

R E V I E W

Flavonoid supplements increase neurotrophin activity to modulate inflammation in retinal genetic diseases

Aysba Karim Kiani¹, Benedetto Falsini², Lucia Ziccardi³, Elena Gusson⁴,
Domenica Mangialavori⁵, Francesca Allegrini⁶, Emma Colao⁷, Matteo Bertelli^{1,8,9}

¹MAGI EUREGIO, Bolzano, Italy; ²Department of Ophthalmology, Catholic University of Sacred Heart, Gemelli Policlinic, Rome, Italy; ³Fondazione Bietti-IRCCS, Rome, Italy; ⁴Unit of Ophthalmology, Azienda Ospedaliera Universitaria Integrata, Verona, Italy; ⁵Institute of Ophthalmology, Department of Medical and Surgical Sciences, Magna Graecia University, Catanzaro, Italy; ⁶Eye Clinic, Department of Neurosciences, Biomedicine and Movement, University of Verona, Verona, Italy; ⁷Medical Genetics Unit, Magna Graecia University, Catanzaro, Italy; ⁸MAGI'S LAB, Rovereto (TN), Italy; ⁹EBTNA-LAB, Rovereto (TN), Italy

Abstract. Retinal degenerative disorders induce loss of photoreceptors associated with inflammation, and negative remodeling and plasticity of neural retina. Retinal degenerative diseases may have genetic and/or environmental causes. Degeneration of retinal pigment epithelium cells initiates a vicious circle increasing the ongoing inflammation in both retina and choroid. Flavonoids are polyphenolic molecules with antioxidant activity and dietary intake, specifically of anthocyanins and flavanols, improves oxidative stress and neuro-inflammation. *In vitro* and *ex vivo* studies have also revealed biological effects of flavonoids on retinal protection against oxidative stress and inflammation. In this brief review, the protective role of flavonoids against retinal degeneration and inflammation will be discussed along with their therapeutic potential for the treatment of retinal degenerative diseases. (www.actabiomedica.it)

Key words: retinal degenerative disorders, neurotrophin synthesis, flavonoids supplementation, anti-inflammation, anti-oxidant

Introduction

Retinal degeneration and remodeling involve genetic diseases with Mendelian inheritance or multifactorial diseases with the contribution of external factors. These factors lead to neuronal reprogramming and rewiring events with gene expression alterations, *de novo* neurogenesis and novel synapses formation. This causes dendritic alterations and supernumerary axons (1). Subsequent degenerative changes of retinal pigment epithelium cells initiate a vicious circle promoting chronic inflammation in both retina and choroid (2).

Though inflammation is an important immune mechanism, chronic inflammation can be harmful and may cause chronic retinal disorders such as age-related macular degeneration (AMD) (3).

AMD is a progressive inflammatory retinal degenerative disease (4) that involves degeneration of the RPE with consequent photoreceptors death leading to unrestrainable central vision loss. RPE is very sensitive to oxidative stress caused by inflammation. The reduction of intracellular inflammation should be the main focus in the development of novel therapeutic options for retinal disorders (2).

Neurotrophins

Neurotrophins (NT) are the growth factors involved in the regulation of central and peripheral nervous systems development and its maintenance. NT belongs to the secreted protein family that includes nerve

growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophic factor 3 and 4 (NTF3, NTF4). NTs have key role in the development, differentiation proliferation and survival of the retinal cells. Moreover, deprivation of NTs has been suggested to have a key role in retinal cell death linked with neurodegenerative disorders (5).

BDNF and NTF4 exert their neuronal functions through the binding of neurotrophic receptor tyrosine kinase 2 (NTRK2) and p75 neurotrophin receptor (p75NTR), whereas NGF binds to NTRK1, and finally NTF3 binds NTRK3 receptor (5).

NTRK2 is expressed in the hippocampus, neocortex, brainstem and striatum, and plays a significant role in plasticity, morphogenesis and neuronal survival (6). BDNF binding to NTRK2 stimulates intracellular signaling pathways like mitogen-activated protein kinase/extracellular signal regulated protein kinase (MAPK/ERK), phospholipase C γ (PLC γ), and phosphoinositide 3-kinase (PI3K) pathways. BDNF plays a significant role in the maintenance of dendritic spine morphology, dendritic branching, synaptic plasticity, long-term potentiation and memory regulation (7). BDNF also prevents glutamate toxicity and apoptosis in cerebellar neurons, decreases ischemic neuronal damage and enhances the functional recovery after post-injury regeneration (8).

NGF has a role in immune-hematopoietic system function and in the function of neuroendocrine system, furthermore it regulates the balance between immune, nervous and endocrine systems (7).

There are evidences that link neurotrophic factors' actions and polyphenol compounds' activity such as flavonoids. The neurotrophic actions of polyphenols include its effects on neuronal growth, survival, differentiation, proliferation and cell signaling. Many polyphenols increase neuronal survival and *in vitro* neurite outgrowth promotion (9).

Flavonoid as a therapeutic option of retinal diseases

Vision loss caused by photoreceptor cell damage is a common experience for young and elder people affected by inherited retinal dystrophies or AMD, respectively. Damaged photoreceptors (rods and cones)

of retina result in the gradual decline of vision or complete blindness. Nature has provided ample sources for the development of herbal medicine for these retinal problems. Several plants secondary metabolites are known as "bioactive compounds" because of their pharmacological effects (10).

Potential therapeutic effects of different fruits such as berries that have very high flavonoids content, particularly anthocyanins, have beneficial effect in the enhancement of vision because of the anthocyanins anti-oxidant potential and these observations are well reported by researchers throughout the world (11).

For several diseases *in silico* approaches are generally used for the identification of lead molecules. Similarly, for retinal diseases researchers have analyzed the efficiency of anthocyanins as potential inhibitor in Best's disease, which is a juvenile inherited macular dystrophy. Therefore, they performed *in silico* docking studies for RP2 protein that could be a potential drug target, with three compounds of anthocyanin, petunidin, malvidin, cyanidin. Moreover, molecular docking as well as binding free energy values obtained from this analysis suggested that out of these compounds cyanidin 3,5 diglucoside have a strong potential to be an effective inhibitor of photoreceptor degeneration in X-linked retinitis pigmentosa (RP). The role of cyanidin 3-glucoside in vision improvement has also been reported (11).

Flavonoids effect on neurotrophin synthesis

Flavonoids have common structural features. They are made of two aromatic rings linked together via three carbon atoms, thereby forming an oxygenated heterocycle. Flavonoids can further be subdivided in six classes, flavonols, flavanones, flavones, isoflavones, flavanols and anthocyanidins. *In vitro* and *ex vivo* studies have revealed many biological effects of these polyphenols such as inhibition of cancer cells proliferation, decreased vascularization, neurons protection against oxidative stress, improvement of insulin secretion, antimicrobial, antiviral, vasorelaxant, and anticoagulant and anti-inflammatory activities (12).

Research studies have proposed that polyphenols might exert their protective effects by increasing neu-

rotrophins action. Numerous natural compounds are involved in the potentiation of NGF action or induction of neurite outgrowth, which could be used for neuronal injury treatment. For instance, experimental data revealed that green tea polyphenols potentiate neuritogenesis induced by NGF (13).

Dietary flavonoids intake, specifically anthocyanins and flavanols, improve endothelial vasodilatation, glucose metabolism, blood pressure, cognitive function, insulin resistance.

Previous animal studies proposed that the improvements of cognitive performance caused by the intake of flavonoid might be linked to an increase of *BDNF* expression. Similarly, *in vitro* studies have proposed that nano-molar concentrations of flavonoids, particularly flavanones and flavanols, might activate the ERK pathway, thus leading to phosphorylation and activation of downstream cAMP response element-binding (CREB) protein (14).

BDNF synthesis also takes place in endothelial and retinal cells. An *ex vivo* study revealed that BDNF synthesis within vascular endothelial cells might contribute to BDNF circulating pool in humans along with its activity-dependent release, and it might also be transported through blood–brain barrier (15).

The neurotrophic effects of a flavonoid in the retina

7,8-Dihydroxyflavone (7,8-DHF) is a natural flavone that can be extracted from primula tree leaves (16). 7,8-DHF acts as direct agonists of NTRK2 receptors and mimics the binding of neurotrophins like BDNF. 7,8-DHF strongly activates NTRK2 and its downstream Akt and ERK pathways, preventing cell death, and promoting neuritogenesis in the retinal ganglion cells (RGC) located in the inner retina (9). In fact, it was observed by Gupta et al that RGC-5 cells pretreated with the 7,8-DHF prior to their exposure to excitotoxic and oxidative stress using glutamate and H_2O_2 show extended neuritis when compared to control (cells exposed to stress but not to 7,8-DHF). To confirm the role of 7,8-DHF as a mimicker of BDNF, a significant decrease in NTRK2 kinase activity is observed in the RGC-5 neurons after exposure to glutamate + H_2O_2 stress, but a high protection of NTRK2

tyrosine kinase activity is observed when cells are pretreated with 7,8-DHF prior to the stress exposure. Interestingly, it seems that 7,8-DHF protects the NTRK2 kinase activity only when cells are exposed to excitotoxic and oxidative stress. Furthermore, rat RGC exposed to the glutamate + H_2O_2 stress die after apoptosis (caspase-3 activation), whereas caspase-3 activation was not observed in stressed cells pretreated with the 7,8-DHF, indicating that this drug protects RGCs from apoptosis under the stress conditions by maintaining the NTRK2 receptor in an activated state (17).

A similar neuroprotective effect was found by Kim et al in 2013 in a natural compound, bakuchiol, extracted from *P. corylifolia*. Although bakuchiol is not a flavonoid, it is effective against in both *in vitro* in RGC-5 cells and *in vivo* rat models with experimentally-damaged retina (18). Therefore, bakuchiol may be proposed to be used as in addition to 7,8-DHF against retinal diseases.

Anthocyanin anti-inflammatory effect in retinitis pigmentosa

Chronic inflammation is one of a prominent etiologic factor of retinitis pigmentosa. Retinal (most likely photoreceptor) autoantibodies could be identified in blood samples of patients suffering from RP. Furthermore, several immune cells like lymphocytes can be found in vitreous samples of RP patients (19).

In the aqueous and vitreous humor samples of RP patients there is an increased level of inflammatory factors and inflammatory cells, like interleukin-1, interleukin-2, interleukin-8 and tumor necrosis factor alpha (20).

In several cell types anthocyanin shows anti-inflammatory effects by the inhibition of COX-1, COX-2, NF- κ B and interleukins expression. Moreover, anthocyanin also reduces the glial fibrillary acidic protein expression, a well-known marker for the retinal neuro-inflammatory response. Because of the association of RP with inflammation, a variety of anthocyanin anti-inflammatory actions justify its potential clinical usage for RP treatment (21).

Conclusions

Retinal inherited diseases and macular degeneration are caused by various genetic and environmental factors like trauma, retinal degeneration and remodeling as well as neuronal factors. Pathogenesis of several retinal diseases, like AMD, has strong relation with inflammation. Neuronal factors like neurotrophins are involved in proper neuronal development and signaling pathways. Polyphenols like flavonoids increase synthesis of neurotrophins. In addition, the antioxidant activity of anthocyanin has neuroprotective role, improves the microcirculation of retina as well as plays role in the photo-transduction and in the visual signaling. Both *in-vivo* and *in-vitro* studies have reported the potential neuroprotective and therapeutic roles of several flavonoids like anthocyanins, Kaempferol and quercetin, against neuroinflammation in retinal and neuro degenerative diseases. Furthermore, *in silico* studies have also showed promising results of flavonoids as therapeutic option for retinal inherited diseases.

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References

- Jones BW, Marc RE, Pfeiffer RL. Retinal degeneration, remodeling and plasticity. In: Webvision: The Organization of the Retina and Visual System. Salt Lake City (UT): University of Utah Health Sciences Center; 2016.
- Kauppinen A, Paterno JJ, Blasiak J, Salminen A, Kaarniranta K. Inflammation and its role in age-related macular degeneration. *Cell Mol Life Sci* 2016; 73: 1765-86.
- Pawelec G, Goldeck D, Derhovanessian E. Inflammation, ageing and chronic disease. *Curr Opin Immunol* 2014; 29: 23-8.
- Ferrington DA, Sinha D, Kaarniranta K. Defects in retinal pigment epithelial cell proteolysis and the pathology associated with age-related macular degeneration. *Prog Retin Eye Res* 2016; 51: 69-89.
- Telegina DV, Kolosova NG, Kozhevnikova OS. Immunohistochemical localization of NGF, BDNF, and their receptors in a normal and AMD-like rat retina. *BMC Med Genomics* 2019; 12: 48.
- Liu C, Chan CB, Ye K. 7,8-dihydroxyflavone, a small molecular TrkB agonist, is useful for treating various BDNF-implicated human disorders. *Transl Neurodegener* 2016; 5: 2.
- Carito V, Ceccanti M, Chaldakov G, et al. Polyphenols, nerve growth factor, brain-derived neurotrophic factor, and the brain. In: *Bioactive Nutraceuticals and Dietary Supplements in Neurological and Brain Disease*. Cambridge, (MA): Academic Press; 2015.
- Du X, Hill RA. 7,8-Dihydroxyflavone as a pro-neurotrophic treatment for neurodevelopmental disorders. *Neurochem Int* 2015; 89: 170-80.
- Moosavi F, Hosseini R, Saso L, Firuzi O. Modulation of neurotrophic signaling pathways by polyphenols. *Drug Des Devel Ther* 2015; 10: 23-42.
- Adisakwattana S, Yibchok-Anun S, Charoenlertkul P, Wongsasiripat N. Cyanidin-3-rutinoside alleviates postprandial hyperglycemia and its synergism with acarbose by inhibition of intestinal α -glucosidase. *J Clin Biochem Nutr* 2011; 49: 36-41.
- Priya SSL, Devi PR, Madeswaran A. In silico docking studies of RP2 (X-Linked retinitis pigmentosa) protein using anthocyanins as potential inhibitors. *Bangladesh Journal of Pharmacology* 2013; 8: 292-9.
- Silva AR, Pinheiro AM, Souza CS, et al. The flavonoid rutin induces astrocyte and microglia activation and regulates TNF-alpha and NO release in primary glial cell cultures. *Cell Biol Toxicol* 2008; 24: 75-86.
- Guo Y, Xu J, Li Y, et al. Iridoids and sesquiterpenoids with NGF-potentiating activity from the rhizomes and roots of *Valeriana fauriei*. *Chem Pharm Bull (Tokyo)* 2006; 54: 123-5.
- Vauzour D, Vafeiadou K, Rodriguez-Mateos A, Rendeiro C, Spencer JP. The neuroprotective potential of flavonoids: a multiplicity of effects. *Genes Nutr* 2008; 3: 115-26.
- Trajkovska V, Marcussen AB, Vinberg M, Hartvig P, Aznar S, Knudsen GM. Measurements of brain-derived neurotrophic factor: methodological aspects and demographical data. *Brain Res Bull* 2007; 73: 143-9.
- Colombo PS, Flamini G, Christodoulou MS, et al. Farinose alpine *Primula* species: phytochemical and morphological investigations. *Phytochemistry* 2014; 98: 151-9.
- Gupta VK, You Y, Li JC, Klistorner A, Graham SL. Protective effects of 7,8-dihydroxyflavone on retinal ganglion and RGC-5 cells against excitotoxic and oxidative stress. *J Mol Neurosci* 2013; 49: 96-104.
- Kim KA, Shim SH, Ahn HR, Jung SH. Protective effects of the compounds isolated from the seed of *Psoralea corylifolia* on oxidative stress-induced retinal damage. *Toxicol Appl Pharmacol* 2013; 269: 109-20.

19. Viringipurampeer IA, Bashar AE, Gregory-Evans CY, Moritz OL, Gregory-Evans K. Targeting inflammation in emerging therapies for genetic retinal disease. *Int J Inflamm* 2013; 2013: 581751.
20. Holan V, Hermankova B, Krulova M, Zajicova A. Cytokine interplay among the diseased retina, inflammatory cells and mesenchymal stem cells - a clue to stem cell-based therapy. *World J Stem Cells* 2019; 11: 957-67.
21. Kwon JY, Lee KW, Hur HJ, Lee HJ. Peonidin inhibits phorbol-ester-induced COX-2 expression and transformation in JB6 P+ cells by blocking phosphorylation of ERK-1 and -2. *Ann N Y Acad Sci* 2007; 1095: 513-20.

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Correspondence:

Stefano Paolacci

Via delle Maioliche, 57/D, Rovereto (TN), Italy

E-mail: stefano.paolacci@assomagi.org