·Review·

Anthocyanin can arrest the cone photoreceptor degeneration and act as a novel treatment for retinitis pigmentosa

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Abstract

• **Retinitis pigmentosa (RP) is a group of heterogeneous inherited retinal diseases that is characterized by primary death rod photoreceptors and the secondary loss of cones. The degeneration of cones causes gradual constriction of visual fields, leaving the central islands that are eventually snuffed out. Studies indicate that the hyperoxia causes oxidative damage in the retina and contributes to the cone death of RP. Moreover, abundant reactive oxidative species (ROS) which are generated in cones may result in mitochondria membrane depolarization, which has been ascribed a central role in the apoptotic process and has been proposed to act as a forward feeding loop for the activation of downstream cascades. Anthocyanin is a potent antioxidant which has been evidenced to be able to counteract oxidative damages, scavenge surplus ROS, and rectify abnormities in the apoptotic cascade. Taken together with its ability to attenuate inflammation which also contributes to the etiology of RP, it is reasonable to hypothesize that the anthocyanin could act as a novel therapeutic strategy to retard or prevent cone degeneration in RP retinas, particularly if the treatment is timed appropriately and delivered efficiently. Future pharmacological investigations will identify the anthocyanin as an effective candidate for PR therapy and refinements of that knowledge would ignite the hope of restoring the visual function in RP patients.**

• **KEYWORDS:** retinitis pigmentosa; reactive oxidative species; apoptosis; cone photoreceptor; anthocyanin
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INTRODUCTION

Retinitis pigmentosa (RP) is a group of heterogeneous inherited neurodegenerative diseases that is characterized by the death of rod photoreceptors, followed by progressive loss of cones and eventually total blindness [1-2]. The genetic heterogeneity among the diseases that constitute RP is problematic for the therapeutic strategies to deal with the primary genetic defects: more than 160 different mutations of rod-related genes which encode proteins with remarkably diverse functions can result in photoreceptors apoptosis [3]. Currently, why various rod-related mutations lose their specificity and lead to the secondary cone death is a hot topic. The death of cones following rods loss causes gradual constriction of visual fields and eventually total blindness. This deterioration seems inevitable as it appears that the cones depend upon the rods for survival. The secondary loss of the cones is really thorny: if these cones could be preserved, RP patients would function very well in bright circumstance and carry on relatively normal lives despite the primary rods loss[4-8]. Therefore, researchers have endeavored to explore the underlying mechanism of the secondary cone cell death in RP and to develop therapeutic measures for the innocent cones. Four pathological hypotheses are prevailing: 1) when rods die they release toxic agents to kill the cones; 2) microglia cells secrete toxic substances after they migrated to the outer nuclear layer; 3) the provision of neurotrophic factors which are essential for cone survival has been interrupted as rods die; 4) hyperoxia introduces oxidative damage to the cones in the absence of numerous rods[9-13].

Significant Role of Reactive Oxidative Species in the Cone Death in Retinitis Pigmentosa The last theory is currently enjoying a substantial popularity and is considered to have potentials to be explored for therapeutic use. Since

rods are most metabolically active in the retina, the oxygen level in the outer retina would significantly increase as rods die [9,12]. This increase goes largely uncompensated because the poor auto-regulation of the choriocapillaries in response to the alterations in tissue oxygen levels. The resulted hyperoxia is toxic as it has been verified that high level of oxygen tension (above 75%) triggers off photoreceptors degeneration[8,12]. Moreover, several studies on the hereditary RP animal models have pointed out that the rods-deletion induced oxidative stress contributes to the progressive cones death regardless of the primary mutation gene: oxidative damage to the mitochondria can result in membrane depolarization, which plays a central role in the apoptotic process and has been has been ascribed as a forward feeding loop for the activation of the downstream cascades[7,14-17]. The oxidative stress also excessively elevates poly adp-ribose polymerase (PARP) activity and triggers off cone apoptosis *via* its interaction with the transcription factors such as nuclear factor-κB (NF-κB), activator protein-1 (AP-1)[18-19]. Furthermore, abundant reactive oxidative species (ROS) generated by the mitochondria could enhance cone apoptosis *via* the up-regulation of Bax, the down-modulation of Bcl-2 in the RP retinas [19-22]. Additionally, a novel study has found that the peroxynitrite generated from ROS and nitric oxide (NO) exacerbates oxidative damage and contributes to the cone death in RP. During the amplification, NADPH oxidases (Nox) serve as critical intermediaries[23]. Therefore, the surplus ROS should be neutralized by antioxidant defense system, otherwise it will interact with the macromolecules including unsaturated lipids, proteins, deoxyribonucleic acid (DNA) and iron, which are critical for cone survival.

Benefits of Antioxidants for the Cones in Retinitis Pigmentosa These metabolic findings cast insights into the etiology of cone loss in RP retinas with the potential of opening up new therapeutic avenues. Series of therapeutic trials against the oxidative damage induced cone degeneration are built on this pathological theory and proven to be beneficial in RP models [24-26]. Administration of cocktails of antioxidants to RP retinas could reduce markers of oxidative damage and cone death to some extent, despite of tremendous variability in the inciting mutations and the rapidity of photoreceptor degeneration. Exogenous antioxidants such as α-tocopherol, stanniocalcin-1,α-ascorbic acid, and lipoic acid can reduce the cone demise in RP animal models. Human clinical trials of long term supplementations of traditional antioxidants such as vitamins A and E, β-carotene, zinc and docosahexaenoic acid have been conducted for RP patients with some beneficial effects noted, especially on the periphery visual function [27-29]. Moreover, it has been found that polymorphisms which result in differences in the activity of antioxidant enzymes and differences in dietary intake of antioxidants could also contribute to the variability in the progression of the visual field loss in RP patients.

Anti –apoptosis Treatment for Retinitis Pigmentosa Given the complex genetic characteristics underlying RP's pathology, the attempt to overcome each individual mutation is confronted with overwhelming challenges. However targeting apoptosis, which represents a highly controlled, final common pathway to photoreceptor cell death of all RP forms, could provide a more practical approach [16,30]. In classic apoptosis pathway, the caspase family is considered as the key executioner of the photoreceptor apoptotic program. Although caspase activity can be sufficient for apoptotic cell death, it is not always necessary. It has been shown that the caspase-3 inhibitor or caspase-3 deletion delayed the onset of RP, but was unable to block the photoreceptor cell death entirely [31]. Even several broad spectrum caspase inhibitors such as z-VAD-fmk, DEVD-CHO and BD-fmk, failed to alter the characteristics or kinetics of apoptosis and to rescue the photoreceptors of RP retinas [20-21]. The caspase-independent apoptosis was described in the photoreceptor degeneration of several RP animal models such as the RCS rat and rd1 mouse: ROS and calpain could also contribute significantly to the apoptotic program without the activation of caspase -9, -8, -7, -3 and -1 which played pivotal roles in the classic caspase-dependant pathway [32-33]. Given the high sensitivity of caspases to redox state alterations, this inactivation of caspase during RP apoptosis might be caused by oxidative modification, most likely at the thiol group of the active sites in these enzymes [34]. Increased ROS generation after rod death prevents the activation of caspases but allows the activation of a caspase-independent apoptotic pathway. Therefore, the cone death of RP models will always be the combined result of multiple apoptotic pathways run in parallel in a complex networks [35]. The cone survival in RP mutants will not only require the inhibition of effectors of cell death machinery, but also of the initiating upstream signals such as ROS. This notion is further supported by the ability of antioxidants to suppress the cone apoptosis in RP retinas, and establishes a role for ROS as mediators of cone apoptosis. The existence of a common cell death mediator and mechanism triggered off by different gene defects may provide a mutation independent therapeutic target which could be generalized to all (or most) RP phenotypes.

Hypothesis When oxidative stress accumulates in the RP retinas and protection by endogenous antioxidants is insufficient for maintaining retinal homeostasis or optimal visual function, it is necessary and reasonable to supply exogenous antioxidants to protect the cones from oxidative

injury and suppress the progression of RP. Anthocyanin is presumed to have the potential to counteract oxidative damages, rectify abnormities in apoptotic cascade, attenuate inflammation response, and act as a natural agent to retard or prevent cone degeneration in RP retinas. Intravenous administration of anthocyanin would be an efficient method to deliver the therapeutic factor to the target retinal tissue. So it is a logical step to test anthocyanin for therapeutic use in various RP animal models, and ultimately in patients.

Anthocyanin can Alleviate Oxidative Stress and Suppress the Reactive Oxidative Species Induced Retinal Apoptosis Anthocyanin is a natural antioxidant widely distributed in various fruits, plants and vegetables. Epidemiological studies report that this water-soluble flavonoid shows protective potency against a variety of pathologies, including cardiovascular diseases, cancer, diabetes mellitus, neurodegeneration, and inflammation[36-37]. Particularly in the retina, anecdotal researchers find that anthocyanin can alleviate symptoms of scotopia and improve the microcirculation of retina. It acts as a stimulator of the rhodopsin resynthesis and plays an important role in the visual signal transduction [38-39]. Recently, the anthocyanin is recognized as a cytoprotective compound because it can suppress the light-induced photoreceptor damage by scavenging ROS both *in vivo* and *in vitro*[40-41]. Furthermore, anthocyanin exerts neuroprotective effects on the retinal ganglion cells and RPE cells at least partly *via* the anti-oxidation mechanism [42-43]. Several instructive studies find that anthocyanin overcomes the photoreceptor apoptosis in the MNU-induced RP animal model, and improves both the photopic and scotopic function[44-45]. More direct evidence shows that anthocyanin can inhibit the AP-1 activation, an important mediator for photoreceptor apoptosis, by preventing p38 phosphorylation[46].

Anthocyanin ranks among the most potent antioxidants: the hydroxyl group on the B ring of anthocyanin can donate hydrogen to free radicals such as (O_2^-), (OH), (H_2O_2), and thus entail this compound tremendous antioxidant potency[47]. Previous study reported that the (O_2^-) scavenging activity of purple rice extracts (anthocyanin as its main antioxidant constituent) was 10-25 times stronger than that of the Trolox (vitamin E analogue) [48]. Besides this direct scavenging ability, anthocyanin can increase the oxygen-radical defensive capacity of cells, stimulate the expression of Phase-II detoxification enzymes, reduce the formation of oxidative adducts in DNA, decrease lipid peroxidation, and inhibit mutagenesis [49]. As formerly mentioned, Peroxynitrite generated from ROS and nitric oxide (NO) amplifies the oxidative damage in RP retinas. *In vitro* studies find anthocyanin efficiently defend the primarily cultured bovine aortic endothelial cells against peroxynitrite mediated apoptosis by counteracting mitochondrial membrane depolarization [50-51]. Furthermore, an *in vivo* study suggests that anthocyanin exerts neuroprotective effect against the N-methyl-D-aspartic acid (NMDA)-induced retinal damage by suppressing the intracellular elevation of peroxynitrite[43].

Possibility on Attenuation of the Inflammation Response of Retinitis Pigmentosa by Anthocyanin Recently, chronic inflammation is considered to be an etiologic factor of RP, although, it is still unclear whether the inflammation is a central or minor contributor to the RP pathogenesis[52-53]. Retinal (probably photoreceptor) autoantibodies can be found in the blood samples of RP patients [54]. It has been shown that vitreous samples from RP patients contain many immune system cells such as lymphocytes [55]. More detailed studies have detected the significant elevated levels of inflammatory cells and inflammatory factors in the aqueous and vitreous humor from RP patients, including multiple cytokines and chemokines such as IL-1, IL-2, IL-8 and TNF-α [52]. More recent studies have highlighted the activation of microglia in RP retina preceding photoreceptor cell death. The highly toxic and inflammatory phenotype microglia, which is designated as the "hyperactive state", can release a variety of highly inflammatory cytokines. Preclinical and clinical trials which target at the activated microglia in the outer retina have suggested some benefits [56-57]. This in turn points to the importance of monitoring inflammation and treating RP patients for stealth infections.

Athocyanin has exhibited anti-inflammatory effects in multiple cell types through its ability to inhibit the expression of COX-2, COX-1, NF-κB and various interleukins, both *in vitro* and *in vivo* [58-59]. Anthocyanin can also reduce the expression of glial fibrillary acidic protein (GFAP), a well-known marker that used for evaluating neuroinflammatory responses in the retina [44]. Given the potential correlation of RP with inflammation, a variety of anti-inflammatory actions of anthocyanin could justify its promising clinical use in RP treatment.

Issues That Need to be Addressed The bioavailability of the anthocyanin to retina is a pharmacological issue yet to be thoroughly addressed. Effective delivery of the therapeutic factors to the target retinal tissue is a formidable task. Repeated intraocular or intravitreal injections are problematic for RP patients due to the delicacy of eye structures and the propensity for cataract formation. On the other hand, pharmacokinetic data indicates that the absorption of anthocyanin is relatively poor in human: less than 1% after oral administration was absorbed [60]. The concentration of anthocyanin in plasma after oral supplements is far below the level required to exhibit cytoprotective effects *in vitro* [49,61]. However, after absorbed into the plasma, the anthocyanin can readily cross the

mammalian blood-retinal barrier and distribute in ocular tissues as intact forms. Especially in rats the concentrations of total anthocyanin in the ocular tissues are even higher than those measured in plasma [62-63]. Therefore, efficient delivery method aimed at enhancing the blood level of anthocyanin should be necessary for the optimal use in RP treatment. The intravenous administration might be a particularly appealing approach for anthocyanin delivery.

We are not that optimistic a single agent such as anthocyanin is potent enough to produce ideal therapeutic effects. It is likely that benefits by reducing oxidative damage to cones and simultaneously increasing the threshold for apoptosis with neurotrophic factors can be synergistic. Anthocyanin can also be utilized as supplements of gene therapy until the oxygen status of outer retina recovers to normal. However, it is essential to keep in mind of possible unexpected effects. Anthocyanin can reduce or increase the bioavailability of co-administered drugs, causing alleviated therapeutic effects or increased side effects [64]. The modulation of certain drug-metabolizing enzymes and transporters by anthocyanins can affect the fate of co-administered drugs and thus exert pharmacological risks [65]. Therefore, the possible pharmacological and toxicological consequences of the combined treatment should be considered.

Pharmacological safety issues need to be addressed before further drug development. Anthocyanin must be present at the right time, correct cellular compartment, and appropriate concentrations to RP models or patients. An important feature of anthocyanin contributing to its wide use in the oxidative stress related disease is the broad spectrum safety [66-67]. Standardized mixtures of anthocyanin fit for cancer chemoprevention and treatment are commercially available. Chronic administration of a prophylactic dose of anthocyanin for various cancers shows no observable adverse effect. The safety of chronic use of anthocyanin is of great importance because the slow progression nature of RP would require long-term administration. However, we can't extrapolate a therapeutic dose of anthocyanin without good evidence of their effectiveness and safety. Potential harms of the high-dose antioxidant supplementation for RP should be evaluated by a randomized, controlled, double-masked clinical trial.

DISCUSSION

Gene therapy for RP remains challenging due to the tremendous genetic heterogeneity and difficulties in accurate genetic characterization. Targeting the patho-physiological process which is common to all the mutation phenotypes (*e.g.* apoptosis, oxidative damages) could serve as a more promising and general alternative for RP treatment. Anthocyanin is a potential drug for RP due to the evidences of its anti-apoptosis and anti oxidation property. It is our hope that the herein presented evidence from numerous independent investigations could provide the impetus for a more extensive evaluation of this treatment for retarding the cone degeneration in RP. Future animal and human studies are warranted to validate this preliminary hypothesis before applying anthocyanin in clinical practice. Elucidation of ROS induced apoptosis pathways by which anthocyanin exerts its protective actions against the photoreceptor degeneration would cast more insights into its potential use as a cone-rescue agent.

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