AcrySof Natural filter decreases blue light-induced apoptosis in human retinal pigment epithelium

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Abstract
Purpose The effect of AcrySof filter (UV light-filtering chromophore; Alcon) and AcrySof Natural filter (UV- and blue light-filtering chromophores) on blue light-induced apoptosis in human retinal pigment epithelial (RPE) cells was evaluated.
Design Laboratory investigation
Clinical relevance Acrysof Natural filter reduces the blue-light toxicity in RPE cells and may have a positive impact on age-related macular degeneration (AMD).
Methods RPE cells were exposed to blue light (430–450 nm) in the presence of either the AcrySof (UV only) filter or Acrysof Natural (UV and blue light) filter for 10 days. The rate of apoptosis was analyzed.
Results Blue light induced significant apoptosis in RPE cells. AcrySof Natural filter significantly reduced the blue light-induced apoptosis when compared to AcrySof filter. The amount of blue-light energy reaching the cells with the AcrySof filter was 4.25 mW/cm² and with the AcrySof Natural filter was 2.5 mW/cm².
Conclusions AcrySof Natural filter significantly reduced blue light-induced apoptosis. This was most likely due to its filtering effect on blue wavelength light, which reduces the energy that reaches the cells. In patients with cataract who are at a high risk for AMD, the implantation of a blue light-filtering intraocular lens may be considered.

Keywords Blue light · RPE · AcrySof

Introduction
Age-related macular degeneration (AMD) is the leading cause of blindness in the population older than 60 years of age in the western world [1]. The patho-physiology of AMD is not well understood; however, it is known that the degeneration of retinal pigment epithelial (RPE) cells plays an important role in the progression of the disease. With increased age, the life-long exposure of RPE cells to light may have a significant impact on their degeneration [2, 3].

The epidemiological evidence for the role of light in AMD is controversial. An Australian case control study could not find any significant relationship between sunlight exposure and AMD [4]. In the Chesapeake Bay study, there was a relationship between high levels of exposure to blue and visible light and development of AMD, especially later in life [5]. In the Beaver Dam Eye Study, a significant association was found between extended exposure to the summer sun and the 10-year incidence of early AMD and increased retinal pigment [6].
Radiant energy in the form of mechanical, thermal, or photochemical reactions can cause significant tissue damage [2]. In AMD the photochemical reaction and oxidative stress induced by light may be a key factor in the degeneration of RPE cells [7–11]. It has already been shown that visible light induces apoptosis in retinal cells [10, 11]. Blue light, the shorter wavelength of the visible spectrum, was found to be at least 30 times more efficient than yellow light in inducing damage to the blood-retinal barrier [12]. Furthermore, blue light induces apoptosis in rat RPE cells by a free radical-associated mechanism [13]. Lipofuscin, also known as age pigment, has long been considered a mediator of photodynamic damage [14–16]. Recently it was shown that the major hydrophobic fluorophore of RPE lipofuscin, A2E, is an initiator of blue light-mediated apoptosis in RPE cells [17, 18]. Blue light also induces the production of reactive oxygen species (ROS) in the mitochondria of RPE cells [19]. It has also been shown shown that melanin free radicals quench ROS in RPE cells [20].

The colorless human lens and cornea filter most of the UV light. With increased age, the human lens develops a yellow color which functions as a filter for shorter wavelengths of visible light [21, 22]. The yellow discoloration of the lens may be a protective mechanism to decrease the exposure of aging RPE cells to high-energy blue light. After cataract surgery, however, this naturally occurring filter is removed and a colorless intraocular lens is implanted.

AcrySof Natural intraocular lens (IOL) protects RPE cells that have accumulated lipofuscin fluorophore A2E from blue light-induced apoptosis [23]. Blue light, however, may be toxic to cells prior to their accumulation of A2E and lipofuscin.

In this study we evaluated whether blue light induces apoptosis in RPE cells in the absence of lipofuscin and A2E. Experiments were performed with human fetal RPE cells which do not have lipofuscin or A2E. The time interval required to induce maximum apoptosis was evaluated by exposing the cells to blue light for various durations (without any filters). The effect of AcrySof and AcrySof Natural filter on blue light-induced apoptosis on RPE cells was compared. RPE cells were exposed to blue light in the presence of either AcrySof filter or AcrySof Natural filter. The rate of apoptosis was analyzed with Annexin V staining and flow cytometry.

Materials and methods

Isolation of RPE cells

RPE cells were obtained from independent human fetal eyes (Advanced Bioscience Resources, Alameda, CA) as described before [24]. Microdissection was performed under sterile conditions using a dissecting microscope. The eyes were opened by a circumferential incision just above the ora serrata near the limbus, and the anterior segment and lens were separated. The posterior segment of the eye was cut into four quadrants and placed in a petri dish containing Dulbecco’s minimum essential medium (DMEM) (Sigma, St. Louis, MO). The neural retina and any remaining vitreous were removed. Sheets of RPE cells were separated from the choroid using fine forceps and immediately placed into a petri dish containing phosphate buffer saline (PBS) without Ca++/Mg++. (Biowhittaker, Walkersville, MD). After the separation of all four quadrants, the sheets were trypsinized (0.25% trypsin, Sigma) for 15 min. Growth medium consisting of DMEM, 15% fetal bovine serum (Sigma), and 1% solution of antibiotics and L-glutamine (Sigma) was added, and the content was centrifuged at 2,000 rpm. The supernatant was discarded and the cells isolated from each eye were resuspended with growth medium into one well of a 24-well plate (Becton Dickinson, Lincoln Park, NJ) and incubated for 1 week in 95% air/5% CO2 at 37°C. The cells were trypsinized and resuspended into a larger culture flask. The cultures were examined on a daily basis and the growth medium was changed twice a week. At confluence the cells were subcultured by trypsinization. Fourth to sixth passage RPE cells were utilized in the experiments. All the culture conditions were exactly the same. The only variable was blue light and the presence or absence of filter.

Source of blue light

We have designed a unique cell culture incubator that allows us to directly expose RPE cell cultures to blue light under controlled conditions (Fig. 1a,b). A dry-wall CO2 refrigerated tissue culture incubator (model 810R, Fisher Scientific, Pittsburgh, PA) was modified as follows. Openings were made in the ceiling of the incubator and the light source was mounted to enable the direct illumination of the cells inside the incubator. Research arc lamp housing equipped with a 200-W mercury-xenon ozone-free bulb was used as the light source. Dichroic mirror (350–450 nm) (Therma Oriel, Stratford, CT) and interference filter (Intor, Socorro, NM) were used to generate the blue wavelength light. The wavelength of the light reaching the cells was 430–450 nm and was measured with a spectroradiometer PR-650 SpectraScan (Chatsworth, CA). The light energy reaching the cells was measured with radiant power and energy meter with a thermopile probe (Therma Oriel). The temperature of the culture medium was measured throughout the experiments with a high precision handheld thermometer with thermocouple probe, type T, equipped...
with hypodermic needle (Omega, Stamford, CT). The temperature in the culture plates ranged from 36.4 to 37.4°C. The cells were incubated in 95% air/5% CO₂.

**Exposure to blue light**

**Without filter**

To assess the time interval needed to reach maximum apoptosis after blue light exposure, RPE cells were irradiated with blue light for 3, 5, 7, and 10 days. During blue-light exposure, RPE cells were cultured in 6-well plates using the following medium: DMEM, HEPES modified, 15% FCS, 2 mM l-glutamine and 1% of penicillin/streptomycin/amphotericin (Sigma). Culture plates with aluminum foil instead of filter (no light exposure) served as control. Experiments were performed on RPE cells isolated from four independent donors.

**With filter**

The blue light-filtering chromophore in AcrySof Natural filter is a yellow compound (N-2-[3-(2-methylphenylazo)-4-hydroxyphenyl] ethyl methacrylamide) which is covalently bound to the AcrySof IOL material. The filters used in these experiments mimic the filters in the intraocular lenses.

Cell culture plates were covered with either AcrySof filter or AcrySof Natural filter. Then they were exposed to blue light with equal energy for 10 days. The rate of

**Table 1** Percentage (mean±SD, n=4 independent donors) of apoptotic RPE cells after exposure to blue light, analyzed with Annexin V staining

<table>
<thead>
<tr>
<th></th>
<th>3 days</th>
<th>5 days</th>
<th>7 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cells exposed to</td>
<td>40.85±3.7</td>
<td>54.27±3.4</td>
<td>84.89±3.48</td>
<td>86.14±3.26</td>
</tr>
<tr>
<td>blue light</td>
<td></td>
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<td></td>
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<tr>
<td>Cells kept in</td>
<td>27.89±3.65</td>
<td>29.62±2.2</td>
<td>19.96±1.63</td>
<td>20.07±5.71</td>
</tr>
<tr>
<td>darkness</td>
<td></td>
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There was a statistically significant difference between the two groups at all time intervals (P≤0.05)
cellular apoptosis was measured as explained below. Culture plates covered with aluminum foil served as control (no light exposure). Experiments were performed on RPE cells isolated from eight independent donors.

### Apoptosis assay

The rate of cell apoptosis was analyzed with Annexin V staining (R & D Systems, Minneapolis, MN). Annexin V binds to phosphatidyl serine (exposed on the cell membrane), which is one of the earliest indicators of cellular apoptosis. After blue-light exposure, RPE cells were collected, washed twice in bead separation buffer and resuspended in Annexin V binding buffer at $1 \times 10^6/ml$. Staining procedures were performed according to the manufacturer’s instructions (R & D). Samples were then diluted in Annexin V binding buffer and analyzed on a FACSscan with the Cell Quest software program (Becton Dickinson). Each sample was run once. The number of cells that were Annexin V positive was counted.

### Statistical analysis

The statistical analysis was performed using the paired $t$-test. $P$ values ≤ 0.05 were accepted as significant.

### Results

#### Morphological analysis

The morphology of RPE cells was assessed with phase-contrast microscopy after a 10-day exposure to blue light in the presence of either the AcrySof filter or AcrySof Natural filter (Fig. 2). RPE cells maintained their tight monolayer structure and their hexagonal morphology in the presence of AcrySof Natural filter (Fig. 2a). In the presence of AcrySof filter, however, they lost their hexagonal shape and their monolayer structure (Fig. 2b).

#### Apoptosis assay

Blue light induced significant apoptosis in RPE cells at all time intervals (3, 5, 7, and 10 days of exposure) when compared to the cells in the dark (Table 1). The amount of RPE apoptosis increased with the exposure time. A 10-day exposure induced the highest number of apoptotic cells (more than 85% of the cells died). Filter experiments were performed with the time interval that induced the highest amount of apoptosis: 10-day exposure.

The percentage of apoptotic cells in the presence of AcrySof filter was 66.05±19.68 and in the presence of AcrySof Natural filter was 37.02±13.33. AcrySof Natural filter significantly decreased blue light-induced apoptosis in RPE cells when compared to the AcrySof filter ($P<0.05$) (Table 2, Fig. 3).

AcrySof Natural filter also reduced the amount of blue-light energy that reached the RPE cells. With the AcrySof filter, the amount of energy reaching the cells was 4.25 mW/cm² (268 Lux) and with the AcrySof Natural filter it was 2.5 mW/cm² (179 Lux). The decrease in the number of apoptotic cells was most likely due to the blocking effect of the AcrySof Natural filter on blue light, reducing the amount of energy reaching the cells.

<table>
<thead>
<tr>
<th>Filter</th>
<th>Apoptotic cells (mean±SD)</th>
<th>$n=8$ independent donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>AcrySof filter</td>
<td>71.16±19.11</td>
<td></td>
</tr>
<tr>
<td>AcrySof Natural</td>
<td>36.70±11.34</td>
<td></td>
</tr>
<tr>
<td>Dark</td>
<td>15.66±5.28</td>
<td></td>
</tr>
</tbody>
</table>

There was a statistically significant difference between the two filters ($P<0.05$).
Discussion

In this study we compared the effect of AcrySof and AcrySof Natural filters on blue light-induced apoptosis in RPE cells. RPE cells in this study did not have lipofuscin or A2E. The specially designed incubator system in this study allowed us to expose a large number of cells to light for a long period of time at a constant temperature. One of the advantages of this system is that the user can modify the duration and wavelength of light exposure without jeopardizing the viability of the cells in culture.

Our findings confirm that blue light induces apoptosis in human RPE cells. The percentage of apoptotic cells is directly related to the duration of exposure. Different mechanisms may be involved in blue light-induced apoptosis. RPE cells are among the most metabolically active cells in the body, filled with mitochondria. Cytochromes in the mitochondria absorb light in the spectrum of the blue wavelength [19]. It has been shown that exposure to blue light leads to an increase in both mitochondrial and nuclear DNA lesions, leading to cell dysfunction [25]. These lesions may explain the documented alterations in RPE mitochondria found in patients with age-related macular degeneration [26].

The exposure to blue light results in the production of reactive oxygen species which subsequently lead to cellular apoptosis [27, 28]. In aged RPE cells, the presence of lipofuscin and its fluorophores, including A2E, further increases the toxicity of blue light [17, 28–30]. Our results confirm that blue light is toxic for human RPE cells in the absence of lipofuscin and A2E.

The role of cataract surgery in the development and progression of AMD is controversial. The pooled findings from the Beaver Dam and Blue Mountains Eye Studies found that the 5-year risk for development of late-stage AMD after cataract surgery may be to two to five times the risk observed in phakic patients of same age, gender, and smoking habits [31]. In the AREDS Trial no association was found between cataract surgery and increased risk of neovascular AMD [32]. In that study the patients were followed for 6 years.

The yellow discoloration of natural lenses with age and the yellow macular pigment may be naturally appearing mechanisms to protect the RPE cells from the high energy blue light. Clinically absent in newborns, the macular pigment gradually accumulates from dietary sources and appears to serve as an optical filter, absorbing the blue wavelength light [33, 34]. Any condition that has an effect on these naturally appearing protective mechanisms may contribute to the progression of AMD.

The removal of the cataract in patients with AMD and implantation of a colorless lens (blocking only UV light) would increase the exposure of already degenerating RPE cells to blue light and may boost their deterioration and their loss of functional viability [35–37]. One may hypothesize that the implantation of a yellow-tinted lens that mimics the color of the natural lens would reduce the risk of blue-light exposure. In this study we showed that AcrySof Natural filter significantly reduced blue light-induced apoptosis in RPE cells when compared to AcrySof filter. This is due to the lens's blocking effect on the blue wavelength light, reducing the energy reaching the cells. RPE cells in our experiments did not contain lipofuscin fluorophore A2E, indicating that even in the absence of lipofuscin the blue light may be toxic. Our results indicate that blue-filtering lenses protect RPE cells from blue light-induced apoptosis.

Our findings need to be analyzed cautiously since they are based on an in vitro model system and may not truly mimic the life-long exposure of RPE cells to blue light. However, though it is impossible to mimic the life-long exposure of RPE cells to light in an in vitro model, our results in this study may have a clinical correlation. Our in vitro model may provide preliminary data that can be used for future clinical studies.

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References