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Ultraviolet Radiation: Cellular Antioxidant Response and the Role of Ocular Aldehyde Dehydrogenase Enzymes

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Abstract

Solar ultraviolet radiation (UVR) exposes the human eye to near constant oxidative stress. Evidence suggests that UVR is the most important environmental insult leading to the development of a variety of ophthalmoheliosis disorders. UVR-induced reactive oxygen species are highly reactive with DNA, proteins and cellular membranes, resulting in cellular and tissue damage. Antioxidant defense systems present in ocular tissues function to combat reactive oxygen species and protect the eye from oxidative damage. Important enzymatic antioxidants are the superoxide dismutases, catalase, glutathione peroxidases, glutathione reductase and members of the aldehyde dehydrogenase (ALDH) superfamily. Glutathione, ascorbic and uric acids, α -tocopherol, NADPH and ferritin serve as small molecule, nonenzymatic antioxidants. Ocular tissues have high levels of these antioxidants which are essential for the maintenance of redox homeostasis in the eye and protection against oxidative damage. ALDH1A1 and ALDH3A1, present abundantly in the cornea and lens, have been shown to have unique roles in the defense against UVR and the downstream effects of oxidative stress. This review presents the properties and functions of ocular antioxidants that play critical roles in the cellular response to UVR exposure, including a focused discussion of the unique roles that the ALDH1A1 and ALDH3A1 enzymes have as multi-functional ocular antioxidants.

Keywords

antioxidants; free radicals; UV radiation; aldehyde dehydrogenase; ALDH1A1; ALDH3A1

Introduction

Continuous exposure to solar radiation challenges the eye with an intense burden of reactive oxygen species (ROS), making it highly vulnerable to oxidative damage. Generation of UVR-derived ROS and the resultant oxidative stress have been implicated in the pathogenesis of numerous eye diseases, including the ocular disorders collectively known as ophthalmoheliosis (*ophthalmo*-eye, *heliosis*-sun). These disorders, including photokeratitis (1), pinguecula and pterygium (2), conjunctival dysplasia, squamous cell carcinoma (2-4) and cataracts (5-7), develop as a consequence of acute or chronic UVR exposure. Such exposure also plays a key role in photokeratoconjunctivitis (8), glaucoma and macular degeneration (9;10). While these disorders are commonly associated with the cornea or conjunctiva, they may also involve deeper ocular structures such as the lens (cataract), iris (uveal melanoma) and retina (macular degeneration).

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The cornea is an avascular and transparent tissue that covers the anterior-most surface of the eye including the iris, pupil and anterior chamber. The two major functions of the cornea are: 1) to protect inner ocular tissues, such as the lens and retina, against external environmental insults by serving as an initial protective physical and biochemical barrier, and 2) in combination with the lens, to serve as the “refraction unit” of the eye in providing greater than 60% of the optical power of the eye to focus incident light on the retina. The human cornea has five distinct layers. The most anterior, the corneal epithelium, is a thin multi-cellular layer of fast-growing and easily-regenerated epithelial cells. Tight junctions between neighboring cells act as a physical barrier against noxious environmental agents. Irregularity or edema of the epithelial cell layer can disrupt the smoothness of the air-tear film interface, the most significant factor in the total refractive power of the eye. The next layer, Bowman's layer, is composed of irregularly-arranged collagen fibers that protect the underlying corneal stroma, a thick transparent middle layer consisting of regularly-arranged type I collagen fibers along with sparsely populated keratocytes. Descemet's membrane is a thin acellular layer that serves as a modified basement membrane for the corneal endothelium. The most posterior layer of the cornea is the corneal endothelium, a simple squamous monolayer of mitochondria-rich cells responsible for regulating fluid and solute transport between the aqueous and corneal stromal compartments. In humans, the corneal endothelium is essentially a monolayer that helps preserve corneal optical properties by eliciting a net fluid transport outward from the stroma into the anterior chamber, thus preventing stromal swelling and preserving corneal clarity. Corneal transparency and optical refraction are dependent both on the continuous renewal of the anterior epithelial layer and on the fluid transport of the endothelial layer. Nutrients and protective molecules are provided to the avascular anterior and posterior tissues of the cornea by the tear film and the aqueous humor, respectively. The tear film and aqueous humor are also important contributors to the defense mechanisms of the ocular surface. The tear film covers the anterior surface of the cornea and is the first line of defense against external insults. It creates a smooth refractive surface on the cornea and protects it from dehydration and environmental damage. It also functions to nourish the anterior cornea and concentrate locally-released biochemical metabolites. The aqueous humor, secreted by ciliary bodies, is a clear and slightly alkaline liquid that occupies the space between the cornea and lens. It plays a crucial role in nourishing and protecting the corneal endothelium and the anterior epithelial lining of the lens. It also removes metabolic wastes and biochemical products generated by the cornea and lens. Accordingly, the ocular surface, comprising the tear film, cornea and aqueous humor, is the first barrier that environmental insults encounter. Not surprisingly, these structures have developed cellular defense systems in the form of various enzymatic and nonenzymatic antioxidants that play a pivotal role in protecting the inner ocular tissues against oxidative damage. Key functions of these antioxidants include the scavenging or neutralization of ROS and the repair of cellular damage caused by free radical ROS.

The lens, located behind the cornea, is a transparent, avascular, flexible, biconvex structure that refracts light onto the retina while also changing the refractive index to allow the focusing on objects at various distances. Unlike the multiple layers of the cornea, the lens consists of an encapsulated tissue comprising an anterior layer of epithelial cells and a posterior array of elongated fiber cells. While the curvature of the cornea is fixed, that of the lens is flexible and can be adjusted in a process called accommodation to fine tune the focus depending upon an object's distance. The lens central fiber cells lack organelles, including their cell nuclei, and are instead primarily composed of a high concentration (>90%) of transparent water-soluble proteins that have a key role in maintaining the refractive properties of the lens while, at the same time, allowing most light to pass through. These proteins, called lens crystallins, are believed to be evolutionarily-related to stress proteins. Similar crystalline proteins are also found in the cornea where they can make up

approximately 40-50% of total water-soluble protein (11). Studies of the various crystallins, including the aldehyde dehydrogenase (ALDH) proteins ALDH1A1 and ALDH3A1, have revealed a variety of diverse functions, including cellular defense against oxidative stress (12) and as direct absorbers of UV light (13). Many of these previously unknown functions have been demonstrated using genetically-altered mice (12).

Normal vision depends on the ability of the cornea and lens to maintain transparency, appropriate curvature and protective defense systems. As such, genetic or environmental factors, such as UVR exposure, that affect these properties can lead to impaired visual acuity and disease states. The purpose of this review is to first introduce the properties of UVR exposure and UVR-induced oxidative damage, second to summarize ocular antioxidants that are critical in the cellular response against UVR and third to provide a focused discussion on the role of the ALDH corneal crystallins, ALDH1A1 and ALDH3A1, in ocular disease and UVR cellular oxidative defense.

1. Properties of UVR Exposure and UVR-Derived ROS

Solar UVR, part of the electromagnetic spectrum that spans 100-400 nm, is considered to be the major environmental stimulus for ROS formation in the eye. UVR consists of UVA (320-400 nm), UVB (290-320 nm) and UVC (220-290 nm). The most genotoxic and damaging of these is the high energy, short wavelength UVC rays because DNA readily absorbs such wavelengths. However, exposure of humans to UVC is minimized due to the protection afforded the Earth's surface by the upper atmosphere, which blocks virtually all wavelengths in this range. The cornea absorbs most UVB, whereas UVA is primarily absorbed by the lens (Figure 1). As such, no UVB and very little UVA (<1%) reach the retina (14). Absorption of UVR by the cornea and lens, particularly the shorter and higher energy wavelengths of UVB, lead to the generation of ROS derived from diatomic oxygen (O_2), including singlet oxygen, superoxide anion and hydroxyl and peroxy radicals (14). While various enzymatic activities produce cellular ROS, the generation of ROS can usually be correlated with cellular metabolic rate as many of these species are produced as byproducts of cellular metabolism (15). Indeed, mitochondrial respiration is primarily responsible for the addition of one electron to dioxygen to form the superoxide anion radical ($O_2^{\bullet-}$) (16). The less reactive species, hydrogen peroxide (H_2O_2), is produced from the superoxide anion radical through dismutation by superoxide dismutase enzymes (17). Both the superoxide anion radical and hydrogen peroxide can also be generated through the activities of various enzymes including cytoplasmic oxidases, such as xanthine oxidase, histamine oxidase, and monoamine oxidase (18). Under normal circumstances, H_2O_2 is scavenged fairly efficiently by various enzymes including glutathione peroxidase (in mitochondria) (19) and catalase (in peroxisomes) (20). However, the breakdown of this molecule by various transition metals, such as Fe^{2+} and Cu^+ *via* the Fenton reaction, generates the highly-reactive hydroxyl radical ($OH^{\bullet}$) (21).

The presence of unpaired electron(s) in free radicals confers high reactivity, which leads to the initiation of reduction-oxidation (redox) reactions with the potential to attack important biomolecules. Cellular redox homeostasis is normally maintained by a delicate balance between ROS generation and antioxidant defense systems. When this balance is disrupted in biological systems, oxidative stress occurs from the overproduction of ROS. Cellular damage resulting from excessive ROS production manifests as a consequence of interference with three major cellular components: 1) cellular membranes, 2) DNA and 3) proteins. In this regard, oxidative stress leads to damage to lipids and DNA and the inhibition and deactivation of proteins with consequent disruption of overall biological function (22). Both the superoxide anion and hydroxyl radical species can initiate the oxidative degradation of cellular membranes in a process known as lipid peroxidation. The

reaction of these radicals with polyunsaturated fatty acids (lipids) in cellular membranes generates peroxy radicals (LOO^{*}) which lead to the downstream production of additional free radicals and over 200 cytotoxic reactive aldehyde species, such as 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) (23). In addition to the cellular membrane, DNA is also a major target of ROS. As the most reactive free radical, the hydroxyl radical can react with a number of DNA components leading to damage to purine and pyrimidine bases and the deoxyribose backbone (24). This type of DNA damage can then initiate the process of DNA mutagenesis and lead to carcinogenesis. All forms of UVR have been shown to cause DNA strand breakage, pyrimidine and thymine dimer formation and protein cross-linking (25;26). The third major target of ROS is proteins, specifically the amino acid residues cysteine and methionine, which are prone to oxidation by ROS. Oxidation of protein sites critical to structural or enzymatic function ultimately results in protein inactivation or misfolding (27). Ocular UVR-induced DNA and protein damage has been associated with a number of eye pathologies, including cataract formation and corneal and retinal degeneration (28). In addition, ocular tissues of patients with certain disease states of the eye, such as inflammation and diabetes where a substantial amount of ROS is produced, are particularly sensitive to oxidative stress (29;30). The prolonged wearing of hydrophilic contact lenses is associated with hypoxia-reoxygenation injury of the cornea, which is due, in part, to xanthine oxidase-mediated ROS formation in the tear film (31).

2. Small Molecule Antioxidants of the Eye

Glutathione (GSH)

GSH is ubiquitously synthesized in all cell types (32). It is an antioxidant tripeptide comprising glutamate, cysteine and glycine. As the most abundant cellular non-protein thiol, the ability of reduced glutathione (GSH) to convert to its oxidized form (GSSG) depending on the oxidative milieu allows it to function as a major cellular redox buffer. Primary functions of GSH include protecting against ROS through the direct scavenging of radicals, including the hydroxyl and superoxide anion radicals, and to act as a cofactor for GSH peroxidases (GPXs) during the metabolism of hydrogen peroxide (to water) or lipid peroxides (to their corresponding alcohol) (Figure 2) (33). In conjunction with the glutathione S-transferase (GST) enzymes, GSH can conjugate with numerous harmful electrophilic endogenous compounds and foreign chemicals, resulting in their detoxification and cellular elimination (34). Moreover, GSH also regenerates important cellular antioxidants, such as vitamins C and E, back to their active forms. Indeed, the cellular redox status of GSH (the ratio of GSH to GSSG) has been suggested to play a key role in various factors determining cellular fate, including proliferation, apoptosis and cellular senescence (35;36). GSH has also been shown to participate in other cellular processes, including the breakdown of nucleotides, the formation of lipid second messengers, the regulation of nitric oxide homeostasis and the post-translational modification of proteins (37). In the cornea, GSH is found in the millimolar range (4-7 mM) and differentially distributed such that highest levels are found in the epithelium (five-fold higher than that in the stroma) (37;38). Glutamate-cysteine ligase (GCL) and glutathione synthetase (GSS), cytosolic enzymes present in the corneal cellular layers, are together responsible for *de novo* GSH (39). Under conditions of oxidative stress, fast turnover of corneal GSH *via* this pathway, in combination with efficient recycling by GSH reductase (GR), are required to counteract free radicals. GSH also plays a pivotal role in maintaining optimum hydration of the cornea (40), protecting the integrity of its cellular membranes and facilitating the degradation of xenobiotics.

Disrupted GSH homeostasis is associated with several corneal diseases in humans, including various viral infections and diabetes (41;42). Rodent models of systemic GSH deficiency have provided evidence to support a protective role of GSH against oxidative damage in

ocular tissues. In mice, disruption of the γ -glutamyl transpeptidase (*Ggt1*) gene, which encodes a key enzyme in GSH re-synthesis, leads to a 90% decrease in GSH levels and the development of cataracts at an earlier age than normal mice (43).

Reduced Nicotinamide-Adenine Dinucleotide Phosphate (NADPH)

NADPH and its oxidized product, NADP⁺, together have the primary function of being an important cellular redox buffer. Cellular levels of NADPH are usually in the micromolar range. Higher levels occur in ocular tissues, perhaps due to the presence of abundant NADPH-dependent enzymes, specifically crystallins, in the cornea and lens (44). Corneal NADPH has been reported to act as an antioxidant through several mechanisms. Firstly, it can serve as the coenzyme of GR in the GPX/GR enzymatic system for the regeneration of GSH from GSSG (45). Secondly, it can act as a direct antioxidant by reducing various radicals generated during oxidative stress, such as glutathyl, tyrosyl and peroxynitrite radicals (46). Thirdly, it can function as a UVR filter (47). Fourthly, it can protect other antioxidant enzymes from ROS-induced inactivation (48). Finally, it can maintain the required reducing potential for various pyridine nucleotide-dependent redox-active enzymes, such as isocitrate dehydrogenase, malic dehydrogenase and 6-phosphate dehydrogenase, which are themselves involved in protecting ocular tissues (49).

Ascorbic Acid

While ascorbate (vitamin C) is known to promote healing of damaged corneal tissues by serving as an essential cofactor for collagen synthesis in corneal fibroblasts, corneal ascorbic acid is also believed to be the most important non-enzymatic antioxidant in the cornea (49-52). It is a strong electron donor with very high reactivity towards a broad spectrum of free radicals, including superoxide anion, hydroxyl and peroxy radicals. Native ascorbate is regenerated from its oxidized product, dehydroascorbate, through the use of GSH or NADPH as electron donors (53). The accumulation of ascorbate into the cornea and lens occurs primarily by active transport through the iris-ciliary body into the aqueous humor with subsequent transport into the cornea and lens (54). In bovine cornea, the highest ascorbate concentration (≈ 1.56 mg/g tissue) was found in the corneal epithelium, particularly in the central region covering the papillary area (55) and lower levels (≈ 0.2 mg/g), although still greater than that of serum, were present in corneal stroma and endothelium. This pattern of corneal distribution, in conjunction with the contribution of corneal fractions to UV absorption, suggests that ascorbate may absorb UVB rays (290-320 nm), conferring protection to internal ocular structures against UVR-induced damage (55). Levels of ascorbate in the corneas of diurnal and nocturnal animals further support an important role of this antioxidant in protecting the eye from UVR and associated stress and tissue damage (56). Higher levels of corneal ascorbate are found in diurnal animals than in nocturnal species (57). Ambient radiation has been suggested to play a major role in sustaining high ascorbate levels in corneal epithelium (58). High concentrations of ascorbic acid are also found in the aqueous humor of diurnal species (59), again consistent with an important role for ascorbic acid in protecting the eye against UVR-mediated damage. Increasing ascorbic acid levels 30-fold in rat aqueous humor has been shown to protect the lens epithelium against UVR-induced DNA damage (60).

Uric acid

Uric acid is a purine metabolite and its antioxidant properties have been documented in extracellular fluids, including the tear film and the aqueous humor (61). The concentration of uric acid in human ocular fluids is only a fraction of the plasma concentration (62;63). This water-soluble molecule serves as a potent scavenger of singlet oxygen and hydroxyl radicals (62;64). In addition, it has been proposed that uric acid regulates the redox state of

the GSH-ascorbate system. In human tears and aqueous humor, uric acid along with ascorbic acid, account for a high percentage of the total antioxidant activity.

L-cysteine and L-tyrosine

In addition to GSH, ascorbic acid and uric acid, other small molecules also contribute to the overall antioxidant capacity within ocular tissues (63;65). The amino acid *L*-tyrosine is electrochemically active and scavenges hydroxyl radicals and singlet oxygen species (63;66). *L*-cysteine replenishes the tissue GSH pool by replenishing the rate-limiting substrate for GSH biosynthesis and acting as a direct antioxidant *via* its thiol group (32).

α -Tocopherol

The fat-soluble antioxidant, α -tocopherol (the most active form of vitamin E), has a unique role in breaking the chain reaction of lipid peroxidation by quenching peroxy radicals (67;68). In addition to helping maintain the integrity of cellular membranes, α -tocopherol can regenerate other antioxidants, including GSH and ascorbate (69). The protective role of vitamin E in ocular tissues is also evidenced by studies demonstrating significant correlation between high plasma α -tocopherol levels and lower prevalence of cataracts (70). Moreover, vitamin E-enriched diets have been reported to mitigate UVR-induced damage in both the cornea and lens (71).

Retinol

While retinol (vitamin A) is probably best known for its physiological role in preserving normal vision, this vitamin also appears to act as an ocular antioxidant by lessening the effects of oxidative stress and preventing apoptosis in corneal endothelial cells. Vitamin A supplementation in cell culture medium confers considerable protection on murine corneal endothelial cells against iron-induced lipid peroxidation and oxidative damage, an effect measurably greater than that of vitamin C and vitamin E combined (72). The mechanism underlying the antioxidant role of vitamin A is believed to involve quenching of ROS, neutralization of radicals and stabilization of peroxy radicals (73).

3. Protein and Enzymatic Antioxidants of the Eye

Albumin

Aside from functioning as a binding and transporting protein, albumin, a major corneal protein, acts as an antioxidant by scavenging hydrogen peroxide (74). In rat cornea, hydrogen peroxide readily oxidizes albumin, suggesting the protein may act as a native antioxidant, scavenging hydrogen peroxide and preventing further damage by this ROS (75). Serum albumin diffuses from peripheral blood vessels present around the cornea and is distributed exclusively in the corneal stromal layer. Levels of albumin at 12-25% of total water soluble proteins have been reported in mammalian corneas (74).

Ferritin

Free iron, in conjunction with UVR-induced ROS, generates the hydroxyl radicals through the Fenton Reaction. Hydroxyl radicals are highly reactive species which trigger lipid peroxidation and ocular tissue injury. Lifetime exposure of ocular tissues to UVR makes it crucial that the level of free iron be tightly regulated and kept at a low level. The iron binding protein, ferritin, is responsible for sequestering iron in the cytoplasm and nucleus in a non-toxic form, thus acting as an indirect antioxidant in the prevention of Fenton reaction-derived ROS (26;76). Indeed, the antioxidant function of nuclear ferritin in corneal epithelial cells has been suggested to be protective against UVR-induced DNA damage (77).

Superoxide Dismutases

SODs (EC 1.15.1.1) catalyze the conversion of the superoxide anion radical to the less reactive hydrogen peroxide (78). Three different biologically-functional SOD isozymes are present in the corneal epithelial and endothelial layers of various species, including humans (79). CuZn-SOD, commonly known as SOD-1, is a cytosolic SOD containing copper-zinc. Mn-SOD, commonly known as SOD-2, is a mitochondrial SOD containing manganese (80). EC-SOD, commonly known as SOD-3, is an extracellular SOD containing copper-zinc (81).

SOD activity appears to be species-dependent, ranging from high levels in rabbits and guinea pigs to low levels in pigs and almost absent from cattle (52). In human corneas, comparable levels of activity have been reported for both EC-SOD and CuZn-SOD, while Mn-SOD had the lowest activity levels among the three (81). Transgenic mice made deficient in SOD1, SOD2 or SOD3 (82-85) exhibit distinct eye phenotypes, indicating differential roles for the respective SOD isozymes of the eye. In particular, the protective role of corneal extracellular SOD against ROS is evident in SOD3 knockout animals, which show increased spontaneous age-related loss of corneal endothelial cells and increased susceptibility to oxidative stress and inflammatory damage (82). SOD1 knockout mice are sensitive to diabetes-induced cataract formation (85) and SOD2 knockout mice show retinal pathologies before they die by 2.5 weeks (83). To date, SOD isozymes are the only reported antioxidant enzymes in the tear film. SOD activity of all three isozymes has also been reported in the aqueous humor, although levels are such that this activity does not appear to contribute significantly to the antioxidant defense of this ocular compartment (81).

Glutathione Peroxidases and Glutathione Reductase

GPXs (EC 1.11.1.9) compose a family of enzymes that catalyze the reduction of hydrogen peroxide or organic hydroperoxides to water or corresponding alcohols using glutathione (GSH) as the electron donor (19;82). In doing so, GPXs serve to terminate the peroxide-dependent chain reaction of free radical generation, thus protecting the cellular membranes of ocular tissues (86). GR functions to maintain levels of GSH by recovering reduced GSH from its oxidized form (GSSG). Corneal GR activity has been reported to be elevated in inflamed corneas, suggesting a potential role of this enzyme in mitigating the effects of inflammation-induced ROS production (87). Both corneal epithelial and endothelial cell layers express GPX and GR (40;88). A deficiency of GPX1 in transgenic mouse models results in increased oxidative damage and associated pathology in the lens and retinas (89); however, no corneal pathology is seen. Transgenic mice devoid of GPX1 show progressive lens pathologies with age and develop mature cataracts after 15 months (89). A role of GR in the corneal defense against UVR-induced oxidative stress is further supported by studies that demonstrate enhanced GR activity in mammalian corneas after exposure to UVA or a small dose of UVC (90).

Catalase

Catalase (CAT) (EC 1.11.1.6), a corneal enzyme present outside the mitochondria in both corneal epithelium and endothelium, is an important scavenger of hydrogen peroxide (87;91). Not only does CAT protect ocular tissues against hydrogen peroxide, this enzyme also serves to protect SODs from hydrogen peroxide-induced inactivation (92). In corneal cell cultures, over-expression of CAT confers protection against ROS-induced injury, while inhibition of CAT confers susceptibility to these toxic species (93;94). While transgenic mice made devoid of CAT display increased sensitivity to ROS-mediated tissue injuries, no eye pathology has been reported in these animals (95).

Glucose-6-phosphate Dehydrogenase

Corneal glucose -6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49) is responsible for maintaining the cellular reductive potential in the form of NADPH through the pentose phosphate pathway (96). This enzyme supplies NADPH as the electron donor for GR-mediated reduction of GSSG to GSH and, consequently, acts to balance the cellular pool of GSH. Nevertheless, no eye pathologies have been reported in human subjects or rodent models with G6PD deficiencies (97;98).

4. Aldehyde Dehydrogenase Enzymes as Ocular Antioxidants

ALDH3A1 (EC 1.2.1.5) and ALDH1A1 (EC 1.2.1.36) belong to a superfamily of NAD(P)⁺-dependent enzymes that catalyze the oxidation of a wide variety of endogenous and exogenous aldehydes to their corresponding carboxylic acids (99). These enzymes are considered to be “corneal and lens crystallins” and are believed to contribute to the transparent and refractory properties of these ocular tissues (100). In addition to serving in such structural roles, ALDH3A1 and ALDH1A1 confer protection against lens oxidative damage and cataract formation associated with UVR exposure in rodent models (12). Similarly, decreased ALDH3A1 activity is associated with pathologic corneas (101). Consistent with an antioxidant role for ALDH3A1 is the observation that overexpression of human ALDH3A1 prevents GSH depletion caused by oxidative agents in rabbit corneal fibroblast cells and protects human corneal epithelial cells from oxidant-induced DNA damage (102;103). Given the abundant expression of these enzymes in the cornea and lens, it is believed that ALDH3A1 and ALDH1A1 play a key role in protecting the eye from UV-induced damage and this may occur through several enzymatic and non-enzymatic mechanisms (104) including: 1) the direct absorption of UVR, 2) acting as antioxidants by directly scavenging UVR-induced free radical ROS, 3) metabolism of toxic aldehydes produced by UVR-induced lipid peroxidation or 4) by producing the antioxidant NADPH (Figure 2).

Mouse models of ALDH3A1 and ALDH1A1 deficiency are viable and such animals display ocular aberrations. The SWR/J strain of mice harbor spontaneous mutations in the *Aldh3a1* allele, which result in amino acid substitutions and consequently loss of ALDH3A1 enzymatic activity; these mice with ‘low activity’ ALDH3A1 are more susceptible to corneal clouding and opacification after UVR exposure (105). In a recent study from our laboratory using transgenic *Aldh3a1*^{−/−} and *Aldh1a1*^{−/−} single and *Aldh3a1/Aldh1a1*^{−/−} double knockout mouse models (12) indicates that: (1) while ALDH3A1 is not detectable in the mouse lens, ALDH3A1 in the cornea may actually serve a critical role in the protection of the lens against environmentally-induced oxidative damage; and (2) both ALDH3A1 and ALDH1A1 are instrumental and have additive functions in protecting the lens against cataract formation. Absence of ALDH1A1 or ALDH3A1 rendered mice highly vulnerable to lens cataracts, with the absence of ALDH3A1 having a more profound effect. Mice devoid of both ALDH1A1 and ALDH3A1 were much more likely to develop cataracts than wild-type animals and at an earlier age. Cataract formation in these double knockout mice was associated with increased corneal and lens levels of HNE- and MDA- adducted proteins and increased protein carbonyl content, a potentially predictable result given the capacity of ALDH3A1 and ALDH1A1 to detoxify lipid peroxidation-derived aldehydes, such as HNE and MDA (Figure 2) (106;107). Aldehydes are generally long-lived compounds that can diffuse to sites some distance from their origin (23). Accordingly, aldehydes accumulating in the cornea as a consequence of ALDH3A1 deficiency may diffuse into the lens. Lens opacification is known to result from aggregation, cross-linking and denaturation of proteins, such as may occur with aldehyde-mediated adduction of proteins. Rapid metabolism of aldehydes by ALDH3A1 and ALDH1A1 would, therefore, be predicted to prevent such opacification (12). Mice devoid of ALDH1A1 and ALDJ3A1 also display

decreased proteasome function relative to wild-type mice. Proper proteasome function is critical for the removal of damaged proteins and the prevention of protein aggregation. Indeed, impaired proteasome function is associated with cataract formation (108). Interestingly, mice devoid of ALDH1A1 and/or ALDH3A1 all show increased levels of GSH that correlate with increased γ -glutamylcysteine synthase (GCS) content in the lens and cornea, with the most significant increase occurring in mice missing both isozymes (12). These results are not surprising given that cellular adaptation to oxidative stress often involves the upregulation of GSH synthesis. Considering that ALDH3A1 is not present in the lens, the primary role of corneal ALDH3A1 in protecting the lens is most likely due to its ability to act as a UVR-filter by absorbing light and thus blunting the amount of potentially damaging UVR that reaches the lens (13). On the other hand, the primary role of ALDH1A1 in protecting the lens against cataract formation is most likely the detoxification of reactive aldehydes in both the cornea and lens (12). In support of this notion, chloroquine, an agent known to bind and inhibit ALDH1A1 (109), causes distinctive anterior cataracts in rats (110). Taken together, mouse models in which levels of ALDH1A1 and ALDH3A1 have been manipulated, have proven instrumental in elucidating the role of ALDH3A1 and ALDH1A1 in protecting the tissues of the eye.

Concluding Remarks

Ocular tissues of the eye including the ocular surface and inner ocular tissues are replete with water-soluble and small molecule antioxidants as well as non-enzymatic and enzymatic antioxidants, all of which enrich the defense mechanisms of the cornea against UVR-induced oxidative stress. The number and diversity of antioxidants with overlapping and redundant functions helps ensure that UVR-induced oxidative stress is effectively and efficiently mitigated. Such redundancy of these antioxidant systems likely explains why a deficiency in a particular antioxidant is not always associated with eye pathology. On the other hand, some ocular surface antioxidants, such as the SOD and ALDH enzymes, may have unique roles that are less easily compensated for by other antioxidants. This may explain the aberrant ocular phenotypes resulting from their genetic disruption. Future studies aimed at understanding the interplay between small molecule, protein and enzymatic antioxidants and the mechanisms underlying these cellular protective systems will provide valuable information about the pathogenesis of ocular diseases associated with UVR and potential targets for therapeutic exploitation.

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Abbreviations

UVR	Ultraviolet radiation
ALDH	aldehyde dehydrogenase
ROS	reactive oxygen species
O₂	diatomic oxygen
O₂^{-•}	superoxide anion radical
H₂O₂	hydrogen peroxide
OH[•]	hydroxyl radical
redox	reduction-oxidation
HNE	4-hydroxy-2-nonenal
MDA	malondialdehyde
GSH	glutathione
GSH	reduced glutathione
GSSG	oxidized glutathione
GPXs	glutathione peroxidases
GSTs	glutathione-S-transferases
GCL	glutamate-cysteine ligase
GSS	glutathione synthetase
GR	glutathione reductase
GGT	gamma-glutamyl transpeptidase
SOD	superoxide dismutase
G6PD	glucose-6-phosphate dehydrogenase
GCS	gamma-glutamylcysteine synthase

Figure 1. The absorption of solar ultraviolet radiation by the eye

Solar ultraviolet radiation (UVR) consists of UVA (320-400 nm), UVB (290-320 nm) and UVC (220-290 nm). UVC rays are precluded from reaching the Earth's surface by the upper atmosphere. The longer wavelengths of UVA and UVB pass through the ozone layer and consist of >90% of the solar UV that reaches the Earth's surface. The ocular surface, composed of the tear film, cornea and aqueous humor, form the first line of defense against UVR while inner ocular structures, such as the lens, serve as additional physical barriers to UVR exposure. The cornea absorbs a high fraction of the UVB rays that reach the ocular surface with the remaining percentage being absorbed by the lens. UVA is primarily absorbed by the lens. No UVB and very little UVA (<1%) reach the retina.

Figure 2. The antioxidant roles of ALDH1A1 and ALDH3A1 in the cornea and lens

Evidence suggests that the aldehyde dehydrogenase enzymes, ALDH1A1 and ALDH3A1, have multiple enzymatic and non-enzymatic antioxidant roles in ocular tissues that protect these structures from the damaging effects of UVR. ALDH3A1 and ALDH1A1 may function to directly absorb UVR (1), scavenge UVR-generated ROS free radicals (2), detoxify downstream oxidative stress and lipid peroxidation-derived reactive aldehyde species (3) and regenerate NADPH from NADP⁺, thus contributing to the cellular GSH antioxidant pool (4).