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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.

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

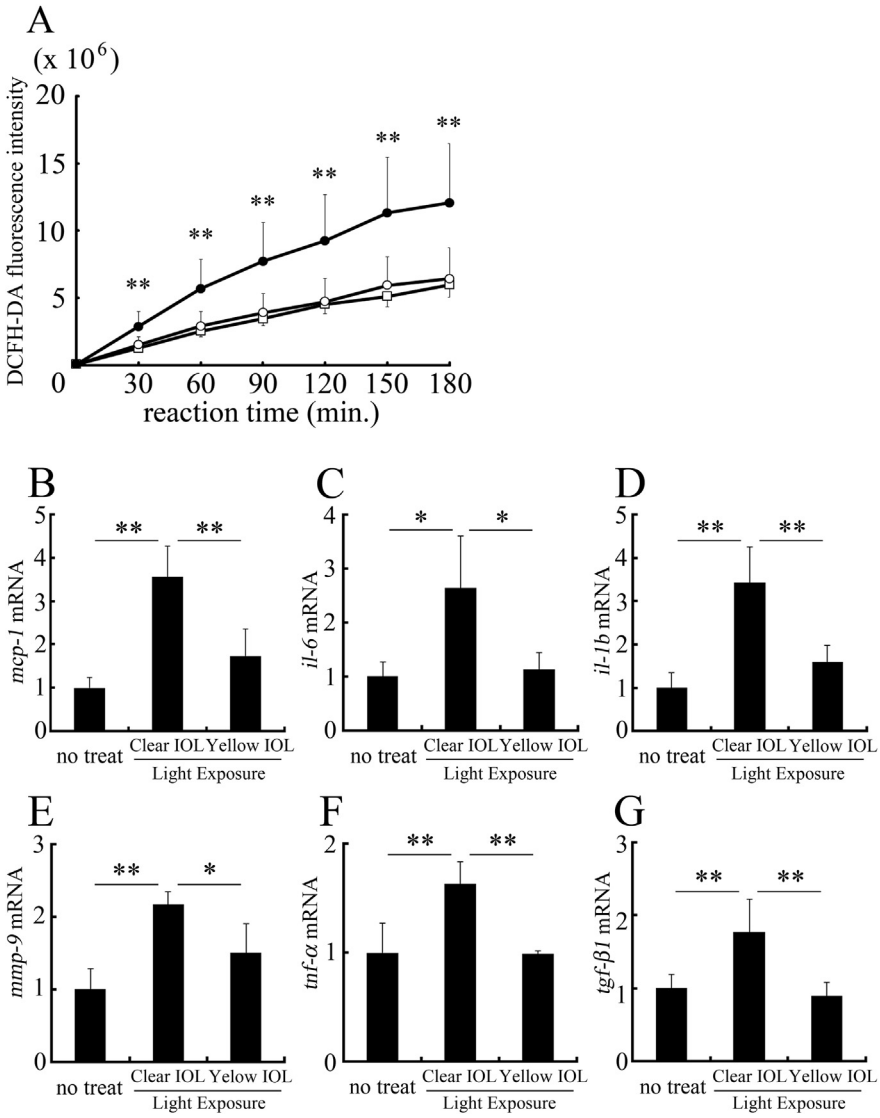

Fig. 1. Suppression of light-induced reactive oxygen species (ROS) and inflammatory cytokine mRNA levels in the RPE-choroid. (A) ROS in the RPE-choroid complex of mice 6 h after light exposure were measured by the fluorescence intensity of 2′, 7′-dichlorodihydrofluorescein diacetate (DCFH-DA), using 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).