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Bisretinoid Photodegradation Is Likely Not a Good Thing

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Abstract

Retinaldehyde adducts (bisretinoids) accumulate in retinal pigment epithelial (RPE) cells as lipofuscin. Bisretinoids are implicated in some inherited and age-related forms of macular degeneration that lead to the death of RPE cells and diminished vision. By comparing albino and black-eyed mice and by rearing mice in darkness and in cyclic light, evidence indicates that bisretinoid fluoro-phores undergo photodegradation in the eye (Ueda et al. *Proc Natl Acad Sci* 113:6904–6909, 2016). Given that the photodegradation products modify and impair cellular and extracellular molecules, these processes likely impart cumulative damage to retina.

Keywords

Retinal degeneration; Age-related macular degeneration; ABCA4-associated disease; Bisretinoid; Visual cycle; Lipofuscin; Retinaldehyde; Vitamin E

49.1 Introduction

Adducts of vitamin A aldehyde having bisretinoid structures form in photoreceptor cells by non-enzymatic reactions between retinaldehyde and amine moieties (Sparrow et al. 2010). These fluorophores are deposited in RPE cells as components of phagocytosed outer segments and constitute the lipofuscin of RPE. Various bisretinoids of RPE lipofuscin have been isolated and structurally characterized (Sparrow et al. 2012).

Bisretinoids form in particular abundance in recessive Stargardt disease (STGD1) caused by mutations in the gene encoding the ATP-binding cassette transporter (ABCA4). This accumulation culminates in the death of RPE. Marked bisretinoid accumulation is replicated in *Abca4* null mutant mice (Weng et al. 1999; Kim et al. 2004), and the relationship between RPE lipofuscin accumulation and retinal disease is evidenced in the *Abca4* mouse model by Bruch's membrane changes and by a progressive loss of photoreceptor cells (Radu et al. 2008; Wu et al. 2010a; Radu et al. 2011; Sparrow et al. 2013; Zhou et al. 2015). *Abca4*^{-/-} mice burdened by elevated levels of bisretinoid lipofuscin are also more susceptible to light

damage than are wild-type mice (Wu et al. 2014). Here we review recent work establishing that photodegradation of bisretinoid and its adverse consequences are ongoing in the eye.

49.2 Bisretinoid Photooxidation and Photodegradation in Vitro

Given the adverse consequences of bisretinoid accumulation, efforts have been made to elucidate mechanisms by which these fluorophores damage cells. For instance, bisretinoids photogenerate reactive oxygen species such as singlet oxygen and superoxide anion (Gaillard et al. 1995; Rozanowska et al. 1995; Ben-Shabat et al. 2002; Jang et al. 2005; Kim et al. 2007; Yamamoto et al. 2011). By quenching these reactive forms of oxygen, bisretinoids are subsequently photooxidized, and with photocleavage at these oxidation sites, aldehyde- and dicarbonyl-carrying fragments are released (Wu et al. 2010b). Proteins modified by these dicarbonyls are constituents of drusen (Farboud et al. 1999; Handa et al. 1999); they cross-link protein and promote resistance to the activity of matrix metalloproteinases (Zhou et al. 2015).

49.3 Bisretinoid Levels in Albino and Black Mice Housed in Cyclic Light or Darkness

The photodegradation of bisretinoid was also demonstrated in mice by comparing levels of bisretinoid in mice raised in cyclic light (12 h on/12 h off) as opposed to darkness and by comparing albino (C57BL/6J^{c2j}) and black (C57BL/6 J) mice. In the absence of melanin, light entering the eye is substantially increased (LaVail and Battelle 1975; van den Berg et al. 1991). The bisretinoid A2E was detected in eyes from both cyclic light- and dark-reared mice (Fig. 49.1) (Boyer et al. 2012). Whereas it might be expected that the added photon catch in the albino eye would drive the formation of these visual cycle adducts (bisretinoids), instead A2E levels were lower in albino C57BL/6J^{c2j} mice maintained under cyclic light than in albino C57BL/6J^{c2j} mice reared in darkness ($p < 0.05$) (Fig. 49.1).

Given in vitro evidence of photodegradation (Wu et al. 2010b) and the presence of oxidized bisretinoid in human and mouse retina (Jang et al. 2005; Kim et al. 2007), the most parsimonious explanation for the light-related differences is photooxidation-associated photodegradative loss of these light-sensitive fluorophores.

It is likely that bisretinoid formation under cyclic light is more pronounced than the levels observed here; for instance, photodegradative loss could mask even greater lipofuscin formation under cyclic light. The comparison of albino versus black-eyed mice presumably allowed photodegradative loss of A2E to be detected over a relatively short period of time. These photobleaching processes have been replicated in cell-based and non-cellular assays (Yamamoto et al. 2012), and examples of photooxidation and photodegradation of RPE bisretinoids (photobleaching) in retinæ of human and nonhuman primates are reported (Hunter et al. 2012; Sparrow and Duncker 2014).

49.4 Vitamin E-Treated Mice: Evidence Supporting Photooxidative Processes in Modulating Bisretinoid Levels

It has been shown previously that the lipid-soluble antioxidant vitamin E can suppress bisretinoid oxidation by quenching singlet oxygen (Sparrow et al. 2003b). In albino *Abca4*^{-/-} mice given a vitamin E-supplemented diet (960 mg (IU)/kg vitamin E as dl- α -tocopheryl acetate) from 1 to 6 months of age, levels of the bisretinoids A2E ($p < 0.05$ one-way ANOVA and Sidak's multiple comparison test), A2-DHP-PE, and all-*trans*-retinal dimer-PE were greater than in control mice. Quantitation of short-wavelength fundus autofluorescence (quantitative fundus autofluorescence, qAF) (Sparrow et al. 2013; Flynn et al. 2014) that originates from bisretinoid lipofuscin also revealed higher levels of fundus autofluorescence in the vitamin E-treated mice ($p < 0.05$, two-tailed *t*-test) (Fig. 49.1). Importantly, the thinning of outer nuclear layer that is indicative of reduced photoreceptor cell viability and that has been observed in albino *Abca4*^{-/-} mice (Wu et al. 2010a; Sparrow et al. 2013; Wu et al. 2014) was less pronounced in the vitamin E-treated mice ($p < 0.05$, two-tailed *t*-test) (Fig. 49.1). The greater levels of bisretinoid and qAF in the vitamin E-treated versus control mice are consistent with a mechanism involving a reduction in photooxidation-associated consumption of bisretinoid in the presence of the antioxidant vitamin E.

49.5 Implications

The findings discussed here indicate that although light deprivation does not prevent the formation of bisretinoids (Boyer et al. 2012; Ueda et al. 2016), limiting light exposure can protect against damaging bisretinoid photodegradation. Thus not surprisingly, a black contact lens that blocked >90% of light in one eye of STGD1 patients was found to reduce the progression of decreased fundus autofluorescence (four of five patients) as compared with the patients' unprotected eyes (Teussink et al. 2015). Sunglasses that attenuate light over a broad range of wavelengths or yellow lenses that reduce "blue" wavelengths might also be used to advantage.

Several studies, most notably the Age-Related Eye Disease Study (AREDS), have demonstrated that dietary antioxidants and intake of antioxidants by supplementation reduces incidence or progression of AMD (Snodderly 1995; Age-Related Eye Disease Study Research 2001; SanGiovanni et al. 2007; Sobrin and Seddon 2014). Given that antioxidants can protect against AMD and that vitamins E and C have also been shown to reduce A2E photooxidation/photodegradation (Sparrow et al. 2003; Zhou et al. 2006), the beneficial effects of antioxidant intake could be mediated at least in part by intercepting bisretinoid photooxidation and degradation. Similarly, a contribution of lifetime light exposure to AMD risk (Cruickshanks et al. 2001; Tomany et al. 2004; Fletcher et al. 2008; Sui et al. 2013; Huang et al. 2014; Klein et al. 2014; Fritsche et al. 2016) could be mediated in part by the cellular damage imposed by bisretinoid photodegradation.

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Fig. 49.1.

The RPE bisretinoid A2E accumulates in both light- and dark-reared mice, levels are reduced in light-reared versus dark-reared albino mice, and levels are modulated by antioxidant status. Analysis by reverse-phase HPLC. **(a)**: Quantitation of the bisretinoid A2E in 6-month-old black C57BL/6J and albino C57BL/6J^{c2j} mice that were dark reared or cyclic light reared from birth. Means $\pm$ SEM of five or seven independent samples six to eight eyes/sample p -values determined by one-way ANOVA and Tukey's multiple comparison test and two-tailed t -test. **(b, c)**: Reduction in bisretinoid photooxidation by vitamin E supplementation is detected as reduced bisretinoid loss and measured as increased quantitative fundus autofluorescence (qAF) (b) and increased HPLC-quantified A2E, A2-DHP-PE, and atRAL di-PE (c). Means $\pm$ SEM of eight mice, $p < 0.05$, two-tailed t -test. **(a)**: Two samples (six eyes per sample), ANOVA and Sidak's multiple comparison test (b). **(d)**: Reduced bisretinoid photooxidation/photodegradation also protects against outer nuclear layer (ONL) thinning. ONL area (μm^2) calculated as the sum of the ONL thicknesses in the superior and inferior retina (0.2–2.0 mm from optic nerve head) multiplied by the measurement interval of 200 microns; means $\pm$ SEM, two-tailed t -test