Effects of Light Exposure and Use of Intraocular Lens on Retinal Pigment Epithelial Cells \textit{In Vitro}

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\section*{ABSTRACT}

To investigate the effect of a blue light-filtering intraocular lens (IOL) and a UV-absorbing IOL on light-induced damage to retinal pigment epithelial (RPE) cells laden with the lipofuscin fluorophore \textit{N}-retinylidene-\textit{N}-retinylethanolamine (A2E). A2E-laden RPE cells were exposed to white light which was filtered by either a blue light-filtering IOL or a UV-absorbing IOL. After 30 min of illumination the cell viability and the level of reactive oxygen species (ROS), free glutathione (GSH), vascular endothelial growth factor (VEGF) and pigment epithelium-derived factor (PEDF) were determined. In the absence of an IOL, the white light exposure decreased cell viability to 37.2\% of the nonirradiated control. The UV-absorbing IOL tended to reduce light-induced cell death; however, the decrease was not significant. The blue light-filtering IOL significantly attenuated light-induced cell damage, increasing cell viability to 79.5\% of the nonirradiated control. The presence of the blue light-filtering IOL significantly increased GSH and PEDF levels, and decreased ROS and VEGF levels. This study suggests that a blue light-filtering IOL may be more effective against A2E-induced light damage and inhibit more light-induced ROS and VEGF production than a conventional UV-absorbing IOL.

\section*{INTRODUCTION}

Exudative age-related macular degeneration (AMD) is a significant cause of irreversible blindness in developed countries (1). Retinal pigment epithelial (RPE) cells are nonreplicating and have a lifetime response to light-induced oxidative stress. \textit{N}-retinylidene-\textit{N}-retinylethanolamine (A2E), the best-characterized component of lipofuscin in RPE cells, is a compound that can neither be degraded by nor eliminated from cells and is toxic as well as phototoxic to the cells. A2E has an excitation maximum of approximately 430 nm, and emission produced at 550 nm. Additionally, when A2E absorbs blue light it is a photogenerator of reactive oxygen species (ROS) (2,3).

Exudative AMD is characterized by abnormal blood vessels growing from the choriocapillaris through the retinal pigment epithelium, resulting in hemorrhage, exudate, scarring and serous retinal detachment. Of several factors upregulated after A2E-laden cells are exposed to light, vascular endothelial growth factor (VEGF) is one of the most potent proangiogenic, suggesting that A2E-mediated VEGF production is involved in the pathogenesis of exudative AMD (4,5). Pigment epithelium-derived factor (PEDF), a glycoprotein belonging to the family of serine proteinase inhibitors, has been found to be an endogenous angiogenic inhibitor (6). Additionally, decreased PEDF levels have been shown to play a role in retinal neovascularization (7).

The crystalline lens of the eye absorbs light between 300 and 400 nm (8). With age it progressively yellows, thus absorbs increasing amounts of blue light, decreasing the amount of blue light passing to the retina (9-11). Conventional intraocular lens (IOL) used to replace the lens on cataract surgery is colorless, \textit{i.e.} ultraviolet absorbing (12), which can only absorb UV. So after cataract removal and implantation of colorless IOL, the amount of blue light reaching the RPE cells increases. Furthermore, epidemiological studies suggest that cataract removal may aggravate exudative AMD (13,14).

The purpose of the present study was to construct an RPE cell culture system to investigate the protective effect of blue light-filtering and UV-absorbing IOLs on A2E-laden RPE cells exposed to blue light. Outcome measures included cell viability, oxidative stress, and production of VEGF and PEDF.

\section*{MATERIALS AND METHODS}

\textit{RPE cell culture}. A human RPE cell line devoid of endogenous A2E (15). ARPE-19 (American Type Culture Collection, Manassas, VA), was used in this study. Cell cultures were maintained in Dulbecco's modified Eagle/Ham's F12 media supplemented with 10\% fetal bovine serum, penicillin (100 U mL\textsuperscript{-1}) and streptomycin (100 mg mL\textsuperscript{-1}) ( Gibco BRL Company). Cells were incubated at 37°C in a humidified chamber under 5\% carbon dioxide and 95\% air.

A2E synthesis and accumulation in RPE cells. A2E was synthesized and purified according to the method of Parish \textit{et al.} (16). After ARPE-19 cells were grown confluent on 96-well plates, they were incubated with A2E at a concentration of 20 \textmu M to allow intracellular accumulation. The amount of A2E uptake in the cells was about 95 ng/10\(^6\) cells as determined by high-performance liquid chromatography analysis, similar to that observed in human RPE cells. The amount of A2E from a 65-year-old human donor eye was about 99 ng/10\(^6\) RPE cells (15,17).

\textit{Light illumination}. The A2E-laden RPE cells were exposed to white light (tungsten halogen lamp, 10 mW cm\textsuperscript{-2}) for 30 min. Power was measured with an Astral AA30 power meter (Sientech, Inc., Boulder, CO). Where irradiated, a UV-absorbing IOL (AcrySof\textsuperscript{\textregistered}, SA60A; Alcon Laboratories, Inc.) (+20.0 diopters \text{[D]}) or a blue light-filtering IOL (Alcon AcrySof\textsuperscript{\textregistered} Natural, SN60AT) (+20.0 D) was attached.
under the surface of the culture well and centered over the light beam. The cultures were exposed to ambient lighting (about 10 lx) during plating and feeding, and while transporting the cells to and from the exposure apparatus. The temperature of the cell cultures was maintained between 36.5 and 37.2°C. Control groups consisted of (1) RPE cells exposed to white light but without an IOL over the light beam and (2) RPE cells kept in the dark.

Cell viability assay. The viability of the RPE cells was determined using a cell counting kit-8 assay (Dojindo Molecular Technologies, Inc., Japan). Briefly, cells were grown to 70% confluence and exposed to white light illumination for 30 min, then each well was incubated with 50 μL of WST-1 solution for 3 h under a humidified atmosphere of 95% air and 5% CO₂. The plate was put on a shaker for 1–2 min and absorbance was measured at 430 nm using a spectrophotometer. Cell viability was calculated as a percentage by dividing the absorbance of treated cells by the absorbance of control cells. This assay is based on the cleavage of WST-1 to formazan by cellular mitochondrial dehydrogenases. As cells die, there is a decrease in the overall activity of mitochondrial dehydrogenases in the sample, which leads to a decrease in the amount of formazan dye formed. The amount of formazan dye produced by viable cells is determined by measuring the absorbance at 430 nm. All groups consisted of 20 wells and were carried out in triplicate. Results were recorded as mean ± SD.

ROS assay. Levels of cytosolic ROS were determined by adding 1 μg/mL of 2',7'-dichlorodihydrofluorescein diacetate (H₂-DCFH-DA, Dojindo) to cell culture medium at 37°C. In the presence of ROS, the reduced form of H₂-DCFH-DA is oxidized to the highly fluorescent dihydrochlorofluorescein (DCF). The fluorescence intensity of the intracellularly trapped DCF indicates the amount of intracellular ROS. The DCF average fluorescence in 2 × 10⁵ cells was measured with a flow cytometer (FACScan; B&D). Seven samples per group were analyzed. The experiment was performed at least three times.

GSH assay. GSH was determined using a Glutathione Quantification Kit (Dojindo Molecular Technologies, Inc.) according to the manufacturer's instructions. In brief, RPE cells were cultured, illuminated, then harvested by centrifugation at 200 g for 10 min at 4°C. Cells were then washed twice with 300 μL of PBS. The cells were lysed by the addition of 80 μL of 10 mM NaCl, frozen, thawed twice, and then 20 μL of 5% sheep serum albumin (SSA) was added and the sample was centrifuged at 800 g for 10 min. The supernatant was used in the GSH assay following the manufacturer's protocol. The absorbance was measured at 405 nm using a microtiter plate enzyme-linked immunosorbent assay (ELISA) reader.

VEGF and PEDF detection. The amount of VEGF and PEDF in the supernatant of RPE culture was determined by ELISA. For the ELISA, the medium was changed to nonserum DMEM when cells were confluent to about 80% for 24 h. The supernatant was collected after light exposure and centrifuged at 800 g for 10 min, after which the concentration of VEGF and PEDF was analyzed by using a commercial VEGF ELISA kit (R&D Company) and PEDF ELISA kit (Millipore Corporation) according to the manufacturer’s instructions. Absorbance at 450 nm was measured using a microplate reader (Bio-Rad). All groups consisted of 10 wells, and the results were averaged.

Statistical analysis. Each experiment was repeated three times. The results were recorded as mean ± SD. One-way analysis of variance followed by the Dunnett post hoc test was used to analyze the results. P < 0.05 was considered statistically significant.

RESULTS

A2E accumulation in RPE cells

After incubation of ARPE-19 cells with 20 μM A2E, uptake by the cells was evidenced by the acquisition of intracellular granules, which appeared yellowish green when viewed by fluorescence microscopy (Fig. 1).

Blue light and IOL effects on A2E-induced damage to RPE cells

In the absence of an IOL, the white light exposure decreased cell viability to (37.2 ± 4.2)% of the nonirradiated control.

The UV-absorbing IOL tended to slightly inhibit this cell damage, with cell viability recovering to (44.1 ± 5.3)% of the nonirradiated control. The inhibitory effect of light-induced cell damage was seen with the blue light-filtering IOL, which enhanced cell survival to (79.5 ± 4.4)% of the nonirradiated control. The inhibitory effect was significantly greater than without an IOL and with a UV-absorbing IOL (P < 0.05).

Detection of ROS and GSH

The ROS level and GSH concentration are presented in Table 1. The presence of the blue light-filtering IOL significantly increased GSH and decreased ROS levels (P < 0.05).
Table 1. The effect of light exposure and use of intraocular lens on the level of ROS and GSH in A2E-containing retinal pigment epithelial cells.

<table>
<thead>
<tr>
<th></th>
<th>ROS (fluorescence intensity)</th>
<th>GSH (μmol L⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>Blue light-filtering IOL</td>
<td>511.53 ± 67.43</td>
<td>15.34 ± 4.77</td>
</tr>
<tr>
<td>UV-absorbing IOL</td>
<td>1000.15 ± 174.88††</td>
<td>2.57 ± 1.96††</td>
</tr>
<tr>
<td>Control (w/o IOL)</td>
<td>1022.23 ± 158.72‡‡</td>
<td>1.58 ± 1.13‡‡</td>
</tr>
<tr>
<td>Nonirradiated control</td>
<td>452.82 ± 77.98</td>
<td>19.73 ± 5.49</td>
</tr>
</tbody>
</table>

ROS = reactive oxygen species; GSH = free glutathione; A2E = N-retinylindene-N-retinylethanolamine; IOL = intraocular lens.
†Compared with blue light-filtering IOL (P < 0.05). ‡Compared with nonirradiated control (P < 0.05).

Detection of VEGF and PEDF

The levels of VEGF and PEDF protein secreted from A2E-laden ARPE-19 cells were measured after exposure to light for 30 min (Table 2). The VEGF level was upregulated without an IOL. The UV-absorbing IOL slightly attenuated VEGF upregulation. Compared with the UV-absorbing IOL, the blue light-filtering IOL had a potent inhibitory effect on the upregulation of VEGF. The VEGF protein levels with a blue light-filtering IOL were significantly lower than those without an IOL (P < 0.05). The PEDF level was downregulated without an IOL. The UV-absorbing IOL slightly attenuated PEDF downregulation. Compared with the UV-absorbing IOL, the blue light-filtering IOL had a potent inhibitory effect on the downregulation of PEDF. The PEDF protein levels with a blue light-filtering IOL were significantly higher than those without an IOL (P < 0.05).

DISCUSSION

Whether light exposure increases the risk of AMD is still controversial. While there is considerable clinical evidence implicating light exposure in exudative AMD development (13,14), our results provide experimental evidence for the damage of white light to RPE cells and the protective effect of a blue light-filtering IOL. Exposure of A2E-laden RPE cells to low intensity white light led to decreased cell viability, production of significant levels of ROS, reduction of GSH, upregulation of VEGF and downregulation of PEDF. In contrast, by absorbing blue light, the blue light-filtering IOL significantly decreased the amount of ROS and VEGF produced.

The present results are consistent with previous reports (18) that a blue light-filtering IOL (Alcon AcrySof® Natural, SN60AT) protects against RPE damage and produces an approximately 80%, 82% and 78% reduction in the death of A2E-laden RPE cells exposed to blue, green and white light, respectively. Similarly, white light damage to A2E-containing RPE cells was less with a yellow IOL (YA60BB, Hoya) than with a conventional UV-absorbing IOL (19). In our study, to demonstrate the effect of the UV-absorbing and blue light-filtering IOLs on A2E-mediated light damage to RPE cells, cell viability was measured after A2E-containing RPE cells were exposed to a low level of white light. The use of the blue light-filtering IOL resulted in less A2E-induced RPE damage.

Sparrow et al. (17) suggested that healthy RPE cells which have accumulated A2E to critical concentrations and are exposed to blue light may undergo an apoptotic form of cell death. Our results provide evidence that white light exposure produces ROS in A2E-laden RPE cells, and that light may be one of the major sources of ROS (i.e. photo-oxidation) in the RPE cells. Although the photochemical events generating apoptosis under conditions of light injury are not fully understood, we suggest that ROS likely serve as mediators.

Our study showed that white light exposure led to upregulation of VEGF and downregulation of PEDF production, which may explain why cataract surgery increases the incidence of exudative AMD (13). VEGF is a hypoxia-induced angiogenic factor and a potent vascular permeability factor that can promote choroidal neovascularization (5). PEDF is a potent angiogenic inhibitor, originally identified from cultured RPE cells (6,20). Studies have demonstrated that decreased retinal levels of PEDF are closely related to the formation and progression of retinal neovascularization (7). Thus, it is reasonable to consider that a blue light-filtering IOL is more protective than a conventional UV-absorbing IOL against the development of exudative AMD. Our results are consistent with those of other studies that show the expression of VEGF is upregulated in A2E-laden RPE cells after light exposure (4,16).

The difference in effects between acute, intense light exposure and chronic, low intensity light exposure on RPE cells and their contribution to the pathogenesis of exudative AMD remain to be determined. Cell death and upregulated VEGF expression were seen in our study with white light for 30 min. Continued illumination for a longer period induced sublethal stress to the cells as well as apoptotic change. Apoptosis in RPE cells may be induced by acute and intense light exposure. In contrast, under milder conditions, i.e. prolonged time and weaker light exposure, the cellular defense system in RPE cells could be easily overcome. The cells might generate ROS, but little apoptosis would occur. Thus, we suggest that white light with varying intensity and duration of exposure may have different effects on A2E-laden RPE cells.

In summary, light exposure that leads to damage in the A2E-containing RPE cell is considered a prelude to the

Table 2. The effect of light exposure and use of intraocular lens on VEGF and PEDF expression of A2E-containing retinal pigment epithelial cells.

<table>
<thead>
<tr>
<th></th>
<th>VEGF (ng mL⁻¹)</th>
<th>PEDF (ng mL⁻¹)</th>
<th>VEGF/PEDF ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue light-filtering IOL</td>
<td>66.97 ± 3.62</td>
<td>47.88 ± 1.93</td>
<td>1.40</td>
</tr>
<tr>
<td>UV-absorbing IOL</td>
<td>110.79 ± 4.13</td>
<td>11.35 ± 2.01</td>
<td>9.76††</td>
</tr>
<tr>
<td>Control (w/o IOL)</td>
<td>153.23 ± 5.27</td>
<td>10.31 ± 1.73</td>
<td>14.86**</td>
</tr>
<tr>
<td>Nonirradiated control</td>
<td>40.92 ± 4.02</td>
<td>57.53 ± 1.76</td>
<td>0.71</td>
</tr>
</tbody>
</table>

VEGF = vascular endothelial growth factor; PEDF = pigment epithelium-derived factor; A2E = N-retinylindene-N-retinylethanolamine; IOL = intraocular lens. *Compared with blue light-filtering IOL (P < 0.05). †Compared with nonirradiated control (P < 0.05).
photoreceptor cell degeneration that characterizes the visual impairment associated with AMD. Our study supports the hypothesis that blue light-filtering IOLs have a greater protective effect against A2E-induced photochemical damage and inhibit light-induced ROS and VEGF production to a greater extent than conventional UV-absorbing IOLs, and thus may reduce the risk for, or progression of, AMD. This concept requires evaluation by a long-term population-based clinical study.

REFERENCES


