10. Antioxidant therapy in retinitis pigmentosa

María Miranda1, Raquel Alvarez-Nölting1, Araiz J2 and Francisco Javier Romero Gómez1,3

1Universidad CEU-Cardenal Herrera, Departamento de Fisiología, Farmacología y Toxicología. Avda Seminario s/n, Moncada, Valencia, Spain; 2Department of Ophthalmology, University of the Basque Country, Spain; 3Fundación Oftalmológica del Mediterráneo (FOM), Bifurcación Pio Baroja-General Avilés s/n 46015 Valencia, Spain

Abstract. Increasing evidence suggests that oxidative stress contributes to the pathogenesis of many neurodegenerative disorders, including retinitis pigmentosa (RP), age related macular degeneration (AMD), glaucoma, diabetic retinopathy, and light damage. RP refers to a group of diseases in which a mutation results in death of rod photoreceptors followed by gradual death of cones. In this review we focus on the importance of oxidative stress alterations in animal models of RP and the possibility of using antioxidants as a new strategy to delay photoreceptor degeneration. It has been suggested that glutathione alterations can also be observed in humans affected with photoreceptors dystrophy. In addition, microarray experiments have shown that in the rd1 retina, genes for products related to protection against oxidative stress are up-regulated when compared to wild type mice. Several studies have shown that the use of antioxidants, in vitro
and in vivo delayed the degeneration process significantly. Among the antioxidants used in these studies are: zeaxanthin, lutein, α-lipoic acid and glutathione, (α-tocopherol, ascorbic acid, Mn(III) tetrakis (4-benzoic acid) porphyrin, docosahexaenoic acid and melatonin. Unless the common upstream initiator for a photoreceptor dystrophy is found, multiple rescue paradigms need to be used to target all active pathways. Furthermore, as the mammalian antioxidant defence system is a complex network and comprise several enzymatic and non-enzymatic entities, it appeared reasonable to combine different antioxidants instead of using them individually.

1. Retinitis pigmentosa and its relation with oxidative stress

Retinitis pigmentosa (RP) refers to a group of diseases in which a mutation results in death of rod photoreceptors followed by gradual death of cones. Mutations in 36 different genes have been found to cause RP, and mutations in many more cause widespread rod cell death in association with syndromes that have extraocular manifestations (www.sph.uth.tmc.edu_RetNet_sum-dis.htm). The enormous genetic heterogeneity among the diseases that constitute RP is a problem for the development of treatments that deal with primary genetic defects.

Despite the diversity of retinal degeneration disorders, apoptosis of photoreceptors seems to be a feature common to all [1-3]. The signalling pathways of apoptosis in photoreceptor cell death are still not fully understood. Several studies have demonstrated that the eye is particularly sensitive to oxidative damage. Because of its high oxygen request and content of unsaturated lipid and its constant exposure to light retina may be an elective site for oxygen radical production and lipid peroxidation [4-7].

After the death of rods, cone photoreceptors begin to die. The mechanism of the gradual death of cones is one of the key unsolved mysteries of RP. Another possible explanation for the slowly progressive death of cones after the death of rods is oxidative damage. Rods are more numerous than cones and are metabolically active cells with a high level of oxygen consumption. As death of rods occurs, the level of oxygen in the retina increases [8, 9].

It has also been demonstrated an increased expression of genes involved in cell proliferation pathways and oxidative stress at PN14 (the peak of rod degeneration), at PN35 (early in cone degeneration) and at PN50 (during cone degeneration) [10]. Carnody et al [11] demonstrated an early and sustained increase in intracellular reactive oxygen species accompanied by a rapid depletion of intracellular glutathione in an in vitro model of photoreceptor apoptosis and that these early changes in the cellular redox state precede disruption of mitochondrial transmembrane potential, nuclear
condensation, DNA nicking, and cell shrinkage, all of which are well-characterized events of apoptotic cell death.

Moreover, the utility of an antioxidant, like CR-6, has been proved to block photoreceptor apoptosis in vitro, probably by preventing the activation of a pathway in which calpains have a key role [12].

The rd1/rd1 mouse has an insertion of viral DNA in the β-subunit of the cGMP phosphodiesterase gene [13]. The mutation leads to toxic accumulation of the second messenger cGMP and subsequent abnormally high Ca\(^{2+}\) levels in the rd1 photoreceptors [1, 14]. This leads to an apoptotic-like rod cell death [15, 16], followed by a mutation independent cone death. A mutation in the same gene has been found in human forms of autosomal recessive retinitis pigmentosa making the rd mouse retina an ideal model for experimental analysis of human retinal dystrophies [17]. Moreover, oxidative stress is a common product of ionic imbalance or elevated Ca\(^{2+}\) levels and it has been found to precede calpain and caspase-3 activation in the rd1 mouse retina [18]. All these findings provide targets for future treatment strategies.

2. Glutathione metabolism

Glutathione (gamma-glutamyl-cysteinyl-glycine; GSH) is the most abundant low-molecular-weight thiol, and GSH/glutathione disulfide is the major redox couple in animal cells. It is formed in a two-step enzymatic process including, first, the formation of gamma-glutamylcysteine from glutamate and cysteine, by the activity of the gamma-glutamylcysteine synthetase; and second, the formation of GSH by the activity of GSH synthetase which uses gamma-glutamylcysteine and glycine as substrates. While its synthesis and metabolism occurs intracellularly, its catabolism occurs extracellularly by a series of enzymatic and plasma membrane transport steps [19]. Glutathione is the most important and effective endogenous antioxidant. It is considered as the body’s first line of defense against oxidative stress.

It is a small molecule found in almost every cell. It cannot enter most cells directly and therefore must be made inside the cell, from its three constituent amino acids. Compelling evidence shows that GSH synthesis is regulated primarily by gamma-glutamylcysteine synthetase activity, cysteine availability, and GSH feedback inhibition.

Protein S-glutathionylation, the reversible binding of glutathione to protein thiols (PSH), is involved in protein redox regulation, storage of glutathione, and protection of PSH from irreversible oxidation [20].
Among the roles attributed to GSH are maintenance of protein thiol groups; protection of cells against oxidative or radiation-induced damages; detoxification of highly reactive xenobiotic metabolites or peroxides; and regeneration of antioxidant vitamins. GSH, ascorbic acid, and vitamin E special is that they interact in a series of coupled oxidation-reduction reactions. GSH is capable of reducing dehydroascorbic acid back to AA, which, in turn, reduces oxidized vitamin E. GSH is normally regenerated from the oxidized disulfide (GSSG) by a mechanism involving the NADPH-dependent glutathione reductase. More recent studies of the functions served by GSH in cells include modulation of protein function via thiolation which may control physiological and pathophysiological pathways to include DNA synthesis and repair, protein synthesis, amino acid transport, modulation of glutamate receptors and neurohormonal signaling [21].

Light and oxygen are essential for vision, but paradoxically these elements also trigger the formation of reactive oxygen species (ROS). Because the eye is continuously exposed to ambient light energy, highly efficient retinal defense mechanisms act to protect against photoinduced damage. It is known that GSH is present in the retina and that this tissue also has enzymatic activity associated with GSH metabolism like glutathione peroxidase, glutathione disulfide reductase, and glutathione S-transferase. Furthermore, continuous phagocytosis and degradation of retinal photoreceptor outer segment material by the retinal pigment epithelium (RPE) mitigates lipid peroxidative damage [22].

Despite this, it has been reported no GSH immunoreactivity in outer segments of rod and cone photoreceptors from rodent, primate and zebrafish retinas[23-25], but, Müller cells and inner retinal neurons appear to contain substantial pools of this compound [26].

Each cellular compartment contains different pools of thiol/disulfide couples, such as glutathione (GSH)/glutathione disulfide (GSSG), cysteine (Cys)/cystine (CySS), and dihydrolipoic acid/lipoic acid [27]. The functions of these redox couples are dependent on both the ratio and total concentrations of the reduced and oxidized forms, the inherent electron
donating/accepting characters, and the kinetics of interactions of the components. Changes in the thiol/disulfide redox state alter signal transduction, DNA and RNA synthesis, protein synthesis, enzyme activation, and cell cycle regulation [28, 29].

2.1. Glutathione and nitric oxide

Nitric oxide (NO) has also been implicated in neurodegenerative diseases [30]. NO is generated in the central nervous system by three isoforms of nitric oxide synthase (NOS) located in the endothelial cells, astroglia, and a few neurons [31]. NO plays an important role in cell-to-cell modulation and vasodilatation via activation of NO-sensitive guanylyl cyclase and the generation of cGMP [31]. Since the measurement of the NO radical by itself is difficult because it is a radical with poor stability and with a very short half-life, measurement of the end products of NO as nitrite and nitrate (NO2−/NO3−) is often used as a marker for the production of NO radicals.

Modifications of the cellular redox state of the eye are believed to contribute to the pathogenesis of many diseases and it has been demonstrated that nitric oxide and reactive oxygen species (ROS) are key signalling molecules in driving apoptosis both in vitro and in vivo models of retinal disease [12].

Recently, Komeima et al [32] demonstrated that peroxynitrite-induced nitrosative damage also occurs in the rd1 mouse model of RP, because they found an increase in S-nitrosocysteine and nitrotyrosine protein adducts that are generated by peroxynitrite. They also found that treatment of rd1 mice with a mixture of nitric oxide synthase (NOS) inhibitors markedly reduced S-nitrosocysteine and nitrotyrosine staining and significantly increased cone survival, indicating that NO-derived peroxynitrite contributes to cone cell death.

NO may interact with oxygen, superoxide anion, and thiol compounds, generating reactive nitrogen species (NOx), peroxynitrite, and S-nitrosothiols including S-nitrosothiols (GSNO) [33]. These NO-derived species may produce biological functions either similar or opposite to that of NO. For example, peroxynitrite may cause oxidative stress and possibly neurotoxicity [34]. It has been proposed that GSNO may be an endogenous NO reservoir that can release NO [35] but also protects against oxidative stress in the endothelium, myocardium, brain tissue, and other cells [36]. It is possible then to think that the only decrease or increase in GSH concentration is able to alter the effects of NO. Another study shows that alterations in GSH levels change the neurotrophic effects of NO in midbrain cultures into neurotoxic [37]. Under these conditions, NO triggers a programmed cell death with...
markers of both apoptosis and necrosis, the kind of death that is seen in this retinitis pigmentosa model. Therefore, restoring GSH levels can help NO to exert its beneficial effects, while the decrease in GSH levels allow more NO to be free and commit neurotoxic actions.

One of the signaling mechanisms of NO is through the S-nitrosylation of cysteine residues on proteins. S-nitrosylation is now regarded as an important redox signaling mechanism in the regulation of different cellular and physiological functions and deregulation of S-nitrosylation has also been linked to various human diseases such as neurodegenerative disorders [38].

2.2. Glutathione metabolism in rd1 retina

We have studied several markers of oxidative stress in retinas from rd1 mice and compared them with its values in control mice. MDA, a lipid peroxidation product, concentration was measured by liquid chromatography according to a modification of the method of Richard et al [39] as previously described [40]. There were no differences between MDA values in retina homogenate from control animals and those from the treated and non treated rd1 mice (Figure 2a). Glutathione peroxidase (GPx) and glutathione reductase (GSSG-R) activities were assayed in retina homogenates (Figure 2a and 2b). GPx activity was assayed as reported by Lawrence et al [41] towards hydrogen peroxide. GSSG-R activity was assayed as reported by Pinto and Bartley [42]. GPx is the key enzymatic activity metabolizing cytosolic and mitochondrial hydrogen peroxide. GPx activity was significantly decreased in retina from rd1 mice at PN11 compared with controls. There was no difference in GSSG-R activity between control mice and the rd1 mice retina.

Furthermore, it is also known that there is a reduction in alpha-GST content in rd1/rd1 retina starting from the second postnatal week and that the addition of alpha GST exogenously to rd1/rd1 explants was able to rescue photoreceptor from death [43]. The authors propose that alpha-GST neuroprotection is mediated by reduction of tissue oxidative stress.

GSH has shown its protective effect on neuronal degeneration by inhibiting glutamate toxicity [44], a situation also present in retinal degenerations in the rd1 mouse [45]. Moreover, it has been suggested that glutathione alterations can also be observed in humans affected with photoreceptors dystrophy. Two sisters with severe glutathione synthetase deficiency, an autosomal recessive inborn error of metabolism resulting in very low intracellular levels of the free-radical scavenger glutathione, showed progressive retinal dystrophy with hyperpigmentations and maculopathy. These findings agree with a rod/cone type of retinal dystrophy [46].
3. Antioxidant treatment in retinitis pigmentosa

Increasing evidence suggests that oxidative stress contributes to the pathogenesis of many neurodegenerative disorders, including RP, age related macular degeneration (AMD), glaucoma, diabetic retinopathy, and light damage [47-49].
Oxidative damage has also been reported to be present in cone photoreceptor degeneration [50, 51]. These studies demonstrated the presence of acrolein- and 4-hydroxynonenal-adducts on protein; specific indicators of lipid peroxidation and biomarkers for oxidative damage to proteins and DNA in experimental animal models of RP at ages in which almost all rods had died. They postulate the hypothesis that the death of rods results in decreased oxygen consumption and hyperoxia in the outer retina resulting in gradual cone cell death from oxidative damage.

In addition, microarray experiments have shown that in the rd1 retina, genes for products related to protection against oxidative stress are up-regulated when compared to wild type mice [52, 53]. Hackman et al [53] studied gene expression in rd1 retina and compared it withagematched control retinas at three time points: postnatal day P14, P35, and P50. At each stage of degeneration, they found there was only limited overlap of the genes that showed increased expression, suggesting the involvement of temporally distinct molecular pathways. But they found increased expression of genes involved in cell proliferation pathways and oxidative stress at each time point.

In previous studies we have shown that the use of a combination of antioxidants (zeaxanthin, lutein, α-lipoic acid and glutathione), in vitro and in vivo drastically reduced the number of rod photoreceptors displaying oxidatively damaged DNA, and delayed the degeneration process significantly [54]. For the in vitro studies, antioxidants were added to the culture medium. For the in vivo studies, postnatal day (PN3) pups of rd1 mice were fed antioxidants either individually or in combination and control rd1 animals received vehicle alone. The number of TUNEL positive and avidin positive cells (indicating nuclear oxidative stress) was considerably decreased upon treatment with the combination of the antioxidants. Rescue of rd1 photoreceptors was significant at PN18 and PN17, respectively, in the in vitro and in vivo studies.

Komeima et al have also showed that injecting another combination of antioxidants (α-tocopherol, ascorbic acid, Mn(III) tetrakis (4-benzoic acid) porphyrin, and α-lipoic acid) in PN18 rd1 mice decreased cone photoreceptor cell death [55]. In this study mice were treated with daily injections of the mixture or each component alone between postnatal day (P)18 and P35. Between P18 and P35, there was an increase in two biomarkers of oxidative damage, carbonyl adducts measured by ELISA and immunohistochemical staining for acrolein, in the retinas of rd1 mice. The staining for acrolein in remaining cones at P35 was eliminated in antioxidant-treated rd1 mice, confirming that the treatment markedly reduced oxidative damage in cones;
this was accompanied by a 2-fold increase in cone cell density and a 50% increase in medium-wavelength cone opsin mRNA [55].

These group has also treated with antioxidants animals with other types of RP, rd10/rd10 mice, a model of more slowly progressive recessive RP, and Q344ter mice, a model of rapidly progressive dominant RP [56]. Compared to appropriate vehicle-treated controls, rd10/rd10 and Q344ter mice treated between P18 and P35 with a mixture of antioxidants previously found to be effective in rd1/rd1 mice showed significantly greater cone survival. These data suggest that oxidative damage contributes to cone cell death regardless of the disease causing mutation that leads to the demise of rods, and that in more slowly progressive rod degenerations, oxidative damage may also contribute to rod cell death [57].

Other studies have also postulated the use of Docosahexaenoic acid (DHA), the major retinal polyunsaturated fatty acid, to prevent photoreceptor apoptosis. DHA has long been known to be critical for proper visual function; its deficiency impairs the electric response to illumination, decreases visual acuity and affects retinal development [57, 58]. DHA effectively prevents photoreceptor apoptosis induced by oxidative stress [59] and induces photoreceptor progenitors to exit the cell cycle [60] and stimulates their differentiation [61]. Probably DHA prevents photoreceptor apoptosis activating the ERK/MAPK(ERK)/mitogen-activated protein kinase pathway to promote photoreceptor survival during early development in vitro and upon oxidative stress avoiding mitochondrial depolarization [62]. Another possible explanation for the neuroprotection exerted by DHA, is its transformation in neuroprotectin DI (NPD1). NPD1 protects retinal pigment epithelial (RPE) cells from oxidative stress-induced apoptosis and photoreceptor survival depends on the integrity of RPE cells [63].

Figure 3. TUNEL staining in retinas from (a) rd1 mice and (b) rd1 mice treated with antioxidants. Note the decrease in the number of TUNEL positive cells in the ONL from the treated rd1 mice.
Finally melatonin has also shown to be effective in delaying photoreceptor loss and reduced the number of apoptotic photoreceptors in the homozygous rds mouse (rds/rds) [64]. The rds/rds mouse is a spontaneous mutant, in which photoreceptor cell death is triggered by a null mutation in the rds/peripherin gene [65]. The mechanisms by which melatonin protects photoreceptor loss in these animals are unknown.

In view of the clear alteration of glutathione metabolism, with its significance in an increase of oxidative stress, protection from oxidative damage may be a broadly applicable treatment strategy in RP. Moreover it has been shown that irrespective of whether photoreceptor degeneration is triggered by gene defects (lack of beta-PDE or rds/peripherin) or environmental stress (light-damage), a number of pro-apoptotic mechanisms are triggered leading to the degeneration of the photoreceptor cells [66]. The temporal pattern of the different pathways suggests that the non-caspase-dependent mechanisms may actively participate in the demise of the photoreceptors. Unless the common upstream initiator for a given photoreceptor dystrophy is found, multiple rescue paradigms need to be used to target all active pathways [66]. Since structurally different compounds with variable antioxidant activity provide additional protection against increased oxidative stress [67], it appeared reasonable to combine different antioxidants instead of using them individually, particularly because the mammalian antioxidant defence system is a complex network and comprise several enzymatic and non-enzymatic entities [68, 69].

We can then conclude that neuroprotection with antioxidants is a feasible therapy opportunity to try to maintain photoreceptor survival in the neuroretina. Anyway, insights into the signalling pathways involved in the degeneration in this illness are crucial for the development of rational antioxidant therapies.

References


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Figure 1 & 3 not mentioned in the text matter. Kindly verify and confirm.