Iron promotes oxidative cell death caused by bisretinoids of retina

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Intracellular Fe plays a key role in redox active energy and electron transfer. We sought to understand how Fe levels impact the retina, given that retinal pigment epithelial (RPE) cells are also challenged by accumulations of vitamin A aldehyde adducts (bisretinoid lipofuscin) that photogenerate reactive oxygen species and photodecompose into damaging aldehyde- and dicarbonyl-bearing species. In mice treated with the Fe chelator deferiprone (DFP), intracellular Fe levels, as reflected in transferrin receptor mRNA expression, were reduced. DFP-treated albino Abca4−/− and agouti wild-type mice exhibited elevated bisretinoid levels as measured by high-performance liquid chromatography or noninvasively by quantitative fundus autofluorescence. Thinning of the outer nuclear layer, a parameter indicative of the loss of photoreceptor cell viability, was also reduced in DFP-treated albino Abca4−/−. In contrast to the effects of the Fe chelator, mice burdened with increased intracellular Fe in RPE due to deficiency in the Fe export proteins hephaestin and ceruloplasmin, presented with reduced bisretinoid levels. These findings indicate that intracellular Fe promotes bisretinoid oxidation and degradation. This interpretation was supported by experiments showing that DFP decreased the oxidative/degradation of the bisretinoid A2E in the presence of light and reduced cell death in cell-based experiments. Moreover, light-independent oxidation and degradation of A2E by Fenton chemistry products were evidenced by the consumption of A2E, release of dicarbonyls, and generation of oxidized A2E species in cell-free assays.

Significance

Cells are subject to metabolic sources of oxidizing species and to the need to regulate Fe, a redox-active metal. Retinal pigment epithelial (RPE) cells have to contend with an additional, unique source of oxidative stress: photooxidative insult from bisretinoids that accumulate as lipofuscin. Here we report that Fe can interact with bisretinoids in RPE to promote cell damage. These findings inform disease processes in both Fe-related and bisretinoid-associated retinal degeneration. The link between Fe and bisretinoid oxidation also highlights opportunities for repurposed and combination therapies. This could include visual cycle inhibitors as a treatment for maculopathy associated with elevated retinal Fe, and Fe chelation to aid in suppressing the damaging effects of bisretinoids in juvenile and age-related macular degeneration.

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We delivered DFP to wild-type agouti 129/SvJ and agouti Abca4<sup>-/-</sup><sub>trans</sub> mice, A2E/iso-A2E were present in amounts that were twofold higher (<0.05) than in control untreated mice (difference as percentage of control) (Fig. 1). A2E levels in the DFP-treated agouti wild-type mice were 49% higher than in untreated mice (<0.05). This treatment effect can be explained as a DFP-mediated reduction in the degradative loss of bisretinoid. Since photodegradation of bisretinoid is more pronounced in the albino eye (17), high-performance liquid chromatography (HPLC)-quantified bisretinoid was not appreciably different in albino Abca4<sup>+/+</sup> versus agouti Abca4<sup>−/−</sup> mice (Fig. 1 B and C).

Fig. 1. The bisretinoids A2E, A2-GPE, and A2-DHP-PE in mice treated with the Fe chelator DFP. Oral DFP was administrated from age 2 to 6 mo. (A) Representative reverse phase HPLC chromatographs illustrating the detection of all trans-retinal (atRAL), A2E (Rt 37.6 m), iso-A2E (Rt 39.4 m), A2-GPE (Rt 39.8 m), and A2-DHP-PE (Rt 45.3 m). (Insets at Right) UV-visible spectra of chromatographic peaks corresponding to A2E (all-trans-A2E), iso-A2E (C13/14 cis-A2E), A2-GPE, and A2-DHP-PE. (B and C) Chromatographic quantitation of bisretinoids in albino Abca4<sup>−/−</sup> and agouti 129 wild-type mice. A2E (the sum of all-trans-A2E and iso-A2E) and A2-DHP-PE were quantified by HPLC. A2-GPE was quantified by UPLC. Values are means ± SEM; four eyes (two mice) or six eyes (three mice) were pooled for each sample of DFP-treated or control albino Abca4<sup>−/−</sup> mice, and eight eyes (four mice) were pooled for agouti 129SvJ mice. P values were determined by one-way ANOVA and Sidak’s multiple comparison test. Rt, retention time.

Quantitative fundus autofluorescence (qAF) levels in mice aged 4 and 6 mo. Mice were treated with DFP beginning at 2 mo of age. Ueda et al. SEM, eight or nine mice per group. Measuring Retinal Fe Levels Through Transferrin Receptor qPCR. When cells need more Fe, transferrin receptor mRNA is stabilized, leading to more Fe uptake (33). Transferrin receptor

Fig. 2. Quantitative fundus autofluorescence (qAF) (488 nm) in Abca4<sup>−/−</sup> albino mice aged 4 and 6 mo. Mice were treated with DFP beginning at 2 mo of age. (A) qAF increases in DFP-treated mice. Values are means ± SEM, eight or nine mice per group. P value was determined by one-way ANOVA and Sidak’s multiple comparison test. (B) Short-wavelength fundus autofluorescence images acquired for qAF analysis. Note that in the two images, the fundus appears to present with similar gray levels; however, the internal reference at the Top of the image of the DFP-treated mouse is darker, indicating higher fundus AF (qAF) levels.

Fig. 2. (A) qAF increases in DFP-treated mice. Values are means ± SEM, eight or nine mice per group. P value was determined by one-way ANOVA and Sidak’s multiple comparison test. (B) Short-wavelength fundus autofluorescence images acquired for qAF analysis. Note that in the two images, the fundus appears to present with similar gray levels; however, the internal reference at the Top of the image of the DFP-treated mouse is darker, indicating higher fundus AF (qAF) levels.
mRNA levels reflect intracellular Fe concentrations (23, 34), since in cells needing more Fe, transferrin receptor mRNA is stabilized. In Abca4<sup>−/−</sup> mice receiving DFP in drinking water from age 2 mo, transferrin receptor mRNA levels, quantified in neural retina by qRT-PCR (35) were 1.73-fold greater than controls (P < 0.05) at 4 mo of age, while the fold change in RPE/choroid/sclera was 1.66 (P < 0.05) (Fig. 3).

**Discussion**

Here in Abca4<sup>−/−</sup> mice treated with the Fe chelator DFP, we observed an increase in HPLC quantified bisretinoid and higher fundus AF intensity measured as qAF. Conversely, elevated Fe in RPE of Cp<sup>−/−</sup>; Heph<sup>−/−</sup> mice (39) resulted in decreased bisretinoid. These findings indicate that Fe can promote the oxidation-based degradation of bisretinoid. This interpretation is further supported by ancillary in vitro data showing that DFP protected against degradative loss of A2E in the presence of light, while Fe and H<sub>2</sub>O<sub>2</sub> promoted the degradation of A2E. The DFP-associated protection against ONL thinning also indicates that the process of Fe-promoted bisretinoid oxidation-associated degradation contributes to photoreceptor cell loss in ABCA4-related disease. These findings are consistent with our previous observation that the antioxidant activity of vitamin E conserved levels of RPE bisretinoid by suppressing photooxidation (17), the
higher levels of RPE bisretinoid being evidenced by quantitative HPLC and gAF. Measurement of outer nuclear layer thickness also revealed photoreceptor cell protection in the vitamin E-treated Abca4/-/- mice. Since photodegradation of bisretinoid is more pronounced in albino versus pigmented mice, HPLC-quantified bisretinoid is more abundant in pigmented mice (17) (Fig. 1).

We have previously demonstrated that lipofuscin bisretinoids can be consumed by the processes of photooxidation and photodegradation even in ambient lighting (17). Bisretinoids are prone to oxidation because of their conjugated systems of double bonds (e.g., C-C = C=C = C). The oxidation of bisretinoids can proceed by multiple independent mechanisms (10, 40, 41). For instance, using A2E as a model it has been shown that bisretinoids are visible light photosensitizers that generate singlet oxygen by energy transfer, and superoxide anion by donation of an electron to ground state molecular O$_2$ (10, 40–42). As with some other photosensitizers (43), A2E can also serve as the substrate for reaction (oxidation) with singlet oxygen and radical oxygen species at double bonds (10, 44). Thus, the side arms of A2E are subsequently oxidized by a molecular singlet oxygen-mediated pathway that adds two oxygen atoms to form a cyclic peroxide (m/z 624) (10, 13, 42, 45). Alternatively addition of one oxygen atom can also occur so as to form epoxide and furanoid moieties (44, 46). Here the produced superoxide anion can generate H$_2$O$_2$ by dismutation [spontaneously or superoxide dismutase (SOD)-catalyzed] followed by the highly reactive hydroxyl radical (OH$^-$) that is generated from H$_2$O$_2$ with Fe as catalyst. The OH$^-$ radical is a powerful oxidant because of its unpaired electron (47, 48). Accordingly, OH$^-$ can readily break carbon double bonds, especially the conjugated double bonds like those that form the side arms of bisretinoids. This reactivity is significant, given the present findings indicating that in the absence of light, the OH$^-$ radical can also oxidize A2E.

Oxidative degradation of bisretinoids such as A2E and all-trans-retinal dimer, releases a mixture of aldehyde- and dicarbonyl-bearing fragments (MG and GO) that elicit cellular damage. This released mixture of aldehyde- and dicarbonyl-bearing fragments (MG and GO) that elicit cellular damage.

The retina is subject to photochemical damage that is readily demonstrable by exposure to light for prolonged periods or at heightened intensities (50, 51). Efforts to understand the molecular mechanisms of light damage have identified both Fe (52) and bisretinoids (53) as participants. In the latter case, we found that light damage-associated ONL thinning and dropout of RPE nuclei were more pronounced in Abca4/-/- mice having elevated levels of RPE bisretinoid than in age-matched wild-type mice. The ONL thinning was also greater in 5-mo-old versus 2-mo-old mice. In Rpe65/-/- mice bisretinoids are not detected chromatically and light damage was not observed (53). Systemic administration of the Fe chelator and antioxidant lipoic acid (alpha-lipoic acid) or DFP protects against light-induced photoreceptor degeneration in the mouse retina (34, 54). DFP, when administered orally to mice (1 mg/mL, 5 mg/d) has been shown to protect against retinal degeneration when the latter is induced by RPE Fe accumulation in Cp/Heph double KO (DKO) mice (55), by sodium iodate-mediated oxidative damage to RPE cells or by the rd6 mutation (24).

Fe and bisretinoids may also have links to AMD. The benefits afforded to patients with AMD by antioxidant intake indicate that oxidative mechanisms are an important factor contributing to AMD pathogenesis (56, 57). It is thus significant that Fe and bisretinoid initiate separate, but perhaps overlapping toxic oxidative processes (49, 58, 59). Perls' staining revealed that Fe levels were increased in AMD-affected maculas compared with healthy age-matched maculas (5) particularly in the RPE and Bruch’s membrane of early AMD, geographic atrophy, and patients with exudative AMD. Examination of the postmortem macula of a 72-year-old white male with geographic atrophy revealed Fe overload in the RPE and photoreceptor cell layers along with ferritin and ferroportin in the photoreceptor cells and internal limiting membrane. Conversely, healthy maculas were only weakly labeled with anti-ferritin and anti-ferroportin antibody (60). Transferrin is also up-regulated in AMD (61). Proteins modified by dicarbonyls are detected in sub-RPE drusen (15, 16) and these dicarbonyls are the same as those released by photodegradation of bisretinoids such as A2E and all-trans-retinal dimer. These findings indicate a possible link between photodegradation of RPE lipofuscin and sub-RPE changes that confer risk of AMD. Evidence that bisretinoid lipofuscin and Fe can initiate damaging light-mediated mechanisms (described above) is supported by epidemiological studies pointing to a relationship between AMD risk and sunlight exposure (62–69).

![Fig. 5. UPLC quantitation of A2E and iso-A2E in mice deficient in ceruloplasmin (Cp) and hephaestin (Heph) (Cp$^{−/−}$; Heph$^{−/−}$) and Cp$^{−/−}$; Heph$^{−/−}$ mice. (A) Detection of A2E and iso-A2E in Cp$^{−/−}$; Heph$^{−/−}$ and Cp$^{−/−}$; Heph$^{−/−}$ mice. Insets at Top) UV-visible spectra of chromatographic peaks corresponding to A2E (all-trans-A2E) and iso-A2E (C13/14 cis-A2E). (B) Picomoles per eye were calculated using calibration curves constructed from authentic standards. ND, not detected. Values based on single samples, four to eight eyes per sample.](image1)

![Fig. 6. Effects of DFP in the presence of bisretinoid photooxidation; cellular assays. ARPE-19 cells that have accumulated A2E were treated with DFP (30, 60, and 100 μM, 30 min) and then illuminated at 430 nm. (A) The Fe chelator DFP reduces photooxidative loss of A2E in ARPE-19 cells (HPLC analysis). (B) The reduced cell viability associated with A2E photooxidation was attenuated by pretreatment with the Fe chelator DFP. P values were determined by one-way ANOVA and Tukey's multiple comparison test.](image2)
These findings point to opportunities for drug repurposing and combination therapies. For instance, Fe chelation alone or in combination with inhibitors of bisretinoid formation (70) could serve as a unique therapy for recessive Stargardt disease (STGD1), a bisretinoid-related disease, and possibly AMD. Conversely, drugs that suppress the synthesis and toxic activities of bisretnoid could be beneficial for Fe-related conditions such as siderosis retinopathy.

**Methods**

**Mouse Models.** Albino Abca4<sup>−/−</sup>, agouti 129S1/SvImJ mice and mice deficient in ceruloplasmin and hephaestin (Cp<sup>−/−</sup>; Heph<sup>−/−</sup>) (38) were studied. Mice received DFP (Ferriprox) (Barr Pharma, pharmaceutical grade) in drinking water (1 mg/mL) from age 2 mo to age 4 or 6 mo. The intake of DFP was ∼3–5 mg per day. Animal protocols were approved by the Institutional Animal Care and Use Committee of Columbia University. Details are provided in Supporting Information.

**Quantitative HPLC and Ultra Performance Liquid Chromatography.** Mouse eyecups (4–8 eyes per sample as indicated) were homogenized, extracted in chloroform/methanol (2:1), and analyzed for bisretinoid by reversed-phase HPLC (A2E, iso-A2E) using an Alliance System (Waters Corp.) or Waters Acquity Ultra Performance Liquid Chromatography (UPLC)-MS System (A2E and iso-A2E in X-bridge, A2-GPE in phenyl) (Waters) as described (17, 29). Molar quantities per eye were calculated by comparison with synthesized standards. The pyridinium bisretinoid A2E and its isomer, iso-A2E, were measured separately and summed (A2E/iso-A2E). To detect DNP-derivatized biscarbonyl, incubated samples were extracted and analyzed by UPLC-MS (71).

**qRT-PCR for Transferrin Receptor Expression.** Neural retina was separated from RPE/photodiscoids, total RNA was isolated using Qiagen RNeasy Plus Micro kit (Qiagen), and reverse transcription was performed (Reverse Transcription kit; Thermo Fisher Scientific) (23, 34). Real-time PCR was performed with Applied Biosystems 7900HT. Probes and primers were as follows: transferrin receptor, Mm00441941_m1; 18s rRNA internal control, Hs99999901_s1.

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19. Morgan JI, Dubra A, Wolfe R, Merigan WH, Williams DR (2009) In vivo autofluorescence imaging of the human and macaque retinal pigment epithelial cell mosaic. Invest Ophthalmol Vis Sci 50:1350–1359.

20. Morgan JI, et al. (2008) Light-induced retinal changes observed with high-resolution autofluorescence imaging of the retinal pigment epithelium. Invest Ophthalmol Vis Sci 49:3715–3729.

21. Pandya VB, Ho IV, Hunyor AP (2012) Does unintentional macular translocation after retinal detachment repair influence visual outcome? Clin Experiment Ophthalmol 40: 88–92.

22. Shiragami C, et al. (2010) Unintentional displacement of the retina after standard vitrectomy for rhegmatogenous retinal detachment. Ophthalmology 117:86–92.e1.

23. Hadziahmetovic M, et al. (2011) The oral iron chelator deferiprone protects against iron overload-induced retinal degeneration. Invest Ophthalmol Vis Sci 52:959–968.

24. Hadziahmetovic M, et al. (2012) The oral iron chelator deferiprone protects against retinal degeneration induced through diverse mechanisms. Trans Vis Sci Technol 1:7.

25. Song D, et al. (2014) The oral iron chelator deferiprone protects against systemic iron overload-induced retinal degeneration in hepcidin knockout mice. Invest Ophthalmol Vis Sci 55:2126–2133.

26. Song D, Duniaef JL (2013) Retinal iron homeostasis in health and disease. Front Aging Neurosci 5:24.

27. Rudu RA, et al. (2008) Accelerated accumulation of lipofuscin pigments in the RPE of a mouse model for ABCA4-mediated retinal dystrophies following Vitamin A supplementa-

28. Weng J, et al. (1999) Insights into the function of Rim protein in photoreceptors and etiology of Stargardt’s disease from the phenotype in abcr knockout mice. Cell 98: 13–24.

29. Yamamoto K, Yoon KD, Ueda K, Hashimoto M, Sparrow JR (2011) A novel bisretinoid of retina is an adduct on glycerophosphoethanolamine. Invest Ophthalmol Vis Sci 52: 9084–9090.

30. Kim SR, et al. (2007) The all-trans-retinal dimer series of lipofuscin pigments in retinal pigment epithelial cells in a recessive Stargardt disease model. Proc Natl Acad Sci USA 104:19273–19278.

31. Sparrow JR, et al. (2013) Quantitative fundus autofluorescence in mice: Correlation with HPLC quantitation of RPE lipofuscin and measurement of retina outer nuclear layer thickness. Invest Ophthalmol Vis Sci 54:2812–2820.

32. Flynn E, Ueda K, Auran E, Sullivan JM, Sparrow JR (2014) Fundus autofluorescence and photoreceptor cell rosettes in mouse models. Invest Ophthalmol Vis Sci 55:5643–5652.

33. He X, et al. (2007) Iron homeostasis and toxicity in retinal degeneration. Prog Retin Eye Res 26:649–673.

34. Hadziahmetovic M, et al. (2012) Microarray analysis of murine retinal light damage reveals changes in iron regulatory, complement, and antioxidant genes in the neurosensory retina and isolated RPE. Invest Ophthalmol Vis Sci 53:5231–5241.

35. Chen P, et al. (2001) A photic visual cycle of rhodopsin regeneration is dependent on Rgr. Nat Genet 28:256–260.

36. Wu L, Nagasaki T, Sparrow JR (2010) Photoreceptor cell degeneration in Abca 4−/− mice. Adv Exp Med Biol 664:533–539.

37. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1:307–310.

38. Hahn P, et al. (2004) Disruption of ceruloplasmin and hephaestin in mice causes retinal iron overload and retinal degeneration with features of age-related macular degeneration. Proc Natl Acad Sci USA 101:13850–13855.

39. Wolkw N, et al. (2012) Ferroxidase hephaestin’s cell-autonomous role in the retinal pigment epithelium. Am J Pathol 180:1614–1624.

40. Pavlak A, et al. (2003) Comparison of the aerobic photoreactivability of A2E with its precursor retinal. Photochem Photobiol 77:253–258.

41. Gaillard ER, et al. (2004) A mechanistic study of the photooxidation of A2E, a component of human retinal lipofuscin. Exp Eye Res 79:313–319.

42. Sparrow JR, et al. (2002) Involvement of oxidative mechanisms in blue-light-induced damage to A2E-laden RPE. Invest Ophthalmol Vis Sci 43:1222–1227.

43. Bonnett R, Martinez G (2001) Photobleaching of sensitisers used in photodynamic therapy. Tetrahedron 43:503–512.

44. Jang YP, Zhou J, Nakanishi K, Sparrow JR (2005) Anthocyanins protect against A2E photodegradation and membrane permeabilization in retinal pigment epithelial cells. Photochem Photobiol 81:529–536.

45. Jang YP, et al. (2012) Ferroxidase hephaestin protects against phototoxic oxidations reactions initiated in retinal pigment epithelial cells by a lipofuscin pigment. Rejuvenation Res 9:256–263.

46. Jang YP, Zhou J, Nakanishi K, Sparrow JR (2005) Anthocyanins protect against A2E photooxidation and membrane permeabilization in retinal pigment epithelial cells. Photochem Photobiol 81:529–536.

47. Dentchev T, Hahn P, Duniaef JL (2005) Strong labeling for iron and the iron-handling proteins ferritin and ferroportin in the photoreceptor layer in age-related macular degeneration. Exp Eye Res 81:134–147.

48. Chowers I, et al. (2006) The iron carrier transferrin is upregulated in retinas from patients with age-related macular degeneration. Invest Ophthalmol Vis Sci 47: 2315–2340.

49. Cruickshanks KJ, Klein R, Klein BEK (1993) Sunlight and age-related macular degeneration. The Beaver Dam Eye Study, Arch Ophthalmol 111:514–518.

50. Tomany SC, Cruickshanks KJ, Klein R, Klein BEK, Knudson MD (2004) Sunlight and the 10-year incidence of age-related maculopathy: The Beaver Dam Eye Study. Arch Ophthalmol 122:750–755.

51. Cruickshanks KJ, Klein R, Klein BEK, Nondahl DM (2001) Sunlight and the 5-year incidence of early age-related maculopathy: The Beaver dam eye study. Arch Ophthalmol 119:246–250.

52. Klein BE, et al. (2014) Sunlight exposure, pigmentation, and incident age-related macular degeneration. The Beaver Dam Eye Study. Arch Ophthalmol 122:10275–10280.

53. Hadziahmetovic M, et al. (2012) Microarray analysis of murine retinal light damage reveals changes in iron regulatory, complement, and antioxidant genes in the neurosensory retina and isolated RPE. Invest Ophthalmol Vis Sci 53:5231–5241.

54. Chen P, et al. (2001) A photic visual cycle of rhodopsin regeneration is dependent on Rgr. Nat Genet 28:256–260.

55. Wu L, Nagasaki T, Sparrow JR (2010) Photoreceptor cell degeneration in Abca 4−/− mice. Adv Exp Med Biol 664:533–539.

56. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1:307–310.

57. Hahn P, et al. (2004) Disruption of ceruloplasmin and hephaestin in mice causes retinal iron overload and retinal degeneration with features of age-related macular degeneration. Proc Natl Acad Sci USA 101:13850–13855.

58. Wolkw N, et al. (2012) Ferroxidase hephaestin’s cell-autonomous role in the retinal pigment epithelium. Am J Pathol 180:1614–1624.

59. Pavlak A, et al. (2003) Comparison of the aerobic photoreactivability of A2E with its precursor retinal. Photochem Photobiol 77:253–258.

60. Gaillard ER, et al. (2004) A mechanistic study of the photooxidation of A2E, a component of human retinal lipofuscin. Exp Eye Res 79:313–319.

61. Sparrow JR, et al. (2002) Involvement of oxidative mechanisms in blue-light-induced damage to A2E-laden RPE. Invest Ophthalmol Vis Sci 43:1222–1227.

62. Bonnett R, Martinez G (2001) Photobleaching of sensitisers used in photodynamic therapy. Tetrahedron 57:9513–9547.

63. Jang YP, Matsuda H, Itagaki Y, Nakanishi K, Sparrow JR (2005) Characterization of perox-A2E and furan-A2E photooxidation products and detection in human and mouse retinal pigment epithelial cell lipofuscin. J Biol Chem 280:39732–39739.

64. Sparrow JR, et al. (2003) A2E-epoxides damage DNA in retinal pigment epithelial cells. Vitamin E and other antioxidants inhibit A2E-epoxide formation. J Biol Chem 278:18207–18213.

65. Dillon J, Wang Z, Avallie LB, Gaillard ER (2004) The photochemical oxidation of A2E results in the formation of a 5,8,5′-biss-furanoid oxide. Exp Eye Res 79:537–542.