Effects of yellow intraocular lenses on light-induced upregulation of vascular endothelial growth factor

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PURPOSE: To investigate the protective effect of a blue-light filtering intraocular lens (yellow IOL) (YA60BB, Hoya) and an ultraviolet (UV)-absorbing IOL (VA60BB, Hoya) on light-induced phototoxicity to retinal pigment epithelial (RPE) cells laden with the lipofuscin fluorophore A2E and on the production of vascular endothelial growth factor (VEGF) after light exposure.

SETTING: University of Tokyo, Tokyo, Japan.

METHODS: The A2E-laden ARPE-19 cells were exposed to white light and a UV-absorbing IOL or a blue-light filtering IOL was placed over the light beam. After 48 hours of irradiation, the viability of the cells was determined with WST-1 (a sodium salt of 4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate) assay, and the secreted protein level of VEGF was determined by enzyme-linked immunosorbent assay.

RESULTS: Without an IOL, the white-light exposure decreased cell viability to 28% of the nonirradiated control. Although the UV-absorbing IOL tended to reduce light-induced cell death, the decrease was not significant. However, the presence of the blue-light filtering IOL significantly attenuated light-induced cell damage, increasing cell viability to 42%. The secreted VEGF protein level increased 3.2-fold after the A2E-laden RPE cells were exposed to white light. In the presence of the UV-absorbing IOL, the VEGF protein level decreased, but not significantly. The presence of the blue-light filtering IOL significantly attenuated the upregulated VEGF expression compared to upregulation without an IOL.

CONCLUSION: This study supports the theory that a blue-light filtering IOL may be more protective against A2E-induced photochemical damage and inhibit more light-induced VEGF production than a conventional UV-absorbing IOL.


Exudative age-related macular degeneration (ARMD) is a leading cause of legal blindness in developed countries. Evidence suggests that light exposure increases the risk for the progression of ARMD. It is generally accepted that short-wavelength light is hazardous to the retina. The brown pigment lipofuscin is involved in the pathogenesis of exudative ARMD, and its role as a photosensitizer is known. Of the several components likely to contribute to lipofuscin photoreactivity, the most well-known is A2E. The lipofuscin fluorophore A2E has a 2-peak absorbance spectrum of 335 nm and 435 nm and triggers apoptosis in retinal pigment epithelial (RPE) cells after blue-light exposure, indicating that it mediates blue-light-induced RPE cell damage. In addition, A2E causes light-induced phenotypic changes in RPE cells. This alteration in the RPE phenotype contributes to the production of proangiogenic factors from RPE cells that may affect the pathogenesis of exudative ARMD (P.A. Campochiaro, Soloway, S.J. Ryan, J.W. Miller. The pathogenesis of choroidal neovascularization in patients with age-related macular degeneration. Mol Vis 1999; 5:34http://www.molvis.org/molvis/v5/p34/). Of the several factors upregulated after A2E-laden cells are exposed to light, vascular endothelial growth factor (VEGF) is one of the most potent proangiogenic factors in the pathogenesis of exudative ARMD. Vascular endothelial growth factor is upregulated in choroidal neovascularization...
lesions in exudative ARMD, and the inhibition of VEGF signaling inhibits choroidal neovascularization, suggesting that A2E-mediated VEGF production is involved in the pathogenesis of exudative ARMD.

The human retina is protected from short-wavelength light because the cornea absorbs light with a wavelength below 295 nm and the crystalline lens absorbs light with a wavelength below 400 nm. Experimental results suggest that light reaching the retina is absorbed by A2E in the RPE cells and remains constant throughout life, whereas a theoretical model estimates that absorption decreases with age. Deposition of A2E increases with age because the crystalline lens yellows to enable absorption of increasing amounts of blue light. Although the results in these studies differ, they agree that yellowing of the crystalline lens attenuates the blue light passing through to the retina. Removing the crystalline lens and implanting an intraocular lens (IOL) increases the blue light reaching the retina, thereby increasing the amount of light absorbed by A2E. These studies support the idea that replacing the crystalline lens with an IOL increases the light absorbance of A2E. In addition, clinical studies suggest cataract surgery may increase the incidence of exudative ARMD.

Blue-light filtering or yellow (ultraviolet [UV]-absorbing) IOLs may have protective effects against exudative ARMD. A recent study showed the protective effects of a blue-light filtering IOL on RPE cell death induced by acute intense light exposure. This study investigated the effects of a blue-light filtering IOL (YA60BB, Hoya) on A2E-laden RPE cells, focusing on cell viability and production of VEGF after less intense light exposure.

MATERIALS AND METHODS

Cell Culture

A human RPE cell line, ARPE-19 (American Type Culture Collection), was used in the study. Cell cultures were maintained in Dulbecco’s Modified Eagle’s/Ham’s F12 media supplemented with 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 μg/mL) (all from Gibco BRL).

Accepted for publication April 21, 2006.

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No author has a financial or proprietary interest in any material or method mentioned.

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A2E-Laden Retinal Pigment Epithelial Cell Preparation

The A2E was synthesized and purified, and A2E-laden ARPE cells were prepared. After ARPE-19 cells were grown confluent on 96-well plates, they were incubated with A2E at a concentration of 50 μM to allow intracellular accumulation. The amount of A2E uptake in the cells was approximately 70 pM/10⁶ cells as determined by high-performance liquid chromatography analysis. Under these conditions, the intracellular concentration of A2E was approximately 80 pM/10⁶, similar to that observed in human RPE cells.

Light Exposure

The A2E-laden ARPE-19 cells were incubated at 37°C in a humid chamber under 5% carbon dioxide/95% air and exposed to white light (NikonTek). Figure 1 shows the spectral irradiance. Light intensity was 2.54 watt/m². Where indicated, a UV-absorbing IOL (VA60BB, Hoya) or the YA60BB blue-light filtering IOL (both +20.0 diopters) was attached under the surface of the culture well and centered over the light beam. After 48 hours of irradiation, the cells’ viability was determined by WST-1 assay (Roche); WST-1 is a sodium salt of 4-[3-4-iodophenyl]-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate. Samples that were not exposed to illumination served as controls. All groups consisted of 20 wells, and the results were averaged.

Enzyme-Linked Immunosorbent Assay

For the enzyme-linked immunosorbent assay (ELISA), the medium was collected after a 48-hour light exposure and centrifuged at 800g for 10 minutes to avoid contamination by cell debris, after which the concentration of VEGF was determined using an ELISA assay kit (R&D Systems). Absorbance at 450 nm was measured using a microplate reader (Bio Rad). Results were recorded as means ± standard error of 6 wells.

Statistical Analysis

All results were expressed as means ± SD. The values were processed for statistical analyses using the 1-way analysis of variance followed by the Dunnett post hoc test. A P value less than 0.05 was considered statistically significant.

RESULTS

Intraocular Lens Effects on A2E-Induced Phototoxicity

To clarify the role of the UV-absorbing and blue-light filtering IOLs on A2E-mediated phototoxicity, cell viability was assessed after A2E-laden cells were exposed to white light for 48 hours. In the absence of an IOL, the white-light exposure decreased cell viability to 28% of the nonirradiated control. The UV-absorbing IOL tended to slightly inhibit this cell damage, with cell viability recovering to 33% of the nonirradiated control (Figure 2). The inhibitory effect of light-induced cell damage was seen with the blue-light filtering IOL, which enhanced cell survival to 42% of...
the nonirradiated control (Figure 2). The inhibitory effect was significantly greater than without an IOL.

Intraocular Lens Effects on VEGF Production

After a 48-hour exposure of white light, VEGF protein levels secreted from A2E-laden ARPE-19 cells were upregulated 3.2-fold with an IOL. The UV-absorbing IOL slightly attenuated VEGF upregulation (Figure 3). Compared to the UV-absorbing IOL, the blue-light filtering IOL had a potent inhibitory effect on the upregulation of VEGF (Figure 3). The VEGF protein levels with a blue-light filtering IOL were significantly lower than those without an IOL.

DISCUSSION

In this study, light filtered by a blue-blocking IOL resulted in less A2E-induced RPE damage than light not filtered by an IOL. Other research shows that another blue-light filtering IOL (AcrySof Natural, Alcon Laboratories, Inc.) protects against RPE damage and produces approximately 80%, 82%, and 78% reduction in the death of A2E-laden RPE cells exposed to blue, green, and white light, respectively. In our study, we used less intense and longer exposure of light. Similar to the previous study, we found that A2E-induced RPE damage was less with a blue-blocking IOL than with a conventional UV-absorbing IOL. Despite the differences in the experimental conditions, our results agree with those in the previous study that a blue-light filtering IOL reduces A2E-mediated light damage to RPE cells.

Another study shows that the expression of a proangiogenic factor, VEGF, is upregulated in A2E-laden RPE cells after light exposure. Our study found that upregulated VEGF production induced by light exposure was inhibited with a blue-light filtering IOL; it is the first to show that light transmitted through an IOL may affect the production of VEGF in the RPE. In addition to photoreceptor cell damage induced by UV light, the upregulated VEGF production in RPE cells may explain why cataract surgery increases the incidence of exudative ARMD. Because VEGF is a potent angiogenic factor and promotes choroidal neovascularization (P.A. Campochiaro, Soloway, S.J. Ryan, J.W. Miller. The pathogenesis of choroidal neovascularization in patients with age-related macular degeneration. Mol Vis 1999; 5:34), it is reasonable to consider that a blue-light blocking IOL is more protective than a conventional UV-absorbing IOL against the development of exudative ARMD.

In another study that found photoactivation of A2E-induced apoptosis and the AcrySof Natural IOL prevented RPE cell death, acute intense light emitted from a tungsten halo source was irradiated for 20 minutes. In contrast, in an earlier study that showed upregulated VEGF expression after light exposure of A2E-laden RPE cells, the cells were illuminated at sublethal irradiation. In our study, with white light (400 to 750 nm, 0.02 mW/cm²) emitted from
a white fluorescent lamp for 48 hours, cell death and upregulated VEGF expression were seen under the same conditions. Thus, the current experimental conditions were different from those in the previous study. Considering that apoptosis induced by A2E is preceded by several events, including a decline of mitochondrial activity and apoptosis-inducing factor in the cytosol and nucleus, respectively, it is reasonable to consider that illumination initially caused sublethal cellular stress, which led to upregulated VEGF secretion from ARPE-19 cells; however, continued illumination for a longer period (48 hours) induced lethal stress to the cells as well as apoptotic change.

In conclusion, our study supports the theory that blue-light filtering IOLs may have more protective effects against A2E-induced photochemical damage and inhibit light-induced VEGF production to a greater extent than conventional UV-absorbing IOLs. Thus, we recommend using a blue-light filtering IOL in patients at increased risk for exudative ARMD.

REFERENCES

LABORATORY SCIENCE: EFFECTS OF YELLOW IOL ON VEGF PRODUCTION