Cytoprotective effects of a blue light-filtering intraocular lens on human retinal pigment epithelium by reducing phototoxic effects on vascular endothelial growth factor-α, Bax, and Bcl-2 expression

Marcus Kernt, MD, Aljoscha S. Neubauer, MD, Raffael Liegl, Kirsten H. Eibl, MD, Claudia S. Alge, MD, Carlo A. Lackerbauer, MD, Michael W. Ulbig, MD, Anselm Kampik, MD

PURPOSE: To compare the possible protective effects of the ultraviolet (UV)-filtering and blue light-filtering SN60AT intraocular lens (IOL) and the untinted UV-filtering SA60AT IOL with regard to light-induced stress on human retinal pigment epithelium (RPE).

SETTING: Department of Ophthalmology, Ludwig-Maximilians-University, Munich, Germany.

METHODS: Primary human RPE cells were exposed to white light, and a tinted or untinted IOL was placed in the light beam. After 15 to 60 minutes of irradiation, cell viability was determined by a colorimetric test (tetrazolium dye-reduction assay) and a microscopic live/dead assay. The expression of vascular endothelial growth factor-α (VEGF-α), Bax, and Bcl-2 and their mRNA was determined by reverse-transcription polymerase chain reaction (RT-PCR) and Western blotting.

RESULTS: Without an IOL, white-light exposure decreased cell viability compared with the decrease with the nonirradiated control in a time-dependent manner. Light-induced cell death was significantly reduced by both the tinted IOL and untinted IOL. The combined UV and blue-light filtering attenuated light-induced cell damage significantly more than UV filtering alone. Results of RT-PCR and Western blotting showed a significant time-dependent decrease in Bcl-2 and increase in Bax and VEGF-α that were significantly less with the tinted IOL than with the untinted IOL.

CONCLUSIONS: Both IOLs reduced light-induced RPE damage. The UV- and blue light-filtering IOL reduced damage more than the conventional IOL. This supports the hypothesis that blue light-filtering IOLs may prevent retinal damage in clinical use.


Age-related macular degeneration (ARMD) is the leading cause of legal blindness in people older than 60 years in developed countries. Approximately 10% of these patients have wet forms of ARMD due to choroidal neovascularization that often lead to a rapid loss of vision.1,2 The other 90% of patients have dry forms of ARMD such as geographic atrophy.1,3-5 Geographic atrophy is characterized by atrophy of retinal pigment epithelium (RPE) cells and consecutive photoreceptor degeneration.6 At present, there is no satisfactory treatment for geographic atrophy.

Even if epidemiologic studies are inconclusive,7,8 there is evidence that cumulative light exposure is significantly associated with progression of ARMD.9-10 Both the cornea and the crystalline lens help protect the retina against the hazards of light by filtering most ultraviolet (UV) radiation.11,12 In older humans, in whom the retina might already be compromised, the crystalline lens also filters potentially phototoxic proportions of light within the blue range by progressive yellowing.13-15 This filtering function of the aging lens is particularly important as this fraction of light is known to induce photochemical damage to the RPE.16,17

Cataract surgery is a safe, common, and beneficial procedure that helps prevent blindness in millions of people. However, aside from the benefits from removing the crystalline lens and implanting an intraocular
lens (IOL), this procedure can result in an increase of radiation reaching the retina and compromising the ocular protective shield against light damage. To protect the retina from possible toxic effects of increased radiation after cataract extraction, yellow-tinted, UV-filtering, and blue light-filtering IOLs have recently been developed. The light-transmission spectrum of UV-filtering and blue light-filtering IOLs differs from that of conventional IOLs that filter UV light only because these IOLs contain chromophores that filter light in the 400 to 500 nm range, adding this light protection to that already provided in the UV range. Within the visible spectrum above 500 nm, both types of IOLs provide even transmission comparable to that of the clear crystalline lens.

The RPE's own pigment, lipofuscin, is known to act as a photosensitizer and to be involved in the pathogenesis of exudative ARMD. To test the protective effects of UV-filtering and blue light-filtering IOLs, experimental studies examined lipofuscin-triggered phototoxic reactions in the RPE. Light-induced toxic effects were studied in RPE cell lines artificially laden with lipofuscin. The results support the theory that UV-filtering and blue light-filtering IOLs may be more protective against photochemical damage.

This study compared the possible protective effects of a UV-filtering and blue light-filtering IOL with those of an untinted UV-filtering IOL with regard to light-induced stress on human RPE. Early passages of primary human RPE cells were studied. Early passages of RPE cells are known to still possess some of the cells' own lipofuscin, which is lost when cells are cultured long term. We exposed the cells to wide-band white light and placed the IOLs in the light beam. The viability of the cells was determined. The expression of vascular endothelial growth factor-α (VEGF-α), a major stimulus in the development of exudative ARMD, and the expression of the pro-apoptotic and anti-apoptotic proteins Bax and Bcl-2 were determined.

**MATERIALS AND METHODS**

**Ethics**

The methods of securing human tissue complied with the Declaration of Helsinki and were approved by the local ethics committee. Written informed consent was obtained from the tissue donors.

**Human Retinal Pigment Epithelium**

Retinal pigment epithelial cells from human donors were obtained 3 to 10 hours postmortem from the eye bank at Ludwig Maximilian University and prepared as previously described. In brief, whole eyes were thoroughly cleansed in 0.9% sodium chloride (NaCl) solution, immersed in 5% polyvinyl pyrrolidone iodine, and rinsed again in the NaCl solution. The anterior segment of each donor eye was removed, and the posteriorm poles were examined with a binocular stereomicroscope to confirm the absence of gross retinal disease. The neural retina was then carefully peeled away from the RPE-choroid-sclera with a fine forceps. The eye cup was rinsed with Ca²⁺- and Mg²⁺-free Hanks balanced salt solution (BSS) and filled with 0.25% trypsin (Invitrogen) for 30 minutes at 37°C. The trypsin was carefully aspirated and replaced with Dulbecco modified Eagle medium (DMEM, Biochrom) supplemented with 20% fetal calf serum (FCS, Biochrom). The medium was gently agitated with a pipette, releasing the RPE cells into the medium without damaging Bruch membrane. The RPE cell solution was transferred to a 50 mL flask (Falcon) containing 20 mL of DMEM supplemented with 20% FCS and maintained at 37°C and 5% carbon dioxide. Epithelial origin was confirmed by immunohistochemical staining for cytokeratin with a pan-cytokeratin antibody (Sigma-Aldrich). The cells were tested and found to be free of contaminating macrophages (anti-CD11, Sigma-Aldrich) and endothelial cells (anti-von Willebrand factor, Sigma-Aldrich) (data not shown). After growing to confluence (100%), primary RPE cells were subcultured and maintained in DMEM supplemented with 10% FCS at 37°C and 5% carbon dioxide. Primary RPE cells of passages 2 and 3 were used in the experiments. All investigated RPE cell cultures still presented intracellular pigmented granules in phase-contrast microscopy. It has been shown that beside melanin and other pigments, these granules contain a certain amount of cell-own lipofuscin. This characteristic is lost when RPE cells are cultured long term.

**Intraocular Lenses**

Two single-piece acrylic foldable IOLs were tested: a yellow-tinted UV- and blue light-filtering IOL (SN60AT; tinted IOL) and a UV-filtering IOL (SA60AT; untinted IOL). Both IOLs (Alcon Laboratories) were 20.0 diopters.

**Illumination of Cells**

A spot-light source (LC-8, Hamamatsu Photonics) from a mercury–xenon lamp using an optic-fiber as the light guide (spectral range 400 nm to 700 nm) was used for illumination.
The cell-culture medium was replaced with phosphate-buffered saline (PBS) solution just before illumination. The plastic cover of the illuminated cell-culture well was removed, and the cells were illuminated from above. They were illuminated for 15 to 60 minutes (350 mW/cm²) in the presence or absence of the IOLs. The IOLs were applied to the light-emitting output of the optic fiber (diameter 3.5 mm), where they remained attached without an aid. The illumination power and spectral range were measured with a spectrometer (C10838MD, Hamamatsu Photonics). Directly after illumination, PBS was replaced by serum-free cell-culture medium and cells were kept in darkness for another 24 hours. Then, the tetrazolium dye-reduction assay (MTT; 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, live/dead assay, phase-contrast microscopy, RNA isolation, or protein extraction was performed. Illumination times of 15, 30, 45, and 60 minutes were chosen for all experiments to evaluate possible protective effects of the untinted IOL and the tinted IOL.

**MTT Assay**

The MTT assay was used to determine the cell survival rate. The MTT test, which is well established for the assessment of cell viability, was performed as described by Mosmann, with some modifications. The medium was removed; the cells were washed with PBS, and 1000 mL of MTT solution (1.5 mL MTT stock, 2.0 mg/mL in PBS, plus 28.5 mL DMEM) was added to each well. The RPE cells were incubated at 37°C for 1 hour. The formazan crystals that formed were dissolved by the addition of 1000 mL of dimethylsulfoxide per well. Absorption was measured by a scanning multwell spectrophotometer at 550 nm (Molecular Probes). The results were expressed as the mean percentage of proliferation in the control. The experiments were performed in triplicate and repeated 3 times. The control cells were the RPE cells of the same passage, incubated with balanced saline, kept in darkness, and without exposure to any radiation.

**Live/Dead Cell Viability Assay**

Confluent RPE cells were prepared and treated as described. Cell viability was quantified based on a 2-color fluorescence assay in which the nuclei of nonviable cells appeared red due to staining by the membrane-impermeable dye propidium iodide (Sigma-Aldrich) while the nuclei of all cells were stained with the membrane-permeable dye Hoechst 33342 (Intergen). Confluent cultures of RPE cells growing on cover slips in 24-well tissue culture plates were exposed to white light in the absence or presence of the IOLs, as described for the MTT assay. To evaluate cell viability, the cells were washed in PBS and incubated with 2.0 mg/mL propidium iodide and 1.0 mg/mL Hoechst 33342 for 20 minutes at 37°C. Subsequently, the cells were analyzed with an epifluorescence microscope (Aristoplan, Leitz). The labeled nuclei were then counted in fluorescence micrographs. Dead cells were expressed as a percentage of the total nuclei in the field. The data are based on counts in 3 experiments performed in duplicate wells, with 3 to 5 documented representative fields per well. The RPE cells of the same passage incubated with DMEM served as controls.

**RNA Isolation and Real-Time Polymerase Chain Reaction**

The RPE cells from 5 donors were used to assess mRNA expression of Bcl-2, Bax, and VEGF-z. Bax, Bcl-2, and VEGF-z messenger RNA expression was detected in every sample. All detected mRNA levels of Bcl-2, Bax, and VEGF-z were normalized to those of 18S RNA, and the values were expressed as the relative ratio of Bcl-2/18S, Bax/18S, or VEGF-z/18S.

Total RNA was isolated from 10 cm Petri dishes by the guanidium thiocyanate-phenol-chloroform extraction method (Stratagene). The structural integrity of the RNA samples was confirmed by electrophoresis in 1% Tris-acetate–ethylenediaminetetraacetic acid (TAE) agarose gels. The yield and purity were determined photometrically (BioPhotometer, Eppendorf). Real-time polymerase chain reaction (PCR) enables quantitative detection of small amounts of mRNA. After the usual isolation of mRNA, the mRNA was transferred to cDNA via reverse transcriptase. This cDNA was then used for the specific PCR. Quantification of Bcl-2, Bax, and VEGF-z mRNA was performed with specific primers (Table 1) with the LightCycler system (Roche Diagnostics). Primers and probes were found with ProbeFinder 2.04 (Roche Diagnostics). All primers and probes were designed to cross intron/exon boundaries to avoid amplification of genomic DNA. All PCR products were sequenced to ensure product validity. Each 14 mL reaction volume contained 1 FastStart DNA Master Hybridization Probes Mix (Roche Diagnostics), 4.0 mM magnesium chloride, 0.5 mM of each primer, 0.2 mM TaqMan probe, and 2.0 mL cDNA.

The amplification signals were detected in real time, which enabled accurate quantification of the amounts of the initial RNA template because the system can select signals easily during the exponential amplification phase of PCR. The cDNA of cells exposed to white light in the absence or presence of the IOLs was amplified with specific primers for 40 cycles. Two oligonucleotides with different labeled fluorophores were hybridized to the amplified fragment during the annealing phase. When the 2 probes came in close proximity, fluorescence resonance energy transfer developed between the 2 fluorophores. The emitted fluorescence was then measured by the LightCycler instrument. Hybridization probes were displaced during the extension step. Depending on the initial concentration of target genes, the signal intensity increased in different cycles; these cycles were used as the crossing point. The standard curve was made with 3

<table>
<thead>
<tr>
<th>Target/Length</th>
<th>Position</th>
<th>AT (°C)</th>
<th>GC</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2</td>
<td>19</td>
<td>3465-3483</td>
<td>60</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>3305-3524</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Bax</td>
<td>18</td>
<td>526-543</td>
<td>59</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>592-609</td>
<td>59</td>
<td>56</td>
</tr>
<tr>
<td>VEGF-z</td>
<td>18</td>
<td>1540-1557</td>
<td>60</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>1592-1609</td>
<td>60</td>
<td>61</td>
</tr>
</tbody>
</table>

AT = annealing temperature; GC = G-C nucleotide pairs; VEGF-z = vascular endothelial growth factor-z
different probes of untreated RPE cells. To normalize for differences in the amount of total RNA added to each reaction, 18S rRNA was simultaneously processed in the same sample as an internal control. The level of Bcl-2, Bax, and VEGF-α mRNA was determined as the relative ratio, which was calculated by dividing the level of Bcl-2, Bax, and VEGF-α mRNA by the level of the 18S rRNA housekeeping gene in the same samples. The ratios are expressed in a decimal format. All experiments were performed at least in triplicate and repeated 3 times.

Protein Extraction and Western Blotting
To verify that the light-induced increase in Bax and decrease in Bcl-2 in mRNA transcription translates into increased protein synthesis, whole cellular protein extracts of nonilluminated control cells and cells that were illuminated in the presence or absence of the tested IOLs (untinted and tinted) were analyzed by Western blotting.

Primary RPE cells grown on 35.0 mm tissue culture dishes were washed twice with ice-cold PBS, collected, and lysed in radioimmunoprecipitation assay cell lysis buffer. After centrifugation for 30 minutes at 19000 × g in a microfuge (5810R, Eppendorf) in the cold, the supernatant was transferred to fresh tubes and stored at −70°C for future use. The protein content was measured by the bicinchoninic acid protein assay (Pierce). Denatured proteins (162 mg) were separated by electrophoresis under reducing conditions using a 5% sodium dodecyl sulfate-polyacrylamide stacking gel and a 12% sodium dodecyl sulfate-polyacrylamide separating gel, transferred with semidy blotting onto a polyvinyl difluoride membrane (Roche), and probed with a mouse anti-Bcl-2-antibody or a mouse anti-Bax-antibody as described previously. Chemiluminescence was detected with the imager (LAS-1000, RayTest) and generated light units. Exposure times ranged between 1 minute and 10 minutes. Quantification was performed on a computer (AIDA software, RayTest). All experiments were performed at least in triplicate in RPE cell cultures from 3 donors.

Statistical Evaluation
All data were analyzed using SPSS for Windows (version 13.0, SPSS, Inc.). For all statistical tests, a P value less than 0.05 was considered significant. Results of the MTT assay are presented as mean ± SD units of absorbance. Ten individual samples per group were measured in triplicate. The Mann-Whitney test was used. Results of the RT-PCR are presented as mean ± SD ratios of the investigated mRNA and 18S rRNA. Again, Mann-Whitney testing was applied; all experiments were performed in triplicate and repeated 3 times. Western blot analysis was performed analogously; experiments were performed at least in triplicate in RPE cell cultures from 3 donors.

RESULTS
The study evaluated RPE cells from 5 human donors aged 42 years, 52 years, 58 years, 64 years, and 72 years.

Viability of Cells
With up to 45 minutes of illumination, no gross abnormalities were detected by phase-contrast microscopy in primary RPE cells in the presence of the tested IOLs or when cells were exposed to unfiltered light. After 60 minutes of light exposure, only cells that were illuminated with unfiltered white light showed distinct morphologic changes and signs of cell death. The number of cells, counted in phase-contrast microscopy, correlated well with the results of the MTT test where quantification was performed (Figure 1).

MTT Assay
When cells were illuminated with unfiltered white light (350 mW/cm²), a time-dependent reduction of RPE cell viability was detected that was rapid and

![Figure 1. Phase-contrast microscopy of primary human RPE cells. There were no morphological changes in the presence of the 2 IOLs (C and E) or with unfiltered illumination (A) after 45 minutes of illumination. When illuminated for 60 minutes, cells exposed to unfiltered white light showed marked signs of destruction and cell death (B) while cells protected by either IOL showed no significant morphologic change (D and F) (IOL = intraocular lens) (scale ×10).](image-url)
significant beginning from 45 minutes of illumination. When an untinted IOL was placed in the light beam during illumination, this decrease in viability was significant less pronounced compared with cells that were illuminated with unfiltered white light; however, this still led to a significant reduction in RPE viability decrease after 60 minutes of illumination. In the presence of the tinted IOL, the reduction in viability decrease was even more distinct. After 60 minutes of illumination, RPE cell viability was significantly less compared with that of cells that were illuminated with unfiltered white light and cells that were illuminated in the presence of the untinted IOL (Figure 2).

**Live/Dead Assay**

When the cells were illuminated 15 or 30 minutes, no significant increase in nonviable cells was detected compared with the control in the presence of the tested IOLs or when cells were exposed to unfiltered light (data not shown). After 45 minutes of unfiltered illumination, the RPE cells showed a significant increase in nonviable cells compared with the nonirradiated control. Cells that were exposed to light that was filtered by the tinted IOL showed no decrease in the number of viable cells after 45 minutes of illumination compared with the nonirradiated control. After 60 minutes, cells that were illuminated in the presence of the tinted IOL showed a significant reduction in viable cells compared with the nonirradiated control. In contrast, cells that were exposed to unfiltered white light showed a significant decrease in viable RPE cells compared with the nonirradiated control. Cells that were illuminated in the presence of the untinted IOL showed significantly fewer dead cells than those with the unfiltered illumination but also showed a significant decrease in viable cells compared with the nonirradiated control (Figure 2).

**Expression of Bax, Bcl-2, and VEGF-α mRNA**

Illumination with unfiltered white light led to a time-dependent decrease in Bcl-2 mRNA expression. In contrast, expression of Bax and VEGF-α mRNA was increased in primary human RPE cells (Figures 4 to 6).

In comparison to unfiltered illumination, filtering light with either tested IOL significantly reduced the light-induced increase in Bax and VEGF-α mRNA after 15, 30, 45, or 60 minutes of illumination. In addition, this reduction of light-induced increase in Bax mRNA was significantly higher when light was filtered by the tinted IOL than when filtered by the untinted IOL after 15, 30, 45, or 60 minutes of illumination. The VEGF-α mRNA overexpression was significantly reduced after 45 or 60 minutes of illumination when light was filtered by the tinted IOL compared with overexpression with the untinted IOL (Figures 4 and 6).

After 15, 30, 45, and 60 minutes of unfiltered illumination of RPE cells, a significant reduction in Bcl-2 mRNA expression was detected. This phototoxic decrease in Bcl-2 mRNA expression was significantly reduced by both tested IOLs. In addition, after 45 and 60 minutes of illumination, the decrease was significantly less when light was filtered by the tinted IOL than when filtered by the untinted IOL (Figure 5).

**Protein-Expression of Bax and Bcl-2**

After 60 minutes of unfiltered illumination, Bcl-2 expression decreased 3.3× in primary human RPE cells compared with the nonirradiated control. This decrease was reduced to 1.4× in the presence of the untinted IOL and to 1.25× in the presence of the tinted IOL (Figure 7). In contrast, Bax expression was increased 1.4× compared with the nonirradiated control, in which cells were exposed to unfiltered irradiation for 60 minutes. In the presence of the untinted IOL, this light-induced overexpression of Bax protein decreased to 1.2×. When light was filtered with the tinted IOL, no increase in Bax expression was detected (Figure 7).

**DISCUSSION**

Several epidemiological studies[^4][^8][^9][^39][^40] seem to indicate that aphakic and pseudophakic eyes have an increased risk for development and progression of ARMD. Photochemical damage and light-induced apoptosis due to cumulative light exposure have been impli-
implicated as risk factors for atrophic degeneration of RPE cells. Light-induced apoptotic cell death within the RPE is even more serious as RPE cells are postmitotic cells that do not divide under normal conditions and should persist for the entire life of an individual.

This in vitro study found that filtering light with a UV- and blue light-filtering IOL or a UV-filtering IOL resulted in reduced RPE cell damage in primary human RPE cells compared with the damage from unfiltered illumination. The light we used for illumination did not include the UV spectrum. Therefore, one explanation of why both IOLs led to a reduction in cell damage could be the light-attenuating properties of the acrylic IOL material, which reduces transmission to approximately 90% within the 500 to 700 nm range of light. In addition, the UV-filtering chromophore that both tested IOLs contain does not cut off exactly at 400 nm wavelength but provides a less pronounced, decreasing absorption up to approximately 430 nm.

In our experimental setup, after 60 minutes of illumination, the reduction in light-induced cell death was significantly less in the presence of the tinted IOL than with the untinted IOL. Apoptosis is a cell-autonomous mechanism that is genetically controlled by the dying cell, resulting in the activation of tumor suppressor proteins (TSP) and apoptosis-initiating caspases. A recent study found that the UV- and blue light-filtering properties of the SN60AT IOL reduced RPE cell apoptosis in vitro. In addition, our results show that the decrease in RPE cell death is accompanied by a significant reduction in phototoxic decrease of the antipapoptotic protein Bcl-2. Bcl-2 is generally considered a custodian of mitochondrial functional integrity, as it stabilizes the mitochondrial membrane against the release of cytochrome c. It is able to interrupt apoptosis through its inhibitory effect on caspase activation. It has been shown that Bcl-2 mediates cell survival and decreases apoptosis in human RPE cells and other ocular and nonocular cell lines.

Figure 3. After 45 minutes of illumination, the number of viable RPE cells was significantly reduced when cells were exposed to unfiltered light (A and B); there was no significant increase in nonviable cells when cells were illuminated in the presence of both IOLs (E, F, I, and K). After 60 minutes of illumination, cells that were illuminated in the presence of the untinted IOL showed significantly fewer dead cells than those with the unfiltered illumination (C, D, G, and H) but also showed a significant decrease in viable cells compared with the nonirradiated control (N and O). Illumination in the presence of the tinted IOL (L and M) did not increase the number of nonviable cells compared with the nonirradiated control (N and O). Comparison to controls is shown above the bars, with significant differences marked by an asterisk (*) (Co = control; IOL = intraocular lens).
In our experimental set-up the light-induced decrease of antiapoptotic Bcl-2 was significantly reduced by both tested IOLs. Furthermore, after 45 or 60 minutes of illumination, the Bcl-2 levels were significantly higher when light was filtered by the tinted IOL than by the untinted IOL.

In contrast, light-exposure led to an increased expression of the apoptotic Bax protein. Bax is a key protein within a major pathway of the cell death program. It is up-regulated by the TSP p53 and is highly associated with apoptotic cell death. An imbalance within the ratio of Bax and Bcl-2 toward an excess of Bax leads to apoptotic cell death in many cellular systems. In our testing, the light-induced overexpression of apoptotic Bax was significantly reduced by both IOLs. However once again, the light-induced overexpression of proapoptotic Bax was significantly more reduced by the tinted than by the untinted IOL.

The main cause for rapid loss of central vision in ARMD patients is choroidal neovascularization within the macular region. Vascular endothelial growth factor-α is known to be a major stimulus for neovascularization in exudative ARMD. Cumulative light exposure is

**Figure 4.** Quantitative RT-PCR analysis of Bax mRNA expression of primary RPE cells after illumination with plain white light (Co) in the presence of an untinted IOL and tinted IOL. The mRNA levels are expressed as the ratio of Bcl-2 mRNA/18s rRNA (Co = control; IOL = intraocular lens; x-axis = relative ratio of bax mRNA normalized to 18s rRNA, expressed in decimal format; y-axis = time of illumination).

**Figure 5.** Quantitative RT-PCR analysis of Bcl-2 mRNA expression of primary RPE cells after illumination with plain white light (Co) in the presence of an untinted IOL and a tinted IOL (Co = control; x-axis = relative ratio of bax mRNA normalized to 18s rRNA, expressed in decimal format; y-axis = time of illumination).

**Figure 6.** Quantitative RT-PCR analysis of VEGF-α mRNA expression of primary RPE cells after illumination with plain white light (Co) in the presence of an untinted IOL and a tinted IOL (Co = control; IOL = intraocular lens; VEGF-α = vascular endothelial growth factor-α; x-axis = relative ratio of bax mRNA normalized to 18s rRNA, expressed in decimal format; y-axis = time of illumination).

**Figure 7.** Representative Western blots showing the expression of Bcl-2 and Bax in non-irradiated RPE cells (Co) and after 60-minute exposure to white light, unfiltered (no IOL) in the presence of a tinted IOL and an untinted IOL. Ten micrograms of protein were loaded per lane (IOL = intraocular lens).
thought to induce proangiogenic factors, such as VEGF-
α, in RPE cells and promotes the development of exuda-
tive ARMD. Studies have shown that unfiltered light ex-
posure can lead to an increased production of VEGF-
α in RPE cells. The reason for this could be that con-
tinuous light exposure induces subthelial cellular stress,
which results in an up-regulation of proangiogenic
VEGF-α secretion. In agreement with these findings,
our results show that unfiltered light exposure directly
stimulates RPE cells to a time-of-irradiation dependent
overexpression of VEGF-α mRNA. This might be a pos-
tible explanation of why cataract surgery is implicated
in the increase in the incidence of exudative ARMD. In
our study, this increase in VEGF-α mRNA expression
was significantly reduced in the presence of either IOL;
however, the effect was significantly stronger in the
presence of the tinted IOL.

In summary, our in vitro findings support the theory
that UV- and blue light-filtering IOLs might be useful
in patients with increased risk for progression of
ARMD. Ultraviolet- and blue light-filtering IOLs proba-
ble have properties that can help to prevent apoptotic
cell death in the human RPE due to extensive light
exposure. Therefore, they might be able to reduce the risk
for development and progression of atrophic ARMD.
Further clinical investigations will have to substantiate
these findings in vivo. Nevertheless, results indicate
that UV- and blue light-filtering IOLs would be ben-
ficial to patients with a high risk for ARMD and to those
that have ARMD that may progress.

REFERENCES

1. Ferris FL III, Fine SL, Hyman L. Age-related macular degenera-
tion and blindness due to neovascular maculopathy. Arch Oph-
thalmol 1984; 102:1640–1642
2. Wilmer AN, Vrensen GFJM, Van Noorden CJF, Schlingemann RO. Vascu-
lar endothelial growth factors and angiogenesis in eye
3. The Eye Diseases Prevalence Research Group. Causes and preva-
ce of visual impairment among adults in the United
4. Klein R, Klein BEK, Linton KL. Prevalence of age-related macu-
lopathy; the Beaver Dam Eye Study. Ophthalmology 1992;
99:933–943
5. The Eye Diseases Prevalence Research Group. Prevalence of
age-related macular degeneration in the United States. Arch
Ophthalmol 2004; 122:564–572
6. Knudtson MD, Klein R, Klein BEK, Lee KE, Muer SM, Tomany SC. Location of lesions associated with age-related macu-
lopathy over a 10-year period: the Beaver Dam Eye Study. Invest
7. Darzins P, Mitchell P, Heiler RF. Sun exposure and age-related
macular degeneration; an Australian case-control study. Oph-
thalmology 1997; 104:770–776
8. Cruickshanks KJ, Klein R, Klein BEK. Sunlight and age-related
macular degeneration; the Beaver Dam Eye Study. Arch
Ophthalmol 1993; 111:514–518
9. Cruickshanks KJ, Klein R, Klein BEK, Nordahl DM. Sunlight and
the 5-year incidence of early age-related maculopathy; the Be-
10. Tomany SC, Cruickshanks KJ, Klein R, Klein BEK, Knudtson MD. Sunlight and the 10-year incidence of age-related
maculopathy; the Beaver Dam Eye Study. Arch Ophthalmol
2004; 122:750–757
11. Norren DV, Vos JJ. Spectral transmission of the human ocular
12. van den Berg TJ, Spekreijse H. Near infrared light absorption
14. Mullerio J. Yellowing of the human lens; nuclear and cortical con-
15. Weale RA. Age and the transmittance of the human crystalline
16. Ham WT Jr, Mueller HA, Ruffolo Jr JJ Jr, Millen JE, Cleary SF,
Guerry RK, Guerry D III. Basic mechanisms underlying the pro-
duction of phototoxic lesions in the mammalian retina. Curr
17. Ham WT Jr, Ruffolo Jr JJ Jr, Mueller HA, Clarke AM, Moon ME.
Histological analysis of photocoagulative lesions produced in rhesus
retina by short-wavelength light. Invest Ophthalmol Vis Sci
1978; 17:1029–1035. Available at: http://www.iovs.org/cgi/
18. Mainster MA, Sparrow JR. How much blue light should an IOL
19. Lawrence HM, Reynolds TR. Erythropilis photocytotoxicity associ-
ated with nonultraviolet-filtering intraocular lenses. J Cataract
20. Komatsu M, Kanagami S, Shimizu K. Ultraviolet-absorbing intra-
ocular lens versus non-UV-absorbing intraocular lens: compari-
son of angiographic cystoid macular edema. J Cataract Refract
21. Braunstein RE, Sparrow JR. A blue-blocking intraocular lens
should be used in cataract surgery [controversies]. Arch
Ophthalmol 2005; 123:547–549
22. Ernst PH. Light-transmission-spectrum comparison of foldable
23. Brockmann C, Schulz M, Laube T. Transmittance characteristics
of ultraviolet and blue-light-filtering intraocular lenses. J Cat-
aract Refract Surg 2008; 34:1161–1166
24. Algvere PV, Seregard S. Age-related maculopathy; pathoge-
netic features and new treatment modalities. Acta Ophthalmol
Scand 2002; 80:136–143
25. Roth F, Bindewald A, Holz FG. Key pathophysiologic pathways
in age-related macular disease. Graefes Arch Clin Exp Ophthal-
mol 2004; 242:710–716
26. Wing GL, Blanchard GD, Weiter JJ. The topography and age rela-
tionship of lipofuscin concentration in the retinal pigment ep-
4, 2008
27. Weiter JJ, Delori FC, Wing GL, Fitch KA. Retinal pigment epithe-
lium lipofuscin and melanin and choroidal melanin in human eyes.
Invest Ophthalmol Vis Sci 1986; 27:145–152. Available at:
4, 2008
28. Holz FG, Bellmann C, Margariti D, Schütt F, Otto TP, Völcker HE. Patterns of increased in vivo fundus autofluores-
cence in the junctional zone of geographic atrophy of the reti-
 nal pigment epithelium associated with age-related macular
237:145–152


34. Waisbourd M, Loewenstein A, Goldstein M, Leibovitch S. By transforming growth factors, by transforming vascular endothelial growth factor; a promising strategy for treating age-related macular degeneration. Drugs Aging 2007; 24:663–662


First author:
MARCUS KERMD
Department of Ophthalmology, Ludwig Maximilian University, Munich, Germany

J CATARACT REFRACT SURG - VOL 35, FEBRUARY 2009