Pharmacological inhibition of lipofuscin accumulation in the retina as a therapeutic strategy for dry AMD treatment

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Abstract

Age-related macular degeneration (AMD) is the leading cause of blindness in the western world. There is no FDA-approved treatment for the most prevalent dry (atrophic) form of AMD. Photoreceptor degeneration in dry AMD is triggered by abnormalities in the retinal pigment epithelium (RPE). It has been suggested that excessive accumulation of fluorescent lipofuscin pigment in the RPE represents an important pathogenic factor in etiology and progression of dry AMD. Cytotoxic lipofuscin bisretinoids, such as A2E, are formed in the retina in a non-enzymatic way from visual cycle retinoids. Inhibition of toxic bisretinoid production in the retina seems to be a sound treatment strategy for dry AMD. In this review we discuss the following classes of pharmacological treatments inhibiting lipofuscin bisretinoid formation in the retina: direct inhibitors of key visual cycle enzymes, RBP4 antagonists, primary amine-containing aldehyde traps, and deuterated analogs of vitamin A.

Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in the United States. There is no FDA-approved treatment for the most prevalent dry (atrophic) form of AMD [1]. Given the lack of treatment and high prevalence, development of drugs for dry AMD is of utmost importance. Clinically, atrophic AMD represents a slowly progressing neurodegenerative disorder in which specialized neurons (rod and cone photoreceptors) die in the central part of the retina called the macula [1]. Histopathological and clinical imaging studies indicate that photoreceptor degeneration in dry AMD may be triggered by abnormalities in the retinal pigment epithelium (RPE) that lies beneath photoreceptors and provides critical metabolic support to these light-sensing neuronal cells [2–6]. Excessive accumulation of lipofuscin in the RPE is frequently cited as one of the causes that may contribute to the demise of the RPE cells in the dry AMD retina [4, 7–11]. It has been suggested that significantly increased phagocytic and metabolic load on the RPE in the...
macula in comparison with the peripheral retina results in higher levels of lipofuscin accumulation in the macular RPE cells [7]. This accumulation may trigger a preferential loss of photoreceptors in the macula region of the AMD retina. One best-known component of RPE lipofuscin is a pyridinium bisretinoid A2E [10]. In many experimental systems A2E was shown to induce a variety of unfavorable effects such as induction of apoptosis in cultured RPE cells [9, 12], inhibition of the critical lysosomal transporter [13], loss of membrane integrity [14, 15], inhibition of phagocytosis [8, 16], disruption of mitochondrial function [16], activation of the complement cascade [17, 18] and oxidative damage [19–22]. In light of these experimental data and given clinical correlations between the progression of dry AMD and parameters of fundus autofluorescence [5, 6] mediated by lipofuscin fluorophores it seems reasonable to assume that excessive bisretinoid accumulation in the retina plays a critical role in pathogenesis of atrophic AMD. Inhibition of toxic bisretinoid production in the AMD retina should be considered as a potential treatment strategy for dry AMD. Chemically, A2E is composed of two molecules of all-trans retinal (vitamin A aldehyde) and one molecule of ethanolamine. It was suggested that formation of A2E and other lipofuscin bisretinoids, such as A2-DHP-PE (A2-dihydropyridinephosphatidylethanolamine) and atRALdi-PE (all-trans-retinal dimer phosphatidylethanolamine), occurs in the retina in a non-enzymatic manner and can be considered a by-product of a properly functioning visual cycle (Figure 1) [10, 23]. The current model of bisretinoid formation specifies that all-trans-retinaldehyde (formed in the outer segments in a light-dependent manner) serves as a direct precursor for A2E synthesis implying that biogenesis of lipofuscin fluorophores is light-dependent [10]. Consistent with this model, complete inhibition of A2E formation in Abca4−/− mice raised in total darkness was previously reported [24]. However, subsequent studies showed that increased retinal luminance is not correlated with increased A2E formation in the Abca4−/− mouse model [25] implying that increased rates of the visual cycle and light-induce all-trans-retinal production may not be linearly proportional to the bisretinoid synthesis in the mouse retina. Additional information on the mechanisms of A2E biogenesis can be inferred from the analysis of knockout mouse models resulting in genetic ablation of the retinoldehydrogenase (RDH) isoforms responsible for the reduction of all-trans-retinal (atRAL) light-dependently generated in the outer segments. It was shown that genetic ablation of Rdh8 which encodes the RDH isoform responsible for reduction of the majority of atRAL generated in the outer segments [26, 27] only modestly increases the atRAL condensation to A2E. [27–29] This indicates that atRAL might not be the sole direct precursor of bisretinoids in the retina. A recent study found no significant difference in lipofuscin and A2E levels between dark- and cyclic light reared mice (either wild type or Abca4−/−) at different ages indicating that light-induced atRAL formation may not be absolutely required for bisretinoid synthesis [30]. Based on these and other observations, it was suggested that light activation may not be necessary for the lipofuscin formation and that atRAL is unlikely to be a main source of lipofuscin fluorophores while 11-cis retinal could be a major direct precursor of A2E in the retina [30, 31]. The potential immediate clinical implication of this new hypothesis relates to the proposed light independence of lipofuscin bisretinoid formation as it has been previously suggested that patients with Stargardt disease and, perhaps, dry AMD may benefit from non-pharmacological inhibition of the visual cycle that can be accomplished by wearing dark sunglasses that limit the photoreceptor exposure to light [32, 33].
impose an unnecessary burden on visually impaired patients if it is definitively established that light-induced aRAL formation in the outer segments does not significantly contribute to bisretinoid synthesis in the human retina. While the detailed mechanisms of bisretinoid formation in the retina may require additional elucidation, four pharmacological classes of compounds have been shown to reduce bisretinoid formation in animal models of enhanced lipofuscinogenesis (Figure 2). The first class comprises direct inhibitors of key visual cycle enzymes such as isomerohydrolase (RPE65) and 11-cis-retinol dehydrogenase [34–37]. The second class of compounds with proven ability to inhibit bisretinoid formation represents RBP4 antagonists capable of inhibiting retinol-stimulated RBP4-TTR interaction [38, 39]. Attesting to the strength of pre-clinical rationale supporting the development of these two groups of compounds as a potential AMD and Stargardt disease treatment, their representatives advanced to different stages of clinical evaluation [37, 40]. Two additional treatment strategies to pharmacological inhibition of lipofuscin bisretinoid formation are currently emerging. Primary amine-containing drugs capable of inactivating aRAL in the retina through the Schiff base formation exemplify another approach to pharmacological reduction of lipofuscin accumulation in the retina [41]. Finally, a deuterated form of vitamin A was hypothesized to have a reduced propensity for non-enzymatic dimerization which could result in the decreased A2E formation [42, 43].

While there are additional treatment approaches to inhibition of lipofuscin accumulation in the retina [44–46], this review will focus on four classes of compounds that demonstrated pre-clinical efficacy in inhibiting bisretinoid formation in animal models of enhanced retinal lipofuscinogenesis.

**Inhibitors of key visual cycle enzymes**

The visual cycle is a chain of biochemical reactions that regenerate visual pigment (11-cis retinaldehyde conjugated to opsin) following exposure to light [1]. A critical step in the visual cycle is the conversion of all-trans retinyl ester to 11-cis retinol by the enzyme called isomerohydrolase (IMH). It has been shown that RPE65 represents the IMH which produces 11-cis retinol from all-trans retinyl ester in the retinal pigment epithelium [47–49]. The IMH reaction is rate-limiting in visual cycle function [47] thus making RPE65 an important drug target for visual cycle inhibition [50]. Due to exclusively restricted pattern of RPE65 expression its specific inhibition is unlikely to result in systemic adverse affects. Experiments conducted in mice validated RPE65 as a drug target for the reduction of bisretinoid formation. Genetic ablation of RPE65 in mice has been shown to drastically reduce the RPE lipofuscin fluorophore accumulation [51]. Additionally, it was reported that the sporadic Leu450Met amino acid substitution in Rpe65 found in certain mouse strains leads to the reduction of the visual cycle rate [52] and inhibition of A2E accumulation [53]. Moreover, in patients with compound heterozygous or homozygous RPE65 mutations there was no or little lipofuscin autofluorescence detected [54] which may indicate that the formation of lipofuscin fluorophores is reduced as a result of RPE65 inactivation. It is known, however, that complete inactivation of RPE65 in patients with Leber congenital amaurosis (LCA) caused by RPE65 mutations is associated with severe retinal degeneration [55]. This underscores the importance of partial pharmacological RPE65 inhibition as a treatment strategy for dry AMD and Stargardt disease given that the residual RPE65 activity
is necessary for basal regeneration of visual pigment required for rod photoreceptor maintenance. Assuming that heterozygous carriers of LCA-associated RPE65 mutations appear to have minimal retinal involvement [56–58], it seems reasonable to suggest that less than 50% inhibition of the RPE65 activity may be safe in humans. Several classes of small molecule RPE65 inhibitors, such as retinylamine derivatives [28, 35, 36] and a synthetic RPE65 antagonist ACU-4429 [37], were suggested as a potential pharmacological treatment for dry AMD and Stargardt disease. Additionally, two farnesyl-containing isoprenoids, TDT and TDH, have been originally shown to inhibit the visual cycle by antagonizing the RPE65 activity [34]. However, the strength of experimental evidence in support of the compound activity as RPE65 antagonists and visual cycle inhibitors has been drastically weakened [59, 60]. As the ability of TDT and TDH to inhibit bisretinoid accumulation in the Abca4−/− mouse model seems to be solidly established [34], the mechanism of action by which these farnesyl-containing isoprenoids reduce bisretinoid formation remains to be defined. In addition to TDT and TDH, preclinical efficacy in the mouse model of enhanced retinal lipofuscinogenesis was demonstrated for retinylamine [28, 34]. Isotretinoin (13-cis retinoic acid) which also significantly reduced lipofuscin accumulation in the Abca4−/− mouse model [61] was suggested to act as an inhibitor of another key visual cycle enzyme, 11-cis-retinol dehydrogenase [61]. However, isotretinoin was later shown to bind to RPE65 and act as an inhibitor of the IMH reaction [50] indicating a broad specificity of this isomer of retinoic acid. The most advanced compound directly inhibiting the visual cycle is the RPE65 antagonist ACU-4429 (Figure 2, A). While no information on the compound structure and its in vitro and in vivo activity is publicly disclosed in the peer-reviewed literature, the results of its Phase I clinical evaluation in normal volunteers have been recently presented [37]. Following a single oral administration the drug dose-dependently suppressed the rod function recovery after the photobleach, as expected of a compound inhibiting the visual cycle. However, starting from the 40 mg dose ACU-4429 dose-dependently suppressed rod ERG amplitudes in the dark adapted eyes. While inhibition of the visual cycle is expected to produce a delay in rod function recovery from the photobleach, rod amplitude itself may remain unaffected following administration of a visual cycle inhibitor. This was convincingly shown in published isotretinoin experiments [62] where 8 weeks of drug administration in rats at the 40 mg/kg daily dose produced no ERG evidence of impaired rod function even though dark-adapted rod b-wave recovery after the bleaching light was significantly inhibited [62]. It remains to be seen whether ERG inhibition by ACU-4499 is compatible with further clinical development of this drug candidate and whether the decision on the range of safe but efficacious doses can be made based on the results of this Phase I trial. It is clear, however, that defining a safe yet efficacious human dose for any RPE65 antagonist may represent a significant challenge for clinical development of this class of compounds as there is no easily measurable biomarker that can be reliably correlated with the desired safe level of RPE65 inhibition. Dose-dependent suppression of the rate of rod function recovery following the photobleach as measured psychophysically or from the ERG b-wave amplitude can potentially be used as pharmacodynamic markers for defining the optimal clinical dose. However, safe levels of suppression of rod function recovery following the photobleach that can be associated with clinical efficacy remain to be defined. In addition, repeated reliable quantitative measurements of kinetics of dark adaptation in the context of the multicenter clinical trial involving many patients may be
challenging. Given that RPE lipofuscin can be quantitatively monitored clinically as fundus autofluorescence (FA) using confocal scanning laser ophthalmoscopy (cSLO), this imaging technique may potentially be used for documenting clinical efficacy of a RPE65 antagonist. As it is not clear whether quantitative FA measurements can provide a proof of clinical efficacy faster than other surrogate markers (e.g., reduction in the rate of growth for geographic atrophy), it seems unlikely that cSLO will be instrumental in defining a suitable dose range early in clinical development. Overall, defining a clinical development strategy for RPE65 antagonists represents a formidable challenge.

Theoretically, partial inhibition of the visual cycle with the following reduction in bisretinoid formation may be accomplished by downregulation of the activity of other members of the visual cycle pathway, such as 11-cis-retinol dehydrogenase (RDH5), lecithin-retinol acyltransferase (LRAT), or cellular retinaldehyde-binding protein (CRALBP). Specific antagonists of RDH5, LRAT and CRALBP are yet to be developed.

Antagonists of retinol-dependent RBP4-TTR interaction

The second class of compounds with proven ability to inhibit bisretinoid formation in the animal model of enhanced lipofusciogenesis comprises RBP4 antagonists. Serum retinol is transported to target tissues, such as RPE, bound to a specific serum carrier protein, Retinol-Binding Protein 4 (RBP4) which has a well defined hydrophobic pocket for retinol binding [63, 64]. Most of the retinol-RBP4 complex in circulation is bound with another serum protein, transthyretin, TTR [64–66]. RBP4-TTR interaction is critical for maintaining serum retinol in circulation as without complexation with TTR RBP4-retinol is rapidly cleared from the bloodstream through glomerular filtration due to its small size, 21 kDa. Retinol binding to RBP4 stimulates the formation of the RBP4-TTR complex while apo-RBP4 poorly interacts with TTR [65, 67]. Taking into account that the visual cycle depends on retinol delivery to the RPE by RBP4, it was suggested that RBP4 ligands antagonizing retinol-dependent RBP4-TTR interaction in circulation may reduce A2E formation in the retina [38]. Fenretinide (Figure 2, C), a prototypic RBP4 antagonist, is known to bind with RBP4, displace all-trans retinol from RBP4 [68], and disrupt the retinol-dependent RBP4-TTR interaction [68, 69]. Fenretinide was shown to reduce serum RBP4 and retinol [70] and, importantly, decrease bisretinoid production in the animal model of excessive lipofuscin accumulation [38]. Over the period of recent decades, fenretinide has been extensively studied as a potential treatment for the variety of cancers [71]. The mechanism of action defining fenretinide’s chemopreventive activity is unrelated to its activity as a RBP4 ligand but seems to be associated with its ability to generate reactive oxygen species and induce apoptosis in malignant cells [72, 73]. Even though cancer clinical trials failed to yield fenretinide approval for any indication, they established a range of generally well-tolerated human doses which allowed clinical evaluation of fenretinide in patients with late-stage atrophic AMD [40]. This Phase II clinical trial studied the efficacy of two fenretinide doses (100 mg and 300 mg per day) in reducing the lesion growth in patients with geographic atrophy (GA). The trial showed a positive correlation between the reduction of serum RBP4 below 1 µM (approximately 70% reduction from the baseline) and inhibition of the GA growth rate in patients from the 300 mg/day cohort [40]. Unfortunately, only 51% of patients from the 300 mg/day group achieved this level of serum RBP4 reduction in the 2-
year trial while there was no correlation between the rates of GA growth and serum RBP4 levels at RBP4 concentrations higher than 1 µM [40]. Because of the limited number of patients achieving the desired level of RBP4 reduction, the GA growth rate difference between the 300 mg/day and placebo groups did not reach statistical significance [40]. Nevertheless, this trial provided critically important information on the desired level of serum RBP4 reduction (below 1 µM) required for suppression of the GA growth. Theoretically, adequate RBP4 lowering could be achieved by increasing the fenretinide dose. However, similar to many other retinoid compounds, fenretinide is non-specific and can be toxic at elevated doses. It has been suggested that some of its adverse effects that can become more evident at higher doses are mediated by its action as a ligand of a nuclear receptor RAR [74–77]. Fenretinide was shown to induce trans-differentiation in cultured RPE cells at a relatively low concentration [78] which seems to be associated with its activity as a nuclear receptor ligand. Independent of its activities as an RAR and RBP4 ligand, fenretinide is reported to be an inducer of apoptosis in many cell types [74, 79–82], including the RPE [75, 83]. Additionally, fenretinide is theratogenic [84, 85] making it unsuitable for the use in Stargardt disease patients of childbearing age. As fenretinide safety profile at higher doses may be incompatible with long-term dosing in individuals with blinding but non-life threatening conditions, identification of new classes of potent and specific RBP4 antagonists is of significant importance. A1120 (Figure 2, B), a potent non-retinoid RBP4 antagonist, was originally developed as a potential treatment for diabetes [86] based on the observation that pharmacological downregulation or genetic ablation of RBP4 enhanced insulin sensitivity [87]. However, administration of A1120 to diet-induced obese mice did not improve insulin sensitivity [86] making the compound or its congeners the unlikely drug candidates for diabetes treatment. We showed that chronic A1120 administration reduced accumulation of lipofuscin bisretinoids by approximately 50% in the Abca4−/− mouse model [88]. This activity correlated with 75% reduction in serum RBP4 and 30–50% reduction in certain visual cycle retinoids [88] confirming the mechanism of action for A1120. In contrast to fenretinide, A1120 does not act as a RARα agonist potentially indicating a more favorable safety profile for this non-retinoid compound [88].

One of the most striking differences between the classes of RBP4 antagonists and RPE65/key visual cycle enzyme inhibitors is the degree of visual cycle inhibition associated with bisretinoid reduction in the animal model. Direct inhibitors of key visual cycle enzymes such as isotretinoin [61], farnesyl-containing isoprenoids [34], and retinylamine [36] induced profound inhibition of the visual cycle as can be judged by the significant delay in recovery of rod function following exposure to the bleaching light [34, 36, 61]. In contrast, chronic administration of fenretinide resulted in a much weaker inhibition of the rod response recovery following exposure to the bleaching light [38]. As it has been suggested in the literature that fenretinide may act as a weak RPE65 inhibitor [89], inhibition of the rod response recovery after the photobleach may potentially be ascribed to this RPE65 inhibitory effect. In our A1120 study [88] we found no statistically significant difference in kinetics of the b-wave recovery after photobleaching in the groups of A1120- and vehicle-treated wild type and Abca4−/− mice. This suggests that the ability of specific RBP4 antagonists to inhibit bisretinoid formation in the Abca4−/− mouse retina requires minimal suppression of the visual cycle which may remain undetected in the ERG b-wave recovery.
experiments. Unaffected rate of rod function recovery after the photobleach following chronic A1120 administration in mice may indicate that the clinical use of A1120 derivatives might not be associated with significant mechanism-based ocular adverse effects (nyctalopia, delayed dark adaptation).

A path for clinical development of RBP4 antagonists for late-stage dry AMD seems to be relatively straightforward in comparison with other 3 classes of compounds inhibiting lipofuscin bisretinoid formation. FDA’s Center for Drug Evaluation and Research (CDER) has accepted a decrease in the rate of growth of an area of retina that no longer has any photoreceptors as an acceptable endpoint in clinical trials [90]. Phase II fenretinide clinical trial [40] documented a positive correlation between inhibition of the GA growth rate and reduction of serum RBP4 below 0.2 mg/dL (1 µM). RBP4 represents an easily measurable serum biomarker that allows demonstration of the clinical proof-of-mechanism (RBP4 lowering) after a single and/or multiple dose administration of a candidate drug in Phase Ia/Ib trials. Selection of the range of efficacious doses that induce RBP4 lowering below the 0.2 mg/dL (1 µM) threshold can also be rapidly accomplished early in clinical development. Based on the data from the fenretinide clinical trial [40], more than 70% RBP4 reduction from baseline may be required for achieving clinical efficacy. One of the general safety concerns for the class of RBP4 antagonists is whether such a reduction in serum RBP4 may induce symptoms of systemic vitamin A deficiency. Based on the available evidence, it would be incorrect to regard partial pharmacological downregulation of serum RBP4 as synonymous to systemic vitamin A deprivation. Patients with compound heterozygous missense mutations in \textit{RBP4} have plasma RBP4 levels below the limit of detection while showing no clinical symptoms of systemic vitamin A deficiency [91]. \textit{Rbp4}^{−/−} mice are phenotypically normal with no systemic abnormalities [92–94] or histological signs of retinal degeneration [95]. At weaning (19–21 days postnatal) the \textit{Rbp4}^{−/−} mice have reduced ERG amplitudes which completely normalize on standard vitamin A sufficient chow by the age of 4–5 months [92, 93] as soon as alternative RBP4-independent routes of vitamin A delivery replenish congenially depleted retinoid stores in the retina to the acceptable level [93]. Human and mouse data illustrate the capacity of several alternative RBP4-independent pathways for retinoid delivery to the target organs, including the RPE [91–94, 96, 97]. Following consumption of vitamin A-containing meal, dietary retinoids are packaged in chylomicrons that are delivered primarily to the liver. However, 25–33% of postprandial retinoid-laden chylomicrons are taken by extrahepatic tissues, such as the RPE [92]. In addition to chylomicron delivery, small amount of vitamin A in the form of retinoic acid may be delivered to the target organs in a complex with serum albumin [96]. Moreover, \textit{de novo} vitamin A biosynthesis from dietary \(\beta\)-carotene has been documented in the RPE cells [96, 98] thus exemplifying yet another RBP4-independent route of retinoid supply to the retina. It seems unlikely that partial pharmacological downregulation of serum RBP4 will be associated with symptoms of systemic vitamin A deficiency in patients on a standard vitamin A sufficient diet. It should be noted that long-term fenretinide administration in cancer patients [99] was associated with transient ocular side effects, such as diminished dark adaptation, in a small subset of patients (yearly prevalence: 5.8–6.7%) [99]. As it has been suggested in the literature that fenretinide may act as a weak RPE65 inhibitor [89], diminished dark adaptation in a sub-population of fenretinide-treated patients may
potentially be ascribed to this RPE65 inhibitory effect. It remains to be determined in future clinical trials whether the use of specific RBP4 antagonists is associated with clinically meaningful inhibitory affect on dark adaptation in treated patients.

Aldehyde traps

Light-induced isomerization of 11-cis retinal to atRAL and the release of all-trans retinal from opsin is a critical step in visual signal transduction. Assuming that atRAL represents a primary source for non-enzymatic formation of A2E and other bisretinoids in the retina, it has been suggested that neutralization of atRAL through the formation of Schiff base adducts using primary amine-containing drugs may reduce biosynthesis of lipofuscin bisretinoids and ameliorate other aspects of aldehyde toxicity in the retina [41]. Several FDA-approved drugs containing primary amines showed photoreceptor protection efficacy in the acute light-induced Abca4−/−Rdh8−/− mouse model [41] in which photoreceptor degeneration is mediated by the massive light-induced release of atRAL. One of the tested compounds, A20 (Figure 2, D), represents γ-aminobutyric acid receptor agonist pregabalin (Lyrica). A20 conferred significant retinal protection in the acute light damage paradigm in Abca4−/−Rdh8−/− mice at two tested doses, 0.5 mg/mouse (~16 mg/kg) and 2 mg/mouse (~65 mg/kg) [41]. Racemic mix of A20 induced approximately 40% reduction in A2E formation after 3 months of daily 65 mg/kg oral administration in Abca4−/−Rdh8−/− mice maintained under standard lighting conditions (12 h 10 lx light, 12 h dark) which provided a proof of efficacy in the animal model of enhanced lipofuscinogenesis [41]. Overall, aldehyde trapping may represent an attractive approach to inhibiting retinal bisretinoid formation as it may lack mechanism-based ocular side effects typical for direct visual cycle inhibitors. However, given that one molecule of a primary amine drug is required to stoichiometrically inactivate one molecule of atRAL it seems that compounds may need to be administered at very high systemic doses in order to act as aldehyde traps in the retina. High systemic doses may raise safety concerns even for a set of FDA-approved drugs. It seems that local retinal delivery may be considered as a viable alternative to systemic administration of aldehyde traps in order to overcome potential systemic toxicities.

Regardless of the route of administration additional studies on metabolism and disposition of Schiff base adducts between atRAL, other biological aldehydes and an administered drug will be required in order to establish treatment safety. One of the overall concerns for the general approach may be a lack of specificity for primary amine compounds as they may react with comparable efficiency with various biologically important aldehydes, such as pyridoxal (vitamin B₆). Results of the randomized trial of vitamin B supplements (B₆, B₁₂, and folic acid) demonstrated statistically significant 35–40% decreased risk of AMD in the treatment group [100]. Because the diagnosis of AMD was based on validated self reports, this study did not result in recommendations for AMD [100, 101]. Nevertheless, this study illustrates the involvement of biological aldehydes in important aspects of retinal biology and underscores the importance of careful safety evaluation of such a broadly specific treatment as aldehyde traps.
Deuterated forms of vitamin A

Deuterium is a stable, safe, and non-radioactive hydrogen isotope that has been used extensively in human metabolic and clinical studies [102–104]. While deuterated compounds have been widely used in humans as metabolic and pharmacokinetic probes, there are very few reports of deuterated derivatives being used as bona fide drug treatments. It has been suggested that non-enzymatic synthesis of A2E in the retina involves cleavage of the C20-H bond in retinaldehyde-PE, and that incorporation of deuterium to vitamin A at the C20 position may stabilize the C20-D bond and inhibit A2E production [42]. Demonstration of efficacy for the deuterated derivatives of vitamin A in animal models required complete replacement of natural forms of vitamin A with its deuterated analogs [42, 43]. The use of C20-D3-retinyl acetate (Figure 2, E) as the sole source of vitamin A in the Abca4-/- mouse model yielded 80% reduction in A2E accumulation after 3 months of treatment [43]. Comparable 68% A2E reduction in wild type mice was induced by the use of intraperitoneally administered C20-D3-atRAL as the sole source of vitamin A [42]. Head-to-head comparison of fenretinide with C20-D3 vitamin A treatment in wild type rats showed superior efficacy for fenretinide (58% A2E reduction) versus complete replacement of vitamin A with the C20-D3 analog (45% A2E reduction) [42]. It should be kept in mind that preclinical efficacy for C20-D3 retinoids was demonstrated only in the context of a complete replacement of natural vitamin A with its deuterated analogs. It remains to be proven whether pre-clinical efficacy demonstrated in animal models in the context of complete vitamin A replacement with the C20-D3 analogs can be translated into clinical efficacy in humans maintained on a standard vitamin A and β-carotene sufficient diet. If complete replacement of natural vitamin A with a C20-D3 analog is required for clinical efficacy, a comprehensive elimination of vitamin A foods (dairy products, eggs, certain fish, etc) may be necessary. Given that vitamin A can be de novo synthesized in the RPE from dietary β-carotene [96, 98] additional dietary exclusion of β-carotene-rich fruits and vegetables may also be required. Despite the obvious concern regarding the dependence of clinical efficacy on unattainable dietary restrictions, C20-D3 treatment represents a potentially attractive approach to inhibiting retinal bisretinoid formation as it seems to lack mechanism-based side effects expected of other classes of drugs inhibiting lipofuscin formation, such as direct visual cycle inhibitors.

Conclusions

Atrophic (dry) AMD is a complex multifactorial retinal disease. Multiple factors such as local inflammation, oxidative stress, and genetic predisposition may play important role in etiology and progression of dry AMD indicating that a variety of biochemical pathways may be pharmacologically engaged in order to prevent the disease or delay its progression. Accumulation of lipofuscin in the RPE is implicated in etiology and progression of dry AMD. Pharmacological inhibition of toxic lipofuscin bisretinoid production in the retina is a sound and clinically testable treatment strategy for dry AMD. Four classes of therapeutics are emerging as potential treatments for inhibition of lipofuscin formation in the retina: direct inhibitors of key visual cycle enzymes, RBP4 antagonists, primary amine-containing aldehyde traps, and deuterated analogs of vitamin A. Recently conducted fenretinide clinical trial established positive correlation between inhibition of the geographic atrophy growth...
rate in dry AMD patients and reduction of serum RBP4 below 0.2 mg/dL (1 µM) thus providing clinical validation for RBP4 as a drug target for dry AMD [40]. While RBP4 lowering with RBP4 ligands antagonizing retinol-dependent RBP4-TTR interaction may be considered a clinically validated approach to dry AMD treatment, three alternative classes of treatments are advancing through a rigorous process of pre-clinical and clinical evaluation.

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Figure 1. The visual cycle and points of intervention for pharmacological inhibition of lipofuscin bisretinoid synthesis in the retina

Enzymes and transporters facilitating visual cycle reactions in photoreceptor outer segments are highlighted in green. Enzymes catalyzing visual cycle reactions conducted in the RPE are shown in orange. Lipofuscin bisretinoid biosynthesis begins when all-trans-retinal leaves the visual cycle and non-enzymatically reacts with phosphatidylethanolamine forming the A2E precursor, A2-PE (blue arrow); 11-cis-retinal is also reported to be a precursor of A2E formation [30] (dotted arrow). Uptake of serum retinol to the RPE (red arrow) fuels the visual cycle. Highlighted in bright blue are four intervention points for suppression of lipofuscin bisretinoid formation: inhibition of key visual cycle enzymes such as RPE65, neutralization of free aldehydes, suppression of bisretinoid formation with deuterated vitamin A analogs, and reduction of retinol uptake to the retina with RBP4 antagonists.

Abbreviations: ABCA4, retina-specific ABC transporter; all-trans-RDH, all-trans-retinol dehydrogenase; LRAT, lecithin-retinol acyltransferase; 11-cis-RDH, 11-cis-retinol-dehydrogenase.
Figure 2. Structure of compounds exemplifying four classes of treatments capable of reducing lipofuscin bisretinoid production in animal models of enhanced retinal lipofuscinogenesis

A, Structure of the RPE65 inhibitor ACU-4429 as can be inferred from the US 2011/0003895-A1 patent application. A1120 (B) and fenretinide (C) exemplify the class of RBP4 antagonists. Racemic mixture of two primary amine-containing pegabalin stereoisomers (D) acts as an aldehyde trap. E, a deuterated C20-D₃ vitamin A analog in a form of retinyl acetate.