

Phototoxic Blue-Violet Wavelengths Determined by Tissue Culture Model of Aging RPE Cells Exposed to Sunlight-Normalized Irradiance Levels

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Background

- Age-related macular degeneration (AMD) is a major cause of visual impairment and morbidity worldwide. The number of US patients with early AMD is expected to double from 9.1 million to 17.8 million by 2050.¹
- Epidemiology research suggests blue light (380 to 500 nm) exposure as a risk factor for AMD.^{2,3}
- Retinal pigment epithelial (RPE) dysfunction and degeneration is a critical pathogenic process in AMD. Animal and in vitro experiments establish that high-energy blue light can cause photochemical damage to RPE cells, leading to apoptotic cell death.⁴
- Though retinal exposure to blue light decreases gradually with the yellowing of the crystalline lens, it remains significant throughout life.
- Due to the potential pathogenic role of blue light phototoxicity in AMD, reducing cumulative lifelong blue light exposure may be of value.

Purpose of Current Study

- Despite accumulating evidence of blue light-mediated retinal cell damage, previous laboratory studies have used broadband blue light illumination, and as a result, none has been able to precisely define the phototoxic action spectrum of blue light.
- The goal of this study was to identify the most hazardous wavelengths within the blue light spectrum (380 to 500 nm) in an in vitro model of aging RPE cells. The model employs RPE cells loaded with A2E, a major chromophore of lipofuscin, the photosensitive pigment granules that accumulate in RPE cells during aging and early AMD.^{5,6}

Methods

The RPE Cell Aging Model

A2E was loaded to primary cultures of swine RPE cells by incubating the cells for 6 hours in serum-free media containing 12.5, 20, or 40 μM A2E. Ingestion of A2E was confirmed by microscopic examination of autofluorescence (Figure 1). To correlate A2E intake to its concentration in the culture medium, intracellular A2E was extracted and quantified by ultra-performance liquid chromatography (UPLC) (Figure 2).

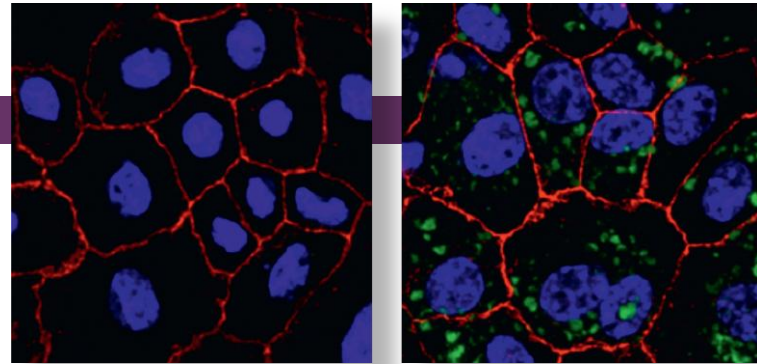


Figure 1. Confocal images showing (left) untreated RPE cells and (right) fluorescent vesicles in the cytoplasm of RPE cells treated with 40 μM A2E (green). Cell membranes were labeled with anti-ZO-1 antibody (red) and nuclei with DAPI stain (blue).

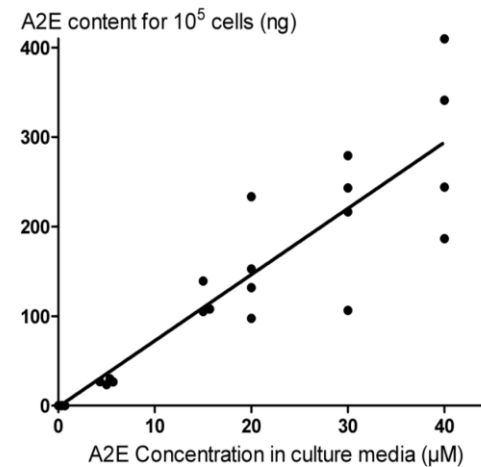


Figure 2. The amount of ingested A2E in RPE cells increases linearly with increasing A2E concentration in culture medium (0, 5, 15, 20, 30, and 40 μM) ($n = 4$, $r^2 = 0.9955$).

Methods

The Illumination System

A computer-controlled LED-based illumination system was custom-built with two optical units: one generates 10-nm illumination bands within the violet to green range of 390 to 520 nm, and one additional 10-nm control band at 630 nm; the other homogenizes light to ensure uniform conditions within each plate subdivision. The two units are linked by optical fiber bundles, which can simultaneously light five 16-well subdivisions of a 96-well plate with five distinct illumination bands (a sixth 16-well subdivision on the plate is maintained in the dark as control) (Figures 3A and 3B).

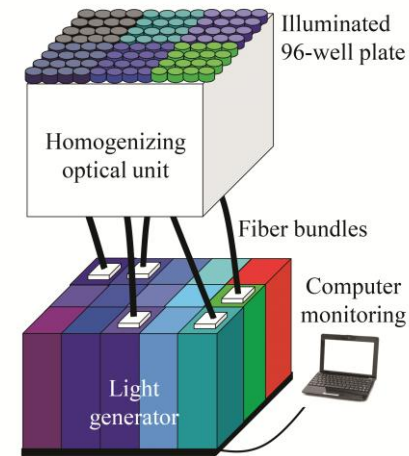


Figure 3A. A schematic of the LED-based fibered illumination device.

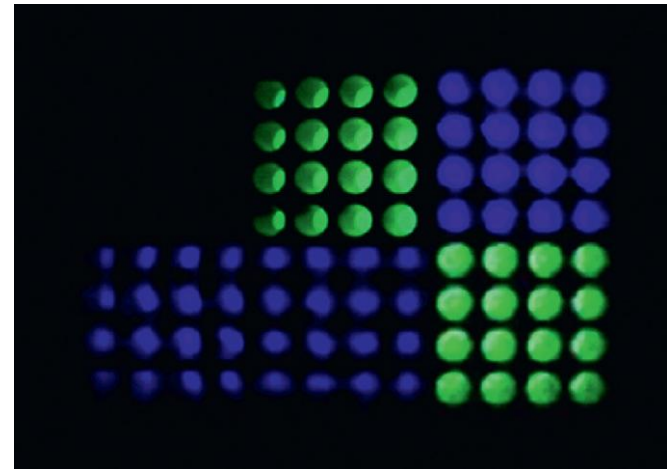


Figure 3B. Representative 96-well plate illumination.

Methods

Irradiance Calibration and Phototoxic Experiment

- Irradiance of each 10-nm illumination band was normalized to simulate typical sunlight exposure level at the retinal plane. Retinal irradiance was calculated by incorporating the transmittance of the ocular media into a reference solar spectrum: ASTM G173-03 (International standard ISO 9845-1, 1992). A multiplicative factor was then introduced to calibrate light irradiances so as to induce detectable RPE cell toxicity within 24 hours (18 hours of light exposure and 6 hours of rest in dark) (Figures 4A and 4B).
- A2E-loaded RPE cells were exposed to each light condition for 18 hours. To quantify phototoxicity, the ApoTox-Glo™ Triplex assay was performed 6 hours after light exposure to assess cell viability, caspase-3/7 activation (to determine apoptosis), and necrosis in each well. Measurements for each light condition and each A2E concentration were averaged from 4 wells and normalized to the dark control.

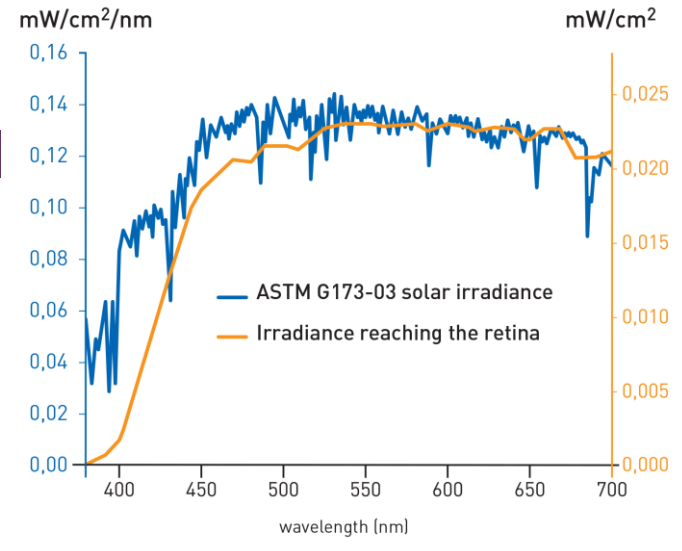


Figure 4A. The retinal irradiances (orange curve, right axis) were calculated by applying the ocular media filtering to the ASTM G173-03 solar spectral irradiance (blue curve, left axis).

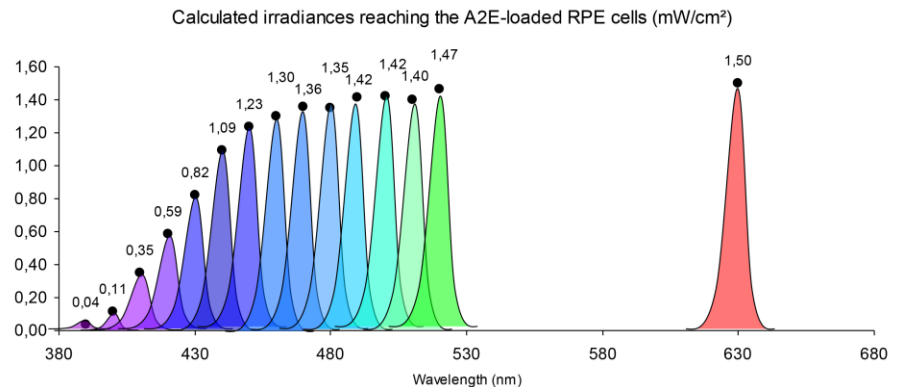


Figure 4B. Irradiances reaching the A2E-loaded RPE cells in 96-well plates (mW/cm²) (Mean \pm SEM, n = 4-6).

Results

Cell Morphology

Six hours after light exposure, morphological changes (including cell rounding, loss of confluence, and decrease of density) were observed by microscopy in cells treated with 20 or 40 μM A2E. These morphological changes were most prominent in the illumination bands centered at 440 nm (Figure 5).

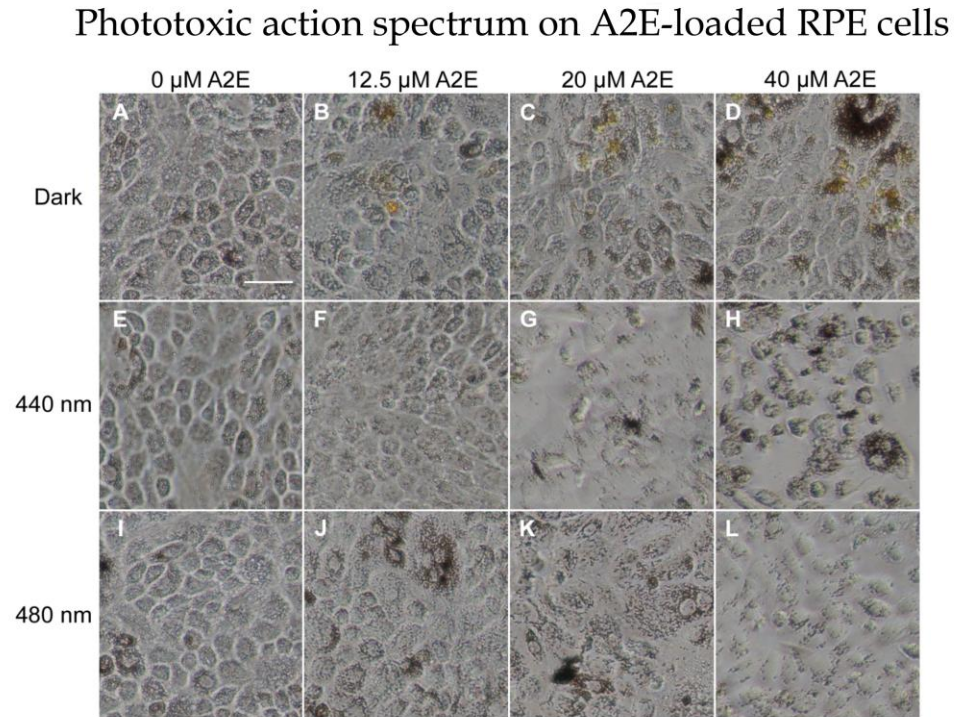


Figure 5. Morphological changes in A2E-loaded RPE cells induced by blue light exposure for 18 hours.

Results

Cell Viability

- A decrease in cell viability was observed with all tested illumination bands, but was more significant at around 440 nm. The viability loss increased with increasing A2E concentration. At 440 nm, a decrease in viability was detectable even in the absence of A2E treatment (Figure 6A).
- Measurements of cell apoptosis demonstrated a consistent wavelength-specific, dose-dependent pattern. The greatest increase occurred in cells treated with 40 μM A2E, with exposure to the 4 narrow illumination bands between 415 to 455 nm (Figure 6B).
- No light-elicited cell necrosis was measured, as compared to control cells maintained in darkness (Figure 6C).
- The phototoxic effect of blue light on cell viability and cell apoptosis, as demonstrated in Figure 6, was not directly correlated to the irradiance levels of each light condition (shown as the red curve).

Phototoxic action spectrum on A2E-loaded RPE cells

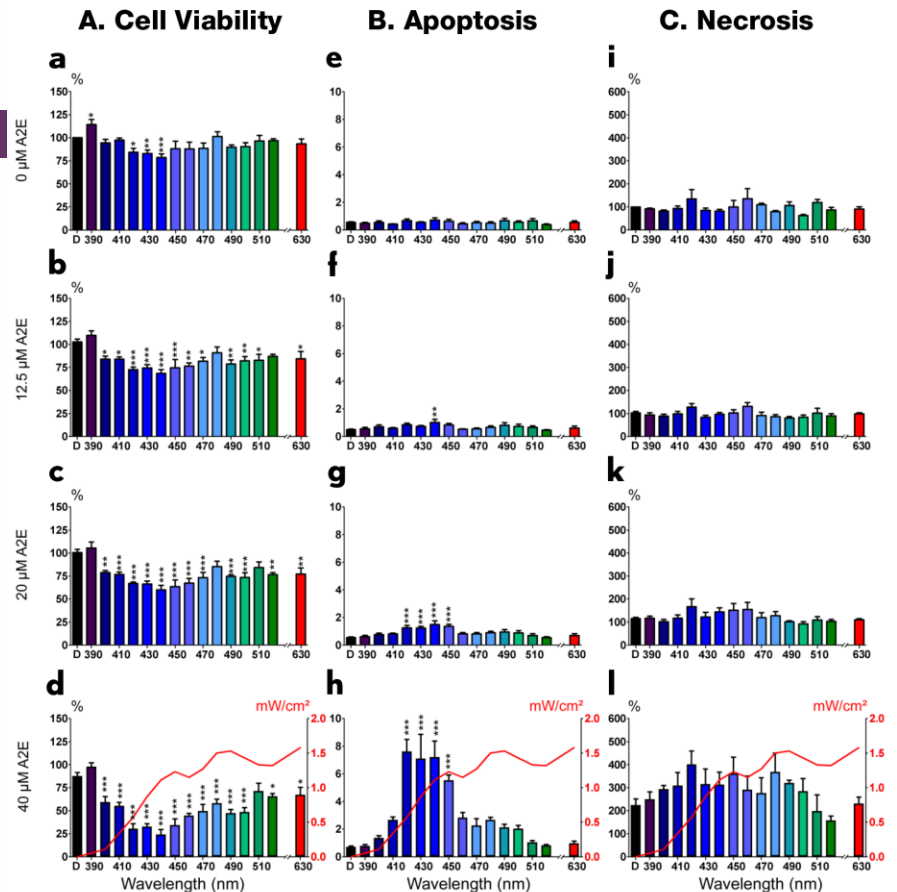


Figure 6. Action spectrum of blue light in RPE cell phototoxicity.

- Cell viability.
- Cell apoptosis (expressed as the ratio of caspase-3/7 activity signal to cell viability signal).
- Cell necrosis.

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Discussion

- The current study addressed limitations of previous experimental research on blue light phototoxicity in RPE cells by:
 1. Using primary cultures of RPE cells instead of immortalized RPE cell lines;
 2. Washing cells prior to measurement with culture medium free of any photosensitizer, such as phenol red, riboflavin, folic acid, or aromatic amino acids;
 3. Normalizing experimental light conditions to mimic the actual physiologic, chronic sunlight exposure at the retinal level; and
 4. Achieving narrow-band illumination (14 bands from 390 to 520 nm, in 10-nm increments) with tight photometric control.

Discussion

- Consistent with previous studies, all tested wavelengths within the blue light range resulted in a loss of cell viability. The greatest phototoxicity, however, was induced by the four illumination bands between 415 and 455 nm, with A2E concentrations at 20 μM and 40 μM —suggesting the phototoxicity observed is not dependent solely on wavelength energy or on light intensity. This is the first time such a precise, narrow action spectrum of blue light phototoxicity has been identified for RPE cells.
- The most hazardous portion of the blue spectrum identified in this study—the narrow band between 415 and 455 nm—does not overlap with the light sensitivity spectrum of intrinsically photosensitive retinal ganglion cells (ipRGCs), which peaks at about 480 nm.^{7,8} ipRGCs have been found to mediate non-visual functions such as pupil constriction and circadian rhythm.

Conclusion

- A2E confers susceptibility to blue light phototoxicity in RPE cells. A2E-loaded primary cultures of porcine RPE cells offer an adequate in vitro model for aging and AMD.
- The narrow band from 415 to 455 nm is associated with the highest risk for blue light phototoxicity in RPE cells.
- This finding provides critical information for developing selective protective filters to prevent ocular blue light hazard without inhibiting the retina's visual and nonvisual functions.

References

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