Review

Use of nutraceuticals in the management of retinopathies

Dr+Vet by Böthmen Pharma

Introduction

Although a large variety of pharmaceutical therapies for treating diseases have been developed in recent years, there has been little progress in retinal disease prevention. In particular, the protection of neural tissue is essential, because it hardly regenerates. The use of nutraceuticals for maintaining health has been supported by several clinical studies, including cross-sectional and interventional studies for age-related macular disease. However, evidence for their effects at the molecular level has been very limited. In this review, we focus on the different ingredients that could help to prevent retinopathies and that are included in our product **Retinae**.

The Retina

The retina is a multiple layered tissue, which lines the back of the eye and connects with the brain via the optic nerve. Light has to cross the entire thickness of the retina until it reaches the photosensitive "rod and cone" photoreceptor cell layer. Blood supply to the retina occurs through retinal blood vessels in the choroid layer. The retina itself is built up of three layers of cellular bodies and two layers of synapses. The **outer nuclear layer** consists of photoreceptor cell bodies (cons and rods), in the **inner nuclear layer** are the cell bodies of bipolar, amacrine, and horizontal cells, and the last layer is formed by **ganglional retinal cells** that form the optic nerve. All these cells contribute to the visual cycle, which may be summarized as the conversion of a photochemical "message" from visible light into a neural signal, which can be interpreted by the brain (1).

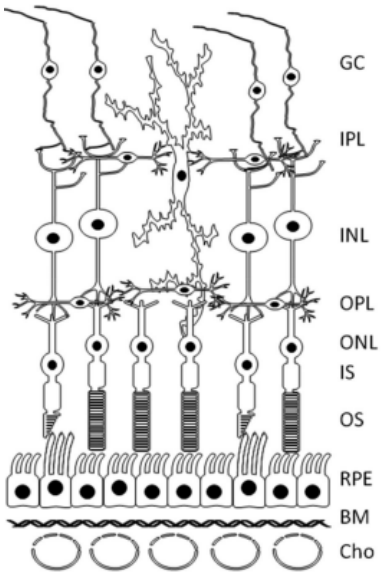

Fig 1. Diagram (1) depicting the cellular organization of the retina-RPE-choroid.
Cho, choroid; **BM**, Bruch's membrane; **RPE**, retinal pigment epithelium; **OS**, outer segment; **IS**, inner segment; **ONL**, outer nuclear layer; **OPL**, outer plexiform layer; **INL**, inner nuclear layer; **IPL**, inner plexiform layer and **GC**, ganglion cell layer.

Junctions between rods, cones, and the photoreceptor inner segments create a barrier called the outer limiting membrane, which separates the retina region from the subretinal space. In the subretinal space, the retinal pigment epithelium creates a single monolayer. Its main function is to support the maintenance of the retina, through reducing the backscattering of light via its high pigment content, and removing by-products of the visual cycle. It also prevents new vessel growth into the retinal layers from the choroidal vasculature underneath. Bruch's membrane, consisting of extracellular matrix proteins, proteoglycans and glycosaminoglycans, separates the RPE from the choroid. Together, the RPE and the Bruch's membrane, form the outer blood–retinal barrier, which prevents the entrance of macromolecules and immune cells from the underlying choroid into the photoreceptor layer. Thus, the integrity of the RPE and Bruch's membrane are essential for maintenance of the blood–retinal barrier and homeostasis in the retina (1).

The **choroid**, located below Bruch's membrane, contains a dense network of blood vessels (the choriocapillaris), which supplies oxygen and nutrients to the RPE, outer retina and optic nerve. The choroid contains tissue-resident melanocytes, fibroblasts, macrophages, mast cells, and dendritic cells (2). Muller cells, a specialized form of local retinal glial cell, are, in contrast, found throughout the retina. As part of the normal aging process, waste material accumulates in the retina-RPE and RPE-choroid interface. Its advanced accumulation can lead to disease, such as **age-related macular degeneration (AMD)** (1).

In humans, AMD is the leading cause of blindness in older individuals in the Western world. The exudative (wet or neovascular) form of AMD is associated most widely with central vision impairment and legal blindness. The management of wet AMD was revolutionized by the introduction of anti–vascular endothelial growth factor (VEGF) therapies. Thus, developing alternative or adjunct therapies to currently available anti-VEGF drugs may increase treatment success, slow AMD progression, and improve VA outcomes (3).

Tagetes erecta (Carotenoids - Lutein & Zeaxanthin)

Carotenoids are fat-soluble plant pigments found in some fruits and vegetables. The carotenoid group substances act as antioxidants. They contain several double bonds, which react with reactive oxygen species (ROS) to scavenge radicals. Lutein and zeaxanthin are selectively concentrated in the macula, where they are hypothesized to protect against AMD by absorbing blue light, quenching free radicals, and stabilizing cell membranes (4).

Lutein is a xanthophyll type of carotenoid. Lutein is not synthesized in mammals and must be obtained from the diet. It is absorbed from the intestinal epithelium into the blood, and circulates systemically to reach the liver, lung, and retina. In the human retina, it is concentrated in the macula, so it is called a macular pigment. The administration of oral supplements with lutein and zeaxanthin improves serum concentration of those products (5).

A large clinical study, the Age-related Eye Disease Study (AREDS) (6), was performed to examine ways to prevent age-related macular degeneration (AMD). In its annexed study, participants reporting the highest dietary intake of lutein/zeaxanthin were statistically less likely to have advanced AMD (either the atrophic or exudative type) or to be at high risk of developing it, than those reporting the lowest dietary intake.

A study using donor eyes showed a negative association between lutein content in the retina and AMD risk (7). Retinas from 56

donors with AMD and 56 controls were analyzed and showed that the levels of lutein and zeaxanthin were lower in the retinas suffering from AMD. Taken together, these observations indicate that lutein obtained from the diet accumulates in the retina and may act locally to prevent disease.

In the study of Yoko Ozawa et al. (8) they have done some trials on endotoxin-induced uveitis (EIU) in mouse models and it concludes that lutein affects the pathological pathways of inflammatory cytokines, such as IL-6 and angiotensin II signaling, and prevents neurodegeneration (e.g., via the loss of function-essential proteins and tropic factors, and via DNA damage) that can be induced by oxidative stress. Lutein protects tissue against pathological stimuli regardless of light exposure.

In the study performed by Wu J. et al. (4) they conclude that higher intakes of bioavailable carotenoids, particularly lutein/zeaxanthin and α-carotene, are associated with reduced risk of advanced AMD.

Omega 3 Fatty Acids

Omega fatty acids, a family of monounsaturated and PUFAs, primarily encompasses three subtypes – Omega-3, Omega-6 and Omega-9 fatty acids. Among these, Omega-3 and Omega-6 are essential PUFAs which cannot be synthesized in mammals' bodies. There are three types of Omega-3 fatty acids: Alpha-linoleic acid (ALA), Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA). In the Omega-6 family, Linolenic acid is a chief member (9).
ALA metabolizes to EPA and DHA, whereas linoleic acid forms arachidonic acid, upon metabolism. As arachidonic acid is a precursor for the formation of pro-inflammatory mediators, prostaglandins, thromboxanes and leukotrienes, linoleic acid is involved in aggravating inflammatory responses in the body. In contrast, Omega-3 fatty acids have been proven to possess anti-inflammatory, antithrombotic, anti-arrhythmic and antiangiogenic properties (9).

A potential strategy to influence and reduce the progression of wet AMD comes from directly modulating the cellular make-up of the retina. In this respect, the outer retina is highly concentrated in diet-derived long-chain polyunsaturated fatty acids (LCPUFAs) (10) such as docosahexaenoic acid (DHA) of the omega-3 family and arachidonic acid of the omega-6 family. The capacity of lipids to play biological roles beyond energy storage and membrane structure long has been recognized (10,11).

Because mammals are limited in their capacity to biosynthesize omega-3 LCPUFAs de novo, their tissue status is modifiable via diet or supplement intake of DHA and EPA(11). The benefits of omega-3 supplementation on wet AMD consistently have been recognized in multiple observational studies (12–14).

Various studies found that ALA possesses both cytoprotective as well as neuroprotective properties. It significantly decreases the levels of several inflammatory mediators, namely VEGF besides inhibiting the production of pro-inflammatory cytokines such as interleukin-6 (IL-6), IL-1b and tumor necrosis factor (TNF-α). They also restitute the altered physiologies of various antioxidant enzymes (15).

In one study conducted in humans, ALA also increases the levels of brain-derived neurotrophic factor, a neuroprotective protein, which protects neurons from various damaging stimuli, such as inflammation and oxidative stress, and enhances their survival, and therefore prevents retinal neuronal degeneration. Hence, ALA reduces damage to endothelial and neuronal retinal cells by inhibiting oxidative stress-induced apoptosis, thereby preventing diabetic retinopathy (16).

EPA possesses anti-inflammatory properties due to its ability to affect the intracellular signaling pathways and, thereafter, the gene expression of various inflammatory mediators involved during an inflammatory response. This mainly occurs via EPA-induced inhibition of NF-jB, subsequently inhibiting the expressions of pro-inflammatory cytokines (17).

The potential of EPA to inhibit the conversion of arachidonic acid into its pro-inflammatory metabolites (mainly prostaglandins and leukotrienes) is the most accepted molecular mechanism behind the portrayal of its anti-inflammatory properties. Besides, EPA greatly reduces inflammation-induced neovascularization. In animal models, EPA markedly reduces the transcription and translation of several inflammatory markers of neovascularization, inhibition of the expression of VEGF is highly significant in preventing retinal angiogenesis (18).

In one clinical trial in humans, Falvio A Rezende et al. (3) investigated the influence of omega-3 supplementation on VEGF-A levels in of patients undergoing anti-VEGF treatment for wet AMD and noted a significant decrease of VEGF-A in patients receiving also a supplement of omega-3. They also demonstrated in other studies (19) that increased omega-3 LCPUFA dietary intake reduces pathologic angiogenesis in experimental animal models of neovascular retinopathies.

Vitamins (C, E, B [B6, B9, B12])

Vitamin C, also known as L-ascorbic acid, is a water-soluble nutrient known to have antioxidant properties. Vitamin C has also been shown to be a cofactor in the regeneration of Vitamin E in the retina (20).

B-vitamins have been shown to reduce serum levels of homocysteine, which has been implicated in increasing the risk of developing AMD. B vitamins can help maintain the cellular response to oxidative stress by acting as cofactors in the enzymatic activation of antioxidants. The role of vitamins B6 and B12 in preventing oxidative damage can be attributed to their role in the metabolic pathway that eliminates homocysteine, in which B12 and B6 act as enzymatic cofactors (21). Homocysteine has been shown to adversely affect vascular endothelial cells, which may also play a role in the development of AMD (20).

The study performed by Subhasish Pramanik et al. (22) demonstrated in diabetic human patients that vitamin B, C, and E supplements in combination with conventional management of hyperglycemia decrease the risk of development of diabetic retinopathy by inhibiting oxidative stress, advanced glycation end products formation, lipid peroxidation and VEGF secretion.

Vitamin E is a lipid-soluble antioxidant that exists in four common forms in nature: α-tocopherol, β-tocopherol, δ-tocopherol, and γ-tocopherol. In the body, α-tocopherol has been shown to be the most abundantly concentrated in both plasma and retinal tissue. Increased dietary levels of Vitamin E have been correlated with increased concentrations in the retina (20).

Minerals (Zinc and Cooper)

Zinc is an essential micronutrient for all organisms, critically required for normal cellular processes as well as for normal metabolism. The eye has an unusually high zinc content, with the highest amount of zinc concentrated in the retinal pigment epithelium (RPE) followed by the retina. Zinc exists in the other ocular tissues, in the following (descending) order of content: the ciliary body, iris, optic nerve, sclera, cornea, and the lens (1).

Zinc plays a key role in fundamental cellular processes such as DNA synthesis, RNA transcription, cell division, and activation, as

well as in prevention of cell apoptosis. So a zinc deficiency may affect negatively to the normal functions of the retina (23,24).

Light-induced retinal degeneration and visual cell loss in rats results in gene expressional changes related to inflammation, apoptosis, cytokine production, and innate immune responses; and these pathways can be suppressed by zinc supplementation, in combination with Age-Related Eye Disease Study (AREDS) antioxidant supplement formula and other antioxidants (25).

Evidence from the large randomized, placebo controlled AREDS clinical trials, which initially evaluated high dose supplementation with vitamins C and E, beta carotene, with or without zinc (zinc oxide 80 mg) and copper, suggested that these components may help protect against the progression to AMD and related vision loss (26). In particular, it has been shown that retinal degenerative pathways are suppressed by zinc supplementation, in combination with the AREDS formula and other antioxidants (25).

Copper is an essential trace element with the specific ability to easily accept and donate electrons; thus, it plays an important role in oxido-reduction and the scavenging of free radicals. Copper is added in various multi vitaminic formulations due to its strong link with zinc levels; high levels of zinc intake may cause copper deficiency anemia (27).

Lactoferrin

Lactoferrin is an iron-binding protein from the transferrin family that has been reported to have numerous functions. It is also found in most exocrine secretions (tears and others) and in the secondary granules of neutrophils. Antimicrobial and anti-inflammatory activity reports on lactoferrin identified its significance in host defense against infection and extreme inflammation (28).

Lactoferrin regulates inflammatory cytokines production in a mode resembling to other anti-inflammatory cytokines by suppressing inflammation interacting with macrophages and restraining the production of inflammatory cytokines by cells (29). Lactoferrin is known to suppress the production of TNF-α, IL-1β, IL-6 and IL-8 in human mononuclear cells (in vitro) (30) and improve production of IL-10 and IL-4 (in vivo) (31).

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