Mini Review

Photo-damage mechanisms and anti-apoptotic effect of lutein in the mouse retina

Seiji Miyake[1, 3)], Mariko Sasaki[1, 2)], Noriko Takahashi[1, 2)], Kazuo Tsubota[2)] and Yoko Ozawa[1, 2, *)]

[1)]Laboratory of Retinal Cell Biology, [2)]Department of Ophthalmology, Keio University School of Medicine, Tokyo, Japan

[3)]Wakasa Seikatsu Co., Ltd., Kyoto, Japan

Photoreceptor cells receive light and transduce it to electrical signals for visual perception. However, excessive exposure to visible light causes photoreceptor cells to undergo apoptosis, which is called photo-damage. This damage involves several biochemical events, including the accumulation of oxidative stress and the elevation of intracellular calcium and nitric oxide (NO). Photo-damage is thought to be related to the progression of retinitis pigmentosa and age-related macular degeneration. Therefore, understanding the molecular mechanisms of retinal photo-damage using model animals may lead to new therapeutic approaches for preventing the progression of these ocular diseases. In this review, we summarize previous reports examining the mechanisms of light-induced retinal damage, and briefly describe the interventional effect of lutein against photo-damage in mice. Lutein is taken from food and systemically delivered to the retina, skin, and certain organs and tissues. It reduces the level of reactive oxygen species and acts as an anti-oxidant in the retina of light-exposed mice, ultimately preventing light-induced DNA double-strand breaks and apoptosis. Although further study is required, lutein may be proposed as a new therapeutic approach for preventing photo-damage in humans.

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*Correspondence should be addressed to:
Yoko Ozawa, M.D., Ph.D., Department of Ophthalmology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Phone: +81-3-3353-1211, Fax: +81-3-3359-8302, E-mail: ozawa@a5.keio.jp

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Introduction

Light is an essential external factor for living things, required for sight in animals as well as photosynthesis and growth in plants. Light is also important for developing visual system; it maturates, receiving light stimuli after birth[1)]. However, light can also induce adverse effects on the eyes; the exposure to excessive and/or intense light induces irreversible visual dysfunction. Noell et al. first demonstrated this effect in light-exposed animals[2)]. That report was followed by extensive studies *in vivo* and *in vitro* on the relationship between light and retinal degeneration[3, 4)].

Light-induced photoreceptor apoptosis is reported to occur in several phases, and many of the contributing factors have been identified[4, 5)], although the entire process of

photo-damage has remained elusive. Interestingly, the first step (the induction phase) of the damage may be triggered by rhodopsin, an essential protein for light perception[2, 6, 7]. For instance, rhodopsin knock-out mice are protected against photo-damage[8]. Furthermore, inhibition of the visual cycle by 13-cis retinoic acid, a putative 11-cis retinal dehydrogenase inhibitor[9, 10], also prevents photo-damage[11]. The chaperone protein RPE65 is distributed in the retinal pigment epithelium, where the photoreceptor cells undergo phagocytosis[12]; it is involved in the conversion of all-trans retinol, to 11-cis retinal[13]. RPE65 knockout mice are also protected against light damage[8, 14]. Taken together, the evidence indicates that excessive stimulation of the visual cycle is an important mediator of photo-damage; moreover, accumulation of a rhodopsin bleaching intermediate, all-trans retinal, is now proposed to be responsible for photo-damage in the retina[15].

Following this induction phase, the death-signal phase can be divided into two sub-phases, early and late[5]. In the early phase, the intracellular calcium level increases, possibly caused by the activation of NO synthase. NO is a gaseous signaling molecule with physiological and pathological actions *in vivo*[16]. While a moderate level of NO in the central nervous system (CNS) is involved in synapse formation, its overexpression is reported to trigger intracellular disorders such as endoplasmic reticulum stress, mitochondrial morphologic change[17], and membrane depolarization[18].

In the late phase, AP-1 activation plays an essential role in mediating photoreceptor apoptosis[17]. AP-1, a major nuclear transcription factor composed of c-Fos and c-Jun heterodimers, regulates various cellular events, including cell transformation, proliferation, differentiation, and apoptosis[19]. Comprehensive gene expression analysis revealed that a component of the AP-1 transcription factor, c-Fos, is upregulated in the photo-damaged retina[20], and the DNA-binding activity of AP-1 is increased after light exposure[21]. Mice that are deficient in c-*fos* exhibit normal retinal function and morphology[22], but are highly resistant to photo-damage, compared with wild-type mice[23]. Many current studies are aimed at understanding the role of AP-1 in retinal light damage; however, the molecules that function downstream of AP-1 activation in photo-damage are still unknown[5].

In addition to AP-1, caspases, a group of cysteine proteases[24], are also believed to contribute to retinal photo-damage. In the light-exposed retina, the caspase-1 mRNA and protein levels increase[25, 26], suggesting that at least caspase-1 participates in the induction of photoreceptor apoptosis.

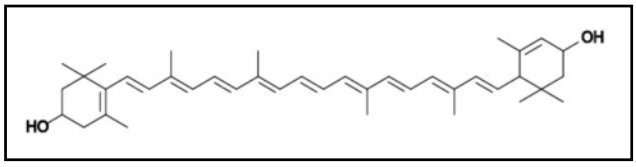

Fig.1 Structure of lutein
Double bonds and hydroxyl groups are believed to play critical roles in its biological functions[39, 40]

An important implication of understanding the mechanism of photo-damage, is the possibility of developing new strategies for neuroprotection, in which these steps of the apoptotic pathway are inhibited. Several cytokines are reported to protect against photo-damage[5]. Interestingly, a recent study revealed that erythropoietin, which stimulates hematopoiesis, exerts a neuroprotective effect on light-induced retinal degeneration[27]. An anti-inflammatory drug, naloxone, also reduces retinal damage[28, 29], consistent with inflammatory events being associated with the light-exposed retina[30-32].

These observations led us to examine whether molecules with antioxidant activity could prevent retinal apoptosis and preserve visual function after light exposure. We recently showed that lutein (Fig.1), an antioxidant also known as a food factor, scavenges reactive oxygen species (ROS) and protects visual function against inflammatory ocular diseases[33,34]. Thus, we next evaluated the beneficial effect of the oral administration of lutein on light-induced retinal degeneration.

Lutein attenuates retinal photo-damage

To elucidate the protective effect of lutein on light-induced retinal degeneration, we exposed lutein-treated and vehicle-treated BALB/c mice to 5000 lux of white light for 3 hours after at least 12 hours of dark adaptation. Five days after the light exposure, we performed electroretinography to assess the biological effect of lutein on visual function. In the vehicle-treated mice, light exposure induced a significant reduction in the amplitude of the a-wave, which reflects photoreceptor cell function, and the b-wave, which reflects the subsequent electrical reaction transmitted from the photoreceptor cells. However, strikingly, in the lutein-

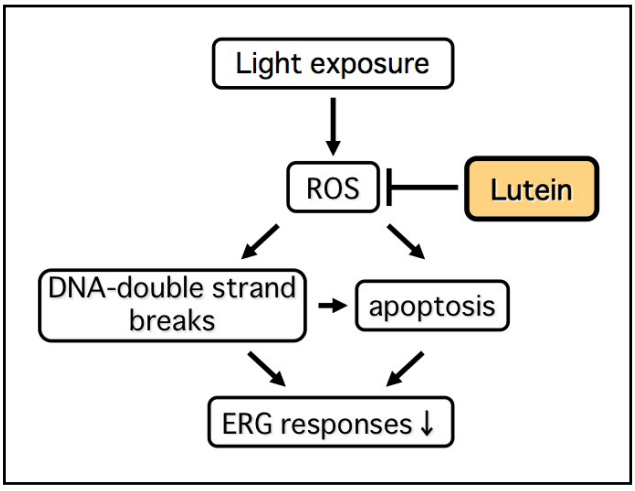

Fig.2 Protective effect of lutein against photo-damage in the retina

Lutein intake reduces the ROS level in the retina. This suppresses DNA-double strand breaks and apoptosis, finally preventing neuronal dysfunction.

treated mice, the reduction of these amplitudes was significantly attenuated. Because photoreceptor cells are susceptible to light exposure[35], we also measured the thickness of the photoreceptor cell layer after light exposure. Consistent with the electroretinography results, the thickness of this cell layer was reduced after light exposure, and this effect was significantly suppressed in the lutein-supplemented mice compared with vehicle-treated mice.

One manifestation of apoptosis is the appearance of double-stranded DNA breaks (DSBs), which is detectable as DNA fragmentation[4]. DNA damage results in the rapid phosphorylation of Histone H2AX, which has an important role in the repair of DSBs, at Ser139 in its C-terminus. To detect the effect of lutein in protecting against DSBs, the expression of phosphorylated H2AX (called gamma-H2AX) was examined by immunohistochemistry. The results showed that lutein-fed mice had fewer gamma-H2AX-positive photoreceptor cells than the controls.

The dephosphorylation of tyrosine142 of H2AX by EYA3 contributes to DNA repair rather than promoting apoptotic processes[36]. Therefore, we further investigated the expression of EYA3 with the concomitant detection of gamma-H2AX. EYA3 was expressed only in the photoreceptor cell layer after light exposure, and there were significantly more EYA3-expressing cells in the mice fed a lutein-supplemented diet than in control mice. This upregulation of EYA3-positive cells was also shown by western blotting.

To elucidate the effect of lutein on the ROS level in retinas after light exposure, the fluorescent probes dihydroethidium (DHE) and BODIPY-C11 were used as indicators of intracellular superoxide radicals[37] and lipid peroxidation[38], respectively. The fluorescence intensity of DHE increased in all the retinal layers after light exposure, but it was clearly suppressed in the mice fed the lutein-supplemented diet. The latter sign of oxidization appeared in the outer segment of photoreceptor cells in light-exposed mice fed control chow compared with non-light-exposed mice. However, this increase was significantly suppressed in the retinas of light-exposed mice fed a lutein-supplemented diet. These observations suggest that lutein's ROS-reducing effect may be protective against the terminal phase of photo-damage, reducing the amounts of DSBs and apoptosis (Fig.2).

Conclusion

The influence of light exposure on retinal damage increases with age, and is involved in the progression of some ocular diseases, such as retinitis pigmentosa (hereditary retinal degeneration) and age-related macular degeneration. Therefore, evidence-based preventive therapies against photo-damage are required. Photo-damage occurs not only in ocular tissues but also in the skin. Because lutein is physiologically obtained from food and delivered to the retina and skin, its therapeutic use against photo-damage of both tissues may be feasible. Further studies aimed at revealing the molecular mechanisms of lutein's effects will help us discover new treatments for protecting tissues from photo-damage.

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Conflict of interests

None

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