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Blue light-induced inflammatory marker expression in the retinal pigment epithelium-choroid of mice and the protective effect of a yellow intraocular lens material *in vivo*

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ABSTRACT

Oxidative stress in the retinal pigment epithelium (RPE) is a well-accepted pathogenic change in vision-threatening diseases such as age-related macular degeneration. One source of oxidative stress is excessive light exposure, which causes excessive activation of the visual cycle. Because short wavelength light (blue light) has more energy, it is reported to be more harmful to photoreceptor cells than the other wavelengths of light. However, the biological effect of blue light in the RPE of living animals and the protective effect of a yellow intraocular lens (IOL) material that blocks blue light is still obscure. Therefore, we compared the pathogenic effect in the RPE-choroid complexes of mice exposed to light in a box made of a clear or a yellow IOL material. We measured the level of reactive oxygen species (ROS) using 2′, 7′-dichlorodihydrofluorescein diacetate, the mRNA levels of inflammatory cytokines and a macrophage marker by real-time polymerase chain reaction, and the protein level of monocyte chemotactic protein-1 (MCP-1) by ELISA. The ROS level after light exposure was suppressed in the RPE-choroids of light-exposed mice in the yellow IOL material box. In parallel, all the inflammatory cytokines that we measured and a macrophage marker were also suppressed in the RPE-choroids of light-exposed mice in the yellow IOL material box. Therefore, a yellow IOL material suppressed, and thus blue light exacerbated, the increase in the ROS level and inflammatory cytokine expression as well as macrophage recruitment in the RPE-choroid *in vivo* after light exposure.

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Oxidative stress is involved not only in systemic diseases such as cancer and diabetes (Halliwell, 2007) but also in vision-threatening diseases such as age-related macular degeneration (AMD) (Ambati et al., 2003a; Grisanti and Tatar, 2008; Mettu et al., 2012). Recruitment of macrophages and secretion of inflammatory cytokines in response to local oxidative stress in the macular area is a well-accepted pathogenetic mechanism for AMD development (Raoul et al., 2010; Sakurai et al., 2003). One source of oxidative stress can be light exposure; excessive light induces excessive activation of the visual cycle, which causes photoreceptor cell apoptosis. The role of oxidative stress in the mechanism was supported by previous findings that antioxidants, *N*-acetyl-L-cysteine (NAC) (Narimatsu et al., 2014) and lutein (Sasaki et al., 2012), can attenuate the apoptosis. Given that AMD is closely related to the condition of the retinal pigment epithelium (RPE) and choroid (Grisanti et al., 1997; Grisanti and Tatar, 2008), the influence of light exposure on the RPE-choroid is important in understanding the underlying mechanism of AMD pathogenesis (Ambati et al., 2003a). In fact, light-induced reactive oxygen species (ROS) induce inflammatory cytokines and macrophage recruitment in the RPE-choroid (Narimatsu et al., 2013).

Short wavelength light has a high level of energy; thus, ultraviolet light and blue light cause more severe damage to the retina (Kurihara et al., 2010; Remé et al., 1966; Tanito et al., 2006). The recently popularized light-emitting diodes (LEDs) produce more

Abbreviations: AMD, age-related macular degeneration; CNV, choroidal neovascularization; IOL, intraocular lens; LED, light-emitting diode; MCP-1, monocyte chemotactic protein-1; ROS, reactive oxygen species; RPE, retinal pigment epithelium.

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blue light than previous forms of artificial lighting. The lens can absorb some blue light; however, many elderly people undergo cataract surgery, which involves lens removal, with the numbers of people undergoing this surgery increasing in recent years as the population ages. Thus, yellow-tinted intraocular lenses (IOLs) that block some blue light are now widely used. The effect of yellow IOLs on attenuating photoreceptor cell death induced by light exposure is well documented in animal experiments (Kurihara et al., 2010; Tanito et al., 2006). However, differences in the biological effects of the materials of the yellow IOL and traditional clear IOL on the RPE, *in vivo*, have not been reported. Considering that inflammation in the RPE-choroid is deeply involved in AMD pathogenesis, we analyzed the influence of these materials on inflammatory changes induced by blue light in the RPE-choroid.

Seven-to eight-week-old *BALB/c* male mice (CLEA Japan, Tokyo, Japan, or Charles River Laboratories Japan, Kanagawa, Japan) were housed in an air-conditioned room (22 ± 2 °C), and maintained under 12-h dark/light cycles (light on from 8:00 to 20:00), with free access to a standard diet (CLEA Japan) and tap water. All animal experiments were conducted in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

The mice were dark-adapted by keeping them in complete darkness for 12 h. Their pupils were dilated with a mixed solution of 0.5% tropicamide and 0.5% phenylephrine (Mydrin®-P; Santen Pharmaceutical, Osaka, Japan) just before exposure to light. The mice were placed in a cage in one of 2 different light-blocking boxes; one was made of the material used in clear IOLs that block ultraviolet (UV) light (SA60AT, Alcon, Hünenberg, Switzerland), and the other was made of the material used for yellow IOLs that block blue light and UV light (SN60AT, Alcon) (Tanito et al., 2006). The transmission profiles of these IOL materials are shown, previously

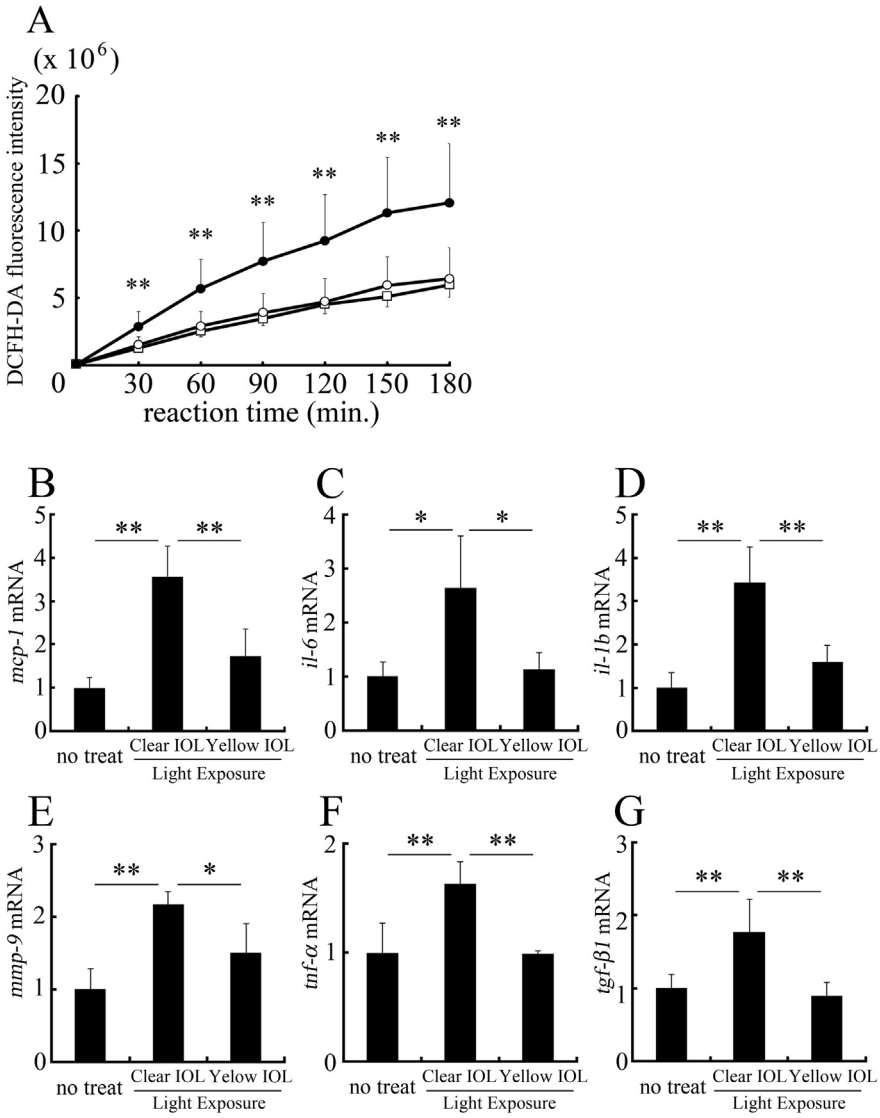

Fig. 1. Suppression of light-induced reactive oxygen species (ROS) and inflammatory cytokine mRNA levels in the RPE-choroid. (A) ROS in the RPE-choroid complex of mice 6 h after light exposure were measured by the fluorescence intensity of 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA), using a multimode plate reader. The fluorescence intensity of DCFH-DA was measured every 30 min up to 180 min after incubation with RPE-choroid samples. The intensity of DCFH-DA fluorescence was increased in the mice exposed to light in the clear IOL material box, but this increase was suppressed in the mice exposed in the yellow IOL material box. (B–G) The mRNA levels of inflammatory cytokines in the RPE-choroid 6 h after light exposure, measured by real-time RT-PCR, shown relative to the values in the mice without light exposure. All the inflammatory cytokine mRNA levels were upregulated in the mice exposed to light in the clear IOL material, but the increases were suppressed in the mice exposed in the yellow IOL material box. □; no treat, ○; Yellow IOL, ●; Clear IOL. $n = 6$. *P < 0.05, **P < 0.01. The values were processed for statistical analyses (one-way ANOVA with Tukey's post hoc test; SPSS 22, IBM, Armonk, NY).

(Tanito et al., 2006). The cages covered with the light-blocking boxes were placed inside a dedicated light-exposure box with stainless-steel mirrors on each wall and the floor (Tinker N, Kyoto, Japan), and exposed to a white fluorescence lamp (FHD100ECW, Panasonic, Osaka, Japan) at 3000 lux for 20 min. The dark-adapted control mice with no light exposure were also prepared.

Because light exposure increases the ROS level in the retina (Narimatsu et al., 2014), we first analyzed whether ROS levels were different in the RPE-choroids of the mice after exposure to light in the box made of clear or yellow IOL material (Fig. 1A). Separating the RPE from the choroid is technically impossible, so the samples were prepared in a complex. ROS levels in the isolated RPE-choroid complexes 6 h after light exposure were measured by incubating the samples with 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA; Life Technologies, Carlsbad, CA) at 37 °C. The fluorescence intensity was measured with a multimode plate reader (Wallac ARVO SX 1420 Multilabel Counter; PerkinElmer, Waltham, MA) every 30 min from the beginning of the incubation. The ROS level was significantly increased in the RPE-choroids of the mice exposed to light in the box made of clear IOL material compared with the level of the mice with no light exposure. However, this change was significantly suppressed when the mice were exposed to the light in the box made of yellow IOL material.

We previously reported that light exposure upregulates inflammatory cytokines in the RPE-choroid in response to light-induced ROS (Narimatsu et al., 2013). Thus, we next measured the mRNA levels of inflammatory cytokines in the RPE-choroid 6 h after light exposure (Fig. 1B–G). Real-time RT-PCR was performed using TaqMan Gene Expression Assays for the specific genes (TaqMan probe assay IDs: *mcp-1*; Mm00441242_m1 and *mmp-9*; Mm00442991_m1, Life Technologies) or the SYBR system (Life Technologies) with the following primers: *il-6*, forward 5′-AAGTCGGAGGCTTAATTACACATGT-3′ and reverse 5′-CCATTGCACAACTCTTTTCTCATTC-3′; *il-1b*, forward 5′-TCCAGGATGAGGACATGAGCAC-3′ and reverse 5′-GAACGTCACACCAGCAGGTTA-3′; *tnf-a*, forward 5′-GCCACCACGCTCTTCTGTCTA-3′ and reverse 5′-GATGAGAGGGAGGCCATTTG-3′; *tgf-b1*, forward 5′-GCCACCACGCTCTTCTGTCTA-3′ and reverse 5′-GATGAGAGGGAGGCCATTTG-3′ (Hokkaido System Science, Hokkaido, Japan). All the mRNA levels were normalized to those of *gapdh* in each system (TaqMan Gene Expression Assays: catalog number 4352339E, Life Technologies; SYBR system: forward 5′-AGGAGCGAGACCCCACTAAC-3′ and reverse 5′-GATGACCCTTTTGGCTCCAC-3′, Hokkaido System Science).

Monocyte chemotactic protein-1 (MCP-1) is the major contributor in recruiting macrophages and has a substantial effect on the pathogenesis of AMD (Suzuki et al., 2012). In fact, chronic exposure to light induces choroidal neovascularization (CNV), but this is suppressed in *mcp-1* knockout mice (Suzuki et al., 2012). Acute exposure to light induces MCP-1 in the retina at both mRNA and protein levels and subsequently recruits macrophages to the RPE-choroid, which suggests that light exposure is a risk factor for increasing AMD development (Narimatsu et al., 2013). In this study, the increase in the *mcp-1* mRNA level observed in the RPE-choroid of mice exposed to the light in the box made of clear IOL material was suppressed in the mice exposed to the light in the box made of yellow IOL material (Fig. 1B).

Several inflammatory cytokines are also involved in promoting CNV in laser-induced CNV models. The levels of IL-6 increase in the serum of AMD patients (Seddon et al., 2005) and IL-6 acts on both macrophages and the vascular endothelium to promote CNV, most likely through STAT3 activation (Campa et al., 2010; Izumi-Nagai et al., 2007). An IL-1β receptor antagonist suppresses laser-induced CNV, without reducing macrophage infiltration, suggesting that IL-1β has a direct effect on vascular endothelium proliferation (Lavalette et al., 2011). Matrix metalloproteinase-9 (MMP-9) is a proteolytic enzyme that breaks down the extracellular matrix, which targets type IV collagen in Bruch's membrane (Xu et al., 2012) and can promote the infiltration of inflammatory cells (Lambert et al., 2002). Surgically removed human CNV tissue also expresses TNF-α (Jasielska et al., 2010) and TGF-β (Amin et al., 1994), supporting the involvement of these cytokines in CNV pathogenesis.

We found that the mRNA levels of all these inflammatory cytokines were upregulated in the RPE-choroids of the mice exposed to light under clear IOL material, but the changes were significantly suppressed in those exposed under yellow IOL material (Fig. 1C–G), indicating that light-induced induction of inflammatory cytokines was suppressed in the RPE-choroid when blue light was blocked by the yellow IOL material.

We also measured the protein level of MCP-1 in the RPE-choroid 24 h after light exposure with ELISA using a Quantikine Mouse CCL2/JE/MCP-1 Immunoassay kit (R&D Systems, Minneapolis, MN). In our previous study, this time point was when MCP-1 protein level increased in the RPE-choroid of light-exposed mice with no intervention (Narimatsu et al., 2013). Consistent with the mRNA results in this study, light-induced increase in the MCP-1 protein level was significantly suppressed in the mice exposed to light under yellow IOL material compared with mice exposed under clear IOL material (Fig. 2A). Moreover, because MCP-1 recruits macrophages (Ambati et al., 2003b; Espinosa-Heidmann, 2003; Sakurai et al., 2003; Takeda et al., 2009), we analyzed the mRNA expression of *f4/80*, a macrophage marker, by real-time RT-PCR using TaqMan Gene Expression Assays for the specific genes (*f4/80*, Mm00802529_m1; and *gapdh*, catalog number 4352339E; Life Technologies). The increase in the level of *f4/80* after light exposure was clearly lower in the RPE-choroids of the mice exposed to light under yellow IOL material than clear IOL material (Fig. 2B). Taken together, blue light blockade suppressed the induction of MCP-1 and subsequent macrophage recruitment in the RPE-choroid after light exposure.

As shown above, light-induced induction of inflammatory cytokines and macrophage recruitment through ROS in the RPE-choroid (Narimatsu et al., 2013) were suppressed by blocking the short wavelength of light, corresponding to blue light, with yellow IOL material. Therefore, blue light significantly increases inflammatory markers (i.e., induction of inflammatory cytokines and

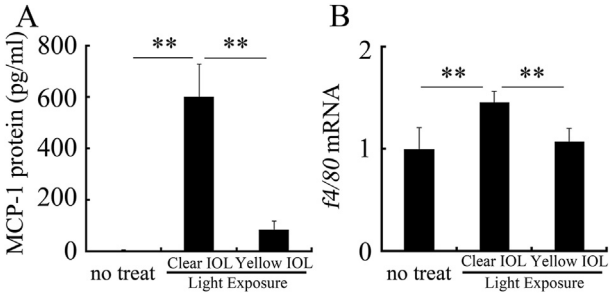

Fig. 2. Suppression of the light-induced monocyte chemotactic protein-1 (MCP-1) protein level and macrophage recruitment in the RPE-choroid. (A) The protein level of MCP-1 in the RPE-choroid, measured by ELISA 24 h after light exposure, was higher in the mice exposed to light in the clear IOL material box than in the mice without light exposure, however, the increase was suppressed in the mice exposed in the yellow IOL material box. (B) The mRNA levels of *f4/80* in the RPE-choroid at 24 h after light exposure were measured by real-time RT-PCR and are shown relative to the values in the mice without light exposure. The light-induced increase in the *f4/80* mRNA level was suppressed in the mice exposed to light in the yellow IOL material box. $n = 6$. **$P < 0.01$. The values were processed for statistical analyses (one-way ANOVA with Tukey'spost hoc test; SPSS 22, IBM).

macrophage recruitment) in the RPE-choroid.

In this study, we demonstrated the effect of blue light in the RPE-choroid for inducing inflammatory markers *in vivo*. The molecular mechanism underlying marker induction involves accumulation of light-induced ROS, as shown in a previous study using mice that were exposed to all wavelengths of fluorescent light (Narimatsu et al., 2013). In this study, the yellow IOL material reduced the ROS level after light exposure in the RPE-choroid, suggesting that the ROS accumulation in the RPE-choroid of the light-exposed mice was substantially mediated by the blue light. This ROS reduction by blue light blockade was the most likely mechanism for the attenuation of the inflammatory markers after light exposure in the box made by yellow IOL material.

The effect of blue light in ROS accumulation was consistent with a previous report showing that accumulation of oxidative stress induced by chronic blue light exposure increases the rate of CNV by inducing MCP-1 (Suzuki et al., 2012). Our results that CNV-related inflammatory markers (including MCP-1 expression) were attenuated by using yellow IOL material to block blue light are consistent with this finding. Therefore, blocking blue light to prevent ROS accumulation could lower the risk of CNV development *in vivo*.

In summary, using yellow IOL material to block blue light reduced the accumulation of ROS and subsequent induction of inflammatory markers in the RPE-choroid *in vivo*. Although our results were obtained from an acute light exposure model, repeated exposure and subsequent accumulation of the inflammatory activity could be involved in CNV pathogenesis. Further study to understand the biological importance of blue light and the effect of materials that block blue light is required.

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