and a ground fused silica plate was used to uniformly irradiate the culture well. Cells were exposed to UV in 8 different wavelength regions centered at 259.5, 265.5, 267.5, 273.5, 282.5, 290.5, 298.5 and 300.5 nm with 10- to 13-nm bandwidth (full-width-at-half-maximum) by using different bandpass filters (254FS10-50, 260FS10-50, 265FS10-50, 270FS10-50, 280FS10-50, 289FS10-50, 297FS10-50, 300FS10-50, Andover) (Table 1). The peak wavelength and bandwidth of UV used for exposure were determined from the spectral irradiance measured at the position of the cells. Irradiance was measured with a radiometer (IL 1400A, International Light) connected to a silicon-photodiode detector (SEL033, International Light) which was calibrated by the manufacturer before the study. Irradiance was measured at the position of the cells before and after exposures at each wavelength and averaged. The exposure dose (radiant exposure) was controlled by varying the exposure duration. To obtain dose-response data, cells in a 96-well plate were exposed to 6 exposure doses ranging from 0 Jm^{-2} to approximately twice or three times the LD_{50} estimated from a preliminary study at each wavelength. Four wells were used for each exposure dose.

Cell viability

Cells in flat-bottomed 96-well plates were fixed with 2.5% glutaraldehyde (Wako Pure Chemical Industries) in PBS for 30 min and washed with water. Then, cells were stained with 0.4% crystal violet at room temperature for another 30 min, washed with water and dried at room temperature. The optical density (OD) of cells at 590 nm, which is proportional to the number of cells[16], was measured in each well using a multiplate scanning photometer (Titertek Uniskan II, Dainippon Pharmaceutical). Cell viability in each well was calculated by dividing the OD by the mean OD of the control (zero-dose) wells of the same plate.

LD_{50} and action spectrum

At each wavelength the dose-response data were fitted, using the least squares method, with a function:

$$f(D)=1-\frac{1}{\sqrt{2\pi}\sigma}\int_0^D \frac{1}{x}\exp\left\{-\frac{(\log x-\mu)^2}{2\sigma^2}\right\}dx,$$

where D is the exposure dose and the second term is the cumulative lognormal distribution function with parameters μ and σ (Fig. 3). The fitting was performed using the Solver function of Excel spread-sheet program (Microsoft). The exposure dose required to kill 50% of cells (LD_{50}) was calculated as the logarithm of the value of μ that provided the best fit. The action spectrum was obtained by plotting the LD_{50} as a function of wavelength.

The experiment was repeated three times to ensure reproducibility.

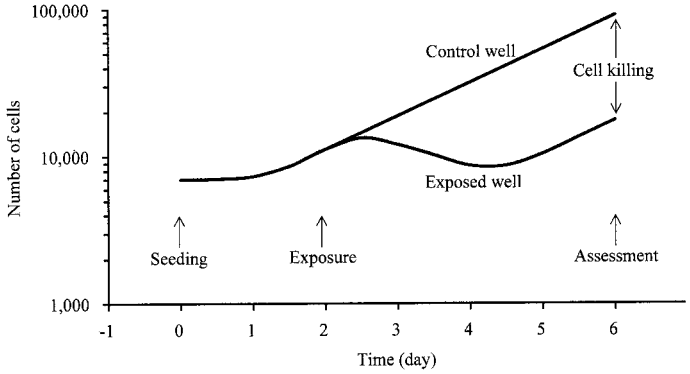

Fig. 1. Schematic cell growth curve.

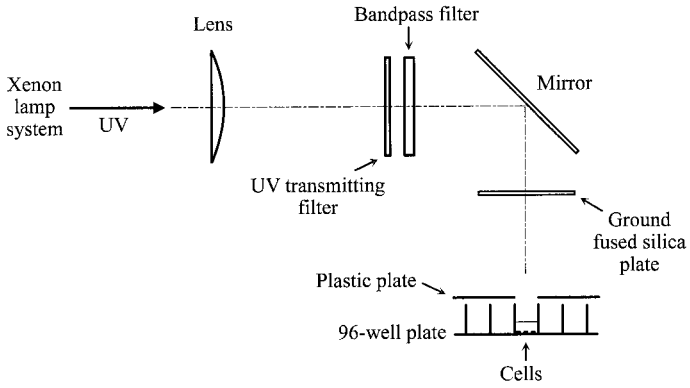

Fig. 2. Schematic experimental setup for UV exposure.

Table 1. UV radiation used for exposure

Peak wavelength (nm)	Bandwidth* (nm)	Irradiance (Wm^{-2})	Bandpass filter
259.5	12.0	0.090–0.123	254FS10-50
265.5	10.5	0.165–0.217	260FS10-50
267.5	12.5	0.193–0.248	265FS10-50
273.5	12.0	0.547–0.706	270FS10-50
282.5	12.0	0.742–0.913	280FS10-50
290.5	12.5	1.22–1.49	289FS10-50
298.5	11.0	1.62–1.92	297FS10-50
300.5	10.5	1.38–1.57	300FS10-50

*Full width at half maximum.

Results

Cell viability decreased in a dose-dependent manner described by a cumulative lognormal distribution function at each wavelength (Fig. 3).

The LD_{50} values obtained from the dose-response data varied with wavelength, ranging from 8.86 Jm^{-2} to 142 Jm^{-2} (Table 2). They were highly reproducible with coefficients of variation of about 10%.

The action spectrum based on LD_{50} is nearly flat or falls slightly with wavelength in the short-wavelength region and then rises increasingly steeply at longer wavelengths, showing that UV is most effective in cell killing around 267.5 nm (Fig. 4).

Discussion

The present *in vitro* action spectrum can be used to derive the action spectrum for killing lens epithelial cells in humans (*in vivo* action spectrum) which may help develop the hazard function for cataract formation, if lens epithelial cells in humans respond to UV in the same way as SRA 01/04 cells. UV incident on the eye is attenuated by the cornea and anterior chamber before reaching lens epithelial cells in humans, and, therefore, the *in vitro* action spectrum was divided at each wavelength by the combined transmittance of the cornea and anterior chamber to obtain the *in vivo* action spectrum (Fig. 5). The transmittance was calculated using the absorption coefficients for the cornea and aqueous humor

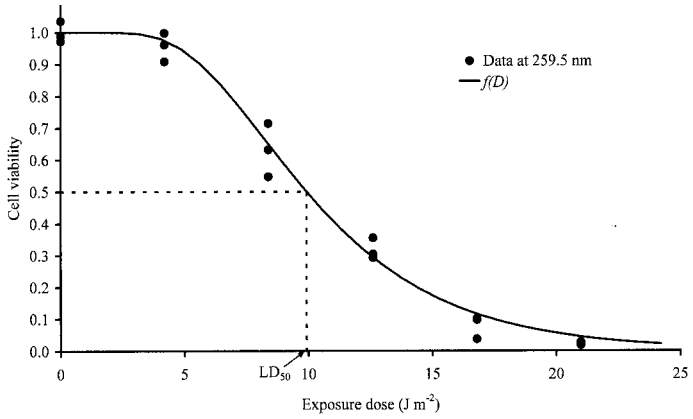

Fig. 3. Example of fitting dose-response data with the function *f(D)*.

Table 2. LD_{50}

Wavelength (nm)	LD_{50} Mean (Jm^{-2})	CV* (%)
259.5	10.1	7.7
265.5	9.34	9.2
267.5	8.86	6.6
273.5	10.0	6.1
282.5	14.3	11.3
290.5	28.3	8.6
298.5	92.1	7.9
300.5	142	7.2

*Coefficient of variation.

of the rhesus monkey eye, which were measured very carefully by Maher[17] and are considered a good baseline for the primate eye. The *in vivo* action spectrum (Fig. 5), unlike the *in vitro* action spectrum (Fig. 4), falls monotonically with wavelength in the range studied, suggesting that UV at wavelengths longer than 300.5 nm may also be highly effective in killing lens epithelial cells in humans and, therefore, in causing cataract. Further studies should be conducted with UV at longer wavelengths.

The derived *in vivo* action spectrum agrees very well with the *in vivo* action spectrum for opacities in the rabbit lens[18] in the wavelength region where both data are available, and also largely agrees with the *in vivo* action spectrum for light scattering in the rat lens[19]. This supports the validity of the obtained *in vivo* action spectrum.

The LD_{50} determined at long wavelengths in this study may be lower than the true value due to the spectral band of 10- to 13-nm width used for exposure. This is because shorter wavelengths within the band are much more effective than longer wavelengths and contribute significantly to the LD_{50} value attributed to the peak (center) wavelength of the band. Thus the determined action spectrum at the long-wavelength end may have been shifted to longer wavelengths from the true action spectrum. The impact of spectral bandwidth should be considered when comparing action spectrum data or using them to develop hazard functions[20].

In the present study an accurate UV action spectrum was determined in a carefully designed and controlled experiment. UV action spectra for various effects on animals, cultured cells and biological molecules should be determined similarly to establish hazard functions that are necessary for UV hazard evaluation.

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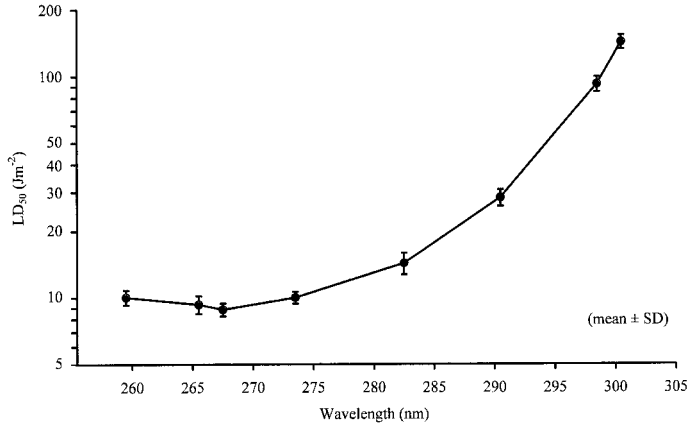

Fig. 4. Action spectrum for cell killing in a human lens epithelial cell line.

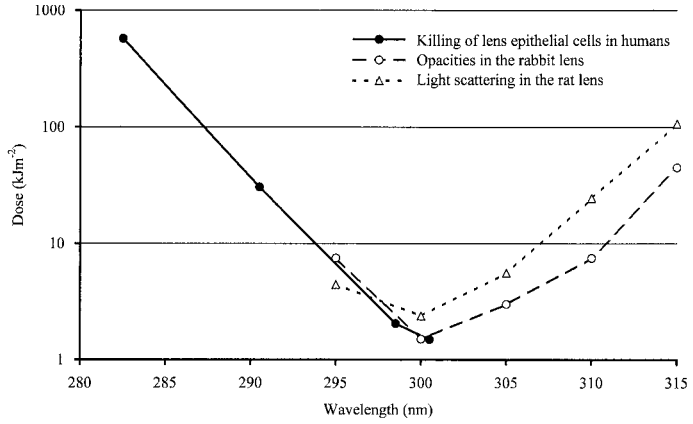

Fig. 5. Derived action spectrum for killing lens epithelial cells in humans (LD_{50}) and *in vivo* action spectra for opacities in the rabbit lens (maximum acceptable dose)[18] and for light scattering in the rat lens (threshold)[19].

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