Light-Induced Oxidative Stress in Choroidal Endothelial Cells in Mice

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Purpose. Although light-induced oxidative stress in the retina has been extensively reported, little information regarding light-induced oxidative stress in choroidal endothelial cells (CECs) is available. In the current study, light-induced DNA oxidation and the activation of nuclear factor-κB (NF-κB), a major oxidative responsive transcription factor, were investigated in mouse CECs.

Methods. Mice were exposed to green light. Light-induced DNA oxidation in CECs was detected by in situ 8-hydroxy-2-deoxyguanosine (8-oxo-dG) immunolabeling. CECs were isolated from the retinal pigment epithelium (RPE)/choroid by using immunomagnetic beads. The isolated CECs were immunohistochemically characterized by the expression of endothelial markers, CD31, and P1H12. The quality of total RNA from CECs was assessed by a bioanalyzer and RT-PCR. NF-κB activation in situ and in isolated CECs was investigated.

Results. After a 3-hour exposure to light, the immunoreactivity to anti-8-oxo-dG antibody or anti-NF-κB p65 antibody in CECs in situ was significantly increased when compared with unexposed mice. Isolated CECs expressed CD31 and P1H12. The 28S/18S rRNA ratio of RNA isolated from CECs was 1.5:1. CD31 and von Willebrand Factor (vWF) transcripts were predominantly expressed in the RNA from isolated CECs. IsxBα was more heavily phosphorylated in light-exposed than untreated CECs. IsxBα expression levels were increased fivefold in isolated CECs after exposure to light compared to unexposed control subjects.

Conclusions. Exposure to light induces oxidative stress in CECs in vivo. A method for CEC isolation from the mouse RPE/choroid with preservation of RNA quality has been developed. The results of this study may facilitate the ability to identify CEC-specific genes and gene products that respond to photo-oxidative stress. (Invest Ophthalmol Vis Sci. 2005;46:1117–1123) DOI:10.1167/iovs.04-0517

Photo-oxidative stress induced by overexposure to sunlight correlates with aging of the RPE/choroid and possibly early age-related macular degeneration (AMD), whereas significant exposure to light can induce neurosensory retinal degeneration. One theory suggests that light induces the formation of reactive oxygen species (ROS), which results in retinal lipid oxidation, decreased docosahexaenoic acid (22:6n-3), and increased peroxides in rod outer segment membranes. In response to photo-oxidative stress, increased retinal levels of anti-oxidative enzymes, such as glutathione reductase, glutathione peroxidase, and glutathione S-transferase have been observed. Antioxidants such as vitamin C and the superoxide dismutase mimic 2,2,6,6-tetramethyl-4-piperidinol-N-oxyl have been shown to prevent light-induced retinal damage. Our previous study indicated that, after an acute 3-hour exposure to light, a small number of apoptotic photoreceptor cells are scattered throughout the outer nuclear layer (ONL) of the retina, although the ONL’s thickness remains unchanged.

Although effects of oxidative stress on the retinal pigment epithelium (RPE) have also been studied, few reports on photo-oxidative stress on the choroid have been published. For example, light activation of choriocapillaris pericytes has been described. Kayatz et al. reported that in albino mice (BALB/c), light-induced lipid peroxides (LPs) as a marker of oxidative stress, were localized in the basal infoldings of the RPE, whereas LPs in the choroid were found in CECs and melanocytes. In addition, Dorey et al. reported that blue-light-induced oxidative stress in cultured porcine aortic endothelial cells. However, the functional and structural differences of macrovascular aortic endothelial cells could produce a different response than microvascular choroidal endothelial cells. In addition, these results in vitro may not accurately represent the events of exposure to light in vivo. These interesting studies, however, suggest that photo-oxidative stress occurs in CECs and that further investigation is warranted.

We hypothesized that overexposure to light induces oxidative stress in CECs, which contributes to choriocapillaris degeneration. To test this hypothesis, we investigated several markers of oxidative stress, including light-induced DNA oxidation by immunohistochemical study with 8-hydroxy-2-deoxyguanosine (8-oxo-dG), a biomarker for DNA oxidative damage, and the activation of nuclear factor-κB (NF-κB), an oxidative responsive transcription factor. It is difficult to perform molecular biological and biochemical oxidative stress analyses on isolated CECs, because these cells are surrounded by many types of cells, which makes their separation difficult by methods such as laser capture microdissection. To overcome this obstacle, we developed a method for isolating CECs from the mouse RPE/choroid, using magnetic beads, which preserved the RNA quality of isolated cells. Ocular melanin presumably plays an important protective role against photo-oxidative stress in the choroid. However, the biological function of melanin in oxidative stress is not well defined. We used albino mice in the present study to eliminate the confounding effects of melanin on photo-oxidative stress in CECs, and to increase the photo-oxidative stress to the choroid. These studies demonstrated that in CECs light-induced markers of oxidative stress such as DNA oxidation, in situ NF-κB activation, and phosphorylation of IκBα protein in vivo and increased expression levels of IκBα in vitro.

Materials and Methods

Mice

Male albino BALB/cj mice aged 6 to 8 weeks (Jackson Laboratory, Bar Harbor, ME) were fed standard laboratory chow (Prolab RMH 1000; Laboratory Products, Inc., Elkridge, MD). All protocols adhered to the
ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the Institutional Animal Care and Use Committee of Johns Hopkins University.

Exposure to Light
All mice were reared in a 12-hour light–dark cycle at a light level of 20 to 40 lux for 14 days after purchase. Before exposure to light, mice were dark adapted for 48 hours. Mice were placed in a chamber of green Plexiglas (model 2092; Dayton Plastics, Dayton, OH) which transmits equidirectional green light (λ<sub>max</sub> = 520 nm) at an illuminance of 3.1 to 3.5 kilolux, a commonly used wavelength and illuminance for light-induced retinal degeneration studies. Mice in groups of four were exposed to light for 0.5, 1, or 3 hours, and four mice dark adapted for 48 hours were used as control mice. The experiments were performed six times, and 96 mice were used. The chamber temperature during experiments was 25 ± 1.5°C. Mice were allowed free access to food and water during exposure to light periods.

CEC and Tissue Preparation
Mice were anesthetized with ketamine (75–100 mg/kg) and xylazine (10 mg/kg) before they were killed by neck dislocation. Eyes from mice exposed to light for 0.5, 1, or 3 hours as well as from dark-adapted mice without exposure to light were enucleated and placed in ice-cold PBS buffer (0.01 M phosphate buffer [pH 7.5], 0.0027 M KCl, and 0.137 M NaCl). A circumferential incision was performed posterior to the ciliary body, and the anterior segment, including the cornea, iris, ciliary body, and lens, was discarded. The retinal was gently lifted away from the RPE with microsurgical forceps. Posterior eyecups consisting of the sclera, choroid, and RPE were prepared for CEC isolation. This experiment was repeated three times, and 48 mice were used in posterior eyecup preparations for subsequent CEC isolation.

For immunocytochemical study, the contralateral eye was embedded in optimal cutting temperature compound (OCT; Tissue-Tek; Sakura Finetek, Torrance, CA), fresh-frozen on dry ice, and stored at −80°C. The blocks were cut vertically at 9 μm through the optic nerve and ora serrata with a cryostat (Histo-Clear; National Diagnostics, Atlanta, GA). Forty-eight mice were used in the study.

8-oxo-dG Immunohistochemistry
Cryosections of the RPE/choroid from light-exposed (n = 3) and nonexposed (n = 3) mice were immunostained with an anti-8-oxo-dG monoclonal antibody (IgG3; MAB 750; Chemicon International, Inc., Temecula, CA) and phosphorylated (p)IκBα antibody (1:100), then incubated with anti-8-oxo-dG antibody (1:300), then biotinylated anti-mouse IgG antibody, and detected with 3,3′-diaminobenzidine; DAB; Vector Laboratories, Burlingame, CA). Antibody preabsorbed with 8-oxo-dG (1 μg/μL; Sigma-Aldrich, St. Louis, MO) was used as a negative control.

NF-κB Immunohistochemistry
Cryosections of the RPE/choroid from two independent experiments were immunostained with a monoclonal antibody that recognizes the active form of NF-κB p65 subunit (12H11; Roche Diagnostics, Indianapolis, IN), as previously described. Briefly, sections were fixed in 70% ethanol, incubated with anti-8-oxo-dG antibody (1:300), then biotinylated anti-mouse IgG antibody, and detected with 3,3′-diaminobenzidine; DAB; Vector Laboratories. An isotype control antibody, a mouse anti-Salmonella poona monoclonal antibody (IgG<sub>2a</sub>; MAB 750; Chemicon International, Inc., Temecula, CA), was used at the same concentration as the anti-NF-κB p65 monoclonal antibody.

Immunoreactivity Grading System
Two independent observers, using a grading system previously described, scored the relative immunoreactive intensity in the choroid for each antibody. The following intensities were assigned: 8, uniformly intense immunoreactivity; 7, patchy and intense; 6, uniform and moderate; 5, patchy and moderate; 4, uniform and weak; 3, patchy and weak; 2, uniform and very weak; 1, patchy and very weak; and 0, comparable to a control section.

Mean scores ± SD from the graders were calculated for each choriocapillaris sample. Significance was determined by comparing mean scores from the control eyes with scores from light-exposed eyes using the Student's t-test and assuming unequal variance and two tails. P ≤ 0.01 was considered significant.

Isolation of CD31-Positive Endothelial Cells from the RPE/Choroid
Posterior eyecups from light-exposed or dark-adapted mice were washed with ice-cold PBS buffer. The RPE/choroid was scraped from the scleral wall of eyecups aseptically with the aid of a dissecting microscope. The dissected RPE/choroid was pooled from eight eyes and digested in 1 mL collagenase type I (2 mg/mL; Roche Diagnostics) and elastase (Grand II; 0.25 mg/mL; Roche Diagnostics) in Dulbecco’s modified Eagle’s medium (DMEM, Cellgro; Mediatech, Herndon, VA) supplemented with 1% fetal calf serum (Invitrogen-Gibco, Grand Island, NY) for 1 hour at 37°C. After digestion, the cellular digests were filtered through a 40-μm nylon mesh (BD Falcon, Bedford, MA). The filtered cellular digests were then isolated by using immunomagnetic beads (Miltenyi Biotec, Auburn, CA) according to the manufacturer’s instructions. Briefly, the digested RPE/choroid was incubated with a biotin-conjugated CD31 monoclonal antibody (MEC13.3; BD PharMingen, San Diego, CA) for 1 hour at 4°C. The cells were washed once with PBS buffer containing 2 mM EDTA and 0.5% BSA (PBS/EDTA/BSA) and resuspended in 0.5 mL of PBS/EDTA/BSA buffer. Ten microliters of anti-biotin microbeads (Milteny Biotec) were added to the cellular digests. After a 0.5-hour incubation, the cellular digests were washed with PBS/EDTA/BSA buffer and then passed over a separation column placed in the magnetic field of a separator (MACS; Miltenyi Biotec). After the column was washed with PBS/EDTA/BSA buffer, the column was removed from the magnet and retained CD31 positively selected cells in the column were flushed with a plunger. Isolated cells were quantified by counting CD31-positive cells per 200× field by fluorescence microscopy. This number was divided by the total number of cells, determined by counting DAPI-stained nuclei in the same field, to yield the percentage of CD31-positive cells within each observed field.

Double Immunolabeling for the Endothelial Cell Markers P2H12 and CD31
Double immunolabeling for CD31 (ebioscience, San Diego, CA) and P2H12 (Chemicon International, Inc.) in CECs was performed, as previously described. Briefly, sections were sequentially incubated with anti-P2H12 antibody, biotinylated anti-mouse IgG antibody, and a streptavidin-Cy-2 fluorochrome complex (1:1000; Jackson Immunoresearch Laboratory, Bar Harbor, ME). Between subsequent steps, sections were washed with PBS. Before the second primary antibody incubation, sections were blocked with streptavidin and biotin blocking solutions (Vector Laboratories) and then incubated with an anti-CD31 antibody (1:200) followed by the same procedures as P2H12 immunolabeling except for using a streptavidin-Cy-3 fluorochrome complex (1:1000; Jackson Immunoresearch Laboratory). Negative controls were performed using mouse normal IgG (1:200; Vector Laboratories). Finally, sections were mounted and analyzed with an epifluorescence microscope (Eclipse, TE 2000-U, Nikon, Tokyo, Japan) with a digital camera any accompanying software (Spot RT and software ver. 3.5; Diagnostic Imaging, Sterling Heights, MI).

Triple Immunolabeling for IkBα, pIkBα, and CD31
Triple immunolabeling for the NF-κB inhibitor, IkBα (1:200; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and phosphorylated (p)IkBα (1:200; Santa Cruz Biotechnology, Inc.) as well as an endothelial marker CD31 (1:500) were performed using a kit (MOM; Vector), according to the manufacturer’s instructions, as described earlier. Streptavidin-Cy-2, -3, and -5 fluorochrome complexes (Jackson Immunoresearch Labora-
**RESULTS**

**DNA Oxidation in the RPE/Choroid In Situ**

To study whether light induces oxidative stress in CECs in situ, 8-oxo-dG immunohistochemical experiments were performed. CECs from both the choriocapillaris and choroidal vessels from mice exposed to light for 3 hours were 8-oxo-dG immunopositive (Fig. 1A), whereas little immunoreaction was observed in untreated controls (Fig. 1B). In addition, light treatment resulted in 8-oxo-dG immunoreaction in RPE cells, melanocytes, and pericytes. Compared with dark-adapted control mice, the immunoreactivity scores for anti-8-oxo-dG antibody were significantly increased in the choriocapillaris of light-treated mice ($P < 0.001$; Fig. 1D).

**Light-Induced NF-κB Activation in the RPE/Choroid In Situ**

The antibody used in this experiment recognizes the active form of NF-κB P65 because it has specificity against the nuclear localization signal epitope, which is masked by its inhibitor, IκBα, when NF-κB is inactive in the cell’s cytoplasm. Immunohistochemical studies showed that reaction to the active form of NF-κB P65 in CECs was increased over control levels after a 3-hour exposure to light (Fig. 2). Mild immunoreactivity to the anti-NF-κB p65 antibody was observed in CECs from dark-adapted control mice. The mean immunoreactivity scores for mouse anti-NF-κB antibody were significantly increased in the choriocapillaris of light-treated mice compared with control mice ($P < 0.001$; Fig. 2D).

**Isolated CECs Doubly Labeled with CD31 and P1H12 Antibodies**

An average of 50 CECs were isolated per eye cup. The purity of isolated cells from the RPE/choroid was examined by CD31 immunolabeling. Greater than 80% of isolated cells were CD31 positive. CD31 positivity was demonstrated before (Fig. 3A) and after (Fig. 3B) CEC isolation with immunomagnetic beads. A few DAPI-stained cells (without CD-31 staining) represented nonendothelial cells, whereas CD-31-stained debris (without DAPI staining) probably represented membranous remnants of disrupted CECs (Fig. 3B). To characterize isolated cells further, we used double immunolabeling with two endothelial markers, CD31 and P1H12, which demonstrated that isolated cells...
were both CD31 (Fig. 3F) and P1H12 (Fig. 3G) positive; CD31 and P1H12 were colocalized on the cell surface (Fig. 3H).

The Quality of the Total RNA from CECs

The 28S/18S rRNA ratio of total RNA from isolated CECs was 1.5:1 (Fig. 4). Although some mild fragmentation peaks were present, both ribosomal peaks were sharp and clean (Fig. 4A). The RNA quality from isolated CECs (Fig. 4A) was equal to that from the RPE/choroid (Fig. 4B). RNA integrity was further confirmed by the expression of CD31 transcripts using primers designed from both the 5' and 3' regions of the gene (Fig. 4C). To determine the purity of isolated CEC RNA, RT-PCR for RPE65, CD31, and vWF was performed. CD31 and vWF transcripts were predominantly expressed in the CEC fraction, whereas the RPE65 transcript was predominantly in the unFractionated RPE/choroid (Fig. 4D).

Light-Induced Activation of NF-κB in Isolated CECs

Light activation of NF-κB in isolated CECs was investigated, which included IκBα phosphorylation, an initial step of the NF-κB activation, and the expression of IκBα, an NF-κB target gene. IκBα was phosphorylated in isolated CECs after mice had a 3-hour exposure to light (Fig. 5B). In contrast, IκBα protein in isolated CECs from control mice was only weakly phosphorylated (Fig. 5F). pIκBα was localized to the cytoplasm of isolated CECs that were labeled with CD31 antibody (Figs. 5C, 5G). The colocalization of IκBα, pIκBα, and CD31 in isolated CECs is also shown in Figures 5D and 5H.

No change in IκBα mRNA expression was seen after a 0.5-hour exposure to light (Fig. 6). However, the expression of IκBα was increased fivefold after 1- and 3-hour exposures to light when compared with control mice (P < 0.005). In addition, vWF expression increased twofold, and the housekeeping...
genes β-actin, GAPDH, and 18S rRNA were increased two- to four-fold after exposure to light.

**DISCUSSION**

Although necessary for vision, light can potentially induce photo-oxidative damage to the eye. We provide evidence of oxidative damage to DNA in the RPE/choroid after a 3-hour exposure to light, with increased immunoreactivity to the DNA adduct 8-oxo-dG in situ. Formed by the hydroxylation of the C-8 position of guanine, 8-oxo-dG is generated by interaction with superoxide radical (O2•-) and singlet oxygen (1O2), hydrogen peroxide (H2O2), and hydroxyl radical (•OH).21 We also found that acute exposure to light induced oxidative stress in the

**FIGURE 4.** The quality of RNA isolated from CECs as assessed by a bioanalyzer and RT-PCR. (A) Bioanalyzer evaluation demonstrated that the 28S/18S ratio in the total RNA from isolated CECs was 1.5:1, indicating that RNA was intact after the isolation procedure. Asterisk: the start of the analysis. (B) The 28S/18S ratio of total RNA from the unfractionated RPE/choroid was also 1.5:1. (C) The CD31 transcript were amplified by two different RT-PCR analyses, one from the 5′ (lane 3) and the other for the 3′ (lane 4) end of the transcript. No signals were detected in the no-template control samples for the CD31 5′ (lane 1) and 3′ (lane 2) end primer pairs. (D) Semiquantitative RT-PCR analysis was performed on cDNA generated from isolated CECs (endothelial fraction) and the RPE/choroid (unfractionated). Two endothelium-specific markers, CD31 and vWF, were amplified in the endothelial fraction, whereas RPE65 was amplified predominantly in the unfractionated RPE/choroid. GAPDH was expressed in all samples. The cDNA templates were diluted 1:10, 1:100, 1:1000, and 1:10,000 (lanes 1–4 for the unfractionated RPE/choroid, and lanes 6–9 for the endothelial portion) as indicated by the declining wedge. No signals were detected in the no-template control (NT; lane 5 for the unfractionated RPE/choroid tissue, and lane 10 for the endothelial fraction).

**FIGURE 5.** Light-induced phosphorylation of IκBα in CECs. Triple immunofluorescence analysis was performed. (A–D) In light-exposed mice, CECs were triply immunostained with biotinylated anti-IκBα, -pIκBα, and -CD31 antibodies followed by fluorescence detection using Cy2-, Cy3-, or Cy5-conjugated streptavidin. Note that IκBα (A) in the cytoplasm of CECs was phosphorylated after a 3-hour exposure to light (B), which is indicated by pIκBα immunolabeling (red). CECs were verified by CD31 (an endothelial cell surface marker) immunolabeling (blue) (C). The two separate panels in (A), (B), (C), and (D) are from two different experiments. (D) Colocalization of IκBα, pIκBα, and CD31 in CECs is shown. (E–H) Triple immunolabeling for IκBα, pIκBα, and CD31 in dark-adapted CECs. CECs isolated from unexposed mice were mildly stained with anti-pIκBα antibody. (I–K) The immunostaining controls for IκBα (I), pIκBα (J), and CD31 (K) showed mild background staining.
RPE, melanocytes, and pericytes. Although light-induced retinal lipid peroxidation has been previously demonstrated, little is known about light-induced DNA oxidation in the RPE/choroid. Because 8-oxo-G is a general marker of cellular oxidative stress, its induction is interpreted as evidence of intracellular ROS formation in the RPE/choroid during exposure to light.

Further evidence of light-induced oxidative stress in the choroid was the activation of the NF-κB signaling pathway in isolated CECs. NF-κB is a primary responsive transcription factor for oxidative stress that could represent an acute protective mechanism against photo-oxidative damage.22,23 Our experiments showed both constitutive activation of NF-κB in dark-adapted control mice and significantly increased immunoreaction to NF-κB p65 in CECs in situ of light-exposed mice. Moreover, exposure to light significantly increased pIκBα levels in isolated CECs, which is an early step in NF-κB activation.24 Finally, we measured the increased IκBα mRNA expression in isolated CECs from light-exposed mice that probably results from an autoregulatory mechanism after IκBα degradation to avoid NF-κB overactivation in response to stimuli.25 The faint immunoreaction to the anti-pIκBα antibody in dark-adapted control mice also suggests that NF-κB is constitutively active in CECs.

Kayatz et al.13 have reported that in albino mice (BALB/cj), light-induced LPs as a marker of oxidative stress, were localized to the photoreceptors, the basal infoldings of the RPE, and the caveolae, but not the cytoplasm, of CECs. This localization suggests that LPs were endocytosed into the cell and were not generated by CECs. Because one biological function of endothelial cells is lipid transportation, Kayatz et al. suggested that LPs were transported from the photoreceptors into the choriocapillaris, and subsequently away by the choroid. In their study, LPs were not observed in pigmented mice (C57BL). In related studies, LPs were detected in the photoreceptors, but not the choroid of pigmented rats.26,27 Although the protective anti-photo-oxidative effect of melanin is an obvious explanation, these different experimental results may alternatively be explained by differences in genetic background between BALB/cj and C57BL mice, or between mice and rats, and/or to different experimental conditions, such as the light source, intensity, and duration of exposure. Similar factors could explain differences in measured oxidative stress in our studies compared with the work by Kayatz et al.13 Finally, we used several different markers of oxidative stress other than LPs that may have improved the sensitivity of oxidative stress detection.

The expression levels of the endothelium-specific gene vWF as well as several housekeeping genes were increased after exposure to light. Light activates not only NF-κB, but other transcription factors. Under oxidative conditions, oxidation of cysteine residues has been shown to alter the activity of specificity protein (SP)-1, a transcription factor necessary for the expression of a number of housekeeping genes.28,29 It is possible that increased SP-1 activity induced the housekeeping gene expression in our experiments. Regardless, we found it necessary to use the number of cells, as outlined in our methods, instead of commonly used housekeeping genes for data normalization.

The choriocapillaris nourishes the RPE and the outer neural retina to the middle-limiting membrane and provides approximately 90% of the oxygen requirements for photoreceptor cells, as well as removing waste from the retina. The choriocapillaris endothelium is fenestrated, indicating active fluid transport between the choriocapillaris, the RPE, and the neurosensory retina. Therefore, endothelial dysfunction through low-level, chronic oxidative stress could disrupt normal RPE and neurosensory retinal functions by altering fluid transport. Alternatively, chronic photo-oxidative stress could result in CEC loss or closure (e.g., chronic constriction) of choroidal blood vessels leading to RPE and photoreceptor degeneration. Visible light overexposure has been proposed as a mechanism for retinal degeneration such as AMD.31,32 It is possible that choroidal endothelial abnormalities could contribute to light-related retinal and RPE degeneration.

The method of CEC isolation described in this study allowed for CEC-specific study. Other microdissection techniques, including laser microdissection, were not feasible due to CEC size, shape, and inherent intermixing with other cell types. With this technique, the RNA quality from isolated cells was preserved and used to quantify gene expression changes in RT-qPCR. The highly sensitive and quantitative nature of RT-qPCR was an advantage over other techniques, such as in situ hybridization. Of equal importance, the isolation method did not appear to alter the CEC response to oxidative stress, which was measured after cells were isolated from light-exposed mice. The drawbacks of this technique include potential contamination by other cell types and the limited quantity of RNA that was isolated from CECs. However, these limitations could be overcome by increasing the sample size or amplifying RNA with 17 RNA polymerase.

Exposure to light caused oxidative stress in CECs in albino mice, as indicated by DNA oxidation and NF-κB activation. Although many factors including ocular pigmentation and ethnic origin have been thought to contribute to aging of the RPE/choroid and possibly AMD, there is presently no established etiology that may serve as a basis for preventive medicine.33 It is important to determine the impact of choroidal melanin on light-induced CEC oxidation and dysfunction. A method for CEC isolation from mouse RPE/choroid has been developed that preserves RNA quality and does not perturb the previously induced oxidative stress response by the cells. These results may facilitate identification of endothelium-specific genes that respond to oxidative stress in vivo and determine the role of CECs in light-induced retinal degeneration. Studying the causal relationship of light-induced choroidal dysfunction to retinal degeneration may lead to a greater understanding of the mechanisms of light-induced endothelial oxidative stress on CECs and aid in the development of preventive and therapeutic approaches for oxidation-associated retinal degeneration.

**Figure 6.** Light-induced upregulation of IκBα mRNA in CECs. IκBα mRNA levels increased approximately fivefold after 1- and 3-hour exposure to light when compared with levels in dark-adapted control mice. There was no change in the expression levels of IκBα after 0.5-hour exposure to light. The data were normalized to the number of cells applied to RT-qPCR and are represented as the mean ± SD of results in three independent experiments. *P < 0.05 compared with dark-adapted control mice, by unpaired Student’s t-test.

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Acknowledgments

The authors thank Brad St. Croix for valuable suggestions on the method for isolation of CECs and Rhonda Grebe and Linping Xu for technical assistance.

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