



# Beneficial Effect of Antioxidants in Retinopathies: A New Hypothesis

Isabella Panfoli, PhD

Department of Pharmacy-DIFAR, Biochemistry Lab. University of Genoa, Genoa, Italy

## ABSTRACT

The retina is the most oxygen consuming tissue of the body. Rod and cone photoreceptors efficiently carry out visual cascades, which are energetically costly processes. Data has recently been published that suggests that the metabolic support to phototransduction in the rod outer segment (OS) may originate directly in the OS, which is able to conduct aerobic metabolism. This oxygen-handling activity of the rod OS, which was never suspected before, appears to be a primary cause of the generation of reactive oxygen species directly inside the OS. Oxidative stress has been hypothesised to contribute to most of the neurodegenerative retinal pathologies, such as diabetic retinopathy, age-related macular degeneration, retinitis pigmentosa and photoreceptor cell death after retinal detachment. Many natural antioxidant compounds are routinely used in experimental or human therapies for preventing or delaying photoreceptor degeneration in those pathologies. Here it is proposed that the ultimate reason for the beneficial actions of antioxidants in preventing or retarding the effect on the retinal degenerative pathologies can be found in their action on reactive oxygen species generated by the ectopic mitochondrial electron transport chain (ETC) coupled to FoF1-ATP synthase in rod OS disks. In fact, if not adequately coupled, the ETC generates reactive oxygen species that, in turn, can act on the polyunsaturated fatty acids which the rod OS is rich in. If correct, the mechanism put forward here would provide a potential for the molecular basis of therapies with antioxidants for retinal degenerative diseases.

## KEY WORDS

Antioxidants; Retinopathy; Oxidative stress

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## Correspondence to:

Professor Isabella Panfoli, DIFAR-Biochemistry Lab., University of Genoa, School of Medical and Pharmaceutical Sciences, V.le Benedetto XV, 3, 16132 GENOVA, Phone + 39 010 353 7397, Fax + 39 010 353 8153, Email: [Isabella.Panfoli@unige.it](mailto:Isabella.Panfoli@unige.it)

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## INTRODUCTION

The vertebrate retina contains two types of photoreceptors, rods and cones, which carry out the first step of vision. Both possess a specialised compartment: the outer segment (OS) that is dedicated to phototransduction, and the inner segment (IS), which contains the subcellular organelles and nucleus. The retina is particularly susceptible to oxidative stress because of its high O<sub>2</sub> consumption [1], its high proportion of polyunsaturated fatty acids [2, 3], and its exposure to visible light. Considering the limited understanding of the origin of the

ATP supply in OS [4-6], where continual ATP consumption is observed particularly during light adaptation, a high cGMP flux rate is observed [7] (i.e. a higher steady PDE activity). In addition, there is a correspondingly higher activity of guanylate cyclase, the enzyme that synthesises cyclic GMP from GTP. Moreover, it was shown that visual transduction is supported by oxidative metabolism [8], and that anaerobic glycolysis is not sufficient to provide enough ATP in the light. This group has conducted proteomic analysis of purified OS disks and reported [9] that these express most of the subunits of the mitochondrial machinery for oxidative phosphorylation, such as Krebs' cycle enzymes and electron transport chain (ETC) proteins, as well as



FoF1-ATP synthase. The ETC complexes are composed of NADH dehydrogenase (ETC I), succinate dehydrogenase (ETC II), ubiquinol-cytochrome c oxidoreductase (ETC III), cytochrome c, cytochrome c oxidase (ETC IV) and F1Fo-ATP synthase (V), performing the oxidative phosphorylation that is currently believed to be exclusive to the mitochondrial membrane in eukaryotes. Rod disks possess active respiratory complexes that may build up a proton gradient also in vivo, which the ectopic ATP synthase can utilise to aerobically synthesise ATP outside the mitochondrion. The OS would supply chemical energy for phototransduction through extra-mitochondrial aerobic ATP synthesis [4, 9-11]. Similar results were obtained on intact myelin vesicles [12-14] and C6 glioma cell plasma membranes [15]. The activity of the Tricarboxylic Acid (TCA) cycle enzymes was also reported to be consistent in the rod OS [11], which is in keeping with the knowledge that many mitochondrial proteins possess dual or multiple localisations, [16] and that mitochondria are dynamic organelles [17]. While the mitochondrial proteome consists of more than 1,000 different proteins, many proteomic analyses of cellular membranes have found the exclusive expression of proteins from the five respiratory complexes [18]. Therefore, a large proportion of the high retinal rate of O<sub>2</sub> consumption would depend on the rod OS, justifying the phenomenon of rod-induced hypoxia on a quantitative basis [6]. Taking into consideration the existence of O<sub>2</sub> consumption in the rod OS, a hypothesis for the involvement of oxidative stress in the pathogenic mechanism for many acquired and inherited retinal degenerations has been recently proposed [6]. Moreover, a decline in the rod OS ETC functioning, along with an increase in reactive oxygen species production and the consequent chronic oxidative stress, would generate hypometabolism, in turn causing an imbalance in the clearance of proteins. This may cause the aggregation of peptides and the generation of drusen [6]. The ETC embedded in the disk membranes would be a primary source of superoxides [19]. Reactive oxygen species are in fact by-products of ETC functioning, and their overproduction is foreseen in any pathological condition that uncouples ATP synthase from the ETC.

Oxidative stress has been implicated in the development of diabetic retinopathy, and has also been shown to be a risk factor for age-related macular degeneration (AMD) [20- and retinitis pigmentosa (RP) [24-26], which are the most common degenerative diseases of the retina. It was also shown to play a fundamental role in photoreceptor cell death after retinal detachment (RD) [27]. Moreover, extensive research suggests that, in those retinal pathologies, naturally occurring compounds with antioxidant actions, that are components of a normal diet, are greatly beneficial. Several studies showed that antioxidants appeared to retard or inhibit the degenerative

pathology [28-30]. Interestingly, curcumin, anthocyanins and catechins are inhibitors of ATP synthase [31].

## HYPOTHESES

Considering the aforementioned facts, the present paper hypothesises that the reason for the efficacy of antioxidants in preventing or retarding the onset of many retinal degenerative pathologies in both experimental and clinical studies is a result of their scavenging action on reactive oxygen species produced inside the OS. These species can be generated by the ectopically-expressed mitochondrial ETC that is coupled to ATP synthase in rod OS disks. In fact, it is quite doubtful that antioxidants can scavenge reactive oxygen species inside the mitochondrion, as they have been found to be unable to penetrate mitochondria [31].

## DISCUSSION

Oxidative stress is also implicated in the development of diabetic complications, in particular diabetic retinopathy [32]. Moreover, the antioxidant mechanisms are impaired in diabetic conditions [33-35]. The administration of antioxidants to diabetic rats prevents oxidative damage in the retina and the development of retinopathy [36]. Lutein prevented the impairment of the electroretinogram observed in controls [37]. The antioxidant lutein, a dietary carotenoid, was also shown to be useful in AMD [28]. The Eye Disease Case-Control Study found that high plasma levels of lutein and zeaxanthin are associated with a reduced risk of neovascular AMD [38, 39].

Oxidative stress plays an important role in photoreceptor cell death after RD. Treatment with a reactive oxygen species scavenger (e.g. Edaravone) was shown to prevent photoreceptor cell death after RD [27]. The model of oxidative stress in photoreceptor cell death after RD is interesting as it rules out a role for retinal pigmented epithelium. Oxidative stress may also act as a mediator of retinal degeneration in RP, and is, in fact, believed to play an important role in photoreceptor cell death [40, 41]. Increased lutein dietary intake increases macular pigment optical density, improving visual function [42]. Anthocyanins are known to be effective ingredients for maintaining eye health, as they are potent antioxidants that have been shown to exert a role against lipid peroxidation [43] and to protect against retinal damage [44, 45]. Extensive research suggests that pro-anthocyanins are beneficial to health in general, especially in improving vision, because of their antioxidant effects [46]. Anthocyanin-rich bilberry extract prevented the impairment of photoreceptor cell function, as measured by electroretinogram, in a mouse model of endotoxin-induced uveitis [47]. Catechins, and EGCG in particular, act as biological antioxidants [48], which are able to scavenge superoxide, hydroxyl radicals, singlet oxygen and peroxynitrite [46, 48]. Curcumin (1,7-bis(4-hydroxy-3-



methoxyphenyl)1,6-heptadiene-3,5-dione), which is a principal component of turmeric, is a promising candidate for the rescue of retinal ischemic diseases [31, 49]. Curcumin is well known for its anti-tumour, antioxidant, and anti-inflammatory properties. Its anti-protein aggregating activity was examined in particular studies on rats expressing the P23H rhodopsin (Rh) mutation, and it rescued photoreceptors from degeneration due to the toxicity of Rh aggregates [49].

Oxidative stress, i.e. the cellular damage caused by reactive oxygen species (free radicals, superoxide, hydrogen peroxide, and singlet oxygen), has been hypothesised to contribute to the development of AMD [23, 39], and diabetic retinopathy [28, 50], which are among the most common cause of blindness in the United States. The retina is considered to be particularly susceptible to oxidative stress, and the contribution of the rod OS should be considered [6] because of its unexpected O<sub>2</sub> consumption ability [1], and the high proportion of polyunsaturated fatty acids in the disk membranes [2], which are highly enriched in docosahexaenoic acid (DHA). DHA is prone to peroxidation, one of the major events induced by oxidative stress, particularly in polyunsaturated fatty acid-rich biomembranes. The photoreceptor OS contain the highest concentration of polyunsaturated fatty acids of any vertebrate tissue, and, in fact, is considered a model for the study of lipid peroxidation [3]. Furthermore, there is strong evidence suggesting that lipofuscin, which is produced during oxidative damage and is a photo-reactive substance, is found in the photoreceptor OS as a consequence of oxidative stress [51]. It was also shown that complex I in particular is the major complex responsible for the generation of reactive oxygen species [19]. In turn, disk membrane lipid peroxidation would be associated with impairment of the protein functions therein located, in particular the ETC, which are extremely sensitive to the state of the membrane environment. Moreover, the disruption of cytochromes might free iron, thereby generating dangerous redox reactions.

## CONCLUSION

The extensive literature data on the protective role of antioxidants utilised in many pharmaceutical preparations for humans, on photoreceptor degeneration, as well as several reports of a pivotal role of oxidative stress in the pathogenesis of degenerating retinal diseases, is suggestive of a role of oxygen radicals on the OS itself. This appears easier to understand when considering the expression of a functional ETC in OS disks, which is an efficient but dangerous location for O<sub>2</sub> utilisation, especially considering the presence of polyunsaturated fatty acids.

## DISCLOSURE

The authors report no conflicts of interest in this work.

## REFERENCES

1. Yu DY, Cringle SJ. Retinal degeneration and local oxygen metabolism. *Exp Eye Res.* 2005 Jun;80(6):745-51. PMID: 15939030.
2. Lamba OP, Borchman D, O'Brien PJ. Fourier transform infrared study of the rod outer segment disk and plasma membranes of vertebrate retina. *Biochemistry.* 1994 Feb 22;33(7):1704-12. PMID: 8110772.
3. Catalá A. An overview of lipid peroxidation with emphasis in outer segments of photoreceptors and the chemiluminescence assay. *Int J Biochem Cell Biol.* 2006;38(9):1482-95. PMID: 16621670.
4. Panfoli I, Calzia D, Bianchini P, Ravera S, Diaspro A, Candiano G, Bachi A, Monticone M, Aluigi MG, Barabino S, Calabria G, Rolando M, Tacchetti C, Morelli A, Pepe IM. Evidence for aerobic metabolism in retinal rod outer segment disks. *Int J Biochem Cell Biol.* 2009 Dec;41(12):2555-65. PMID: 19715769.
5. Pepe IM. Recent advances in our understanding of rhodopsin and phototransduction. *Prog Retin Eye Res.* 2001 Nov;20(6):733-59. PMID: 11587916.
6. Panfoli I, Calzia D, Ravera S, Morelli AM, Traverso CE. Extra-mitochondrial aerobic metabolism in retinal rod outer segments: new perspectives in retinopathies. *Med Hypotheses.* 2012 Apr;78(4):423-7. PMID: 22284635.
7. Pugh EN Jr, Nikonov S, Lamb TD. Molecular mechanisms of vertebrate photoreceptor light adaptation. *Curr Opin Neurobiol.* 1999 Aug;9(4):410-8. PMID: 10448166.
8. Ames A 3rd, Li YY, Heher EC, Kimble CR. Energy metabolism of rabbit retina as related to function: high cost of Na<sup>+</sup> transport. *J Neurosci.* 1992 Mar;12(3):840-53. PMID: 1312136.
9. Panfoli I, Musante L, Bachi A, Ravera S, Calzia D, Cattaneo A, Bruschi M, Bianchini P, Diaspro A, Morelli A, Pepe IM, Tacchetti C, Candiano G. Proteomic analysis of the retinal rod outer segment disks. *J Proteome Res.* 2008 Jul;7(7):2654-69. PMID: 18489131.
10. Smith HG Jr, Litman BJ. Preparation of osmotically intact rod outer segment disks by Ficoll flotation. *Methods Enzymol.* 1982;81:57-61. PMID: 7047994.
11. Panfoli I, Calzia D, Ravera S, Bruschi M, Tacchetti C, Candiano S, Morelli A, Candiano G. Extramitochondrial tricarboxylic acid cycle in retinal rod outer segments. *Biochimie.* 2011 Sep;93(9):1565-75. PMID: 21683117.
12. Ravera S, Panfoli I, Calzia D, Aluigi MG, Bianchini P, Diaspro A, Mancardi G, Morelli A. Evidence for aerobic ATP synthesis in isolated myelin vesicles. *Int J Biochem Cell Biol.* 2009 Jul;41(7):1581-91. PMID: 19401152.
13. Morelli A, Ravera S, Panfoli I. Hypothesis of an energetic function for myelin. *Cell Biochem Biophys.* 2011 Sep;61(1):179-87. PMID: 21455684.
14. Morelli A, Ravera S, Calzia D, Panfoli I. Impairment of heme synthesis in myelin as potential trigger of multiple sclerosis. *Med Hypotheses.* 2012 Jun;78(6):707-10. PMID: 22398388.
15. Ravera S, Aluigi MG, Calzia D, Ramoino P, Morelli A, Panfoli I. Evidence for ectopic aerobic ATP production on C6 glioma cell plasma membrane. *Cell Mol Neurobiol.* 2011 Mar;31(2):313-21. PMID: 21082238.
16. Gregersen N, Hansen J, Palmfeldt J. Mitochondrial proteomics—a tool for the study of metabolic disorders. *J Inher Metab Dis.* 2012 Jul;35(4):715-26. PMID: 22526845.
17. McBride HM, Neuspiel M, Wasiak S. Mitochondria: more than just a powerhouse. *Curr Biol.* 2006 Jul 25;16(14):R551-60. PMID: 16860735.



18. Panfili I, Ravera S, Bruschi M, Candiano G, Morelli A. Proteomics unravels the exportability of mitochondrial respiratory chains. *Expert Rev Proteomics*. 2011 Apr;8(2):231-9. PubMed PMID: 21501016.
19. Genova ML, Baracca A, Biondi A, Casalena G, Faccioli M, Falasca AI, Formiggini G, Sgarbi G, Solaini G, Lenaz G. Is supercomplex organization of the respiratory chain required for optimal electron transfer activity? *Biochim Biophys Acta*. 2008 Jul-Aug;1777(7-8):740-6. PMID: 18454935.
20. Rattner A, Nathans J. Macular degeneration: recent advances and therapeutic opportunities. *Nat Rev Neurosci*. 2006 Nov;7(11):860-72. PMID: 17033682.
21. Jin H, Randazzo J, Zhang P, Kador PF. Multifunctional antioxidants for the treatment of age-related diseases. *J Med Chem*. 2010 Feb 11;53(3):1117-27. PMID: 20078105.
22. Brennan LA, Kantorow M. Mitochondrial function and redox control in the aging eye: role of MsrA and other repair systems in cataract and macular degenerations. *Exp Eye Res*. 2009 Feb;88(2):195-203. PMID: 18588875.
23. Hollyfield JG, Bonilha VL, Rayborn ME, Yang X, Shadrach KG, Lu L, Ufret RL, Salomon RG, Perez VL. Oxidative damage-induced inflammation initiates age-related macular degeneration. *Nat Med*. 2008 Feb;14(2):194-8. PMID: 18223656.
24. Shintani K, Shechtman DL, Gurwood AS. Review and update: current treatment trends for patients with retinitis pigmentosa. *Optometry*. 2009 Jul;80(7):384-401. PMID: 19545852.
25. Eckmiller MS. Energy depletion hypothesis for retinitis pigmentosa. *Adv Exp Med Biol*. 2003;533:277-85. PMID: 15180274.
26. Berson EL. Retinitis pigmentosa: unfolding its mystery. *Proc Natl Acad Sci U S A*. 1996 May 14;93(10):4526-8. PMID: 8643437.
27. Roh MI, Murakami Y, Thanos A, Vavvas DG, Miller JW, Edaravone, an ROS scavenger, ameliorates photoreceptor cell death after experimental retinal detachment. *Invest Ophthalmol Vis Sci*. 2011 Jun 1;52(6):3825-31. PMID: 21310909.
28. Zhao L, Sweet BV. Lutein and zeaxanthin for macular degeneration. *Am J Health Syst Pharm*. 2008 Jul 1;65(13):1232-8. PMID:18574012.
29. Ranchon I, Gorrand JM, Cluzel J, Droy-Lefaix MT, Doly M. Functional protection of photoreceptors from light-induced damage by dimethylthiourea and Ginkgo biloba extract. *Invest Ophthalmol Vis Sci*. 1999 May;40(6):1191-9. PMID: 10235553.
30. Lam S, Tso MO, Gurne DH. Amelioration of retinal photic injury in albino rats by dimethylthiourea. *Arch Ophthalmol*. 1990 Dec;108(12):1751-7. PMID: 2124103.
31. Zheng J, Ramirez VD. Inhibition of mitochondrial proton F0F1-ATPase/ATP synthase by polyphenolic phytochemicals. *Br J Pharmacol*. 2000 Jul;130(5):1115-23. PMID: 10882397.
32. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diabetes*. 1991 Apr;40(4):405-12. PMID: 2010041.
33. Kowluru RA, Tang J, Kern TS. Abnormalities of retinal metabolism in diabetes and experimental galactosemia. VII. Effect of long-term administration of antioxidants on the development of retinopathy. *Diabetes*. 2001 Aug;50(8):1938-42. PMID: 11473058.
34. Kowluru RA, Abbas SN. Diabetes-induced mitochondrial dysfunction in the retina. *Invest Ophthalmol Vis Sci*. 2003 Dec;44(12):5327-34. PMID: 14638734.
35. Kowluru RA, Koppolu P. Diabetes-induced activation of caspase-3 in retina: effect of antioxidant therapy. *Free Radic Res*. 2002 Sep;36(9):993-9. PMID: 12448825.
36. Feldman EL. Oxidative stress and diabetic neuropathy: a new understanding of an old problem. *J Clin Invest*. 2003 Feb;111(4):431-3. PMID: 12588877.
37. Muriach M, Bosch-Morell F, Alexander G, Blomhoff R, Barcia J, Arnal E, Almansa I, Romero FJ, Miranda M. Lutein effect on retina and hippocampus of diabetic mice. *Free Radic Biol Med*. 2006 Sep 15;41(6):979-84. PMID: 16934681.
38. Beatty S, Boulton M, Henson D, Koh HH, Murray IJ. Macular pigment and age related macular degeneration. *Br J Ophthalmol*. 1999 Jul;83(7):867-77. PMID: 10381676.
39. Beatty S, Koh H, Phil M, Henson D, Boulton M. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol*. 2000 Sep-Oct;45(2):115-34. PMID: 11033038.
40. Baumgartner WA. Etiology, pathogenesis, and experimental treatment of retinitis pigmentosa. *Med Hypotheses*. 2000 May;54(5):814-24. PMID: 10859693.
41. Krishnamoorthy RR, Crawford MJ, Chaturvedi MM, Jain SK, Aggarwal BB, Al-Ubaidi MR, Agarwal N. Photo-oxidative stress down-modulates the activity of nuclear factor-kappaB via involvement of caspase-1, leading to apoptosis of photoreceptor cells. *J Biol Chem*. 1999 Feb 5;274(6):3734-43. PMID: 9920926.
42. Sasaki M, Ozawa Y, Kurihara T, Noda K, Imamura Y, Kobayashi S, Ishida S, Tsubota K. Neuroprotective effect of an antioxidant, lutein, during retinal inflammation. *Invest Ophthalmol Vis Sci*. 2009 Mar;50(3):1433-9. PMID: 18997089.
43. Narayan MS, Naidu KA, Ravishankar GA, Srinivas L, Venkataraman LV. Antioxidant effect of anthocyanin on enzymatic and non-enzymatic lipid peroxidation. *Prostaglandins Leukot Essent Fatty Acids*. 1999 Jan;60(1):1-4. PMID: 10319911.
44. Tanaka J, Nakamura S, Tsuruma K, Shimazawa M, Shimoda H, Hara H. Purple rice (*Oryza sativa* L.) extract and its constituents inhibit VEGF-induced angiogenesis. *Phytother Res*. 2012 Feb;26(2):214-22. PMID: 21633982.
45. Tanaka J, Nakanishi T, Ogawa K, Tsuruma K, Shimazawa M, Shimoda H, Hara H. Purple rice extract and anthocyanidins of the constituents protect against light-induced retinal damage in vitro and in vivo. *J Agric Food Chem*. 2011 Jan 26;59(2):528-36. PMID: 21175193.
46. Shi J, Yu J, Pohorly JE, Kakuda Y. Polyphenolics in grape seeds-biochemistry and functionality. *J Med Food*. 2003 Winter;6(4):291-9. PMID: 14977436.
47. Miyake S, Takahashi N, Sasaki M, Kobayashi S, Tsubota K, Ozawa Y. Vision preservation during retinal inflammation by anthocyanin-rich bilberry extract: cellular and molecular mechanism. *Lab Invest*. 2012 Jan;92(1):102-9. PMID: 21894150.
48. Nanjo F, Mori M, Goto K, Hara Y. Radical scavenging activity of tea catechins and their related compounds. *Biosci Biotechnol Biochem*. 1999 Sep;63(9):1621-3. PMID: 10610125.
49. Vasireddy V, Chavali VR, Joseph VT, Kadam R, Lin JH, Jamison JA, Kompella UB, Reddy GB, Ayyagari R. Rescue of photoreceptor degeneration by curcumin in transgenic rats with P23H rhodopsin mutation. *PLoS One*. 2011;6(6):e21193. PMID: 21738619.
50. Kowluru RA, Engerman RL, Case GL, Kern TS. Retinal glutamate in diabetes and effect of antioxidants. *Neurochem Int*. 2001 Apr;38(5):385-90. PMID: 11222918.
51. Finnemann SC, Leung LW, Rodriguez-Boulan E. The lipofuscin component A2E selectively inhibits phagolysosomal degradation of photoreceptor phospholipid by the retinal pigment epithelium. *Proc Natl Acad Sci U S A*. 2002 Mar;99(6):3842-47. PMID: 11904436.