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Lipid metabolites in the pathogenesis and treatment of neovascular eye disease

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Abstract

Lipids and lipid metabolites have long been known to play biological roles that go beyond energy storage and membrane structure. In age-related macular degeneration and diabetes, for example, dysregulation of lipid metabolism is closely associated with disease onset and progression. At the same time, some lipids and their metabolites can exert beneficial effects in the same disorders. This review summarises our current knowledge of the contributions of lipids to both the pathogenesis and treatment of neovascular eye disease. The clinical entities covered are exudative age-related macular degeneration, diabetic retinopathy and retinopathy of prematurity, with a special emphasis on the potential therapeutic effects of ω 3-(also known as *n*-3) polyunsaturated fatty acids.

INTRODUCTION

Angiogenesis research began to evolve into an academic specialty in its own right as early as the 1970s.^{1,2} An impressive body of literature has since accumulated, comprising close to 50 000 articles in PubMed. However, only about 2000 (4%) of these publications relate to lipids (PubMed search September 2010). This is in stark contrast to the increasing evidence of a potent role for lipid mediators particularly in the development of neovascular eye disease. Progression of diabetic retinopathy, for example, can be modulated by altering a patient's lipid profile.^{3–5} Similarly, progression of age-related macular degeneration (AMD) and retinopathy of prematurity (ROP) have been linked to differences in lipid intake and metabolism.^{6–14}

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One class of lipid-based mediators found to modulate neovascular eye disease particularly are ω 3-(also known as *n*-3) polyunsaturated fatty acids (PUFAs).⁷⁸¹¹¹⁵¹⁶ ω 3-PUFAs are essential fatty acids that cannot be synthesised in sufficient amounts by humans and must therefore be obtained from the diet. The retina has the highest ω 3-PUFA concentration of all tissues (20%) and ω 3-PUFAs serve vital functions in normal retinal architecture and function.¹⁷ Conversely, excess dietary intake of the structurally similar ω 6-PUFAs appears to convey very different, even opposing effects in the retina: while ω 3-PUFAs appear to be associated with a reduced risk of AMD,¹⁸ ω 6-PUFAs seem to be associated with an increased risk.⁸

These observations illustrate that it is crucial to understand the role of lipid pathways and lipid-derived mediators in retinal health and disease in order to develop targeted, lipid-based therapies. It may well be that among the multitude of ω 3-PUFA-derived lipid metabolites, only few are relevant for conveying beneficial effects during proliferative retinopathy or AMD. A direct analogy may be drawn from proteins, where vascular endothelial growth factor (VEGF) stands out as a major angiogenic mediator from a plethora of other, less relevant proteins. This review will provide an overview of our current knowledge of lipid-based mediators and their relative contribution to both the pathogenesis and potential treatment of neovascular eye diseases. The clinical entities covered will comprise wet AMD, diabetic retinopathy and retinopathy of prematurity (ROP), three of the most prevalent blinding eye diseases.^{19–21}

NON-ENZYMATIC LIPID PATHWAYS: LIPID PEROXIDATION

Late-stage neovascular AMD (exudative or wet AMD) is characterised by progressive dysfunction of macular retinal pigment epithelium (RPE) and formation of choroidal neovascularisation (CNV).²²²³ While the complex pathogenesis of AMD is not yet fully elucidated, clinical and experimental evidence support a key role for reactive lipid mediators generated by oxidative damage in the outer retina.²⁴²⁵ The retina exceeds all other human tissues in its concentration of PUFAs. Due to their polyunsaturated structure, these PUFAs are particularly susceptible to oxidative degradation by non-enzymatic lipid peroxidation. Within the retina, PUFAs are especially enriched in the photoreceptor outer segment (POS) membranes, putting the outer retina at high risk for damage by lipid peroxidation products.

During non-enzymatic lipid peroxidation, oxygen-derived free radicals interact with unsaturated PUFA double bonds²⁶ to generate a variety of highly reactive aldehyde intermediates, the most widely studied being malondialdehyde (MDA), 4-hydroxynonenal (HNE) and carboxyethylpyrrole (CEP).²⁵²⁷ These molecules rapidly attach to cellular proteins forming covalent adducts (advanced lipid peroxidation end products, ALE), thereby impairing protein stability and function. Accumulation of lipid peroxidation products and ALEs occurs in vivo as a result of both photo-oxidative damage and ageing.^{28–30} ALEs are also found in RPE-derived lipofuscin isolated from human donor eyes³¹ as well as in drusen and Bruch's membrane of AMD patients.³²

Photoreceptor cells cope with damage by continuous shedding and renewal of their PUFA-rich outer segments (POS). The shed POS are phagocytosed by RPE cells that metabolise and clear the toxic lipid peroxidation products, mainly through lysosomal enzymes. Thus RPE lysosomal enzymes are important modulators in this process as well as likely targets for lipid peroxidation-mediated damage. This notion is supported by in vitro observations that phagocytosis of lipid peroxidation-modified POS by RPE can induce profound lysosomal dysfunction,³³³⁴ resulting in increased lipofuscin generation, impaired cellular self-renewal by autophagy, and release of undegraded POS proteins into the sub-RPE space.⁹³⁵ Furthermore, lipid peroxidation-related lysosomal dysfunction induces VEGF

secretion by RPE cells in vitro (Bergmann *et al*, unpublished results) and subretinal injection of ALEs exacerbates laser-induced CNV in vivo.³⁶ Interestingly, ALEs also serve as haptens that induce autoantibody formation against lipid peroxidation-modified retinal proteins inducing a subsequent inflammatory response in the sub-RPE space that may contribute to complement activation and RPE damage in AMD.²⁷³⁷

Combined, these results from in vitro and in vivo studies suggest that lipid peroxidation products contribute to the pathogenesis of exudative AMD. In accordance with this, the Age-Related Eye Disease Study (AREDS)1 demonstrates a protective effect of antioxidative treatment on AMD progression,³⁸ indicating that under specific circumstances oxidative processes in AMD are susceptible to therapeutic intervention. However, other clinical studies targeting lipid peroxidation, for example by carbonyl scavenging, have not yielded conclusive results.³⁹ Further research is needed to evaluate if interference with lipid peroxidation is useful for prophylactic and therapeutic intervention in AMD.

Similar processes as illustrated above for AMD may also play a role in proliferative retinopathies. While less research has been done in these areas, non-enzymatic lipid peroxidation initiated by reactive oxygen species has been implicated in the pathogenesis of both diabetic retinopathy and ROP.^{40–42} Similarly, the conversion of arachidonic acid (AA) into *trans*-AA under nitrate stress was found to mediate apoptosis of microvascular cells via upregulation of thrombospondin-1 (TSP-1) and activation of the CD36 receptor on endothelial cells.⁴³ Approaches to reduce oxidative and nitrate stress using antioxidative therapy would attenuate these detrimental non-enzymatic lipid peroxidation processes. However, antioxidative therapies have produced inconsistent results thus far^{44–46} and more studies are needed to evaluate if antioxidants can play a significant role in attenuating proliferative retinopathies.

ENZYMATIC LIPID PATHWAYS: ATX, PLA₂, COX, LOX AND CYP450

Beyond non-enzymatic lipid peroxidation, many specific lipid-metabolising enzymes play a role in angioproliferative diseases.⁴⁷ One example is autotaxin (ATX), an enzyme that converts lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA), a bioactive signalling molecule. Once generated, LPA can upregulate VEGF-C expression and induce tube formation in endothelial cells.⁴⁸ One other important example is phospholipase A₂ (PLA₂), an enzyme that becomes activated following ischaemia/ reperfusion injuries.^{49–51} PLA₂ catalyses the hydrolysis of lipids from cell membranes, liberating them for further metabolism and signalling. The amount of each fatty acid released from the cell membrane depends on the relative amount of that particular fatty acid present in the membrane. For essential fatty acids (which cannot be adequately synthesised by humans) this available amount of lipid substrate, in turn, is dependent on dietary intake.¹⁸

An important lipid that is liberated by PLA₂ from the cell membrane is AA, a member of the family of ω 6-PUFAs. Once liberated, AA is rapidly further metabolised by three major pathways: the cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (Cyp450) pathways (figure 1). COX-2 in particular is among the immediate-early gene products that is upregulated in response to retinal ischemia, for example in diabetic retinopathy.⁵²⁵³ The same enzymes (PLA₂, COX, LOX, Cyp450) that metabolise ω 6-PUFAs also process the structurally similar ω 3-PUFAs, which can be exploited for potential therapeutic approaches (see below).

ω 6-PUFA metabolites TXA₂, PGE₂ and 5-HETE

Increased PLA₂ and COX-2 activities can have important implications for proliferative retinopathies. For example, increased production of ω 6-PUFA-derived thromboxane A₂

(TXA²) via PLA₂ and COX-2 can lead to a time- and concentration-dependent death of retinal endothelial cells.^{54–57} Interestingly, TXA² is generated more abundantly in the stressed newborn retina compared with adults, potentially giving this lipid-derived metabolite an important role in the pathogenesis of ROP.⁴⁷ In contrast to the endotheliotoxic effects of TXA₂, other ω 6-PUFA-derived mediators from the COX-2 pathway have potent pro-angiogenic properties during retinopathy. Prostaglandin (PG) E², for example, can stimulate the formation of pathological retinal neovessels through binding to its PGE receptor 3 (EP³).^{53,58} Importantly, ω 6-PUFA-derived PGE₂ and ω 3-PUFA-derived PGE₃ exert opposing effects on endothelial cell proliferation. While PGE₂ increases the production of angiotensin-2 (ANG2) and matrix metalloproteinase-9 to stimulate endothelial tube formation, PGE₃ inhibits the same processes.⁵⁹

It is not only cyclooxygenase-dependent metabolites that are differentially regulated during neovascular eye diseases. The lipoxygenase-dependent ω 6-PUFA product 5-hydroxyeicosatetraenoic acid (5-HETE), for example, is increased in vitreous of patients with diabetic retinopathy.⁶⁰ These findings, along with reports on ω 6-PUFA-derived 5-HETE and epoxyeicosatrienoic acids (EETs) being involved in mediating both inflammatory and angiogenic processes^{61–63} illustrate that not only COX-dependent metabolites of arachidonic acid but also lipoxygenase- and/or Cyp450-dependent lipid metabolites of ω 6-PUFAs may be potent modulators of proliferative retinopathies. This contribution of enzymatically created lipid mediators to the pathogenesis of retinopathy appears especially relevant in patients with diabetes where strong evidence points towards a significant contribution of dyslipidaemia to retinopathy progression.^{364–67} In AMD, the AREDS1 indicates that higher intake of ω 6-PUFAs may be associated with a higher prevalence of exudative AMD.⁸

LIPID MEDIATORS AS THERAPEUTICS FOR NEOVASCULAR EYE DISEASE

As illustrated above, many lipid-derived mediators can potently induce or amplify neovascular eye disease. Increasing evidence, however, indicates that other lipid-derived mediators can improve the same pathologies. This is especially the case with ω 3-PUFAs, which convey beneficial effects in several models of neovascular eye disease.^{11,15,16} Structurally, ω 3-PUFAs are very similar to ω 6-PUFAs and differ solely in the location of their double bonds: ω 3-PUFAs have the first double bond after the third carbon atom when counting from the ω end; ω 6-PUFAs have the first double bond after the sixth carbon atom (figure 2).

The minor differences in structure between ω 3- and ω 6-PUFAs translate into significant differences in biological function. Although ω 3-PUFAs are metabolised by the same enzymes as ω 6-PUFAs, unique metabolites are generated from ω 3-PUFAs (figure 3). Pioneering work by Serhan and colleagues identified resolvins and neuroprotectins as potent anti-inflammatory and anti-angiogenic ω 3-PUFA metabolites.^{68–70} Studies in animal models of proliferative retinopathy¹¹ and choroidal neovascularisation¹⁵ found that these ω 3-PUFA metabolites significantly attenuated neovascular eye disease. In addition, the ω 3-PUFA metabolite neuroprotectin promotes RPE survival during oxidative stress,^{71,72} a process thought to be involved in the pathogenesis of AMD.

Lipid mediators as therapeutics for exudative AMD

Both ω 3- and ω 6-PUFAs are essential fatty acids obtained from diet.⁷³ This has led to studies investigating the correlation between dietary ω 3-PUFA intake (mainly in the form of fish) and the risk for neovascular eye disease. The largest set of data on ω 3-PUFAs and AMD risk comes from the AREDS1 study. While AREDS1 was not initially designed to evaluate the effect of ω 3-PUFAs on progression of AMD, retrospective data analysis found

that ω 3-PUFA intake is inversely associated with neovascular AMD.⁸ Conversely, higher intake of AA (an ω 6-PUFA mainly obtained from ingestion of meat) is associated with greater prevalence of neovascular AMD.⁸ As retrospective data and reliance on patient-reported data may introduce confounding variables,⁷⁴ the ongoing AREDS2 study was specifically designed to prospectively evaluate the impact of ω 3-PUFA supplementation on AMD prevalence and progression. With definitive results from the placebo-controlled, double-blind AREDS2 study not available before 2013, the currently available data from both pre-clinical¹⁵⁷¹⁷² and retrospective clinical studies⁷⁸ must be interpreted carefully, but on the whole, they point towards beneficial effects of ω 3-PUFAs in AMD. In RPE cell cultures ω 3-PUFAs reduced oxidative stress-induced apoptosis by over 70%⁷² and in a mouse model of laser-induced CNV, neuroprotectin D1 (an ω 3-PUFA metabolite) reduced CNV areas by 68%.¹⁵ Clinically, the retrospective AREDS1 data analysis suggests higher intake of ω 3-PUFAs to be associated with a decreased likelihood for AMD progression⁸ and participants who reported the highest ω 3-PUFA intake were 30% less likely to develop late stage AMD over a 12-year period.⁷ No apparent risks were associated with increased ω 3-PUFA intake in patients at risk of AMD.

Lipid mediators as therapeutics for diabetic retinopathy and ROP

While exudative AMD, diabetic retinopathy and ROP all share the final common pathway of retinal (or subretinal) neovascularisation, it has to be stated clearly that very disease-specific variables influence disease onset and progression in these three entities. While in AMD degenerative changes in RPE and Bruch's membrane play an important pathogenic role,⁷⁵⁷⁶ diabetic retinopathy is characterised by severe metabolic dysregulation⁴⁵ and ROP by an overall immaturity not only of the retina but the whole organism.⁷⁷⁷⁸ All these disease-specific factors can significantly alter lipidomic profiles and the expression of lipid-processing enzymes and thus may differentially affect the success of lipid-based therapies. Therefore, it will be important to evaluate independently whether the positive findings from the AREDS1 cohort⁷⁸ can be extended to diabetic retinopathy and ROP.

With regard to diabetic retinopathy, it is intriguing that recent studies have identified peroxisome proliferator-activated receptor (PPAR) γ as one of the target receptors of ω 3-PUFAs.¹⁶ Activation of the nuclear receptor PPAR γ by ω 3-PUFAs was found to be crucial in mediating the beneficial effect of ω 3-PUFAs in experimental retinopathy.¹⁶ Interestingly, activation of the same receptor by rosiglitazone, a drug that is used to treat insulin resistance in type 2 diabetes, was found to have similar beneficial effects on progression of diabetic retinopathy.⁷⁹ An independent study with over 10 000 patients also reported beneficial effects on progression of diabetic retinopathy when patients were treated with fenofibrate, a pharmacological activator of PPAR γ .³ PPAR γ shares high structural homology with PPAR γ and can similarly be activated by ω 3-PUFAs.⁸⁰ The prospect that ω 3-PUFAs exert their beneficial effects on retinopathy, at least in part, through activation of PPARs¹⁶ may offer the prospect of using ω 3-PUFAs as alternative or supplement to established pharmacological PPAR activators in the treatment of diabetic complications, including retinopathy.³¹⁶⁷⁹

With regard to ROP, data from the mouse model of oxygen-induced retinopathy (OIR)^{81–84} strongly indicates a beneficial effect of ω 3-PUFAs on both functional vascularisation of avascular retina¹¹ as well as reduction of pre-retinal neovascularisation.¹⁶ The effect of ω 3-PUFAs in this model is profound and comparable in its extent to the effects that are achievable with anti-VEGF therapy during OIR (figure 4).⁸⁵

Importantly, in premature infants ω 3-PUFA supplementation may have implications that reach far beyond ROP. During normal third trimester development, ω 3-PUFAs are provided in utero from the mother to her fetus. This physiological supply is interrupted by premature

birth. Recent studies found that newborn infants may exhibit daily deficits of up to 44% in docosahexaenoic acid (DHA; an ω 3-PUFA).⁸⁶ However, total parenteral nutrition (TPN) for premature infants is in many cases completely devoid of ω 3-PUFAs, thus not replenishing this deficit. Clinical studies that evaluate the effects of increased ω 3-PUFAs in TPN protocols have suggested a beneficial effect of ω 3-PUFAs on the progression of TPN-associated liver disease.^{87,88} Combined with the results from OIR animal studies^{11,16} these data suggest that supplementing ω 3-PUFAs in preterm infants to levels that are seen in utero during a healthy third trimester pregnancy might improve not only ROP but be beneficial for overall postnatal development.

Lipid mediators and over-the-counter COX inhibitors

Inhibitors of the COX pathways are among the most frequently used over-the-counter drugs. These COX inhibitors significantly alter lipid metabolism by blocking parts of the major enzymatic lipid pathways (figures 1 and 3). However, no concordant results have been reported from studies investigating the effect of COX inhibitors on neovascular eye disease.^{89–91} One explanation might be that inhibiting COX activity decreases production of both potentially beneficial as well as potentially negative metabolites (figure 5A,B) or that beneficial metabolites are produced through another enzyme system. Altering the substrate pool of available lipids (figure 5C) or administering beneficial lipid metabolites (figure 5D) might be a more efficient approach to shift the retina towards a more advantageous composition in lipid-derived mediators.

CONCLUSION

Results from animal studies and clinical trials suggest that lipid-based mediators are likely to emerge as a novel group of modifiable variables in neovascular eye disease. As lipid mediators can convey both positive and negative effects on retinopathy, inhibition of lipid-metabolising enzymes (eg, by COX inhibitors) may reduce both positive and negative metabolites. Whether the optimal approach for effective lipid-based treatments will lie in supplementation of beneficial lipid substrates such as ω 3-PUFAs or rather in the selective use of beneficial lipid metabolites or enzyme inhibitors will largely depend on ongoing research aimed at identifying the specific lipid metabolites that convey beneficial effects in retinopathy. Given our current knowledge, an important conclusion at this stage is that lipid mediators can play important roles in both pathogenesis and treatment of neovascular eye disease and that a thorough understanding of retinal lipid metabolism must form the foundation of any lipid-based intervention. The clinical potential of this relatively new field in ophthalmology research and the promising findings obtained so far render lipid-based therapies a very interesting target to investigate further in order to harness lipids and their metabolites as future therapies of neovascular eye disease.

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Figure 1.

Schematic of the major enzymatic pathways metabolising ω6-polyunsaturated fatty acids (PUFAs). Phospholipase A₂ (PLA₂) liberates ω6-PUFAs (eg, arachidonic acid (AA) or linoleic acid (LA)) from the cell membrane. These lipids are rapidly further metabolised by cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (Cyp450) enzymes, generating the pathway-specific lipid metabolites thromboxane A₂ (TXA₂), prostaglandin (PG) E₂, PGF_{2α}, hydroxyeicosatetraenoic acids (HETEs), epoxyeicosatrienoic acids (EETs), dihydroxyeicosatrienoic acids (DiHOMEs) and others.

Figure 2.
Chemical structure of arachidonic acid (AA), an ω6-polyunsaturated fatty acid (PUFA), and docosahexaenoic acid (DHA), an ω3-PUFA. Coloured numbers indicate the third (green) and sixth (red) carbon atom from the omega end after which the first double bond is located.

Figure 3.

Schematic of the major enzymatic pathways metabolising ω3-polyunsaturated fatty acids (PUFAs). Phospholipase A₂ (PLA₂) liberates ω3-PUFAs (eg, docosahexaenoic acid (DHA) or eicosapentaenoic acid (EPA)) from the cell membrane. These lipids are rapidly further metabolised by cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (Cyp450) enzymes, generating the pathway-specific lipid metabolites 3-series prostaglandins, hydroxydocosahexaenoic acids (HDHAs), resolvins, neuroprotectins, epoxyeicosatetraenoic acids (EpETEs) and epoxydocosapentaenoic acids (EpDPEs).

Figure 4. ω3-PUFAs attenuate proliferative retinopathy in the mouse model of oxygen-induced retinopathy (OIR) by more than 40%, an effect comparable to anti-vascular endothelial growth factor (VEGF) therapy in the same model. NV, neovascularization; P17, postnatal day 17; ****p<0.00001. Figure reproduced with permission from Stahl *et al.*¹⁷

Figure 5.

The cyclooxygenase pathways used as examples for different lipid-based treatment approaches. (A) With both ω 3- and ω 6-polyunsaturated fatty acids (PUFAs) available, the enzymatic cascade of phospholipase A_2 (PLA_2) and cyclooxygenase (COX)-1 and -2 create anti-inflammatory 3-series prostaglandins and inflammatory 2-series prostaglandins (thromboxane A_2 (TXA_2), prostaglandin (PG) E_2 and $\text{PGF}_{2\alpha}$). (B) Pharmacological inhibition of COX enzymes with COX-inhibitors limits both the generation of ω 3- and ω 6-derived metabolites thus reducing the pool of both inflammatory and anti-inflammatory mediators in the retina. (C) In contrast, increasing the amount of available ω 3-PUFA substrates for enzymatic processing yields more anti-inflammatory ω 3-derived mediators

and less inflammatory ω 6-metabolites. (D) A different treatment approach would be to directly administer lipid metabolites that have been identified as having beneficial effects during retinopathy. Similar rationales apply to other lipid-metabolising pathways (lipoxygenase (LOX) or cytochrome P450 (Cyp450)) where the pool of lipid metabolites can be shifted towards a more beneficial profile. AA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid.