Apolipoprotein A-I Mimetic Peptide L-4F Removes Bruch’s Membrane Lipids in Aged Nonhuman Primates

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PURPOSE. Multiple evidence lines support Bruch’s membrane lipid deposition as a major precursor of soft drusen and age-related macular degeneration as including a potentially treatable atherosclerosis-like progression in the subretinal pigment epithelium (RPE)-basal lamina space. We evaluated the effect of anti-inflammatory, antiatherogenic peptide L-4F on Bruch’s membrane of aged nonhuman primates in a dose-escalating study.

METHODS. Macaca fascicularis ≥20 years of age evaluated by color fundus photography and optical coherence tomography received monocular intravitreal injections of L-4F (n = 7) or a placebo-scrambled peptide (n = 6) in 6 doses of 25 to 175 µg over 6 months. Eyes were processed for detection and masked semiquantitative assessment of macular Bruch’s membrane neutral lipid (oil red O staining), esterified cholesterol (filipin histochemistry), membrane attack complex (immunofluorescence), and paramacular thickness (transmission electron microscopy).

RESULTS. Bruch’s membrane neutral lipid, esterified cholesterol, and membrane attack complex were cleared and ultrastructure was improved in L-4F-injected eyes, compared to placebo-injected eyes. Fellow eyes were also affected to the same degree as the injected eyes. Punctate yellow fundus lesions without corresponding RPE elevations on optical coherence tomography correlated to RPE lipoidal degeneration (engorgement with lipid droplets), which was unchanged by this treatment.

CONCLUSIONS. Clinical-stage apolipoprotein A-I mimetic peptide L-4F delivered intravitreally in repeated doses, produced a substantial pharmacologic reduction of Bruch’s membrane lipid and restoration of ultrastructure in a nonhuman primate model that exhibits an important precursor of soft drusen, if not soft drusen themselves.

Keywords: age-related macular degeneration, lipids, Bruch’s membrane, retinal pigment epithelium, drusen, histochemistry, electron microscopy, lipoproteins, apolipoprotein mimic, L-4F

Age-related macular degeneration (AMD) is a common cause of legal blindness among older persons in industrialized countries.1 Neovascular complications are only partly managed with antiangiogenic agents.2 Recent clinical trials failed to meet primary endpoints of slowed expansion of geographic atrophy (GA),3–5 a stage that may be too late for intervention.6,7 An agent targeting earlier AMD stages8 could prevent both neovascularization and atrophy.

Multiple evidence lines, including human eye pathology, clinical imaging, cell biology, and epidemiology, support the targeting of soft drusen,9 the largest intraocular risk factor for AMD progression.10–12 Soft drusen are focal deposits of extracellular material between the basal lamina of the retinal pigment epithelium (RPE-BL) and the inner collagenous layer of Bruch’s membrane (BrM). The principal component of soft drusen (“membranous debris” of Sarks et al.)10,13,14 is now considered partially preserved large lipoprotein particles of RPE origin.15–18 Starting in early adulthood, BrM universally accumulates ultrastructurally and histochemically detectable lipid, including esterified cholesterol (EC), a hallmark lipid of lipoprotein particle cores. These back up and fill the sub-RPE-BL space,19–21 a pathogenic model that received recent experimental confirmation.22 Deposits are believed to represent a biomechanically fragile cleavage plane of proinflammatory, proangiogenic lipids,23–26 which, with VEGF gradients,27 facilitate entry and ramification of neovessels.28–30 Furthermore,
and soft drusen because it is small and because lipoprotein-instigated disease occurs in both atherosclerosis and AMD.46 We recently showed in an AMD-relevant mouse model47 that a single intravitreal injection of L-4F reached target tissues, dramatically reducing EC and restoring BrM ultrastructure.

Because therapeutically removing, reducing, or preventing BrM lipid accumulation could obviate downstream sequelae,48 this proof of concept (POC) study describes the effect of escalating doses of intravitreally-injected L-4F on BrM in aged nonhuman primates (NHPs). By color fundus photography (CFP) and OCT,49 NHPs in some closed colonies exhibit drusen9,40 L-4F abates atherosclerosis in animal models,31,41–43 and is well tolerated by humans.44,45 L-4F is attractive for targeting BrM lipids and soft drusen because it contains membranous debris,50 and they harbor human AMD lesions on CFP, thought to be drusen, were screened. Criteria of age; body weight, 4–7 kg) with punctate yellow fundus lesions on CFP, and high-sensitivity C-reactive protein (hs-CRP). Due to a clerical error, serum low-density lipoprotein (LDL) was not assayed.

### Compliance

We complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the German Public Health Service Policy on Humane Care and Use of Laboratory Animals. Animal studies were conducted at Covance facilities in Münster, Germany with approval from the German federal state of North Rhine-Westphalia. Only Covance personnel were allowed to perform intraocular injections, under supervision (MR).

### Peptides


### Animal Husbandry

Twenty-six Macaca fascicularis of Mauritian origin (>18 years of age; body weight, 4–7 kg) with punctate yellow fundus lesions on CFP, thought to be drusen, were screened. Criteria for study entry were the absence of medical history or signs of systemic or confounding eye diseases. Study NHPs received general health checks and blood analysis. They consumed a standard low-fat NHP diet supplemented daily with fruit and bread (Supplementary Material: Methods). All NHPs were considered healthy and exhibited mild age-related cataracts. One week before the first injection and 3 weeks after every injection, examinations of both eyes included CFP, fundus autofluorescence, slit lamp examination, tonometry, and fundoscopy. NHPs were imaged at baseline by spectral domain OCT (Supplementary Material: Methods). A blood checkup 2 days after injection included liver enzymes (aspartate aminotransferase, alanine aminotransferase, and gamma glutamyltransferase), lipids (total cholesterol, triglycerides, and HDL), and high-sensitivity C-reactive protein (hs-CRP). Due to a clerical error, serum low-density lipoprotein (LDL) was not assayed.

### Intravitreal Injections

Ten NHPs were enrolled in two treatment groups (sL-4F, M1-M2; L-4F, M3–M10). Each NHP received a total of 6 intravitreal injections in ascending doses (25–175 μg, total 625 μg) (Table) between April and August 2012. NHPs were sedated (intramuscular medetomidine [Domitor, Orion Corp., Espoo, Finland] and ketamine hydrochloride) and received a mydriatic (1%...
tropicamide; Mydriatikum, Pharma Stulln, Stulln, Germany). L-4F and sL-4F were freshly dissolved in sterile 0.9% balanced salt solution (Bausch and Lomb GmbH, Berlin, Germany) and used within 6 hours. Intravitreal injections were performed as recommended by the Retinal Society of Germany for humans. A lid speculum was emplaced, and a 31-gauge needle was inserted 2 mm from the corneo-scleral limbus via pars plana in the vitreous body center. Dexamethasone ointment (Bepanthen Bayer Vital GmbH, Leverkusen, Germany) was administered.

Follow-Up Assessments

As elaborated in the Results, the lack of RPE elevations consistent with drusen on OCT necessitated the use of histologic outcome measures to assess treatment effects. Detailed histologic methods, which were used in previous studies, are found in Supplementary Material: Methods.

In September 2012, NHPs were sedated with intramuscular ketamine hydrochloride, euthanized with intravenous sodium pentobarbital overdose, and transcardiac-perfused with fixatives. At necropsy, eyes and aorta were harvested. All organs appeared macroscopically unremarkable. M6 in the L-4F group was removed from the study on veterinarian recommendation 2 weeks after the first dose due to high blood sugar levels. M6 later developed diabetic shock and was euthanized. Eyes were opened via corneotomy and preserved by immersion, and due to compromised eye shape, submitted for qualitative lipid histochemistry only.

After anterior segment removal in all animals, lenses were examined under a preparation microscope (M125; Leica Microsystems, Wetzlar, Germany). In two cases, lens capsules were stained with 0.1% trypan blue for 30 seconds to detect injuries due to intravitreal injections. Macular plus optic nerve head and vascular arcades were removed with an 11.5-mm cornea trephine (Geuder AG, Heidelberg, Germany) for fluorescence and light microscopic evaluation (Supplementary Material: Methods).

Four tissue-level evaluations were performed on cryosections of the macula (Supplementary Material: Methods). The classic lysochromic lipid stain oil red O (ORO) binds to triglycerides, EC, free fatty acids, and vitamin A esters. Filipin is a polyene antibiotic that can be used to visualize both unesterified cholesterol and EC, depending on pretreatment. We focused on EC due to its specificity for BrM and drusen (compared to unesterified cholesterol, which localizes to all cellular membranes), and its significance as a lipoprotein core lipid. Indirect immunofluorescence was used to assess membrane attack complex (MAC), previously localized to human BrM. MAC (C5b-9) is the terminal effector of complement pathways of known AMD importance due to associations of complement gene sequence variants and complement proteins in drusen. For ultrastructural evaluation of the RPE-BrM-choroid complex and BrM integrity and thickness, 4-mm-diameter punches centered at 2 to 4 mm temporal to the fovea were shipped to University Eye Hospital Erlangen for transmission electron microscopy (US-S).

Statistics and Data Analysis

The null hypotheses were that L-4F-injected, placebo-injected, and noninjected eyes had identical outcomes on the four histologic assessments. Statistical evaluations used t-tests in BioStat 2009 Professional (v. 5.8.4.3; AnalystSoft, Inc., Walnut, CA, USA), SPSS 20 (IBM, Armonk, NY, USA) and Excel Add-in XLSTAT (v. 2018.2; XLSTAT, New York, NY, USA). P ≤ 0.05 was considered significant.

Results

Multimodal Imaging Revealed No Drusen

By ophthalmoscopy and CFP, fundus lesions appeared as small yellow round puncta of uniform size at the level of the RPE (Fig. 1). The number of lesions varied between NHPs and did not obviously change during the study (Fig. 1). The fundus lesions did not manifest as RPE elevations by OCT (Fig. 2), which revealed only normal RPE and macular anatomy in all NHPs. Fundus autofluorescence was unremarkable (not shown).

By Clinical Observation, Injections Were Well Tolerated

A localized slight redness or conjunctival bleeding at the injection site was associated with the procedure. No significant intraocular inflammatory reaction was observed. Intraocular pressure was within normal limits (<21 mm Hg). One NHP from the placebo group had pressures up to 23 mm Hg in the injected eye (Supplementary Fig. S2) and borderline high...
pressure in the fellow eye (up to 24 mm Hg). All NHP had binocular yellow cataracts that progressed over time, without relation to the drug or injection. In two animals, a lens touch was found.

**Lipid Histochemistry Demonstrates Binocular Lipid Clearing After Monocular Injections**

We observed neither gross alterations of eyes nor pathologic changes visible by light microscopy. The main target was extracellular lipid in and around BrM. In NHPs receiving placebo, both injected and noninjected eyes had intense ORO staining of BrM and intercapillary pillars (Figs. 3A, 3B), resembling older humans. In contrast, NHPs receiving multiple escalating doses of L-4F showed a significant reduction of BrM staining in the injected eye (Fig. 3C). BrM was also cleared in the second, noninjected eye of these same NHPs (Fig. 3D). Prior lipid extraction abolishes BrM staining (Fig. 3E). In the early terminated M6 with only one injection, BrM in both eyes stained intensely (Supplementary Fig. S3), strongly suggesting that the treatment effect is a function of dose and/or time. ORO staining intensity, quantified in eyes of 3 groups (L-4F-injected, placebo-injected, and noninjected) (Fig. 4), dropped 61% in L-4F-injected and noninjected fellow eyes.

The ORO findings were corroborated by filipin fluorescence for EC, both qualitatively (Fig. 5) and quantitatively via fluorescence intensity, for treatment groups (Fig. 6A) and for individual NHPs (Fig. 6B). Mean decline in intensity in L-4F-injected eyes and fellow noninjected eyes was estimated at 68.2% after 6 months, compared to placebo-injected NHPs (Fig. 6A).

Histochemically detectable neutral lipid and EC in NHP photoreceptor outer segments (Figs. 3, 5) support recent findings in mice and contrast with long-standing concepts about low EC concentration in outer segments based on chromatography. Whether EC localizes to outer segments themselves, interphotoreceptor matrix, or both, remains to be determined.

By histologic examination, no soft drusen were detected in any eye. Rather, histologic examination revealed single RPE cells intensely stained with ORO (Figs. 7A–C) (and not filipin) that corresponded in number and distribution to the punctate fundus lesions. By electron microscopy, these lesions localized to the sub-RPE-BL space and contained amorphous granular and membranous materials (Fig. 8). Thus, confirmed as tiny hard drusen by laminar location, shape, and ultrastructure, these deposits measured ≤8 μm in height and ≤30 μm in width at the base.

**Monocularly Injected L-4F Binocularly Clears C5b-9 MAC Immunoreactivity**

To investigate MAC, we used indirect immunofluorescence and semiquantitative grading of fluorescence intensity. In the placebo-injected and noninjected fellow eyes of the same animals, prominent immunoreactivity was found in choriocapillaris endothelium and adjoining BrM, including intercapillary...
pills (Figs. 9A, 9B). In the L-4F injected eyes and noninjected fellow eyes of the same NHPs, immunoreactivity was markedly reduced (Figs. 9C, 9D; 64% by assessment of fluorescence intensity, Fig. 10).

Fine Structure and Thickness of BrM is Improved

BrM at the macula edge near the temporal retinal vascular arcades was examined by transmission electron microscopy. In this region of placebo-injected eyes, BrM was thick with accumulation of electron-lucent vacuoles (Fig. 11A, 11B), likely resulting from processing-related extraction of lipid. BrM was thinner in L-4F-injected eyes and noninjected fellow eyes of the same animals, compared to placebo-injected eyes (Figs. 11C, 11D, 12).

Serum Lipids and Proteins, Aorta Are Unremarkable

Consistent with previous L-4F studies, total cholesterol, triglycerides, and HDL were within normal limits for all but M6, which was abnormal even before dosing (Supplementary Fig. S3). The only known side effect from human L-4F trials is an early increase of hs-CRP (of hepatic origin), interpreted as secondary to increased L-4F-mediated transport of oxidized lipids from peripheral tissues to the liver. Here, the placebo group had relatively high hs-CRP values at baseline and higher values than the L-4F group at all time points. hs-CRP decreased in both groups over time, sometimes below detection (Supplementary Fig. S4). All liver enzymes were within normal limits except for M6 (Supplementary Fig. S5). Finally, aortic valves lacked evidence of atherosclerosis (Supplementary Fig. S6).

DISCUSSION

We provide a POC pharmacology study of repeated intravitreal L-4F injections in aged NHPs that exhibited abundant extracellular lipids in BrM. We demonstrate that L-4F is a novel, well-tolerated, and effective means to remove these lipids and improve BrM ultrastructure in NHPs. Supported by strong biologic rationale in human eye pathology, epidemiology, and genetics, these positive results have important clinical implications for treating AMD at an early stage. As elaborated below, L-4F potentially neutralizes the effects of accumulated lipids, a major step in soft drusen biogenesis. We previously elaborated a rationale for targeting soft drusen to forestall progression to type 1 neovascularization and GA in AMD. Because study animals lacked soft drusen and their sequelae, we cannot address this possibility directly. Yet, the current results represent strong progress toward this goal.
been proposed as retinyl ester stores.\textsuperscript{52,53,78} These cells were degeneration resembles steatosis, and the lipid droplets have elus.\textsuperscript{79–81} Because we tested an agent of initially unknown contrast to single injections previously used in these mod-

Future studies using NHPs should be designed accordingly.\textsuperscript{\textendash}is possible that both pathologies may exist in other animals.

Interestingly, the fundus lesions correlated to isolated, intense-
to confine the macula, and containing previously seen heterogeneous cellular debris.\textsuperscript{73–75} These were too small for clinical visibility (<30 \mu m).\textsuperscript{76,77} Interestingly, the fundus lesions correlated to isolated, intensely ORO-positive RPE-cells with little EC.\textsuperscript{52} Lipoidal RPE degeneration resembles steatosis, and the lipid droplets have been proposed as retinyl ester stores.\textsuperscript{52,53,78} These cells were unchanged by L-4F administration, even while BrM was cleared. Our conclusions about lipidol degeneration were facilitated by the absence of soft drusen in the study NHP, but it is possible that both pathologies may exist in other animals. Future studies using NHPs should be designed accordingly.

In NHP, we performed multiple injections over 6 months, in closed colonies can have soft drusen and are considered the closest model of early AMD.\textsuperscript{70,71} However, as reported in rhesus macaques\textsuperscript{49} and in humans,\textsuperscript{72} these Macaca nemestrina had punctate yellow lesions on CFP that did not manifest as RPE elevations on OCT. Light and electron microscopy revealed tiny drusen, not confined to the macula, and treatments.\textsuperscript{85–87} We targeted extracellular lipids, a cardinal feature of aging human BrM and a critical process in soft drusen biogenesis.\textsuperscript{9} We suspect that surface-active agents like L-4F would be advantageous in eyes that have these loosely packed lipid deposits, unlike the study NHP. Strong evidence supports the idea that soft drusen result from the accumulation of apolipoprotein B- and E-containing lipoprotein particles secreted by the RPE to recycle dietary and outer segment lipids and impeded in egress to the circulation by a physical barrier at the aged BrM-choriocapillary complex.\textsuperscript{9} Our approach contrasts with others also involving lipids. For example, increased offloading of cellular cholesterol to circulating HDL via liver X receptor (LXR) agonists\textsuperscript{86} may reduce substrate availability for apoB lipidation. Another route is to stimulate RPE uptake of lipids, possibly to clear drusen (e.g., via the CD36 scavenger receptor).\textsuperscript{89} Recently a single-arm trial reported regression of large drusen after 1 year of 80 mg/day atorvastatin\textsuperscript{8,88} which may involve intraocular as well as systemic mechanisms because the RPE

![Figure 6](image-url)

**FIGURE 6.** Filipin fluorescence for EC decreased after L-4F treatment. (A) As a group, L-4F injected eyes had 68.2% less EC than eyes injected with sl-4F. Statistical tests: between injected and noninjected eyes in the same group, paired \textit{t}-test; between injected and placebo group, unpaired \textit{t}-test. (B) Individual L-4F-treated animals exhibited reduced fluorescence intensity, in both eyes, compared to placebo-treated animals. Eyes of the early terminated animal M6 were not submitted for filipin histochemistry.

![Figure 7](image-url)

**FIGURE 7.** Lipoidal degeneration of retinal pigment epithelium corresponding to drusenoid fundus lesions in aged NHPs. CC, choriocapillaris; PR, photoreceptors; Arrowhead 1. BrM. (A, B) Lipid-filled RPE (2, 3). (C) Diffuse staining (4). (D) Tiny drusen (5); filipin for EC, differential interference contrast and 488-nm epifluorescence microscopy.

binocular treatment effect on all outcome measures despite monoclonal injection. This noteworthy systemic effect of L-4F was not found in our prior study using single doses in the apoE\textsuperscript{77} AMD-relevant mouse model,\textsuperscript{47} nor was it seen by clinical observation of these NHP. However, it was documented objectively by semiquantitative analysis of histochemical markers in postmortem tissue-level studies. Reasons for this finding are currently unclear. Because L-4F reached the BrM target, it could have also entered the systemic circulation via the choriocapillaris, as reported for antiangiogenic agents.\textsuperscript{82,83} The study NHPs did not have atherosclerotic lesions that could bind L-4F and lower plasma concentrations, at least in the aorta, which was examined histologically. Other organs were grossly normal at necropsy.

Data allow a preliminary assessment of safety. L-4F was well tolerated even at the highest dose. Adverse events were mild and related to injections rather than the drug. We chose intravitreal injection for delivery to assess the validity of the therapeutic strategy, while also permitting precise dosing and use of fellow eyes for comparison. Injections in macaques are challenged by small eyes and access limited by a pronounced orbital ring. These factors, plus nonspecialist-administered injections, explain why 2/9 animals experienced lens touch, a rare event in humans.\textsuperscript{84} High plasma glucose like that in one early terminated NHP (M6) has been reported in older animals receiving the sedatives we used for eye examinations and treatments.\textsuperscript{85–87}

We targeted extracellular lipids, a cardinal feature of aging human BrM and a critical process in soft drusen biogenesis.\textsuperscript{9} We suggest that surface-active agents like L-4F would be advantageous in eyes that have these loosely packed lipid deposits, unlike the study NHP. Strong evidence supports the idea that soft drusen result from the accumulation of apolipoprotein B- and E-containing lipoprotein particles secreted by the RPE to recycle dietary and outer segment lipids and impeded in egress to the circulation by a physical barrier at the aged BrM-choriocapillary complex.\textsuperscript{9} Our approach contrasts with others also involving lipids. For example, increased offloading of cellular cholesterol to circulating HDL via liver X receptor (LXR) agonists\textsuperscript{86} may reduce substrate availability for apoB lipidation. Another route is to stimulate RPE uptake of lipids, possibly to clear drusen (e.g., via the CD36 scavenger receptor).\textsuperscript{89} Recently a single-arm trial reported regression of large drusen after 1 year of 80 mg/day atorvastatin\textsuperscript{8,88} which may involve intraocular as well as systemic mechanisms because the RPE
has LDL receptors and statins directly reduce apoB secretion.

L-4F came from research about the structure and lipid-sequestering properties of apolipoproteins, especially apoA-I. L-4F combats atherosclerosis in model systems by pleomorphic effects in the vascular endothelium, intima, and intestine. These include limiting LDL oxidation and aggregation to reduce monocyte adhesion to proteoglycans and improving HDL function by increasing pre-

HDL, decreasing lipid hydroperoxides in HDL, and enhancing anti-inflammatory properties. Amphiphilic L-4F quells inflammation by scavenging oxidized phospholipids and fatty acid hydroperoxides that might otherwise partition into cellular membranes. Our initial expectation was that L-4F would sequester oxidized lipids and remove them into the circulation. Thus, it was surprising that so much neutral lipid including EC disappeared after L-4F administration. Known proangiogenic and proinflammatory lipids in aging BrM and drusen include 7-ketocholesterol and linoleate hydroperoxides. These may also be scavenged by L-4F and account for some of the disappeared lipids. L-4F affinity for oxidized phospholipids is several-fold higher than that for native phospholipids in membranes, potentially allowing the removal of toxic phospholipids without markedly impacting local cells, a notion supported (but not proven) by differential effects on RPE and BrM (Figs. 3, 5). Animal studies testing L-4F efficacy for cardiovascular disease assessed fatty streaks rather than intimal lipoprotein deposition and cannot elucidate the identity of eliminated extracellular lipids. Finally, L-4F action in the eye does not preclude an impact on intestinal absorption and delivery of lipids of interest due to recent evidence that the gut biome modulates AMD risk.

We found substantially reduced MAC immunoreactivity in eyes receiving intravitreal L-4F. Although not tested directly, MAC immunoreactivity in NHP, as in humans, was likely adjacent to and not in the choriocapillary endothelium. Bruch’s membrane of monkeys treated with L-4F or placebo were assessed. Comparisons between injected and noninjected eyes in the same group, paired t-test; between injected and non-injected eyes in the placebo group, unpaired t-test.

**FIGURE 8.** Very small drusen with heterogeneous contents. (A–C) Examples of drusen too small to substantially elevate the RPE, temporal to macula of NHP (Macaca fascicularis). Deposits are extracellular, with heterogeneous contents, located between the RPE-BL (orange arrowbeads) and the inner collagenous layer of BrM. ChC, choriocapillaris lumen.

**FIGURE 9.** Membrane attack complex immunofluorescence declines binocularly after monocular L-4F injection. (A, B) Hematoxylin-stained sections. (C, D) Intense green signal (arrowbead) in injected (C) and noninjected fellow eyes of placebo group (D). (E, F) Reduced intensity in injected and noninjected fellow eyes of L-4F group. Blue, ONL nuclei; ONL, outer nuclear layer; PR, photoreceptors; CC, choriocapillaris; Ch, choroid.

**FIGURE 10.** Semiquantitative assessment of MAC immunoreactivity. Bruch’s membrane of monkeys treated with L-4F or placebo were assessed. Comparisons between injected and noninjected eyes in the same group, paired t-test; between injected and non-injected eyes in the placebo group, unpaired t-test.
immunoreactivity is strong in small but not large soft drusen. Why does MAC localize to the subendothelial space, and why is it undetectable after L-4F treatment? One way in which nucleated cells such as RPE and choriocapillary endothelium survive MAC is the physical removal of MAC from cell surfaces by shedding of vesicles with membranes enriched in unesterified cholesterol and diacylglycerol. The presence of MAC in BrM may signify that protective mechanisms are functional, regardless of which cells are MAC targets. Perhaps these vesicles partition into or associate with lipids accumulating in nearby BrM. Supporting this idea, MAC immunoreactivity in early atherosclerotic lesions colocalizes exclusively with insudated plasma lipoproteins. Furthermore, in AMD eyes, MAC immunoreactivity is lightly scattered throughout or confined to the basal aspect of thick basal laminar deposits, where membranous material is present. In this scenario, L-4F abrogates MAC immunoreactivity by removing solubilizing lipids in BrM.

Our study has general relevance to AMD models, none of which to date exhibit the entire AMD spectrum. Yet, within a comprehensive theory of AMD pathogenesis (for example, Refs. 9 and 119), specific processes such as neovascularization and RPE migration can be successfully explored in animal models. NHP can have ultrastructurally confirmed drusen, as do ours, and in at least one colony, drusen with contents resembling membranous debris of Sarks et al. We recommend that fundus lesions by CFP be verified as sub-RPE-BL extracellular deposits by OCT, histology, and electron microscopy. This recommendation also applies to mouse models, in which pale fundus spots can correspond to cells rather than extracellular deposits.

This POC study was not designed to assess levels and pharmacokinetics of L-4F in plasma, plasma-oxidized lipid markers, identity of removed lipids, mechanisms of lipid clearance, the effect of L-4F on clinically visible drusen, function or lipid content of retinal cells, and lipid deposition in animals of specific genotypes. Future directions could include an exploration of lower dose intravitreal injections and alternative delivery routes, such as intravitreal sustained release, topical, or systemic administration. This study’s significant strength is a well-tolerated, bilateral, highly effective pharmacologic reduction of BrM lipids, an important precursor to soft drusen, in NHPs that lacked soft drusen themselves. Together with prior results in mice also demonstrating efficacy of BrM lipid clearing, the data support L-4F as a promising candidate for eventual advancement to trial in patients with AMD.
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