

## Oxidative Stress and the Eye

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The eye is a highly metabolically active structure, continually bathed in light, the absorption of which is its very function. Thus, oxidative and particularly photo-oxidative processes are critical factors in ocular pathologic conditions but are often poorly recognized by those investigating ocular disease. The author discusses oxidative stress in inflammatory processes of the conjunctiva, cornea, and uvea; in cataract formation in the lens; in retinal degeneration; and in optic nerve pathologic conditions, inflammatory in optic neuritis and degenerative in glaucoma. As can be seen from that list, oxidative stress occurs throughout the eye and is involved in many different types of tissue damage. Indeed, although oxidative stress has been increasingly recognized as important in pathologic conditions generally and in ocular pathologic conditions specifically in the past decade, reports documenting the centrality of this in ophthalmic disease have been in the literature for more than 20 years [1]. From a therapeutic perspective, countering these oxidative stresses may be an important and as yet poorly recognized treatment target in many ocular diseases.

### THE OCULAR SURFACE

Although the lens and retina are the tissues most clearly affected by oxidative stress, the author starts his considerations at the front of the eye with the conjunctiva and cornea, the ocular surface. The metabolic rate is not particularly high here, reducing oxidative stress from that quarter; however, clearly, the ocular surface is the most exposed part of the eye, and oxidative stress associated with actinic radiation and allergens is thus seen here.

Allergic conjunctivitis is, in this author's opinion, a much underreported condition in the dog. The seasonal incidence of much apparently idiopathic canine conjunctivitis and the lack of infectious agents in most cases suggest that allergy

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may play an important role. Much of the tissue damage in such cases can be attributed to oxidative stress. Experimental studies of allergic eye disease in rodent models have shown that. Oxidative stress is an important factor in ocular surface disease.

Other ocular surface effects of certain pollens can be generated independent of immune system involvement. Ragweed pollen (RWP) grains contain nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, generating a superoxide anion that can be converted to hydrogen peroxide by pollen grain-associated superoxide dismutase (SOD). These diffusible oxygen radicals from pollen grains increase intracellular levels of reactive oxygen species in cultured epithelial cells and conjunctiva in mouse models [2]. The same effect was observed in sensitized and naive mice, suggesting that the RWP-induced oxidative stress in conjunctival epithelium is independent of adaptive immunity. It is unclear how widespread such effects may be with other plant-derived allergens. Lactoferrin reduces the associated tissue responses in allergic airway disease in rodent models; thus, we might expect tear-film lactoferrin to have similar effects in the eye [3].

Lactoferrin protects ocular surface cells from the deleterious effects of ultraviolet (UV) radiation [4] and, experimentally, from the effects of reoxygenation after hypoxia [5], although the clinical relevance of this is unclear. Tear lactoferrin has been reported to decrease in the postoperative period after human cataract surgery, and we might expect the same potentially deleterious reduction after canine intraocular surgery [6]. More critical is the damaging effect of low ocular surface lactoferrin levels in individuals with keratoconjunctivitis sicca. Reduced levels of tear lactoferrin in human cases of dry eye and the close correlation between lactoferrin levels and degree of ocular surface damage in dry eye [7] are sufficiently strong that the lactoferrin tear test is a recognized diagnostic tool in human ophthalmology [8,9].

Ironically, one of the most common conditions associated with oxidative stress in the ocular surface is the iatrogenic response to topical eyedrops containing the preservative benzalkonium chloride [10]. In vitro studies on preserved and unpreserved eyedrops containing drugs as diverse as beta-blockers [11] and fluoroquinolone [12] showed that it was the benzalkonium chloride that was cytotoxic and that it acts through P2X7 cell death receptor activation and is associated with oxidative stress and apoptosis. Oxidative stress, as evidenced by enhanced production of reactive oxygen species and mitochondrial injury rather than by cellular glutathione depletion, is a mechanism involved in apoptosis induced by preservative-containing eyedrops.

## CORNEAL DISEASE

The matrix metalloproteinase-mediated pathologic condition of melting corneal ulcers might be considered a prime example of oxidative stress in the ocular surface. Indeed, this would seem to be the case, but only two reports document the involvement of tissue damage through oxidative processes. In one report, cationic glucose oxidase injected into rabbit corneas, with the

resulting sustained release of hydrogen peroxide, yielded corneal opacification through attack on corneal glycol conjugates by reactive oxygen species and infiltration of phagocytes, further compromising corneal integrity through the oxidative sequelae of their respiratory burst [13]. Ulcerative keratitis can be treated successfully with topical antioxidants, demonstrating the importance of oxidative stress in ulcer pathogenesis. This is the case in ulcers with alkali burn as a cause [14] and in those with an infectious cause [15]. Recently, researchers have shown that the use of mobile telephones close to rat eyes increases oxidative stress in the cornea and lens [16]. In corneal tissue, levels of malondialdehyde, a key marker of oxidative stress, together with catalase activity, significantly increased in the mobile telephone group compared with the use of a mobile telephone plus vitamin C group and the control group ( $P < .05$ ), whereas SOD activity was significantly decreased ( $P < .05$ ). At the other face of the cornea, that of the endothelial surface, oxidative stress can likewise have deleterious effects. Incubation of the cornea with hydrogen peroxide reduced endothelial barrier function, with an increase in dextran permeability and concurrent reductions in active fluxes across the endothelium [17]. These changes resulted in corneal edema, with this effect potentiated by pretreatment with 3-aminotriazole to inhibit catalase activity or with buthionine sulfoximine to inhibit glutathione synthesis [18]. Hydrogen peroxide should not, however, be seen only as an aggressor molecule in the anterior segment of the eye; it has an important immunoregulatory role as is considered elsewhere in this article. Endothelial protection from oxidative stress comes in many guises, with vasoactive intestinal peptide (VIP), a neurotrophic peptide present in aqueous humor, being an important factor [19]. VIP is generally immunosuppressive.

### Uveitis

Oxidative stress is a key player in the drama of inflammation; thus, we should not be surprised that it is important in uveitis. Experimental rodent models of uveitis generally involve the posterior segment; as such, it is retinal mitochondria that exhibit signs of oxidative stress, which seems to result from the upregulation of inducible nitric oxide synthase (iNOS) in photoreceptor mitochondria and retinal cytokine generation by antigen-specific infiltrating T cells [20]. Macrophages in these uveitic environs show upregulated iNOS when in the presence of  $\gamma$ -interferon [21]. Such models may be of relevance to uveitic disease involving the posterior segment of the eye in human beings, such as Behçet's disease, in which oxidative stress is of importance [22]. Their relevance to the anterior uveitis seen more commonly in our companion animal species is less clear. Yet, oxidative stress is also seen in models of anterior uveitis, such as that induced by endotoxin [23]. Aqueous humor levels of malondialdehyde, a key marker of oxidative stress [24], were markedly increased in endotoxin-mediated uveitis, whereas SOD, glutathione peroxidase, and catalase, enzymes acting to inhibit oxidative stress, were reduced. As one might expect, in the same way that it aims to reduce or curtail uveitic tendencies by the influence of anterior chamber acquired immune deviation

[25], the eye has several mechanisms to reduce the oxidative stress caused by intraocular inflammation. The trophic factor pigment epithelial-derived factor (PEDF) is produced by the retinal pigment epithelium and also by the epithelium of the ciliary body, from whence it is secreted into the aqueous humor [26]. Increased levels of PEDF are reported in cases of uveitis [27], with this factor having several beneficial effects, ranging from reducing the apoptotic effects of reactive oxygen species in the eye [28] to ameliorating the effects of oxidative stress on barrier function so critical in separating the intraocular milieu from the rest of the body [29].

### Cataract

One might not expect the lens to be site wherein oxidative stress plays a major part in pathologic conditions; metabolic activity here is quite low, because the lens is mostly crystalline protein with a paucity of cell organelles, such as mitochondria, which are the center of so much oxidative stress in the rest of the body. Yet, in fact, the lens is perhaps the most oxidatively stressed tissue in the body. Lens issue is, after all, exposed to light all the time that the eyelids are open, and this means that photo-oxidation occurs at a high rate with major effects [30]. The thiol groups on lens crystallins are readily oxidized to a disulfide bridge joining the proteins [31]. The resulting protein aggregation results in cataract, a condition that blinds millions of people globally [32] and many aging animals as well [33,34], although we have much less data on the condition in companion animals compared with that in people. In human beings, age-related cataractogenesis first starts in the lens nucleus with brunescence (browning) of the lens tissue [35]. Lens proteins experimentally irradiated with UV light develop a similar browning associated with loss of the amino acid tryptophan. Brunescence aged lenses *in vivo* do not show this loss of tryptophan, and the hydroxylated amino acids that occur in age-related cataract do not occur in artificially irradiated lens proteins [36]. The solution of this conundrum is that UV filters in the lens are responsible for the lens changes seen *in vivo*. In skin, melanin is the compound, a polymer of tyrosine, that protects against UV irradiation damaging effects. UV protection in the lens is based on a group of molecules synthesized from tryptophan, with the main protectant being 3-hydroxykynurenine glucoside (3-OHKG) [37]. This glucoside and several relatives break down on UV irradiation and are then scavenged by the antioxidants NADPH and glutathione, protecting other lens molecules from damage. This is important, because unlike any other proteins in the body, crystallins in the center of the lens do not turnover through life. Your nuclear crystallins are the ones you were born with. The UV filters are, however, themselves damaged irreversibly once, in later life, when the scavenging antioxidant systems begin to fail. Loss of reduced glutathione through oxidation and lack of dietary antioxidant protection for the lens lead to accelerated formation of hydroxyl radical formation in the lens. The UV filters that should prevent this damaging irradiation seem to accumulate on the lens proteins and become oxidized by low levels of oxygen in the center of the lens. These UV filters are not only

lens-specific but are primate-specific, explaining why we do not see brunescence in aging lenses in other mammal groups and may also be why the age-related cataract seen in dogs and cats is more posterior cortical rather than nuclear [38]. Here, the key feature protecting these aging lens proteins is the predominant lens protein itself.  $\alpha$ -Crystallin has not only a key structural role in the lens but acts as a chaperone molecule here and elsewhere in the body. These molecules, closely linked to heat-shock proteins throughout the animal kingdom even down the evolutionary tree as far back as bacteria, act to protect other proteins from damage and misfolding. Because the lens proteins must stay in solution to yield a transparent lens, cross-linking and misfolding result in protein aggregation and lens opacification. In regard to thiol photo-oxidation, lens crystallins can be altered to include carbonyl (ie, oxygen double-bonded to the carbon backbone) adducts or glycosylated in the presence of sugars through the Maillard and Amadori reaction to produce advanced glycation end products [39]. These changes are particularly important in diabetic individuals, in whom several pathways lead to lens change, with many acting finally through the production of the hyperglycemia-induced process of overproduction of superoxide by the mitochondrial electron transport chain [40]. Other lens proteins, such as aquaporins, can undergo posttranslational modifications, such as oxidation, glycation, or deamidation, which may lead to cataract [41].

Oxygen is certainly important in the generation of cataract; patients treated with hyperbaric oxygen do develop nuclear cataract; the loss of glutathione, protein insolubilization, and membrane changes all seem to be an accelerated form of age-related cataract [42]. Mitochondria seem to be vital in keeping oxygen levels low in the center of the lens. Interestingly, the porcine lens is particularly highly exposed to oxygen, because a countercurrent system in the choroid concentrates oxygen there to supply the metabolically highly active retina. Even in fish with pathologically high posterior segment oxygen tensions, cataract does not seem to be a major ophthalmic lesion [43], presumably because of a specifically adapted lens redox system. In the same way, as is detailed elsewhere in this article, oxygen retinotoxicity does not occur in fish in the same way that it does in experimental rodents subjected to hyperbaric oxygen for long periods.

Recovery from oxidative damage in the lens depends on the removal of oxidized lens proteins. The ubiquitin-proteasome pathway used throughout the body to protect against the adverse events of oxidation is increased in lens cells during recovery from oxidative stress, such as that provided *in vitro* by the use of hydrogen peroxide. Proteins with carbonyl adducts are found to be ubiquitinated [44], and the age-related reduction in lens ubiquitin is paralleled by an increase in oxidative damage [45].

Lipid peroxidation is also a factor in cataractogenesis [46], although the focus of oxidative action in this case is the cell membranes of the lens epithelial cells [47,48]. The changes induced in these studies were seen in lens fiber cultured in atmospheres with increased oxygen levels but mirror the changes seen in older

lenses [49]. Lens membranes are unique in that they contain high levels of cholesterol and plasmalogen and high levels of dihydrosphingomyelin. Lens lipid composition changes substantially with age, with particular effects on membrane fluidity. Lipid peroxidation results in the generation of lipid-derived aldehydes, and in extralenticular tissues, these have been shown to mediate many of the effects of oxidative stress by acting as toxic messengers. The relative and absolute amounts of sphingolipids increase with age, whereas glycerolipids, including phosphatidylcholine, decrease. These changes are exacerbated in the presence of cataract and are substantial, greater than the changes in lipid levels reported in any other organ system in association with other aging diseases [49,50].

DNA is also a target of oxidative stress, and DNA damage and apoptosis occur in lens epithelial cells exposed to oxidative stress, a factor causing cataract in experimental rodents [51] ultraviolet B (UVB) irradiation causes DNA fragmentation and apoptotic cell death in oxidative stress-induced immortalized lens cell lines when the stressor was UV irradiation, whereas necrosis occurred when the stressor was hydrogen peroxide or t-butyl hydroperoxide [52]. The same is true of x-ray-irradiated lenses, in which antioxidants, such as carnitine, can be instrumental in reducing pathologic effects [53].

Dietary antioxidants are important in reducing the incidence of lens opacification, as demonstrated in *in vitro* models [54–56] in numerous retrospective analyses in human and rodent models of cataractogenesis, with these having been reviewed in detail recently [57]. Two important rodent models are those of the OXYS rat, selected for high oxidative stress. These rats had been bred for a high incidence of cataract formation when fed high levels of galactose; however, after five generations of selective breeding, the rats developed cataract without the requirement for high dietary galactose. Enhanced transport of glucose into the cells of the rat was causing the cataract and also leading to overgeneration of reactive oxygen species intracellularly without diabetes and with normal blood sugar levels. Lens opacification starts at the interface between the nucleus and cortex by 2 months of age, progressing to total nuclear cataract with increasing cortical involvement by 6 months of age [58]. A wide variety of morphologic changes occur at different areas within the lens: cell swelling and globule formation occur at the corticonuclear interface, redistribution of cytoplasmic contents occurs in the nucleus, and cell fusion sites are common in the cortex. Evidence for the involvement of reactive oxygen species in the pathologic changes comes from histochemical determination of oxidative breakdown products from damaged DNA, lens epithelial hyperplasia, and altered membrane structures [59]. Another rodent model is the Emory mouse, which has been studied since the mid-1980s [60] and, more recently, particularly investigated with regard to the effect of caloric restriction of cataract formation [61], which is especially relevant given the close association between caloric restriction, aging, and the effects of long-term oxidative stress [62,63].

## RETINAL DEGENERATION

The fact that the mutations responsible for inherited degenerations, such as generalized progressive retinal atrophy in the dog and similar disease in experimental models like the rd-1 mouse [64] or environmental factors in rodents exposed to continuous light, should not blind us (if one would excuse the pun) to the importance of oxidative stress in the degenerating retina. Elevated malondialdehyde and low glutathione peroxidase activity detected in the degenerating retina in rd-1 mice indicate higher oxidative load [65]. Decreased levels of glutathione transferase in the degenerating retina increase the effects of reactive oxygen species produced by the degenerating retina, and replenishment of this enzyme reduces the degree of photoreceptor death [66]. Indeed, it is oxidative stress that may well be the cause of cone death in a condition that is focused on metabolic change in the rod. Rods are the primary users of oxygen in the retina, and once they begin to die, excess oxygen can have catastrophic deleterious effects on the remaining photoreceptors [67]. This hypothesis is supported by the ameliorative and protective effect of antioxidants on retinal degeneration [68].

Although the efficacy of antioxidants in generalized progressive retinal atrophy is at present unclear, the retinal degeneration that used to be called “central retinal atrophy” and is now called “retinal pigment epithelial dystrophy” clearly has an oxidative background, not to say center. Subnormal retinal pigment epithelial activity, whether through a genetic mutation [69] or through reduced levels of dietary vitamin E intake [70], renders the normal phagocytic and antioxidant function of the retinal pigment epithelium defective. Lipofuscin is deposited in the retina when photoreceptor pigment is insufficiently dealt with by the antioxidant biochemical pathways in the retinal pigment epithelium. Lipofuscin and ceroid are fluorophore products formed during the reaction of cell metabolites with secondary aldehydic products of oxygen free radical-induced oxidation, particularly lipid peroxidation. Ceroid and lipofuscin progressively accumulate as a result of phagocytosis and autophagocytosis of modified biomaterials within postmitotic cells. Lipofuscin is generally considered the classic age pigment of postmitotic cells, whereas ceroid is held to accumulate as a result of pathologic processes. Even so, lipofuscin deposition can occur in the retina to the extent that it compromises retinal function [71]. The fluorophores in lipofuscin, and particularly A2E, generate reactive oxidative species when exposed to light, particularly at the blue end of the spectrum [72–74]. A2E and other fluorophores are formed as a result of reactions of *all-trans* retinal with phosphatidylethanolamine when reduction to retinol does not occur [75]. In this way, oxidative stress, which prevents reduction of *all-trans* retinal, results in further photo-oxidative reactions. The retinal pigment epithelium is thus a hotbed of oxidative activity. It lives on a knife edge of apoptotic potential and is only saved from this oxidative catastrophe by the presence of  $\alpha$ -crystallin, discussed previously in the context of the ocular lens [76]. In our short-lived companion animal species, these reactions are probably not critical to maintaining vision, apart from in dog breeds with inherited vitamin E defects, such as the Briard [77], and those with ceroid lipofuscinosis, such as

the miniature schnauzer [78] and Owczarek Nizinny (Polish lowland sheepdog) [79]. We have little information on retinal oxidative stress in these latter conditions, but the *mnd* mouse, a classic model of ceroid lipofuscinosis, shows oxidative damage in the retina [80], and it would not be unreasonable to suggest that the same changes are likely to be occurring in the dog. In human beings, oxidative stressors play a key part in the pathogenesis of age-related macular degeneration [81].

## Glaucoma

If one approaches glaucoma as an optic neuropathy in which damage to the optic nerve and subsequent ganglion cell loss is the key feature, oxidative stress can readily be built into the picture of disease initiation and progression. Retinal ganglion cell death in glaucoma has been shown to be directly associated with the generation and effects of reactive oxygen species [82]. Axonal injury caused by increased intraocular pressure and resulting ganglion cell apoptosis results in the generation of reactive oxygen species that can then contribute to the death of previous undamaged ganglion cells. Experiments demonstrating reduced apoptosis under the influence of reactive oxygen species scavengers, such as SOD and catalase, show that oxidative stress is an important if not crucial factor in cell loss through apoptosis. Reactive oxygen species can also act as cell signaling molecules, which leads to glial cell dysfunction and also the stimulation of antigen presentation [83].

Increased intraocular pressure is, however, only one factor, albeit an extremely important one, in ganglion cell death in glaucoma. Vascular factors leading to reductions in optic nerve head perfusion mean that hypoxia and oxidative stress are key features of the optic nerve and retina in many cases of chronic glaucoma [84]. Quite how important these factors are in canine glaucoma is unclear, and further research needs to address this whole question. Hypoxia-induced expression of genes for molecules, such as hypoxia-inducible factor-1 $\alpha$ , a master regulator of oxygen homeostasis, leads to the upregulation of a wide range of genes in the retina and optic nerve, such as those encoding erythropoietin, glycolytic enzymes, and vascular endothelial growth factor. In addition, activation of optic nerve glial cells by hypoxia seems to be an important feature in determining their ultimate role in glaucomatous neurodegeneration [85]. Yet, how does this relate to oxidative stress? Surely, hypoxia and oxidative stress are at opposite ends of the redox spectrum?

The same paradox is seen in the role of oxidative stress and reactive oxygen species in cerebral ischemia: hypoxia damages neurons, but it is reactive oxygen species that also play a key role in neuronal death during ischemia, and more particularly during reperfusion injury [86]. Reactive oxygen species, such as nitric oxide, can also play an important role as intracellular signaling molecules acting in a proapoptotic manner to be key players in the retinal ganglion cell degeneration characteristic of glaucoma [87–89].

Oxidative stress is not only active in the glaucomatous optic nerve head, however. It was more than a quarter of a century ago that Alvarado and

colleagues [90,91] postulated that oxidative stress was instrumental in the loss of trabecular meshwork cells in progressive open-angle glaucoma in human beings. More recently, researchers have dissected the changes in trabecular meshwork connective tissue morphology and endothelial cell function occasioned by reactive oxygen species [92].

### Optic Neuropathies

The clearest example of oxidative stress playing a central role in an ocular disease is that of Leber's optic neuropathy in human beings. This ganglion cell degeneration is caused by one of three point mutations in mitochondrial DNA encoding the NADH/ubiquinone oxidoreductase of the oxidative phosphorylation chain in mitochondria [93]. These mutations decrease ATP synthesis yet increase oxidative stress [94]. Ganglion cell death occurs by apoptosis, which may be linked to a concurrent impairment of glutamate transport and to increases in retinal reactive oxygen species caused by disruption of the mitochondrial electron transfer chain. Although several experimental animal models, mostly transgenic [95], have been investigated, no spontaneously occurring animal model of Leber's optic neuropathy (not to be confused with Leber's congenital amaurosis, for which the Briard and long-haired miniature dachshund stand as valuable canine models [96,97]) has been recognized and reported.

Oxidative stress also seems to play an important part in optic neuritis [98]. Again, it is experimental animal models that have led the way in defining this, but evaluation of optic nerves from mice with experimental autoimmune encephalomyelitis using dichlorofluorescein diacetate (DCFDA), dihydroethidium, and cerium chloride to probe for reactive oxygen species showed their presence, and ribozyme suppression of SOD increases nerve damage, underlining the importance of oxidative stress in the disease [98].

### SUMMARY

It might be argued that most of the disease states described in this article are those encountered in human beings or in experimental rodent models. How do they relate to the clinical world of veterinary ophthalmology? The author would argue that many of the molecular interactions modeled here are likely to be reproduced in the diseases of the ocular surface, cornea, uvea, lens, retina, and optic nerve seen in the companion dogs and cats we treat. It is for us to investigate further the involvement of oxidative stress in clinical disease with the long-term aim to use antioxidants to reduce the impact of oxidative stress in these diseases. The author hopes that this review will stimulate such research in basic science and clinical practice.

### References

- [1] Mittag T. Role of oxygen radicals in ocular inflammation and cellular damage. *Exp Eye Res* 1984;39:759–69.
- [2] Bacsi A, Dharajiya N, Choudhury BK, et al. Effect of pollen-mediated oxidative stress on immediate hypersensitivity reactions and late-phase inflammation in allergic conjunctivitis. *J Allergy Clin Immunol* 2005;116:836–43.

- [3] Kruzel ML, Bacsi A, Choudhury BK, et al. Lactoferrin decreases pollen antigen-induced allergic airway inflammation in a murine model of asthma. *Immunology* 2006;119:159–66.
- [4] Fujihara T, Nagano T, Endo K, et al. Lactoferrin protects against UV-B irradiation-induced corneal epithelial damage in rats. *Cornea* 2000;19:207–11.
- [5] Shimmura S, Shimoyama M, Hojo M, et al. Reoxygenation injury in a cultured corneal epithelial cell line protected by the uptake of lactoferrin. *Invest Ophthalmol Vis Sci* 1998;39:1346–51.
- [6] Kumar R, Parmar IP, Chhillar N, et al. Tear lactoferrin concentration during postoperative ocular inflammation in cataract surgery. *Acta Ophthalmol Scand* 1997;75:142–4.
- [7] Danjo Y, Lee M, Horimoto K, et al. Ocular surface damage and tear lactoferrin in dry eye syndrome. *Acta Ophthalmol (Copenh)* 1994;72:433–7.
- [8] Da Dalt S, Moncada AS, Priori R, et al. The lactoferrin tear test in the diagnosis of Sjögren's syndrome. *Eur J Ophthalmol* 1996;6:284–6.
- [9] Solomon A, Dursun D, Liu Z, et al. Pro- and anti-inflammatory forms of interleukin-1 in the tear fluid and conjunctiva of patients with dry-eye disease. *Invest Ophthalmol Vis Sci* 2001;42:2283–92.
- [10] Pauly A, Brignole-Baudouin F, Guenoun JM, et al. Comparative study of topical anti-allergic eye drops on human conjunctiva-derived cells: responses to histamine and IFN-gamma and toxicological profiles. *Graefes Arch Clin Exp Ophthalmol* 2007;245:534–46.
- [11] Debbasch C, Pisella PJ, De Saint Jean M, et al. Mitochondrial activity and glutathione injury in apoptosis induced by unpreserved and preserved beta-blockers on Chang conjunctival cells. *Invest Ophthalmol Vis Sci* 2001;42:2525–33.
- [12] Dutot M, Pouzaud F, Larosche I, et al. Fluoroquinolone eye drop-induced cytotoxicity: role of preservative in P2X7 cell death receptor activation and apoptosis. *Invest Ophthalmol Vis Sci* 2001;47:2812–9.
- [13] Carubelli R, Nordquist RE, Rowsey JJ. Role of active oxygen species in corneal ulceration. Effect of hydrogen peroxide generated in situ. *Cornea* 1990;9:161–9.
- [14] Alio JL, Ayala MJ, Mulet MF, et al. Antioxidant therapy in the treatment of experimental acute corneal inflammation. *Ophthalmic Res* 1995;27:136–43.
- [15] Alio JL, Artola A, Serra A, et al. Effect of topical antioxidant therapy on experimental infectious keratitis. *Cornea* 1995;14:175–9.
- [16] Balci M, Devrim E, Durak I. Effects of mobile phones on oxidant/antioxidant balance in cornea and lens of rats. *Curr Eye Res* 2007;32:21–5.
- [17] Hull DS, Green K, Thomas L, et al. Hydrogen peroxide-mediated corneal endothelial damage. Induction by oxygen free radical. *Invest Ophthalmol Vis Sci* 1984;25:1246–53.
- [18] Costarides A, Recasens JF, Riley MV, et al. The effects of ascorbate, 3-aminotriazole, and 1,3-bis-(2-chloroethyl)-1-nitrosourea on hydrogen peroxide levels in the rabbit aqueous humor. *Lens Eye Toxic Res* 1989;6:167–73.
- [19] Koh SW, Waschek JA. Corneal endothelial cell survival in organ cultures under acute oxidative stress: effect of VIP. *Invest Ophthalmol Vis Sci* 2000;41:4085–92.
- [20] Rajendrum R, Sarawathy S, Rao NA. Photoreceptor mitochondrial oxidative stress in early experimental autoimmune uveoretinitis. *Br J Ophthalmol* 2007;91:531–7.
- [21] Zhang J, Wu LY, Wu GS, et al. Differential expression of nitric oxide synthase in experimental uveoretinitis. *Invest Ophthalmol Vis Sci* 1999;40:1899–905.
- [22] Taysi S, Demircan B, Akdeniz N, et al. Oxidant/antioxidant status in men with Behçet's disease. *Clin Rheumatol* 2007;26:418–22.
- [23] Satici A, Guzey M, Gurler B, et al. Malondialdehyde and antioxidant enzyme levels in the aqueous humor of rabbits in endotoxin-induced uveitis. *Eur J Ophthalmol* 2003;13:779–83.
- [24] Rahman I, Biswas SK. Non-invasive biomarkers of oxidative stress: reproducibility and methodological issues. *Redox Rep* 2004;9:125–43.
- [25] Neiderkorn JY. The induction of anterior chamber-associated immune deviation. *Chem Immunol Allergy* 2007;92:27–35.

- [26] Ortego J, Escribano J, Becerra SP, et al. Gene expression of the neurotrophic pigment epithelium-derived factor in the human ciliary epithelium. Synthesis and secretion into the aqueous humor. *Invest Ophthalmol Vis Sci* 1996;37:2759–67.
- [27] Yoshida Y, Yamagishi S, Matsui T, et al. Increased levels of pigment epithelium-derived factor in aqueous humor of patients with uveitis. *Br J Ophthalmol* 2007;91:149–50.
- [28] Tsao YP, Ho TC, Chen SL, et al. Pigment epithelium-derived factor inhibits oxidative stress-induced cell death by activation of extracellular signal-regulated kinases in cultured retinal pigment epithelial cells. *Life Sci* 2006;79:545–50.
- [29] Ho TC, Yang YC, Cheng HC, et al. Pigment epithelium-derived factor protects retinal pigment epithelium from oxidant-mediated barrier dysfunction. *Biochem Biophys Res Commun* 2006;342:372–8.
- [30] Varma SD, Chand D, Sharma YR, et al. Oxidative stress on lens and cataract formation: role of light and oxygen. *Curr Eye Res* 1984;3:35–57.
- [31] Lou MF. Thiol regulation in the lens. *J Ocul Pharmacol Ther* 2000;16:137–48.
- [32] Thylefors R, Negrel AD, Pararajasegaram R, et al. Global data on blindness. *Bull World Health Organ* 1995;73:115–21.
- [33] Ohia SE, Opere CA, Leday AM. Pharmacological consequences of oxidative stress in ocular tissues. *Mutat Res* 2005;579:22–36.
- [34] Williams DL, Wallis CM, Heath MF. Prevalence of canine cataract: preliminary results of a cross-sectional study. *Vet Ophthalmol* 2004;7:29–35.
- [35] Truscott RJW. Human cataract: the mechanisms responsible; light and butterfly eyes. *Int J Biochem Cell Biol* 2003;35:1500–4.
- [36] Streete IM, Jamie JF, Truscott RJW. Lenticular levels of amino acids and free UV filters differ significantly between normals and cataract patients. *Invest Ophthalmol Vis Sci* 2004;45:4091–8.
- [37] Van Heyningen R. Fluorescent derivatives of 3-hydroxy-L-kynurenine in the lens of man, the baboon and the grey squirrel. *Biochem J* 1971;123:30P–1P.
- [38] Williams DL, Heath MF. Prevalence of feline cataract: results of a cross-sectional study of 2000 normal animals, 50 cats with diabetes and one hundred cats following dehydrational crises. *Vet Ophthalmol* 2006;9:341–9.
- [39] Pokupec R, Kalauz M, Turk N, et al. Advanced glycation endproducts in human diabetic and non-diabetic cataractous lenses. *Graefes Arch Clin Exp Ophthalmol* 2003;241:378–84.
- [40] Brownlee MR. Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;414:813–20.
- [41] Truscott RJW. Age-related nuclear cataract-oxidation is the key. *Exp Eye Res* 2005;80:709–25.
- [42] Giblin FJ, Schrimsher L, Chakrapani B, et al. Exposure of rabbit lens to hyperbaric oxygen in vitro: regional effects on GSH level. *Invest Ophthalmol Vis Sci* 1988;29:1312–9.
- [43] Williams DL, Brancker WM. Intraocular oxygen tensions in normal and diseased eyes of farmed halibut. *Vet J* 2004;167:81–6.
- [44] Shang F, Nowell TR Jr, Taylor A. Removal of oxidatively damaged proteins from lens cells by the ubiquitin-proteasome pathway. *Exp Eye Res* 2001;73:229–38.
- [45] Shang F, Gong X, Palmer HJ, et al. Age-related decline in ubiquitin conjugation in response to oxidative stress in the lens. *Exp Eye Res* 1997;64:21–30.
- [46] Babihayev MA, Costa EB. Lipid peroxide and reactive oxygen species generating systems of the crystalline lens. *Biochim Biophys Acta* 1994;1225:326–37.
- [47] Huang L, Estrada R, Yappert MC, et al. Oxidation-induced changes in human lens epithelial cells. 1. Phospholipids. *Free Radic Biol Med* 2006;41:1425–32.
- [48] Huang L, Tang D, Yappert MC, et al. Oxidation-induced changes in human lens epithelial cells. 2. Mitochondria and the generation of reactive oxygen species. *Free Radic Biol Med* 2006;41:926–36.
- [49] Huang L, Grami V, Marrero Y, et al. Human lens phospholipid changes with age and cataract. *Invest Ophthalmol Vis Sci* 2005;46:1682–9.

- [50] Choudhary S, Xiao T, Srivastava S, et al. Role of aldehyde dehydrogenase isozymes in the defense of rat lens and human lens epithelial cells against oxidative stress. *Invest Ophthalmol Vis Sci* 2005;46:259–67.
- [51] Li WC, Kusak JR, Dunn K, et al. Lens epithelial cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals. *J Cell Biol* 1995;130:169–81.
- [52] Long AC, Colitz CM, Bomser JA. Apoptotic and necrotic mechanisms of stress-induced human lens epithelial cell death. *Exp Biol Med (Maywood)* 2004;229:1072–80.
- [53] Kocer I, Taysi S, Ertekin MV, et al. The effect of L-carnitine in the prevention of ionizing radiation-induced cataracts: a rat model. *Graefes Arch Clin Exp Ophthalmol* 2007;245:588–94.
- [54] Karslioglu I, Ertekin MV, Kocer I, et al. Protective role of intramuscularly administered vitamin E on the levels of lipid peroxidation and the activities of antioxidant enzymes in the lens of rats made cataractous with gamma-irradiation. *Eur J Ophthalmol* 2004;14:478–85.
- [55] Cornish KM, Williamson G, Sanderson J. Quercetin metabolism in the lens: role in inhibition of hydrogen peroxide induced cataract. *Free Radic Biol Med* 2002;33:63–70.
- [56] Chitchumroonchokchai C, Bomser JA, Glamm JE, et al. Xanthophylls and alpha-tocopherol decrease UVB-induced lipid peroxidation and stress signaling in human lens epithelial cells. *J Nutr* 2004;134:3225–32.
- [57] Williams DL. Oxidation, antioxidants and cataract formation: a literature review. *Vet Ophthalmol* 2006;9:292–8.
- [58] Chen HM, Gonzalez RG. The effect of high glucose and oxidative stress on lens metabolism, aldose reductase, and senile cataractogenesis. *Metabolism* 1986;35(4 Suppl 1):10–4.
- [59] Marsili S, Salganik RI, Albright CD, et al. Cataract formation in a strain of rats selected for high oxidative stress. *Exp Eye Res* 2004;79:595–612.
- [60] Varma SD, Devamanoharan PS, Mansour S, et al. Studies on Emory mouse cataracts: oxidative factors. *Ophthalmic Res* 1984;26:141–8.
- [61] Taylor A, Zuliani AM, Hopkins RE, et al. Moderate caloric restriction delays cataract formation in the Emory mouse. *FASEB J* 1989;3:1741–6.
- [62] Gong X, Shang F, Obin M, et al. Antioxidant enzyme activities in lens, liver and kidney of calorie restricted Emory mice. *Mech Ageing Dev* 1997;99:181–92.
- [63] Pamplona R, Barja G. Mitochondrial oxidative stress, aging and caloric restriction: the protein and methionine connection. *Biochim Biophys Acta* 2006;1757:496–508.
- [64] Boves C, Li T, Danciger M, et al. Retinal degeneration in the rd mouse is caused by a defect in the  $\beta$ -subunit of rod cGMP-phosphodiesterase. *Nature* 1990;347:677–80.
- [65] Ahuja-Jensen P, Johnsen-Soriano S, Ahuja S, et al. Low glutathione peroxidase in rd1 mouse retina increases oxidative stress and proteases. *Neuroreport* 2007;18:797–801.
- [66] Ahuja P, Caffé AR, Ahuja S, et al. Decreased glutathione transferase levels in rd1/rd1 mouse retina: replenishment protects photoreceptors in retinal explants. *Neuroscience* 2005;131:935–43.
- [67] Shen J, Yang X, Dong A, et al. Oxidative damage is a potential cause of cone cell death in retinitis pigmentosa. *J Cell Physiol* 2005;203:457–64.
- [68] Komeima K, Rogers BS, Lu L, et al. Antioxidants reduce cone cell death in a model of retinitis pigmentosa. *Proc Natl Acad Sci U S A* 2006;103:11300–5.
- [69] McLellan GJ, Cappello R, Mayhew IG, et al. Clinical and pathological observations in English cocker spaniels with primary metabolic vitamin E deficiency and retinal pigment epithelial dystrophy. *Vet Rec* 2003;53:287–92.
- [70] Davidson MG, Geoly FJ, Gilger BC, et al. Retinal degeneration associated with vitamin E deficiency in hunting dogs. *J Am Vet Med Assoc* 1998;213:645–51.
- [71] Sparrow JR, Boulton M. RPE lipofuscin and its role in retinal pathobiology. *Exp Eye Res* 2005;80:595–606.
- [72] Lamb LE, Simon JD. A2E: a component of ocular lipofuscin. *Photochem Photobiol* 2004;79:127–36.

- [73] Jang YP, Matsuda H, Itagaki Y, et al. Characterization of peroxy-A2E and furan-A2E photo-oxidation products and detection in human and mouse retinal pigment epithelial cell lipofuscin. *J Biol Chem* 2005;280:39732–9.
- [74] Sparrow JR, Zhou J, Ben-Shabat S, et al. Involvement of oxidative mechanisms in blue-light-induced damage to A2E-laden RPE. *Invest Ophthalmol Vis Sci* 2002;43:1222–7.
- [75] Sparrow JR, Fishkin N, Zhou J, et al. A2E, a byproduct of the visual cycle. *Vision Res* 2003;43:2983–90.
- [76] Alge CS, Priglinger SG, Neubauer AS, et al. Retinal pigment epithelium is protected against apoptosis by alphaB-crystallin. *Invest Ophthalmol Vis Sci* 2002;43:3575–82.
- [77] Lightfoot RM, Cabral L, Gooch L, et al. Retinal pigment epithelial dystrophy in Briard dogs. *Res Vet Sci* 1996;60:17–23.
- [78] Palmer DN, Tynnela J, van Mil HC, et al. Accumulation of sphingolipid activator proteins (SAPs) A and D in granular osmiophilic deposits in miniature Schnauzer dogs with ceroid-lipofuscinosis. *J Inherit Metab Dis* 1997;20:74–84.
- [79] Narfstrom K, Wrigstad A, Eksten B, et al. Neuronal ceroid lipofuscinosis: clinical and morphologic findings in nine affected Polish Owczarek Niziny (PON) dogs. *Vet Ophthalmol* 2007;10:111–20.
- [80] Guarneri R, Russo D, Cascio C, et al. Retinal oxidation, apoptosis and age- and sex-differences in the mnd mutant mouse, a model of neuronal ceroid lipofuscinosis. *Brain Res* 2004;1014:209–20.
- [81] Beatty S, Koh H, Phil M, et al. The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 2000;45:115–34.
- [82] Moreno MC, Campanelli J, Sande P, et al. Retinal oxidative stress induced by high intraocular pressure. *Free Radic Biol Med* 2004;37:803–12.
- [83] Tezel G, Li LY, Patil RV, et al. TNF-alpha and TNF-alpha receptor-1 in the retina of normal and glaucomatous eyes. *Invest Ophthalmol Vis Sci* 2001;42:1787–94.
- [84] Chung HS, Harris A, Kagemann L, et al. Vascular aspects in the pathophysiology of glaucomatous optic neuropathy. *Surv Ophthalmol* 1999;43(Suppl 1):S43–50.
- [85] Tezel G. Oxidative stress in glaucomatous neurodegeneration: mechanisms and consequences. *Prog Retin Eye Res* 2006;25(5):490–513.
- [86] Sugawara T, Chan PH. Reactive oxygen radicals and pathogenesis of neuronal death after cerebral ischemia. *Antioxid Redox Signal* 2003;5:597–607.
- [87] Polak K, Luksch A, Berisha F, et al. Altered nitric oxide system in patients with open-angle glaucoma. *Arch Ophthalmol* 2007;125:494–8.
- [88] Sacca SC, Izzotti A, Rossi P, et al. Glaucomatous outflow pathway and oxidative stress. *Exp Eye Res* 2007;84:389–99.
- [89] Alvarado JA, Murphy CG, Polansky JR, et al. Age-related changes in trabecular meshwork cellularity. *Invest Ophthalmol Vis Sci* 1981;21:714–27.
- [90] Sacca SC, Paschetto A, Camicione P, et al. Oxidative DNA damage in the human trabecular meshwork: clinical correlation in patients with primary open-angle glaucoma. *Arch Ophthalmol* 2005;123:458–63.
- [91] Alvarado JA, Murphy CG, Juster R. Trabecular meshwork cellularity in primary open-angle glaucoma and non-glaucomatous normals. *Ophthalmology* 1984;91:564–79.
- [92] Izzotti A, Bagnis A, Sacca SC. The role of oxidative stress in glaucoma. *Mutat Res* 2000;612:105–14.
- [93] Carelli V, Ross-Cisneros F, Sadun A. Mitochondrial dysfunction as a cause of optic neuropathies. *Prog Retin Eye Res* 2004;23:53–89.
- [94] Carelli V, Rugolo M, Sgarbi G, et al. Bioenergetics shapes cellular death pathways in Leber's hereditary optic neuropathy: a model of mitochondrial neurodegeneration. *Biochim Biophys Acta* 2004;1658:172–9.
- [95] Qi X, Sun L, Lewin AS, et al. The mutant human ND4 subunit of complex I induces optic neuropathy in the mouse. *Invest Ophthalmol Vis Sci* 2007;48:1–10.

- [96] Acland GM, Aguirre GD, Ray J, et al. Gene therapy restores vision in a canine model of childhood blindness. *Nat Genet* 2001;28:92–5.
- [97] Mellersh CS, Bourns ME, Pettitt L, et al. Canine RPGRIP1 mutation establishes cone-rod dystrophy in miniature longhaired dachshunds as a homologue of human Leber congenital amaurosis. *Genomics* 2006;88:293–301.
- [98] Qi X, Lewin AS, Sun L, et al. Suppression of mitochondrial oxidative stress provides long-term neuroprotection in experimental optic neuritis. *Invest Ophthalmol Vis Sci* 2007;48:681–91.