Docosanoid signaling modulates corneal nerve regeneration: effect on tear secretion, wound healing, and neuropathic pain

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Abstract The cornea is densely innervated, mainly by sensory nerves of the ophthalmic branch of the trigeminal ganglia (TG). These nerves are important to maintain corneal homeostasis, and nerve damage can lead to a decrease in wound healing, an increase in corneal ulceration and dry eye disease (DED), and neuropathic pain. Pathologies, such as diabetes, aging, viral and bacterial infection, as well as prolonged use of contact lenses and surgeries to correct vision can produce nerve damage. There are no effective therapies to alleviate DED (a multifunctional disease) and several clinical trials using ω-3 supplementation show unclear and sometimes negative results. Using animal models of corneal nerve damage, we show that treating corneas with pigment epithelium-derived factor plus DHA increases nerve regeneration, wound healing, and tear secretion. The mechanism involves the activation of a calcium-independent phospholipase A2 that releases the incorporated DHA from phospholipids and enhances the synthesis of the docosanoids, neuroprotectin D1 (NPD1) and a new resolvin stereoisomer, resolvin D6i (RvD6i). NPD1 stimulates the synthesis of brain-derived neurotrophic factor, nerve growth factor, and semaphorin 7A. RvD6i treatment of injured corneas modulates gene expression in the TG resulting in enhanced neurogenesis, decreased neuropathic pain, and increased sensitivity. Taken together, these results represent a promising therapeutic option to reestablish the homeostasis of the cornea.

Supplementary key words cell signaling • gene expression • lipoxygenase • omega 3 fatty acids • phospholipase A2 • dry eye • stereoisomer of resolvin D6 • neuroprotectin D1 • docosahexaenoic acid • pigment epithelium-derived factor

CORNEA ANATOMY

The transparent cornea accounts for 70% of the refractive power of the human eye by allowing light to pass through and be projected to the retina. In addition, the cornea also provides an important barrier to regulate immune response and to prevent pathogens from entering the ocular globe. Anatomically, the cornea can be divided into five sublayers: epithelium, Bowman’s layer, stroma or substantia propria, Descemet’s membrane, and endothelium (1, 2) (Fig. 1A).

The epithelium consists of five to seven layers of nonkeratinized squamous epithelial cells, which are classified into three morphological cell types: superficial epithelial cells, intermediate wing cells, and the innermost basal epithelial cells with high rates of proliferation (2). The epithelial cells are connected by tight junctions that block the passage of foreign materials, such as dust, water, and bacteria, into the eye and provide a smooth surface that absorbs oxygen and cell nutrients. Moreover, the outermost layer of the epithelium is in contact with the tear film, which facilitates the moistness maintenance of the ocular surface that protects against damage from drying (dry eye [DE]). Corneal epithelial cells regularly undergo a “turnover” with movement of stem cells from the limbal epithelium to the basal layer. These basal cells move...
toward the surface to generate two to three layers of wing cells and then begin terminal differentiation and desquamation. On average, the turnover time of human corneal epithelial cells is between 7 and 10 days (3).

The Bowman’s layer is a thin acellular layer that separates the epithelium from the stroma. It mainly contains collagen IV and laminin. The organization of these proteins is important to maintain the transparency of the tissue.

The stroma layer is built up by quiescent keratocytes and a well-organized extracellular matrix composed primarily of highly ordered collagen type 1 fibrils, called lamella, and proteoglycans, and also constitutes the largest portion of the cornea (about 90% of corneal thickness). The stroma provides structural support to the cornea as well as transparency by facilitating the passage of light through collagen fibrils in a manner that prevents scattering. Keratocytes (the flat cells situated between collagen fibers) are the main cell residents of corneal stroma.

The Descemet’s membrane is an acellular thin layer synthesized by the endothelium that is composed of fibronectin, laminin, and collagen IV and VII as well as proteoglycans. Damage to the Descemet’s membrane produces corneal edema and loss of vision.

The last layer of the cornea is the endothelium, which is in contact with the aqueous humor. It is a monolayer of cells responsible for pumping fluid to regulate corneal stromal dehydration. Without endothelial pumps, there will be stroma edema, which produces opacity and decrease in vision. The human corneal endothelial cells have a very low capacity for proliferation, resulting in age-related reduction in cell density.

An important characteristic of the cornea is its dense innervation (Fig. 1B). Most corneal nerve fibers are sensory in origin and derived mostly from neurons of the ophthalmic branch of the trigeminal ganglia (TG) (4–6). Anatomically, the corneal nerve network originates when stromal nerves enter the corneal sclera limbus in a radial fashion. To maintain corneal transparency, the arriving nerves lose their myelin sheaths and are surrounded by Schwann cells alone. In the stroma, the thick branches are divided into smaller nerve branches. Most of the branches penetrate the Bowman’s layer in the periphery and run to the center of the epithelium to form the epithelial nerve network (Fig. 1C), giving life to a dense network of nerve terminals.

Corneal nerves stimulate tear secretion and blinking to maintain the integrity of the ocular surface (7). Alterations in corneal innervation occur in aging, diabetes, immunological diseases, such as rheumatoid arthritis and Sjögren’s syndrome, viral and bacterial infection, prolonged use of contact lenses, and refractive surgeries, such as laser in situ keratomileusis (LASIK) and photorefractive keratectomy (PRK) (8–13). Complications from nerve damage diminish sensitivity, decrease tear secretion and blinking, and as a consequence, result in DE disease (DED) that produces
neuropathic pain and corneal ulceration in severe cases. Due to the abundance of sensory nerves, the cornea is also a potent generator of pain in the human body.

**PEDF+DHA TREATMENT FOR CORNEA-RELATED DAMAGE: DISCOVERY OF A RESOLVIN D6 STEREOISOMER**

As mentioned, after damage, corneal nerve density slowly and incompletely recovered with decrease in sensitivity and DE symptoms. Early studies from our laboratory have shown that application of nerve growth factor (NGF) in conjunction with the ω-3 fatty acid DHA results in faster recovery of corneal nerve density after experimental PRK in rabbits (14). At that time, we proposed that the mechanisms could be mediated by the DHA-derived lipid mediator neuroprotectin D1 (NPD1), a docosanoid with potent anti-inflammatory and neuroprotective actions (15). Synthesis of NPD1 in retinal pigment epithelial cells is stimulated by several growth factors with pigment epithelium-derived factor (PEDF) being 10 times more potent than NGF (16). PEDF is a broad-acting neurotrophic and neuroprotective factor that regulates processes associated with angiogenesis, neuronal cell survival, and cell differentiation (17) and is released from corneal epithelium after injury (18). Posterior studies have shown that treatment with PEDF+DHA decreases inflammation and stimulates corneal wound healing and nerve regeneration in rabbit and mouse cornea models of experimental surgery, as well as in pathologies like diabetes and herpes simplex virus infection (19–23). It is important to mention that the action requires treatment with both PEDF and DHA (19). A 44-amino acid fragment of PEDF has neuroprotective activity, while an adjacent 34-amino acid peptide has anti-angiogenic activity (24, 25). Comparing the effect of the two peptides with the whole PEDF protein plus DHA in a rabbit model of corneal stroma dissection, we found that, unlike 34-mer-PEDF, 44 mer-PEDF+DHA decreases inflammation and increases tear secretion and corneal sensitivity and also promotes regeneration of corneal nerves by activating a PEDF receptor (PEDF-R) (21). This transmembrane receptor is expressed in the cornea and has calcium-independent phospholipase A2 (iPLA2) activity (26, 27) that releases DHA, which is enriched in the sn-2 position of membrane phospholipids by DHA supplementation.

Early studies on calf corneas identified PC, PE, and sphingomyelin as the main phospholipids in the tissue (28). Among these phospholipids, PC is the most abundant with the highest content in the epithelium. Similar observations were reported in human (29) and rabbit corneas (30). In the rabbit, oleic acid (18:1) is the dominant fatty acid esterified in phospholipids in all of the corneal layers (about 50% of total fatty acids in phospholipids) followed by palmitic acid (16:0), which comprises about 16–18%. With respect to the PUFAs esterified in phospholipids, the higher percentage (about 9% of total fatty acids) corresponds to AA, while the percentage of EPA and DHA esterified in phospholipids is much lower (around 1.6% of total fatty acids) (30).

DHA topical treatment of mouse corneas, in which stromal nerves had been damaged, produced a rapid incorporation of the fatty acid in PC and PE molecular species containing 18:1-DHA (27), demonstrating that the addition of the PUFAs created a significant enrichment of DHA in the lipid membrane composition (Fig. 2A).

Tissue damage activates phospholipase A2 that releases PUFAs, such as AA, EPA, and DHA, from the sn-2 position (31, 32). Several early studies from our laboratory and others have demonstrated that the cornea responds to injury, increasing the synthesis of prostaglandins by activation of cyclooxygenase (COX)-2 (33–36) and HETEs and lipoxin A4 (LxA4) by activation of lipoxigenases (LOXs) (37–39). Because the concentration of DHA in membrane lipids is very low (Fig. 2A) (30), we found that the addition of DHA to the corneas treated with PEDF was important to increasing the synthesis of lipid derivatives of DHA (docosanoids)
with strong anti-inflammatory properties (19, 40, 41). Therefore, activating the calcium-independent phospholipase A2 (iPLA2) of the PEDF-R by treating the corneas with PEDF+DHA leads to a more than 3,000-fold increase of free DHA released from the cornea (Fig. 2B).

Free DHA is then the substrate for the synthesis of 14- and 17-hydroperoxy-DHA that are rapidly converted in the more stable hydroxy-DHA (HDHA) derivatives (Fig. 2B). These results confirmed our hypothesis that PEDF+DHA treatment stimulates the formation of docosanoids derived from DHA.

Figure 3 shows a scheme of bioactive lipids resulting from AA, EPA, and DHA. While many AA lipid mediators, as well as some EPA lipid mediators, have strong pro-inflammatory properties, all known DHA mediators (the docosanoids) act to protect and resolve inflammation (42, 43). They constitute part of a family named specialized pro-resolvin mediators that includes NPD1 and other protectins, maresins, and resolvins of the D series (43) and the newer sulfide conjugates of protectins (PCTR), maresins (MCTR), and resolvins (RCTR). The synthetic mechanism to produce the specialized pro-resolvin mediators involves LOXs (including 15-LOX as primary catalyzer and 5-LOX as secondary catalyzer), COX (in the presence of aspirin), and cytochrome P450 enzymes (44). Information about the signaling mechanisms of DHA lipid mediators is still limited, especially identification of their receptors (Table 1). Most of the known receptors belong to the family of G protein-coupled receptors. In addition, some docosanoids share the same receptor, but their activation exerts specific biological activities (43).

**Table 1. List of reported receptors of docosanoids**

| Name          | Receptors                  | References | Expression in the Cornea |
|---------------|----------------------------|------------|--------------------------|
| Resolvin D1   | ALX/FPR2, GPR32 (DRV1)     | (44)       | Yes                      |
| Resolvin D2   | GPR38 (DRV2)               | (78)       | ND                       |
| Resolvin D3   | ALX/FPR2, GPR32 (DRV1)     | (79)       | Yes                      |
| Resolvin D4   | N/A                        | —          | —                        |
| Resolvin D5   | GPR32                      | (80)       | ND                       |
| Resolvin D6   | N/A                        | —          | —                        |
| Neuroprotectin D1 | GPR37 (Pacl-R)          | (81)       | ND                       |
| Maresin 1     | LGR6                       | (82)       | ND                       |
| Maresin 2     | N/A                        | —          | —                        |

ND, not determined; N/A, not available.
Recently, we discovered a novel docosanoid, a stereoisomer of resolvin D6 (RvD6), named RvD6i (Fig. 4), that is released in mouse tears after injury and treatment with PEDF+DHA (40). The fragmentation pattern of this new lipid shows at least six matched product ions that coincide with RvD6. Resolvin D6 had been found in some tissues, and studies in plasma from healthy individuals showed that RvD6 is a biomarker that decreases with aging (45). RvD6 is also released from stem cells isolated from human periodontal ligaments, which is important in tissue regeneration (46). However, RvD6 is not detected in normal human tears (47). Compared with treatments with PEDF+DHA and RvD6, the new RvD6i accelerated corneal wound healing and sensitivity, demonstrating a higher bioactivity (Fig. 4A, B).

**USE OF DHA FOR DED**

DED affects between 5% and 40% of adults older than 40 years (48, 49) with an estimated 16.4 million people impacted in the United States (50). In a recent Dry Eye Workshop (DEWS II), DE was defined as “a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tearfilm, and accompanied by ocular symptoms, in which tearfilm instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles.” (Ref. 49; p. 278).

Within the last decade, there have been a number of clinical trials of DED patients with different etiologies using ω-3 fatty acid, DHA and EPA, supplementation with the argument that dietary fatty acids can be incorporated in the lacrimal gland as well as in plasma phospholipids (51). However, the effect of oral PUFA supplementation in DED is controversial. While some studies showed improvement, others showed insignificant effects. In Table 2, we summarized clinical trials conducted in the last 10 years in which supplementation with DHA was used to treat DED of different etiologies.

One of the most important trials, the DREAM study, which involved a total of 499 patients with 329 receiving 12 months of supplementation with EPA and DHA and 170 patients treated with refined olive oil as a placebo (52), suggested that there was no improvement. This study increases the doubtfulness about the benefit of DHA in the treatment of DED. For this reason, in this review, we point out problems that may explain the controversial results of DHA supplementation.

One concern is the form of DHA supplementation. Most of the studies employed natural enriched fish oil. However, analysis of fish oil composition showed that the PUFA are mainly esterified in triglycerides, DHA from the diet needs to be taken up by the liver before being esterified in the sn-2 position of membrane phospholipid, mainly PC (53). DHA-phospholipids are then packaged in VLDLs or other lipoproteins before being released into the blood stream (53, 54). Therefore, the possibility that supplementation of DHA or EPA from fish oil reaches the ocular surface, especially the cornea, is very low. This is supported by previous studies where krill oil, which mainly contains PC with long chain PUFA, showed a higher absorption rate in rat blood and brain than fish oil (55). There is only one study that uses krill oil to treat DED, a small clinical trial (18 participants per group) in which Deinema and colleagues showed lower Ocular Surface Disease Index and IL-17A levels in krill oil supplementation than in fish oil after 90 days of treatment (56) and Table 2.

In addition, it is important to note that the cornea is avascular, therefore, the probability that dietary fatty acids are incorporated into the corneal cellular membrane is limited. This is supported by a study using 14C-labeled DHA given orally to rats, which showed a very small amount (less than 0.03% of the oral dose) of DHA that reached the eye compartment (57). Of this quantity, the amount that might get into the cornea is very low because the retina takes most of the DHA from subretinal blood vessels. Therefore, PUFA enrichment in the lacrimal gland is insufficient to ensure a beneficial treatment in the cornea.

To our knowledge, there is only one clinical trial using topical DHA (58) (Table 2). This trial was based on previous studies showing that AA, DHA, and EPA were found in the tears of patients with DED and that the ratio of ω-6 (AA)/ω-3 (DHA+EPA) correlates with the severity of the tear film dysfunction (59). The small trial (19 patients treated topically with DHA) demonstrated that treatment with eye drops containing omega-3 fatty acids increases lipid layer thickness of the tear film up to 1 h after instillation (58).

Lastly, our animal studies show that DHA is rapidly incorporated in the corneal phospholipids, mainly in PE and PC, to increase nerve density. A decrease in nerve density is a well-documented alteration in DED that requires both PEDF and DHA to regenerate the nerves.
| Study                        | Number of Patients/Treatment | Randomized/Controlled | Masking | Effect                | Comments                                                                 |
|-----------------------------|-----------------------------|-----------------------|---------|-----------------------|--------------------------------------------------------------------------|
| Brignole-Baudouin et al., 2011 (83) | DE, n = 127, time = 90 days | Yes/Yes               | Double  | No significant effect | Only decrease in the percentage of HLA-DR-positive cell was detected in treated group |
|                             | Group 1, n = 61             |                       |         |                       |                                                                          |
|                             | 142.5 mg EPA, 95 mg DHA, and |                       |         |                       |                                                                          |
|                             | supplements, three times daily |                       |         |                       |                                                                          |
|                             | Group 2, n = 66             |                       |         |                       |                                                                          |
|                             | Placebo, medium-chain triglyceride, three times daily |                       |         |                       |                                                                          |
| Wojtowicz et al., 2011 (84) | DE, n = 36, time = 90 days  | Yes/Yes               | Double  | No significant effect | No changes in aqueous tear evaporation                                   |
|                             | Group 1                     |                       |         |                       |                                                                          |
|                             | 450 mg EPA, 300 mg DHA, and |                       |         |                       |                                                                          |
|                             | 1,000 mg flaxseed oil, one time daily |                       |         |                       |                                                                          |
|                             | Group 2                     |                       |         |                       |                                                                          |
|                             | Placebo, wheat germ oil     |                       |         |                       |                                                                          |
| Bhargava et al., 2013 (85)  | DE, n = 528, time = 3 months| Yes/Yes               | Double  | Improved              |                                                                          |
|                             | Group 1                     |                       |         |                       |                                                                          |
|                             | 325 mg EPA and 175 mg DHA, two times daily |                       |         |                       |                                                                          |
|                             | Group 2                     |                       |         |                       |                                                                          |
|                             | Placebo, two times daily    |                       |         |                       |                                                                          |
| Kangari et al., 2013 (86)   | DE, n = 64, time = 30 days  | Yes                   | Double  | Slightly improved     | No significant differences in corneal staining from placebo              |
|                             | Group 1                     |                       |         |                       |                                                                          |
|                             | 180 mg EPA and 120 mg DHA, two times daily |                       |         |                       |                                                                          |
|                             | Group 2                     |                       |         |                       |                                                                          |
|                             | Placebo, medium-chain triglyceride |                       |         |                       |                                                                          |
| Oleňík et al., 2013 (87)    | Meibomian gland dysfunction, n = 64, time = 3 months | Yes/No               | Double  | Slightly improved     | No significant differences in corneal staining from placebo              |
|                             | Group 1                     |                       |         |                       |                                                                          |
|                             | Brudysec (350 mg DHA, 42,5 mg EPA, 30 mg DPA), three times daily |                       |         |                       |                                                                          |
|                             | Group 2                     |                       |         |                       |                                                                          |
|                             | Placebo, 500 mg sunflower oil, three times daily |                       |         |                       |                                                                          |
|                             | All patients received cleaning the lid margins with neutral baby shampoo and artificial tears without preservatives |                       |         |                       |                                                                          |
| Ong et al., 2013 (88)       | Healthy PRK patients, n = 18, time = 6 weeks | Yes/Yes               | Single  | Improved              | Treatment was 2 weeks before PRK surgery through 1 month after surgery |
|                             | Group 1                     |                       |         |                       |                                                                          |
|                             | 250 mg EPA and DHA, 333 mg flaxseed oil, and 61 IU vitamin E, three times daily |                       |         |                       |                                                                          |
|                             | Group 2                     |                       |         |                       |                                                                          |
|                             | Placebo                     |                       |         |                       |                                                                          |
| Sheppard et al., 2013 (89)  | Post-menopausal women with DE, n = 38, time = 6 months | Yes/Yes               | Double  | Improved              | Placebo treatment also increased HLA-DR intensity by 36 ± 9% and CD11c by 34 ± 7% when compared with supplement treatment |
|                             | Group 1                     |                       |         |                       |                                                                          |
|                             | 49 mg ALA, 315 mg EPA, 375 mg DPA, 25 mg DHA, 177.5 mg LA, 60 mg GLA, <0.75 AA, and supplements, four times daily |                       |         |                       |                                                                          |
|                             | Group 2                     |                       |         |                       |                                                                          |
|                             | Placebo                     |                       |         |                       |                                                                          |
|                             | Baseline (T0), 1 month of treatment (T1), 2 months of treatment (T2), 3 months of treatment (T3) |                       |         |                       |                                                                          |
| Oleňík, 2014 (90)           | DE, n = 905, time = 12 weeks| No/No                 | No      | Improved              | A total of 68.1% of patients reported better tolerance to contact lenses after treatment |
|                             | Brudysec (350 mg DHA, 42,5 mg EPA, 30 mg DPA), three times daily |                       |         |                       |                                                                          |
| Georgakopoulos et al., 2017 (91) | Diabetic patients with DE, time = 3 months | No/No                 | No      | Improved              | —                                                                          |
|                             | Group 1                     |                       |         |                       |                                                                          |
|                             | 170 mg EPA and 115 mg DHA, three times daily |                       |         |                       |                                                                          |
| Bhargava et al., 2015 (92)  | Computer-related DE, n = 456, time = 3 months | Yes/Yes               | Double  | Improved              | —                                                                          |
|                             | Group 1                     |                       |         |                       |                                                                          |
|                             | 180 mg EPA and 120 mg DHA, two times daily |                       |         |                       |                                                                          |
|                             | Group 2                     |                       |         |                       |                                                                          |
|                             | Placebo containing olive oil, two times daily |                       |         |                       |                                                                          |

(continued)
The treatment releases DHA and stimulates the synthesis of RvD6i, and this docosanoid increases wound healing and sensitivity (Fig. 5A, B) and could be of better therapeutic use than DHA for DED (40).

The effectiveness of docosanoids in decreasing inflammation and increasing corneal wound healing, nerve regeneration, and tear secretion has been demonstrated clearly on several different models of...
TABLE 3. In vivo studies using PEDF+DHA or docosanoids for corneal damage

| Animal                          | Model                                                                 | Docosanoids                  | Administration                       | Key Result                                                                 |
|---------------------------------|-----------------------------------------------------------------------|------------------------------|---------------------------------------|-----------------------------------------------------------------------------|
| Mouse                           | Corneal epithelium removal up to the corneal/limbal border (94)       | NPDI, 17S-HDHA               | Topical eye drops, three times daily for 96 h | Increased rate of re-epithelialization. Increased PMNs in the cornea. Decreased formation of the pro-inflammatory chemokine KC |
| Mouse                           | Suture-induced inflammatory corneal angiogenesis (95)                 | RvD1                         | Subconjunctival injection every 48 h; time = 14 days | Reduced numbers of infiltrating neutrophils and macrophages and reduced mRNA expression levels of TNF-α, IL-1β, IL-18, VEGF-A, VEGF-C, and VEGFR2. Suppressed suture-induced or IL-1β-induced hemangiogenesis but not lymphangiogenesis |
| Rabbit                          | Experimental PRK (19–21)                                              | PEDF+DHA, PEDF domains + DHA | Topical using collagen shield, twice a week; time = 8 weeks | Increased nerve density and tear secretion in treated group for 8 weeks, with PEDF+DHA. NPDI synthesis peaked at 1 week and was four times higher in the PEDF+DHA-treated group than in the controls. The 44-mer domain of PEDF is more potent than the 34-mer domain |
| Rabbit                          | Experimental PRK (41)                                                 | NPDI                         | Topical eye drops of NPDI (33 ng/eye) three times daily for 6 weeks | Increased subepithelial corneal nerves and tear secretion. Decreased neutrophil infiltration after 2 and 4 days of treatment |
| Mouse                           | Corneal allotransplantation (96)                                      | RvD1 analog                  | Intravenous injection                 | Reduced allosensitization. Reduced angiogenesis at the graft site. Enhanced graft survival |
| Mouse                           | Type 2 diabetes (97)                                                  | RvD1, RvD1-methyl ester, RvD2-methyl ester | Daily intraocular injections of 1 ng/g body weight for 8 weeks | Reduced the diabetes-induced corneal nerve loss. Methyl ester version is less bioactive than free fatty acid |
| Rabbit                          | HSV1 corneal infection (22)                                           | PEDF+DHA                     | Topical eye drops, three times daily for 2 weeks. Topical using collagen shield, twice a week for 10 weeks more | Stronger infiltration of CD4+T cells, neutrophils, and macrophages at 7 days, then decreased by 14 days. Corneal nerve density increased at 12 weeks with functional recovery of corneal sensation |
| Mouse                           | Type 1 diabetes. Corneal epithelium removal inside 2 mm diameter central area (23) | PEDF+DHA                     | Topical eye drops, three times daily for 14 days | Increased in corneal epithelial nerve regeneration, SP-positive nerve density and tear volume. Accelerated corneal wound healing, selectively recruited type 2 macrophages, and prevented neutrophil infiltration |
| Mouse                           | Corneal nerve cutting (27)                                            | PEDF+DHA                     | Topical eye drops, three times daily for 7 days | Increased nerve regeneration and tear secretion. Phospholipase A2 activity of the PEDF-R is required for the working mechanism |
| Mouse                           | Type 1 diabetes. Corneal epithelium removal inside 2 mm diameter central area (98) | RvD1                         | Topical eye drops, four times daily for 14 days | Promotes corneal epithelial wound healing and nerve regeneration |
| Mouse                           | Corneal epithelium removal inside 2 mm diameter central area (40)     | RvD6i                        | Topical eye drops, three times daily for 12 days | Discovered the RvD6i underlying the mechanism of PEDF+DHA. Increased corneal wound healing, sensitivity, and nerve regeneration. Reduced inflammatory- and pain-related neuropeptides, increased ion channel gene expression in TG |

Bold type indicates studies from our laboratory. HSV1, herpes simplex virus.

injury, infection, diabetes, corneal angiogenesis, and transplantation (Table 3). These results emphasized the action of docosanoids as potent drugs.

**RvD6i REGULATES GENES INVOLVED IN NEUROGENESIS AND PAIN IN THE TG**

Previous studies have showed that cornea treatment with PEDF and DHA also stimulated the synthesis of the docosanoid NPDI. However, the synthetized amount is much lower than RvD6i (19, 40). When adding NPDI to injured corneas, there is an increase in the gene expression and protein levels of the neurotrophins NGF, brain-derived neurotrophic factor (BDNF), and semaphorin 7A (Sema7A) that stimulate axon growth (27). These proteins are secreted into tears and activate receptors in the corneal nerve terminals to facilitate downstream signaling as well as retrograde to the neurons of the TG.

Using RNA-sequencing to analyze the gene expression in TG from the injured corneas of mice, we reveal that the product of PEDF+DHA, RvD6i, applied topically to the cornea induces the expression of two interesting genes in TG. A chromosome 9 open reading frame (C9orf72) and Gm6A (Gpm6A) (40). These genes stimulate neurogenesis and growth cone formation (60, 61).
Ocular pathologies that damage corneal nerves in many cases produce neuropathic pain (62). In addition, there are a significant number of patients who have symptoms of DED and experience neuropathic pain, suggesting that there is an active cornea-TG relationship (63). Two genes involved in pain were decreased in corneas treated with RvD6i: *Tac1* that encodes substance P (SP), which is one of the most abundant neuropeptides expressed in corneal nerves (4, 64, 65), and *Calcb*, which encodes calcitonin gene-related peptide (also abundant in corneal nerves) (4, 20) (Fig. 5C). Both neuropeptides have important roles in neurogenic inflammation and pain (66, 67). In addition, corneal treatment with RvD6i increased the gene expression of transient receptor potential melastatin 8 (*Trpm8*) (Fig. 5D). TRPM8 ion channels are cool sensors that regulate the wetting of the ocular surface and produce an analgesic effect on chronic pain (68–72). Our prior studies in a mouse model where the nerves had been damaged at the level of the anterior stroma, showed that cornea TRPM8-positive nerve fibers only reach 50% of their normal density after 3 months of injury, suggesting that there is a decrease in TRPM8 may contribute to DE-like pain (73). Therefore, decreased expression of SP and calcitonin gene-related peptide and increased expression of TRPM8 after injury and treatment with RvD6i suggests that the new docosanoid could protect corneas from pain. It also opens avenues of potential therapeutic exploration for ocular surface damage, especially corneal neurotrophic ulcers, because previous studies have shown ocular pain as a side effect of increased corneal nerve regeneration caused by topical treatment with NGF (74). Previous studies using RvD1 and RvD5 had shown pain attenuation in a mouse model of tibia bone fracture, while RvD3 and RvD4 had no effect (75). These differences could be due to different expression of its receptors. In an osteoporosis mouse model, the precursor of RvD6i, 17R-HDHA, decreases pain behavior probably through activation of AXL receptors (76). Another important finding is that RvD6i is a strong inducer of the gene expression of Rictor in the TG (40) (Fig. 5D). RICTOR is a key component of the mammalian target of rapamycin-insensitive complex 2 (mTORC2) and plays a role in anti-inflammation and axon growth of sensory neurons after injury (77).

A summarized scheme of the signaling pathways of docosanoids stimulated by PEDF and DHA is shown in Fig. 6.

**CONCLUSIONS**

Cornea innervation plays a pivotal role in maintaining the homeostasis of the ocular surface and tissue clarity (7). Damage to corneal nerves produces a decrease in tear production and blinking reflex and can impair epithelial wound healing resulting in loss of transparency and vision (8–13). Therefore, better knowledge on corneal nerve function and repair will increase therapeutic strategies for pathologies that affect corneal innervation. DHA-derived docosanoids, such as the new mediator RvD6i, could serve as potential treatment options to reduce cornea-related inflammation. The effect of this lipid in accelerating nerve regeneration and modulating the gene expression of components of neuropathic pain in the TG could provide a new alternative in the treatment of patients with DE following refractive surgery as well as possible cotreatment to...
several pathologies that decrease corneal nerve density. Prospective human clinical trials will be needed to confirm optimal dosing, modes of administration, efficacy, and safety of these promising new treatments for DE and ocular surface diseases.

Author contributions

T. L. P. and H. E. P. B. contributed to the conception and design of the article and to interpreting the relevant literature. T. L. P. wrote the first draft, and H. E. P. B. reviewed the manuscript.

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Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations

BDNF, brain-derived neurotrophic factor; COX, cyclooxygenase; DE, dry eye; DED, dry eye disease; HDHA, hydroxy-DHA; LOX, lipoxygenase; NGF, nerve growth factor; NPDl, neuroprotectin DI; PEDF, pigment epithelium-derived factor; PEDF-R, pigment epithelium-derived factor receptor; PRK, photorefractive keratectomy; RvD6, resolvin D6; SP, substance P; TG, trigeminal ganglia.

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REFERENCES

1. DelMonte, D. W., and Kim, T. (2011) Anatomy and physiology of the cornea. J. Cataract Refract. Surg. 37, 588–598
2. Meek, K. M., and Knupp, C. (2015) Corneal structure and transparency. Prog. Retin. Eye Res. 49, 1–36
3. Hanna, C., Bicknell, D. S., and O’Brien, J. E. (1961) Cell turnover in the adult human eye. Arch. Ophthalmol. 65, 695–698
4. Müller, L. J., Marfurt, C. F., Kruse, F., and Tervo, T. M. T. (2003) Corneal nerves: structure, contents and function. Exp. Eye Res. 76, 521–542
5. He, J., Bazan, N. G., and Bazan, H. E. P. (2010) Mapping the entire human corneal nerve architecture. Exp. Eye Res. 91, 513–523
6. Al-Aqaba, M. A., Dhillon, V. K., Mohammed, I., Said, D. G., and Dua, H. S. (2019) Corneal nerves in health and disease. Prog. Retin. Eye Res. 75, 100762
7. Shaheen, B. S., Bakir, M., and Jain, S. (2014) Corneal nerves in health and disease. Surv. Ophthalmol. 59, 263–285
8. He, J., and Bazan, H. E. P. (2012) Mapping the nerve architecture of diabetic human corneas. Ophthalmolog. 119, 956–964
9. Hamrah, P., Cruzat, A., Duesterji, M. H., Zheng, L., Shahatit, B. M., Bayhan, H. A., Dana, R., and Pavan-Langston, D. (2010) Corneal sensation and subbasal nerve alterations in patients with herpes simplex keratitis: an in vivo confocal microscopy study. Ophthalmolog. 117, 1930–1936
10. Cruzat, A., Witkin, D., Baniassadi, N., Zheng, L., Ciolino, J. B., Jurkunas, U. V., Chodosh, J., Pavan-Langston, D., Dana, R., and Hamrah, P. (2011) Inflammation and the nervous system: the connection in the cornea in patients with infectious keratitis. Invest. Ophthalmol. Vis. Sci. 52, 5156–5163
11. He, J., and Bazan, H. E. P. (2013) Corneal nerve architecture in a donor with unilateral epithelial basement membrane dystrophy. Ophthalmolog. Res. 49, 185–191
12. Pham, T. L., Kakazu, A., He, J., and Bazan, H. E. P. (2019) Mouse strains and sexual divergence in corneal innervation and nerve regeneration. PLoS ONE 23, 4508–4609
13. Garcia-Gonzalez, M., Cañadas, P., Grov-Otero, J., Rodriguez-Perez, E., Califanes-Zafra, R., Kozobolis, V., and Teus, M. A. (2019) Long-term corneal subbasal nerve plexus regeneration after laser in situ keratomileusis. J. Cataract Refract. Surg. 45, 966–971
14. Esquenazi, S., Bazan, H. E. P., Bui, V., He, J., Kim, D. B., and Bazan, N. G. (2005) Topical combination of NGF and DHA increases rabbit corneal nerve regeneration after photorefractive keratectomy. Invest. Ophthalmol. Vis. Sci. 46, 3121–3127
15. Mukherjee, P. K., Marcheselli, V. L., Serhan, C. N., and Bazan, N. G. (2004) Neuroprotectin DI: a docosahexaenoic acid-derived docosatriene protects human retinal pigment epithelial cells from oxidative stress. Proc. Natl. Acad. Sci. USA. 101, 8491–8496
16. Mukherjee, P. K., Marcheselli, V. L., Barreiro, S., Ho, J., Bok, D., and Bazan, N. G. (2007) Neurotrophins enhance retinal pigment epithelial cell survival through neuroprotectin D1 signaling. Proc. Natl. Acad. Sci. USA. 104, 15152–15157
17. Gumbricht, L., and Barnes, J. C. (2003) PEDF: a multifaceted neurotrophic factor. Nat. Rev. Neurosci. 4, 628–636
18. Khenchegowda, S., He, J., and Bazan, H. E. P. (2015) Involvement of pigment epithelium-derived factor, docosahexaenoic acid and neuroprotectin DI in corneal inflammation and nerve integrity after refractive surgery. Prostaglandins Leukot. Essent. Fatty Acids. 88, 27–31
19. Cortina, M. S., He, J., Li, N., Bazan, N. G., and Bazan, H. E. P. (2010) Neuroprotectin DI synthesis and corneal nerve regeneration after experimental surgery and treatment with PEDF plus DHA. Invest. Ophthalmol. Vis. Sci. 51, 804–810
20. Cortina, M. S., He, J., Li, N., Bazan, N. G., and Bazan, H. E. P. (2012) Recovery of corneal sensitivity, calcitonin gene-related peptide-positive nerves, and increased wound healing induced by pigment epithelial-derived factor plus docosahexaenoic acid after experimental surgery. Arch. Ophthalmol. 130, 76–83
21. He, J., Cortina, M. S., Kakazu, A., and Bazan, H. E. P. (2015) The PEDF neuroprotective domain plus DHA induces corneal nerve regeneration after experimental surgery. Invest. Ophthalmol. Vis. Sci. 56, 3505–3513
22. He, J., Neumann, D., Kakazu, A., Pham, T. L., Musarrat, F., Cortina, M. S., and Bazan, H. E. P. (2017) PEDF plus DHA modulate inflammation and stimulate nerve regeneration after HSV-1 infection. Exp. Eye Res. 161, 153–162
23. He, J., Pham, T. L., Kakazu, A., and Bazan, H. E. P. (2017) Recovery of corneal sensitivity and increase in nerve density and wound healing in diabetic mice after PEDF plus DHA treatment. Diabetes. 66, 2511–2520
24. Houenou, L. J., D’Costa, A. P., Li, L., Turgeon, V. L., Enoyakide, C., Alberdi, E., and Becerra, S. P. (1999) Pigment epithelium-derived factor promotes the survival and differentiation of developing retinal motor neurons. J. Comp. Neurol. 412, 506–514
25. Amaral, J., and Becerra, S. P. (2010) Effects of human recombinant PEDF protein and PEDF-derived peptide 34-mer on choroidal neovascularization. Invest. Ophthalmol. Vis. Sci. 51, 1518–1526
26. Notari, L., Baladron, V., Aracca-Aguilar, J. D., Balko, N., Heredia, R., Meyer, C., Notario, P. M., Sarvamanyuthu, S., Nueda, M.-L., Sanchez-Sanchez, P., et al. (2006) Identification of a lipase-linked cell membrane receptor for pigment epithelium-derived factor. J. Biol. Chem. 281, 38022–38037
27. Pham, T. L., He, J., Kakazu, A. H., Jun, B., Bazan, N. G., and Bazan, H. E. P. (2017) Defining a mechanistic link between pigment epithelium-derived factor, docosahexaenoic acid, and corneal nerve regeneration. J. Biol. Chem. 292, 18486–18496
28. Brockhuyse, R. M. (1968) Phospholipids in tissues of the eye. Isolation, characterization and quantitative analysis by
two-dimensional thin-layer chromatography of diacyl and vinyl-ether phospholipids. Biochem. Biophys. Acta. 152, 307–315

29. Tschetter, R. T. (1966) Lipid analysis of the human cornea with and without arcus senilis. Arch. Ophthalmol. 76, 403–405

30. Bazan, H. E., and Bazan, N. G. (1984) Composition of phospholipids and free fatty acids and incorporation of labeled arachidonic acid in rabbit cornea. Composition of epithelium, stroma and endothelium. Curr. Eye Res. 3, 1313–1319

31. Katsura, K., Rodríguez de Turco, E. B., Siesjo, B. K., and Bazan, N. G. (2004) Effects of hyperglycemia and hypercapnia on lipid metabolism during complete brain ischemia. Brain Res. 1030, 158–169

32. Rodríguez de Turco, E. B., Belavev, L., Liu, Y., Busto, R., Parkins, N., Bazan, N. G., and Ginsberg, M. D. (2002) Systemic fatty acid responses to transient focal cerebral ischemia: influence of neuroprotectant therapy with human albumin. J. Neurochem. 83, 515–524

33. Bazan, H. E., Birkle, D. L., Beuerman, R., and Bazan, N. G. (1985) Corynebacterium alters the metabolism of arachidonic acid in rabbit cornea layers. Invest. Ophthalmol. Vis. Sci. 26, 474–480

34. Bazan, H. E., Tao, Y., DeCoster, M. A., and Bazan, N. G. (1997) Platelet-activating factor induces cyclooxygenase-2 gene expression in corneal epithelium. Requirement of calcium in the signal transduction pathway. Invest. Ophthalmol. Vis. Sci. 38, 1198–1201

35. Lichicau, E. L., Nguyen, V., Sullivan, A. B., and Gronert, K. (2010) Selective activation of the prostaglandin E2 circuit in chronic injury-induced pathologic angiogenesis. Invest. Ophthalmol. Vis. Sci. 51, 6311–6320

36. Amico, C., Yakimov, M., Catania, M. V., Giuffrida, R., Pistone, M., and Enea, V. (2004) Differential expression of cyclooxygenase-1 and cyclooxygenase-2 in the cornea during wound healing. Tissue Cell. 36, 1–12

37. Hurst, J. S., Balazy, M., Bazan, H. E., and Bazan, N. G. (1991) The epithelium, endothelium, and stroma of the rabbit cornea generate (12S)-hydroxyeicosatetraenoic acid as the main lip-oxygenase metabolite in response to injury. J. Biol. Chem. 266, 15766–15769

38. Sharma, G. D., Ottino, P., Bazan, N. G., and Bazan, H. E. P. (2005) Epidermal and hepatocyte growth factors, but not keratinocyte growth factor, modulate protein kinase Calpha translocation to the plasma membrane through 15(S)-hydroxyeicosatetraenoic acid synthesis. J. Biol. Chem. 280, 7917–7924

39. Leedion, A. J., Sullivan, A. B., Dong, B., Lai, D., and Gronert, K. (2010) Endogenous LXA4 circuits are determinants of pathologic angiogenesis in response to chronic injury. Am. J. Pathol. 176, 74–84

40. Pham, T. L., Kakazu, A. H., Jeff, J., Jun, B., Bazan, N. G., and Bazan, H. E. P. (2020) Novel RvD6 stereoisomer induces corneal nerve regeneration and wound healing post-injury by modulating trigeminal transcriptomic signature. Sci. Rep. 10, 4582

41. Cortina, M. S., He, J., Russ, T., Bazan, N. G., and Bazan, H. E. P. (2015) Neuroprotectin D1 restores corneal nerve integrity and function after damage from experimental surgery. Invest. Ophthalmol. Vis. Sci. 54, 4109–4116

42. Bazan, N. G. (2018) Docosanoids and epilovanoids from omega-3 fatty acids are pro-homeostatic modulators of inflammatory responses, cell damage and neuroprotection. Mol. Aspects Med. 64, 18–33

43. Serhan, C. N., and Levy, B. D. (2018) Resolvins in inflammation: emergence of the pro-resolving superfamily of mediators. J. Gerontol. A Biol. Sci. Med. Sci. 78, 2657–2669

44. Krishnamoorthy, S., Recchiuti, A., Chiang, N., Yacoubian, S., Lee, C-H., Yang, R., Petasis, N. A., and Serhan, C. N. (2010) Endogenous LXA4 circuits are determinants of patho- logical angiogenesis in response to chronic injury. J. Mol. Cell. Neurosci. 41, 338–348

45. Jove, M., Mate, I., Naudí, A., Mota-Martorell, N., Portero-Otín, M., and Pamplona, R. (2016) Human aging is a hexaenoic acid signalomist in nutrition: significance in neurodegeneration, Alzheimer’s, and other neurodegenerative diseases. Annu. Rev. Nutr. 31, 321–351

46. Ahn, S. H., Lim, S. J., Ryu, Y. M., Park, H. R., Suh, H. J., and Han, S. H. (2018) Absorption rate of krill oil and fish oil in blood and brain of rats. Lipids Health Dis. 17, 102

47. Deinema, L. A., Vingrys, A. J., Wong, C. Y., Jackson, D. C., Chinney, H. R., and Davalos, E. A. (2017) A randomized, double-masked, placebo-controlled clinical trial of two forms of omega-3 supplements for treating dry eye disease. Ophthalmol. 124, 43–52

48. Graf, B. A., Dutchateau, G. S. M. J. E., Patterson, A. B., Mitchell, E. S., van Bruggen, P., Kock, J. H., Melville, S., and Verkade, H. J. (2018) Absorption rate of krill oil and fish oil in blood and body tissues after dosing various 14C-DHA-esters. Prostaglandins Leukot. Essent. Fatty Acids. 83, 89–96

49. Fogg, J. S., Fogg, N., King-Smith, P. E., Liu, H., and Barr, J. T. (2019) Changes in tear lipid layer thickness and symptoms following the use of artificial tears with and without omega-3 fatty acids: a randomized, double-masked, crossover study. Clin. Ophthalmol. 13, 2553–2561

50. Walter, S. D., Gronert, K., McClellan, A. L., Levitt, R. C., Sar- antopoulos, K. D., and Galor, A. (2016) ω3 Tear film lipids correlate with clinical measures of dry eye. Invest. Ophthalmol. Vis. Sci. 57, 2472–2478

51. Savadassan, R., Hornburg, D., Drepper, C., Frank, N., Jablonka, S., Hansel, A., Lojewski, X., Sternekert, J., Hermann, A., Shaw, P. J., et al. (2016) C5ARF72 interaction with coflin modulates actin dynamics in motor neurons. Nat. Neurosci. 19, 1610–1618

52. Formoso, K., Garcia, M. D., Frasch, A. C., and Scorticati, C. (2016) Evidence for a role of glycoprotein M6a in dendritic spine formation and synaptogenesis. Mol. Cell. Neurosci. 77, 95–104

53. Goyal, S., and Hanahar, P. (2016) Understanding neuropsychiatric pain–gaps and current therapeutic approaches. Semin. Ophthalmol. 31, 59–70

54. Ferrari, G., Bignami, F., Giacomin, C., Capitolo, E., Comi, G., Chaabane, L., and Rama, P. (2014) Ocular surface injury induces inflammation in the brain: in vivo and ex vivo evidence of a corneal-trigeminal axis. Invest. Ophthalmol. Vis. Sci. 55, 6289–6300

55. He, J., and Bazan, H. E. P. (2016) Neuroanatomy and neurochemistry of mouse cornea. Invest. Ophthalmol. Vis. Sci. 57, 664–674

56. He, J., Pham, T. L., and Bazan, H. E. P. (2019) Mapping the entire nerve architecture of the cat cornea. Vet. Ophthalmol. 22, 345–352

57. Ziegglänsbäger, W. (2010) Substance P and pain chronicity. Cell Tissue Res. 375, 227–241
67. Iyengar, S., Ossipov, M. H., and Johnson, K. W. (2017) The role of calciotinin gene-related peptide in peripheral and central pain mechanisms including migraine. *Pain* 158, 543–559.

68. Belmonte, C., and Gallar, J. (2011) Cold thermoreceptors, unexpected players in tear production and ocular dryness sensations. *Invent. Ophthalmol.* 8, 3888–3892.

69. Parra, A., Madrid, R., Echevarria, D., del Olmo, S., Morellina-Palao, C., Acosta, M. C., Gallar, J., Dhaka, A., Viana, F., and Belmonte, C. (2010) Ocular surface wetness is regulated by TRPM8-dependent cold thermoreceptors of the cornea. *Nat. Med.* 16, 1396–1399.

70. Proudfoot, C. J., Garry, E. M., Cottrell, D. F., Rosie, R., Anderson, H., Robertson, D. C., Fleetwood-Walker, S. M., and Mitchell, R. (2006) Analgesia mediated by the TRPM8 cold receptor in chronic neuropathic pain. *Curr. Biol.* 16, 1591–1605.

71. Liu, B., Fan, L., Balakrishna, S., Sui, A., Morris, J. B., and Jordt, S. E. (2020). *Fernandéz-Peña*, C., and Viana, F. (2013) Targeting TRPM8 for pain relief. *Open Pain J.* 6, 154–164.

72. He, J., Pham, T. L., Kakazu, A. H., and Bazan, H. E. P. (2019) Remodeling of substance P sensory nerves and transient receptor potential melastatin 8 (TRPM8) cold receptors after corneal surgical surgery. *Invent. Ophthalmol.* 8, 2449–2460.

73. Lambiase, A., Rama, P., Bonini, S., Caprioglio, G., and Aloe, L. (1998) Topical treatment with nerve growth factor for corneal neurotrophic ulcers. *N. Engl. J. Med.* 338, 1174–1180.

74. Zhang, L., Terrando, N., Sui, A., Morris, J. B., and Jordt, S. E. (2018) Distinct analgesic action of DHA and DHA-derived specialized pro-resolver mediators on post-operative pain relief. *Open Pain J.* 6, 154–164.

75. Huang, J., Burst, J. M., Li, M., Ashraf, S., Mapp, P. I., Bennett, A. J., Ravipati, S., Poussin, P., Barret, D. A., Scammell, B. E., et al. (2017) Targeting the D series resolvin receptor system for the treatment of osteoarthritic pain. *Arthritis Rheum.* 69, 996–1008.

76. Chen, N., Zhou, P., Liu, X., Li, J., Wan, Y., Liu, S., and Wei, F. (2020) Overexpression of Rictor in the injured spinal cord promotes functional recovery in a rat model of spinal cord injury. *FASEB J.* 34, 6984–6998.

77. Chiang, N., Dall, J., Colas, R. A., and Serhan, C. N. (2015) Identification of resolvin D2 receptor mediating resolution of infections and organ protection. *J. Exp. Med.* 212, 1203–1217.

78. Dall, J., Winkler, J. W., Colas, R. A., Araurdottir, H., Cheng, C. Y., C., Chiang, N., Petasis, N. A., and Serhan, C. N. (2013) Resolvin D3 and aspirin-triggered resolvin D3 are potent immunosuppressors. *Chem. Biol.* 20, 188–201.

79. Chiang, N., Fredman, G., Eckhed, F., Oh, S. F., Vickery, T., Schmidt, B. A., and Serhan, C. N. (2012) Infection regulates pro-resolving mediators that lower antibiotic requirements. *Nature* 484, 524–528.

80. Bang, S., Xie, Y.-K., Zhang, Z.-J., Wang, Z., Xu, Z.-Z., and Ji, R.-R. (2018) CPR37 regulates macrophage phagocytosis and resolution of inflammatory pain. *J. Clin. Invest.* 128, 3506–3522.

81. Chiang, N., Libreros, S., Norris, P. C., de la Rosa, X., and Serhan, C. N. (2019) Maresin 1 activates LGR6 receptor promoting phagocyte immunoreactive functions. *J. Clin. Invest.* 129, 5294–5311.

82. Brignole-Baudouin, F., Baudouin, C., Aragona, P., Rolando, M., Labeloueille, M., Pisella, P. J., Barabino, S., Siou-Mermet, R., and Grezor-Garcher, C. (2011) A multicentre, double- masked, randomized, controlled trial assessing the effect of oral supplementation of omega-3 and omega-6 fatty acids on a convulsant inflammatory marker in dry eye patients. *Acta Ophthalmol.* 89, e591–e597.

83. Wojtowicz, J. C., Butovich, I., Uchiyama, E., Aronowicz, J., Agee, S., and McGulley, J. P. (2011) Pilot, prospective, randomized, double-masked, placebo-controlled clinical trial of an omega-3 supplement for dry eye. *Cornea* 30, 308–314.

84. Bhargava, R., Kumar, P., Kumar, M., Mehra, N., and Mishra, A. (2013) A randomized controlled trial of omega-3 fatty acids in dry eye syndrome. *Int. J. Ophthalmol.* 6, 811–816.

85. Kangari, H., Eftekhari, M. H., Sardari, S., Hashemi, H., Salama-deh, J., Ghassemi-Broumand, M., and Khabazkhoob, M. (2013) Short-term consumption of oral omega-3 and dry eye syndrome. *Ophthalmologe* 120, 2191–2196.

86. Oelek, A., Jiménez-Alfaro, I., Alejandre-Alba, N., and Mahillo-Fernández, I. (2015) A randomized, double-masked study to evaluate the effect of omega-3 fatty acids supplementation in meliobion gland dysfunction. *Clin. Interv. Aging* 8, 1133–1138.

87. Ong, N. H., Purcell, T. L., Roch-Leveque, A.-C., Wang, D., Istrodo, M. A., Bottos, K. M., Heichel, C. W., and Schanzlin, D. J. (2013) Epithelial healing and visual outcomes of patients using omega-3 oral nutritional supplements before and after photorefractive keratectomy: a pilot study. *Cornea* 32, 761–765.

88. Sheppard, J. D., Singh, R., McCellan, A. J., Weikert, M. P., Scoper, S. V., Joly, T. J., Whitley, W. O., Kakkar, E., and Pflugfelder, S. C. (2015) Long-term supplementation with n-6 and n-3 pufas improves moderate-to-severe keratoconjunctivitis sicca: a randomized double-blind clinical trial. *Cornea* 32, 1297–1304.

89. Oelek, A. (2014) Effectiveness and tolerability of dietary supplementation with a combination of omega-3 polyunsaturated fatty acids and antioxidants in the treatment of dry eye symptoms: Results of a prospective study. *Clin. Ophthalmol.* 8, 169–176.

90. Georgakopoulos, C. D., Makri, O. E., Pagoulatos, D., Vasilakis, P., Peristeropoulou, P., Koulis, V., Eliopoulou, M. L., and Psachoulia, C. (2017) Effect of omega-3 fatty acids dietary supplementation on ocular surface and tear film in diabetic patients with dry eye. *J. Am. Coll. Nutr.* 36, 38–43.

91. Bhargava, R., Kumar, P., Phogat, H., Kaur, A., and Kumar, M. (2015) Oral omega-3 fatty acids treatment in computer vision syndrome related dry eye. *Cont. Lens Anterior Eye* 38, 206–210.

92. Goyal, P., Jain, A. K., and Malhotra, C. (2017) Oral omega-3 fatty acid supplementation for laser in situ keratomileusis-associated dry eye. *Cornea* 36, 169–175.

93. Gronert, K., Maheshwari, N., Khan, N., Hassan, I. R., Dunn, M., and Schwartzman, M. L. (2005) A role for the mouse 12/15-lipoxygenase pathway in promoting epithelial wound healing and host defense. *J. Biol. Chem.* 280, 15267–15278.

94. Jin, Y., Arita, M., Zhang, Q., Sahani, D. R., Chauhan, S. K., Chiang, N., Serhan, C. N., and Dana, R. (2009) Anti-angiogenesis effect of the novel anti-inflammatory and pro-resolving lipid mediators. *Invest. Ophthalmol.* 8, 4743–4752.

95. Obrosov, A., Coppey, I. J., Shevalev, H., and Yorek, M. A. (2017) Effect of fish oil vs. resolin D1, E1, methyl esters of resolvins D1 or D2 on diabetic peripheral neuropathy. *J. Neurol. Neurophysiol.* 8, 453.

96. Zhang, Z., Hu, X., Qi, X., Di, G., Zhang, Y., Wang, Q., and Zhou, Q. (2018) Resolvin D1 promotes corneal epithelial wound healing and restoration of mechanical sensation in diabetic mice. *Mol. Vis.* 24, 274–285.