Blue light-absorbing intraocular lens and retinal pigment epithelium protection in vitro

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Purpose: To compare the Alcon AcrySof® Natural (SN60AT) and AcrySof (SA60AT), the AMO Sensar® (AR40e) and ClariFlex®, and the Pfizer CeeOn® Edge 911A intraocular lenses (IOLs) as to their ability to protect retinal pigment epithelial (RPE) cells from light damage mediated by the lipofuscin fluorophore A2E.

Setting: Department of Ophthalmology, Columbia University, New York, New York, USA.

Methods: Cultured human RPE cells (ARPE-19 cell line) that had accumulated A2E were exposed to blue (430 nm ± 30), green (550 ± 10 nm), or white (390 to 750 nm) light with and without an IOL in the light path.

Results: The blue light-absorbing AcrySof Natural IOL was associated with significant reduction (78% to 82%; P<.01) in the death of A2E-laden RPE that were exposed to blue, white, and green light. The decrease in the incidence of cell death was greater in magnitude than would be expected from the amount of light that was absorbed by the IOL. The considerably smaller declines in cell death observed with the AcrySof, Sensar, ClariFlex, and CeeOn Edge IOLs were likely due to nonspecific reductions in light transmittance.

Conclusions: By absorbing blue light, the AcrySof Natural IOL shields RPE cells that have accumulated the aging lipofuscin fluorophore A2E from the damaging effects of light. A long-term population-based clinical trial would determine whether a blue light-absorbing IOL can reduce the risk for or progression of age-related macular degeneration.


The design of intraocular lenses (IOLs) should be based on the properties of the human ocular lens, especially in the transmission properties of the IOL. Notably, the human crystalline lens absorbs most ultraviolet light between 300 nm and 400 nm. Thus, to provide the same protection to the retina afforded by the natural lens, the use of ultraviolet light-absorbing IOLs became the standard of practice once these lenses were developed. However, the transmission properties of most IOLs are not comparable to those of the natural human lens, since the latter yellows with age while IOLs in current use are colorless. The color change in the natural lens is likely attributable to oxidation products of tryptophan (n-formyl-kynurenine) and to glycosylation of lens proteins. It results in a progressive increase in absorbance within the blue range of the visible spectrum.

Filtering the shorter wavelengths of the visible spectrum is particularly significant because it is also this portion of the spectrum that produces photochemical damage to the retinal pigment epithelium (RPE). It is now generally accepted that at least 1 of the intracellular chromophores responsible for the blue light sensitivity of RPE cells is the lipofuscin constituent A2E. This fluorophore is unique to RPE cells and...
has been shown to accumulate in human RPE cells throughout the lifetime of an individual, with levels being highest in the aged eye. Thus, replacement of a senile cataractous crystalline lens with a colorless IOL may leave the RPE vulnerable at an age when its content of blue light sensitive A2E is already high.

The RPE fluorophore A2E is maximally excited by light in the blue region of the spectrum. When irradiated, A2E generates singlet oxygen that proceeds to add to carbon–carbon double bonds of A2E to generate highly reactive epoxides (A2E-epoxides) along the side-arms of the molecule. The cellular injury induced by the illumination of A2E-laden RPE includes oxidative DNA base changes and it is likely that as electrophiles that can readily react with many cellular molecules, A2E-epoxides may account for much of the cellular damage accrued. The photochemical events provoked by the irradiation of A2E-laden RPE ultimately result in the initiation of a cell death program. The sensitivity to blue light conferred by the lipofuscin fluorophore A2E may explain why atrophic age-related macular degeneration (AMD) has been linked to both RPE lipofuscin and cumulative light exposure. Moreover, the loss of RPE cells in atrophic AMD is a critical event as it leads to photoreceptor cell degeneration.

Given that the attenuation of blue light afforded by the yellowed senescent lens may defend lipofuscin-filled RPE cells against blue light damage, we are interested in efforts being made to develop IOLs that replicate the transmission characteristics of the aging crystalline lens. In this study, we constructed a cell culture system that allowed us to compare several IOLs as to their ability to protect A2E-laden RPE from blue light damage. In this cell culture system, A2E accumulates in the lysosomal compartment of a human RPE cell line, as it does in RPE of the eye. Moreover, the levels of A2E are comparable to the level occurring in vivo. This model also allows us to study RPE cells with and without intracellular deposits of A2E.

Materials and Methods

The IOLs studied were the Alcon AcrySof® Natural (SN60AT) (acrylic, 20.0 diopters [D], 6.0 mm optic diameter) and AcrySof (SA60AT) (acrylic, 20.0 D, 6.0 mm optic diameter); the AMO ClariFlex® (CLRFLXB) (silicone, 20.0 D, 6.0 mm optic diameter) and Sensar® (AR40e) (acrylic, 20.0 D, 6.0 mm optic diameter); and the Pharmacia CeeOn® Edge (911A) (silicone, 20.0 D, 6.0 mm optic diameter).

A2E Accumulation in Culture

Human RPE cells (ARPE-19, American Type Culture Collection), which are devoid of endogenous A2E, were grown in 8-well, plastic chamber slides (Laboratory-Tek, Nunc), as described. Once confluent, the cells were allowed to accumulate synthesized A2E from a 20 μM concentration added to the medium. With this protocol, A2E accumulates in the lysosomal compartment of the cells to levels that are comparable to amounts present in vivo.

Illumination and Placement of the IOL

Immediately before illumination, the culture medium was replaced with phosphate-buffered saline containing calcium, magnesium, and glucose. For quantitative measurements of light-induced cell death, the cells were exposed to blue light (430 nm ± 30 [SD], 8 mW/cm²), green light (550 ± 10 nm, 8 mW/cm²), or white light (246 mW/cm²) over a 0.8 mm × 8.5 mm field. The light was delivered from a tungsten halogen source for 20 minutes, and power was measured with a Newport optical power meter (model 840). The spectral range of the lamp was 390 to 750 nm, with a lower output at the shorter wavelengths.

The IOL to be tested was applied to the undersurface of the culture well, where it remained attached, unaired. It was centered over the light path. To obtain a visual representation of IOL protection, 1.0 mm diameter disks were cut from the center of the IOL using a trephine blade (Katena Products, Inc.). The IOL disks were positioned on the undersurface of the well, which was then irradiated (430 ± 30 nm, 16 mW/cm²) from below.

Detection of Nonviable Cells

The nuclei of dead RPE cells were labeled with a membrane-impermeant dye (Dead Red, Molecular Probes), and the nuclei of all cells with 4′,6′-diamino-2-phenylindole (DAPI), as reported. Blue light-illuminated A2E-containing RPE labeled in this way are undergoing an apoptotic form of cell death. Digital images (5 fields per illumination zone) were obtained using a Zeiss Axioplan II microscope with Axiocam camera and KS400 image processing software. Subsequently, Dead Red and DAPI-stained nuclei were counted. Dead cells were quantified as a percentage of the total number of cells in a field. Means are based on 3 experiments.

Statistical analysis was by an analysis of variance followed by the Newman-Keul multiple comparison test (Prism, GraphPad Software). A P value of 0.05 or less was considered significant.
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that did not contain A2E (no A2E, no IOL) remained viable, while blue light-illuminated (430 nm peak with a bandwidth of 60 nm, 8 mW/cm²) RPE that had previously accumulated A2E underwent marked cell death. In the present experiments, 41.1% ± 4.1% (3 experiments) of the A2E-laden cells in a field of illumination became nonviable after blue light exposure in the absence of an IOL (Figure 1). When the yellow-tinted AcrySof Natural IOL was placed in the center of the light path, transmission of the 430 nm light was reduced by approximately 50% and cell death was reduced by 80% (P < .001) compared to irradiation in the absence of an IOL. The yellow-tinted AcrySof Natural IOL was placed in the center of the light path, transmission of the 430 nm light was reduced by approximately 50% and cell death was reduced by 80% (P < .001) compared to irradiation in the absence of an IOL. The smallest but consistent declines in cell death that were detected with these IOLs (Figure 1) were undoubtedly due to the small reductions in light transmission (~5%) that were measured.

The ability of the AcrySof Natural IOL to attenuate blue light-mediated cell death was evident when fields of irradiated cells were imaged. In the absence of an IOL, the nuclei of nonviable cells were uniformly distributed across the illuminated field (Figure 2). Conversely, placement of the blue light-absorbing AcrySof Natural IOL (a 1.0 mm disk) within the light path spared a circular zone of cells, the diameter of this area corresponding to the diameter of the IOL disc. Within this sector, all nuclei were labeled with DAPI but labeled nuclei of nonviable cells were only occasionally observed.

With the use of wide-band white light (390 to 750 nm; 246 mW/cm²) that included all wavelengths toward which A2E exhibits sensitivity, the death of A2E-laden RPE reached a frequency of 35% (35.4% ± 2.7% of the cells in an illumination zone; 3 experiments). In the presence of the blue light-absorbing AcrySof Natural IOL, cell death from white light was reduced by 82% (P < .001) compared to that without an IOL (Figure 3). In comparison, the AcrySof IOL diminished the incidence of nonviable cells by 22% (P < .05); with the Sensar, ClariFlex, and CeeOn Edge IOLs, cell death was decreased by 37% to 39% (P < .01). Again, the decreases observed with the colorless IOLs were likely related to the reductions in spectral transmittance associated with all IOLs.

When compared with blue and white light, green light (550 ± 20 nm) delivered at an intensity that was...
Figure 3. (Sparrow) Quantitation of nonviable cells after white light (390 to 750 nm) illumination of A2E-laden RPE with and without the indicated IOL placed in the light path. The percentage of nonviable cells was normalized to values obtained in the absence of an IOL. Values are mean ± SEM.

Figure 4. (Sparrow) Quantitation of nonviable cells after green light illumination (550 nm) of A2E-laden RPE with and without the indicated IOL placed in the light path. Values for percentage of nonviable cells were normalized to values obtained in the absence of an IOL. Means ± SEM are presented.

Discussion

The study shows that by absorbing predominantly in the blue region of the spectrum, the AcrySof Natural IOL shields RPE cells that have accumulated the lipofuscin fluorophore A2E from the damaging effects of light. The protection afforded by the AcrySof Natural IOL was pronounced for wide-band white light just as it was for narrow-band blue light. The reduction in cell death afforded by this blue light-filtering IOL was greater in magnitude than would be expected from the decline in transmitted blue light. That a reduction in blue light transmission of approximately 50% resulted in an 80% decline in cell death probably occurred because blue light levels were brought below the threshold for lethal damage for a significant proportion of the cells. Small but consistent declines in cell death observed with the other IOLs were probably due to IOL-associated nonspecific decreases (5% to 10%) in light transmission that have been reported.2,7,8,37

The AcrySof Natural IOL did not completely abolish cell death in this model. However, this is not surprising since by design, this IOL does not block all blue light. Rather, the yellow tint of the lens is intended to confer a spectral transmission similar to that of the crystalline lens of an adult.1 That A2E-containing RPE are less sensitive than to green light is to be expected: A2E has an excitation maximum of approximately 430 nm with the emission elicited at 550 nm being less than 5% of that elicited at 430 nm.18

Ham et al.15 were the first investigators to demonstrate that retinal injury from wavelengths that peak around 425 nm is initiated by photochemical processes in the RPE. The action spectrum of this damage corresponds to the absorption spectrum of the aging fluorophore A2E,18 and there is now an abundance of experimental evidence that A2E is a sensitizing molecule that can mediate light injury to RPE, leading to RPE atrophy.18,23,25,26,36 Since lipofuscin fluorophores, including A2E, accumulate with age, the natural yellowing of the aging human lens is fortunate as it may dampen the damaging potential of short wavelength blue light.

For some time, it has been postulated that cumulative photochemical damage from lifetime exposures to light are a cause of AMD,18 although the results of
epidemiological studies are inconclusive. Nevertheless, it is of interest that the Chesapeake Bay Waterman Study reported an association between the incidence of advanced AMD and blue light exposure during the 20-year period that preceded the study. Other reports suggest a relationship between the incidence of AMD and cataract extraction. For instance, the National Health and Nutrition Survey observed 3087 patients between 1970 and 1974 and reports that aphakic subjects had an increased risk for developing AMD. In a study of 47 patients who presented with bilateral drusen and pigmentary changes, choroidal neovascularization was observed in 19.1% of the eyes that had had cataract extraction with implantation of a colorless IOL compared with an incidence of 4.3% in the fellow phakic eyes. The Beaver Dam Study comprised 4926 patients from 1988 to 1990 and found a positive association between cataract extraction and clinical indications of early AMD. Indeed, both the 5-year and 10-year follow-up studies confirm that eyes that had had cataract surgery before baseline evaluation were more likely to exhibit progression of AMD. Nevertheless, similar associations were not reported by the Age-Related Eye Disease Study (DF Martin, ARVO abstract 1907, 2002).

Circumstances that lead to damage in the aging RPE cell are considered as a prelude to the photoreceptor cell degeneration that characterizes the visual impairment associated with AMD. We speculate that a yellow-tinted IOL that simulates the adult natural lens and protects lipofuscin-containing RPE from blue light damage may reduce the risk for or progression of AMD. This concept warrants evaluation by a long-term population-based clinical study.

References
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