PERSPECTIVE

Vitamin A-aldehyde adducts: AMD risk and targeted therapeutics

Janet R. Sparrow[a,b,1]

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Although currently available treatment options for age-related macular degeneration (AMD) are limited, particularly for atrophic AMD, the identification of predisposing genetic variations has informed clinical studies addressing therapeutic options such as complement inhibitors and anti-inflammatory agents. To lower risk of early AMD, recommended lifestyle interventions such as the avoidance of smoking and the intake of low glycemic antioxidant-rich diets have largely followed from the identification of nongenetic modifiable factors. On the other hand, the challenge of understanding the complex relationship between aging and cumulative damage leading to AMD has fueled investigations of the visual cycle adducts that accumulate in retinal pigment epithelial (RPE) cells and are a hallmark of aging retina. These studies have revealed properties of these compounds that provide insights into processes that may compromise RPE and could contribute to disease mechanisms in AMD. This work has also led to the design of targeted therapeutics that are currently under investigation.

age-related macular degeneration | vitamin A-aldehyde adducts | bisretinoids | retinal pigment epithelium | photoreceptor cells

Age-related macular degeneration (AMD) is a complex disorder that is predicted to have a growing impact on elderly populations. Although the cause of central vision loss in AMD is the progressive impairment of photoreceptor cells, the disease is generally thought to begin with dysfunctioning of retinal pigment epithelium (RPE) and adverse changes in subjacent Bruch's membrane (1, 2). The early stage of the disease is typically marked by extracellular accumulations (drusen) between RPE and Bruch's membrane. Progression to advanced disease is defined by delineated areas devoid of RPE and photoreceptor cell loss (atrophic AMD) and/or abnormal growth of blood vessels underneath RPE or within the subretinal space (neovascular AMD).

AMD Risk Factors

AMD susceptibility is influenced by multiple factors of both genetic and environmental origin. Predisposing genetic factors account for 71% of the variation in disease risk among individuals (3). Currently, 52 independently associated genetic variants at 34 loci are known to account for ∼50% of AMD heritability (4). The genes implicated by these variants belong to multiple systems some of which are associated with the complement pathway, lipid metabolism, and maintenance of extracellular matrix (5, 6). Nevertheless, many individuals carrying risk variants do not develop AMD.

Two loci, CFH (complement factor H; 1q31) and ARMS2/HTRA1 (age-related maculopathy susceptibility 2/high-temperature requirement factor A1; 10q26), make the greatest contribution to AMD risk. Variants at the ARMS2/HTRA1 and CFH loci significantly increase risk for progression to both the atrophic and neovascular forms of AMD, although the magnitude of the association of the ARMS2/HTRA1 risk allele is somewhat greater for the neovascular phenotype of late AMD, whereas CFH risk variants favor progression toward geography atrophy (5). Due to strong disequilibrium, genetic studies cannot determine whether ARMS2 or HTRA1 is the causal gene, and the functions of the genes at this locus are a matter of investigation (7, 8).

Loci near the complement genes (CFH, C2/CFB, C3, and CFI) are credited with ∼57% of the contribution of known variants to disease risk. Given the numbers of genes encoding complement system factors, together with the presence of complement factors and immune system proteins in drusen, complement dysregulation and inflammation are thought

[a]Department of Ophthalmology, Columbia University Medical Center, New York, NY 10032; and [b]Department of Pathology and Cell Biology, Columbia University Medical Center, New York, NY 10032

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[1]Email: jrs88@cumc.columbia.edu.

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to play a major role in AMD pathogenesis (9). Accordingly, these known genetic risks have guided some of the therapeutic options for atrophic AMD that are currently under investigation (10).

Nongenetic factors such as age and smoking also make a substantial contribution to AMD risk. The strongest modifiable AMD risk factor is smoking (11, 12). Nutritional status particularly in regard to dietary antioxidants (13) and glycemic index (14) also impacts AMD risk. Intake of the antioxidant vitamins E and C and zinc together or zinc alone [Age-Related Eye Disease Study (AREDS) supplements] (15) was shown to reduce the rate of progression to advanced AMD. Although lutein and zeaxanthin are more appropriate than β-carotene for inclusion in the AREDS formulation (16), no additional benefit was derived if patients took these nutrients in addition to the AREDS supplement (17). This is also the case for the intake of ω-3 fatty acids. The benefits of AREDS nutritional supplements (vitamins E, C, and zinc) appear to be the same regardless of whether the patients carry the risk variants in *CFH* Y402H and/or *ARMS2* A69S (18).

Efforts to unravel the pathogenesis of AMD have for many years given consideration to intracellular deposits of vitamin A-aldehydes, a prominent feature of aging RPE that unleash chronic mechanisms consistent with late-onset disease (19). Thus, the remaining sections of this article will explore biological mechanisms through which this material could modulate the development of AMD pathogenesis and address associated therapeutic implications. The interplay among these visual cycle adducts, lifetime light exposure, and oxidative stress will also be discussed in relation to AMD risk.

What Are Vitamin A-Aldehyde Adducts?

RPE cell aging is marked by intracellular accumulations of a family of autofluorescent compounds. These vitamin A aldehyde adducts have a bisretinoid structure and form by nonenzymatic reactions in photoreceptor outer segments, particularly condensations of retinaldehyde and phosphatidylethanolamine (PE). RPE phagocytosis serves to transfer bisretinoid-burdened outer segment discs to the RPE, but phagocytosis is not necessary for the formation of these fluorophores (20). The spectral characteristics of bisretinoids can account for the distinct fluorescence of RPE lipofuscin and for the fluorescence emission that is recorded noninvasively as fundus autofluorescence (AF) in clinical and experimental work (21). The AF emitted by these fluorophores is of highest intensity in the macula (22).

Bisretinoid fluorophores accumulate with age in RPE cells in all healthy eyes but form in abundance in recessive Stargardt disease (STGD1) due to mutations in the ATP-binding cassette transporter 4 (*ABCA4*). The toxicity of these vitamin A-aldehyde adducts is likely attributable to their propensity to photogenerate reactive oxygen species and to photodecompose into aldehyde- and dicarbonyl- (glyoxal and methylglyoxal) bearing fragments (23, 24). Evidence that proteins modified by the same dicarbonyls are detected in drusen, is indicative of a link between photodegradation of RPE lipofuscin and sub-RPE aging changes that confer risk of AMD. These damaging photodegradative processes and associated bisretinoid loss may also explain the predilection of the macula for disease. In support of this association are results showing that mice burdened with augmented bisretinoid formation monitored as elevated A2E and all-*trans*-retinal dimer, exhibit accentuated carbonyl-adduct deposition in Bruch's membrane, excessive complement activation, Bruch's membrane thickening due to basal laminar deposits, and accelerated loss of photoreceptor cells compared with WT mice (25–30). Additionally, albino $Abca4^{-/-}$ mice exhibit an increased susceptibility to retina light damage (31).

AMD Risk Factors That Intersect with Elements of Bisretinoid Deposition

Oxidative mechanisms are widely considered to be a major factor contributing to AMD pathogenesis (5, 32). Indeed the oxidant content of cigarette smoke may explain the impact of the latter on AMD risk (32). Numerous clinical and observation studies have demonstrated that dietary antioxidants and intake of antioxidants by supplementation reduces incidence or progression of AMD (15, 33–36). Because antioxidants can protect against AMD, and vitamins E and C have also been shown to reduce A2E photooxidation/photodegradation (37–39) (Fig. 1), the beneficial effects of antioxidant intake could, in some measure, be attributable to the suppression of photooxidative processes that are known to precede bisretinoid photodegradation. Similarly, it is significant that recent epidemiological studies (12, 40–45) and a meta-analysis (46) have supported a relationship between AMD and sunlight exposure. The potential contribution of lifetime light exposure to disease risk could be, at least partially, understood from the perspective of the cellular damage imposed by bisretinoid photodegradation.

Therapeutic Approaches That Target Bisretinoid: Clinical and Preclinical Studies

Limiting Vitamin A. One approach to reducing bisretinoid formation is to limit the delivery of vitamin A (all-*trans*-retinol) to the RPE (Fig. 1). For delivery in serum to RPE and other tissues, retinol is held within a carrier complex of retinol binding protein 4 (RBP4) and transthyretin (TTR); uptake by RPE cells is receptor mediated (47). Fenretinide [4-hydroxy(phenyl)retinamide], a retinoid derivative, competes with retinol for binding to RBP; as a consequence systemic retinol is reduced. In preclinical studies in $Abca4^{-/-}$ mice, investigators demonstrated that fenretinide reduced the formation of A2E during treatment for 1 mo. Based on these data, a 2-y phase 2 double-masked, randomized, placebo-controlled multicenter trial (ClinicalTrials.gov identifier; NCT00429936) was initiated to determine safety and efficacy of oral fenretinide at 100 and 300 mg daily in subjects with geographic atrophy (GA) (48, 49). Serum RBP levels were reduced by 50% (100-mg dose) and 70% (300-mg dose). In keeping with the reduced availability of retinol, delayed dark adaptation was observed (50). Compared with placebo, significant differences in GA lesion size was not observed, and although there was a trend for reduced lesion growth rates when RBP levels dropped below 2 mg/dL, statistical significance was not achieved. Whether the effects of RBP4 antagonists on vitamin A delivery to retina are short-lived due to compensation by alternative RBP4-independent mechanisms, such as occurs in the $Rbp4^{-/-}$ mouse (51), is not known.

A similar platform is the focus of work testing the efficacy of the RBP4 antagonist A1120, a nonretinoid that was shown to reduce serum RBP4 levels by 75% and bisretinoid lipofuscin levels in $Abca4^{-/-}$ mice by 50% (52). Unlike fenretinide, A1120 does not activate retinoic acid receptors. Structural modifications made to the core of the model have improved its metabolic stability (53).

Deuterated Vitamin A. In yet another strategy aimed at reducing bisretinoid formation, deuterium isotope replacement at the carbon 20 position of all-*trans*-retinol (C20-D_3-vitamin A) has been designed to reduce the rate at which retinaldehyde reacts (54, 55) (Fig. 1) without slowing the visual cycle. In vitro experiments revealed several fold slower formation of the retinaldehyde

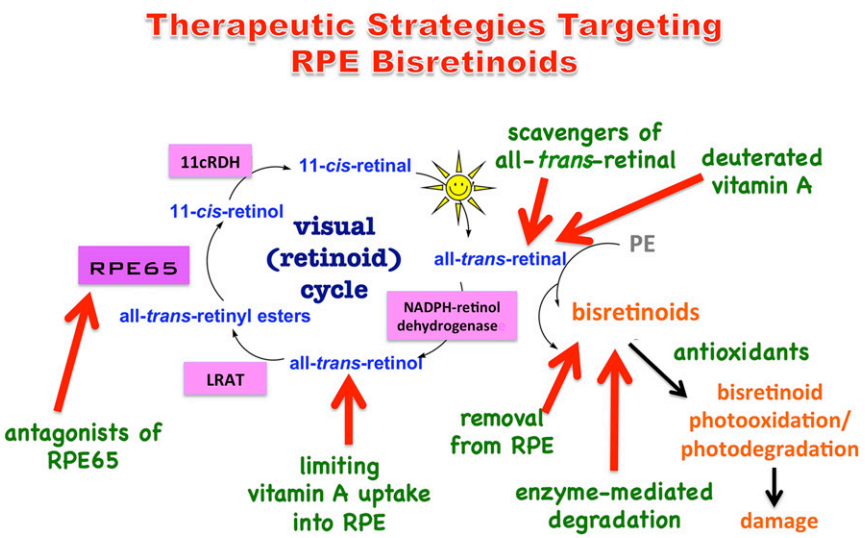

Fig. 1. Therapeutic strategies targeting RPE bisretinoids. Bisretinoids are visual cycle adducts having a variety of structures. These fluorophores form randomly in photoreceptor cell outer segments due to reactions of retinaldehyde (all-*trans*- and 11-*cis*-retinal) with amines such as PE. Bisretinoids are transferred to RPE by phagocytosis and accumulate as lipofuscin. Vitamin A (all-*trans*-retinol) enters the visual cycle by uptake into RPE. On photoisomerization of 11-*cis*-retinal in photoreceptor cells, all-*trans*-retinal is released from rhodopsin and is reduced to all-*trans*-retinol by NADPH-dependent all-*trans*-retinol dehydrogenase (NADPH-retinol dehydrogenase). Within RPE, LRAT converts all-*trans*-retinol to all-*trans*-retinyl esters; the isomerase RPE65 generates 11-*cis*-retinol, and 11-*cis*-retinol is oxidized to 11-*cis*-retinal by 11-*cis*-retinol dehydrogenase (11-cRDH). Bisretinoids are photosensitizers; they also photooxidize and photodegrade, releasing damaging dicarbonyls and aldehyde-containing fragments.

adducts A2E and all-*trans*-retinal dimer. In addition, treatment of rats and mice with C20-D_3-vitamin A administered either in the diet or by i.p. injection resulted in reduced bisretinoid. A phase 1 safety study (ClinicalTrials.gov identifier: NCT02230228) oral administration of C20-D_3-vitamin A (ALK-001) in healthy volunteers has been completed. Recruitment is now ongoing for a phase 2 placebo-controlled study (ClinicalTrials.gov identifier: NCT02402660) that will examine tolerability and effects of ALK-001 in STGD1. As indicated by the authors, the efficiency with which native vitamin A is replaced by C20-D_3-vitamin A remains to be determined (55). An increase in total vitamin A intake might also potentiate the drive toward bisretinoid formation (56).

Targeting RPE65. Another approach to modulating the visual cycle for the purposes of inhibiting bisretinoid formation is exemplified by emixustat (previously known as ACU-4429; ClinicalTrials.gov identifier: NCT01802866) (57), an amine-carrying drug and derivative of all-*trans*-retinylamine (58). Emixustat is a retinoid mimic developed to inhibit the activity of the isomerohydrolase RPE65 (Fig. 1). RPE65 is expressed in RPE cells and serves to generate 11-*cis*-retinol from all-*trans*-retinyl esters (59). Acylation of retinylamine by lecithin:retinol acyltransferase (LRAT) may facilitate retention of the drug in RPE cells (60). In WT mice, treatment with emixustat inhibited the production of 11-*cis*-retinal by ~80% (57, 61), whereas oral administration of emixustat to *Abca4*$^{-/-}$ mice for 3 mo reduced A2E in a dose-dependent manner (61). Complete arrest of RPE65 activity is not desirable as this would prevent visual pigment regeneration and create a drug effect comparable to severe retinopathy. In a multidose phase 1 trial (14 d; ClinicalTrials.gov identifier: NCT00942240) in healthy subjects and a phase 2 (90 d; NCT01002950) study of atrophic AMD, patients undergoing oral treatment with emixustat experienced dyschromatopsia and delayed dark adaptation as would be expected (62). Emixustat is currently being tested in a phase 2b/3 multicenter, randomized, double-masked, placebo-controlled dose-ranging study that is comparing its efficacy and safety with placebo for the treatment of atrophic AMD (63).

Support for a paradigm aimed at targeting RPE65 was also obtained using small molecule farnesyl-containing nonretinoid isoprenoids, one in which a ketone replaced the ester linkage (TDT) and a second that used an amide (TDH). Both antagonized the isomerase activity of RPE65 by competing with all-*trans*-retinyl esters. In rats and WT mice, a single injection of TDT and TDH slowed the regeneration of 11-*cis*-retinal after bleaching light. Chronic treatment of *Abca4*$^{-/-}$ mice with TDT had a pronounced effect on A2E accumulation; after the course of treatment, levels of this bisretinoid were the same as before treatment was begun (64).

Scavengers of All-Trans-Retinal. To reduce the production of retinaldehyde adducts, primary amine-containing compounds are being studied for the purpose of trapping free all-*trans*-retinal (65) (Fig. 1). To be effective, candidate molecules should compete with PE and penetrate into outer segments at sufficient concentration. In mice carrying double null mutations in retinol dehydrogenase (*Rdh8*$^{-/-}$) and *Abca4*$^{-/-}$, both of which are essential to the conversion of toxic all-*trans*-retinal to all-*trans*-retinol, screening of FDA-approved drugs has revealed some that can protect against acute light-induced retinal degeneration while maintaining 11-*cis*-retinal levels. The *Rdh8*$^{-/-}$/*Abca4*$^{-/-}$ mouse, under acute light exposure, provides a high throughput testing platform within which aberrant free all-*trans*-retinal is abundant.

Reversing Bisretinoid Accumulation. Treatment strategies geared toward removing bisretinoid from the RPE have taken two forms (Fig. 1). Because these bisretinoid fluorophores are refractory to lysis by native lysosomal enzymes, the first approach aimed to degrade bisretinoid by delivery of exogenous enzyme (66). This design was borrowed from enzyme replacement therapies aimed at reversing lysosomal storage disease (67). As proof of principle, horseradish peroxidase (HRP) was used in noncellular and cell-based assays to

demonstrate enzymatic degradation of A2E; this approach would be applicable to all of the bisretinoid visual cycle adducts as they all present with polyene side-arms. One limitation would rest with the products of bisretinoid degradation and whether they carry toxic moieties e.g., aldehydes.

Still other designs have been explored for the purpose of removing bisretinoids from RPE cells by small molecule tetrahydropyridoethers (68) or β-cyclodextrins that encase bisretinoids in hydrophobic cavities formed by seven D-glucose units for elimination (69). Additional research is required to elucidate the safety and efficacy of clearing bisretinoid not only from the RPE cells but also from the surrounding tissues.

Light Attenuation. Light deprivation does not suppress the formation of bisretinoids (70), probably because 11-*cis*-retinal (the light-sensitive configuration) and all-*trans*-retinal both serve as bisretinoid precursors. Nevertheless, reducing light exposure can defend against damaging bisretinoid photodegradation. For instance, sunglasses that attenuate over a broad range of wavelengths or yellow lenses that reduce blue wavelengths could be expected to provide protection (71). A black contact lens that blocked >90% of light in one eye of STGD1 patients was found to reduce the progression of decreased fundus AF (72); the latter decrease is likely attributable to bisretinoid photooxidation and photodegradation.

Other Considerations

It is too early to know whether some or all of these therapeutic schemes will be effective. Although the discussion here has focused on AMD, interventions aimed at bisretinoids would also be applicable to less common diseases such as STGD1. In the latter early-onset form of macular degeneration, gene therapy promises to correct the accelerated bisretinoid deposition that is a consequence of most *ABCA4* mutations (73, 74), whereas cell-based therapies aim to replace the damaged RPE (75).

Bisretinoids likely impart chronic insult that at any given time is subtle (76). Thus, therapies that target bisretinoids are probably appropriate for suppressing early and intermediate stages of cellular damage but may not be as effective for ameliorating existing disease (76). In most cases the target cell for drug delivery would be the RPE; however, scavengers of all-*trans*-retinal would have to gain access to photoreceptor cell outer segments. Because some of these drugs would have to be continuously active over the long term, drug delivery systems that maintain therapeutic levels of the drugs are desirable.

The choice of appropriate outcome measures is essential to the design of clinical trials. Ongoing clinical trials targeting RPE bisretinoids typically use the rate of enlargement of GA measured in color fundus or fundus AF images, as the primary end point for judging treatment efficacy. Another meaningful efficacy end point for studies addressing vitamin A-aldehyde adduct formation is the measurement of fundus AF. Retinal bisretinoids are the source of fundus AF that is imaged noninvasively by confocal laser scanning ophthalmoscopy; as such, fundus AF is suited to the definition of a biomarker (77). The introduction of protocols for quantifying fundus AF [quantitative fundus AF (qAF)] (78) also raises the possibility of acquiring qAF values as direct indicators of the response to therapeutic intervention. The robustness of this approach in the setting of clinical trials awaits testing.

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