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MINI REVIEW

Glutathione-Related Enzymes and the Eye

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ABSTRACT Glutathione and the related enzymes belong to the defence system protecting the eye against chemical and oxidative stress. This review focuses on GSH and two key enzymes, glutathione reductase and glucose-6-phosphate dehydrogenase in lens, cornea, and retina. Lens contains a high concentration of reduced glutathione, which maintains the thiol groups in the reduced form. These contribute to lens complete transparency as well as to the transparent and refractive properties of the mammalian cornea, which are essential for proper image formation on the retina. In cornea, gluthatione also plays an important role in maintaining normal hydration level, and in protecting cellular membrane integrity. In retina, glutathione is distributed in the different types of retinal cells. Intracellular enzyme, glutathione reductase, involved in reducing the oxidized glutathione has been found at highest activity in human and primate lenses, as compared to other species. Besides the enzymes directly involved in maintaining the normal redox status of the cell, glucose-6-phosphate dehydrogenase which catalyzes the first reaction of the pentose phosphate pathway, plays a key role in protection of the eye against reactive exygen species. Cornea has a high activity of the pentose phosphate pathway and glucose-6-phosphate dehydrogenase activity. Glycation, the non-enzymic reaction between a free amino group in proteins and a reducing sugar, slowly inactivates gluthathione-related and other enzymes. In addition, glutathione can be also glycated. The presence of glutathione, and of the related enzymes has been also reported in other parts of the eye, such as ciliary body and trabecular meshwork, suggesting that the same enzyme systems are present in all tissues of the eye to generate NADPH and to maintain gluthatione in the reduced form. Changes of glutathione and related enzymes activity in lens, cornea, retina and other eye tissues, occur with ageing, cataract, diabetes, irradiation and administration of some drugs.

KEYWORDS glutathione; glucose-6-phosphate dehydrogenase; glutathione reductase

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INTRODUCTION

Enzymaticand nonenzymatic defense systems protect the eye against external or internal chemical stress, which is involved in various degenerative diseases. Glutathione (γ-glutamyl-cysteinyl-glycine; GSH) is involved in many cellular functions including scavenging free radicals and other reactive species, regulation of DNA and protein synthesis, signal transduction, cell-cycle regulation,

proteolysis, immune response and cytokines, as well as in various metabolic pathways.[1,2] The synthesis of GSH from its constituent amino acids requires ATP and it is catalyzed in the first step by γ-glutamylcysteine synthetase (GCS), a rate-limiting enzyme, and by GSH synthetase in the second step. GCS activity, cysteine availability, and GSH feedback inhibition are the main factors regulating GSH synthesis. The decreased GSH synthesis, as well as its use in detoxification, the increased breakdown, or the failure to regenerate GSH from the oxidized form GSSG induces lowered GSH levels, contributing to oxidative stress, which is involved in the pathogenesis of many diseases.

Glutathione-dependent enzymes glutathione peroxidase (GSH-Px) and glutathione reductase (GSH-R), as well as the NADPH producing enzymes G6PD (glucose-6-phosphate dehydrogenase) and 6-phosphogluconate dehydrogenase (6PGD) are part of the mechanism of defense against oxidation. Other enzymes such as superoxide dismutases (Cu-, Zn-, or Mn-dependent SODs) and catalase are also very important for protection against reactive oxygen species.

GSH-Px reduces H_2O_2 to water or alkyl peroxides to alcohols, at the expense of reduced glutathione. GSH oxidation provides therefore an important way of protection against endogenous peroxides. The oxidized glutathione GSSG is reduced back to GSH by a NADPH-dependent reductase, glutathione reductase (GR) (Fig. 1). GR is important not only for the maintaining the required GSH level but also for reducing protein thiols to their native state.

We have focused on GSH and two enzymes, GR and G6PD, in lens, cornea, and retina in this review. Other aspects of GSH-related enzymes in the eye were covered in recent reviews.[3,4]

LENS

The eye lens has very special properties, one being the complete transparency in spite of the high protein content, and another one the high thiol content (glutathione-SH and protein-SH). It has been assumed that the reaction of the exposed thiol groups in proteins with other thiol groups to form disulfide bonds would lead to cross-linking and aggregation. Lens contains a high concentration of reduced glutathione (GSH), which maintains the thiol groups in the reduced form. Decreased GSH levels have been reported in human lens with age[5] and in human cataract lenses,[6] especially in diabetic cataract lenses.[7] One cause of the lower glutathione level could be a decreased GSH synthesis, as the activity of glutathione synthesis enzymes GCS and GS has been found lower in old clear human lenses[8] and in human subcapsular cataract[9] than in normal young lenses. Age-related deficiency in γ-cystathionase

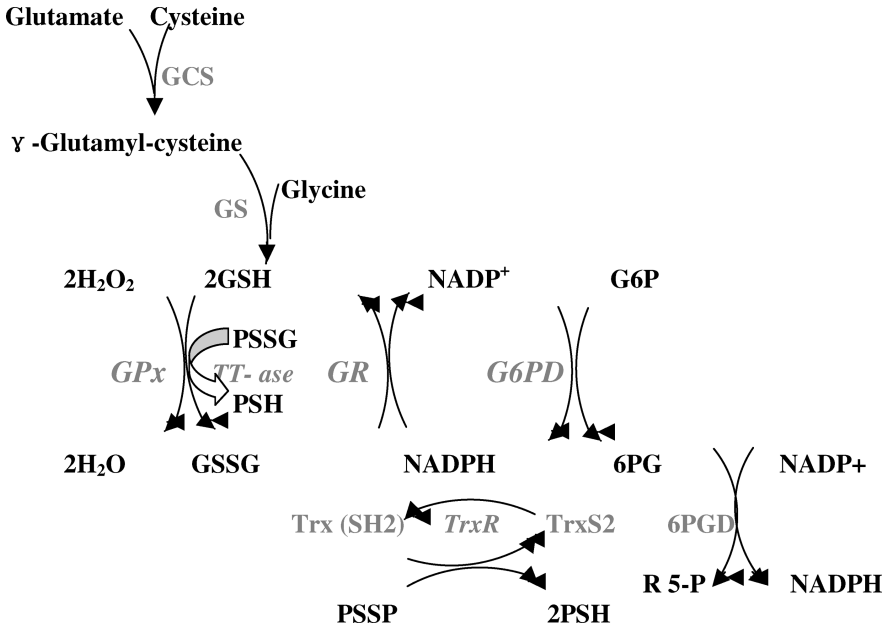

FIGURE 1 **Enzymes involved in glutathione synthesis and metabolism. GCS, gamma-glutamyl-cysteine synthetase; GS, glutathione synthetase; GPx, glutathione peroxidase; GR, glutathione reductase; G6PD, glucose-6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; R 5-P, ribulose 5-phosphate; TT-ase, thiol transferase; TrxR, thioredoxin reductase; Trx (SH2), thioredoxin; TrxS2, oxidized thioredoxin; PSSG, protein-S-S-glutathione; PSSP, protein-S-S-protein; GSSG, oxidized glutathione; PSH, protein-SH.**

TABLE 1 **Changes in Lens Glutathione Reductase Activity**

Condition/ Disease	Activity	Species	Reference
Aging	Decreased activity, but no effect on gene expression	Rat	14, 15
Cataract	Fall of mRNA level and enzyme activity	Rat	16
		Human	5
Senile cataract	Significant decreased activity	Human	6, 17
Diabetes	significant decreased activity	Rat	18
UV- AB irradiation	Decreased activity and LEC density	Rat	19
UV-A irradiation	70% enzyme inactivation	Human	20, 21

LEC, lens epithelial cell.

activity, essential for cysteine synthesis from methionine, through the trans-sulfuration pathway could also contribute to a decreased GSH level in the lens.[10]

The failure to regenerate GSH from GSSG via glutathione reductase and NADPH may be another cause of lowered glutathione levels. A decrease in GR activity has been found parallel with GSH loss in patients with senile cataract.[11] The intracellular flavoenzyme glutathione reductase (EC 1.6.4.2) has been found in high levels in eye tissues, its expression in the lens epithelium being detected from prenatal to early postnatal stages.[12] In lens, the specific activity of the enzyme decreases gradually from the epithelium, toward the center of the lens, and this distribution is common for various species studied so far, although there are marked differences between activity levels.[13] It has been found that human and primate lenses have the highest GR activity; in primate lens GR activity was 10 times higher than in other species and decreased with age[14,15] (Table 1). However, the lowered activity with advancing age was not accompanied by a change of the mRNA level for GR. It has been reported that some of the activity was recovered by *in vitro* addition of flavin adenine dinucleotide (FAD), the prosthetic part of enzyme.[15] Studies on GR activity in concentric layers of lens, in human and in various species, as a function of age suggested that the decrease in activity is related to the maturation of fiber cells, and not to the aging.[13]

A decrease in GR activity occurs in human cataract and in most experimental cataract.[22] Although a severe loss of activity has been found in RCS rat lenses, it is not clear if this is a cause of cataract or an effect of it.[23]

Diabetic rats develop cataract, an opacification of the lens, which is insulin independent. Glutathione reductase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase decreased significantly in lenses from diabetic rats.[18] Aspirin protected against cataract in diabetic rats and against lens opacification *in vitro*.[24]

There is increasing evidence that products of the Maillard reaction may play an important role in the etiology of diabetes and age-related cataract. Advanced glycation end products (AGEs) accumulate in the intracellular and/or extracellular environment of ocular structures, contributing to the development of diseases such as diabetic retinopathy, age-related macular degeneration, and cataract formation.[25] The *in vitro* experiments demonstrate that glycation alters the enzyme activity in much shorter times (days, hours) than necessary for AGE formation. Glutathione reductase loses almost half of the activity after 5 days of incubation with low glucose concentration (5 mM), and almost 80% of initial activity after 15 days.[26] The inactivation was prevented by the molecular chaperone α-crystallin,[27] suggesting that the high content of α-crystallin in vertebrate lenses may help to maintain lens transparency.

The effects of eye lens irradiation, especially by UV radiation, are reported by various studies, but the interaction of UV light with glutathione-related lens enzymes is not entirely elucidated. UV-AB irradiation of rat pups, for various time intervals, induced biochemical and morphological changes, a decrease in the soluble thiols, and a decrease in the activity of antioxidant enzymes, GR included.[19] It was suggested that lens epithelial cells (LECs) became vulnerable to oxidative stress because of the decreased activity of glutathione reductase. There are also data indicating an inactivation of GR caused by UV-A irradiation of lenses. A loss of GR activity was found both in the water-soluble fraction[20] and in intact human or fetal calf lenses[21] exposed to UV-A radiation. The inactivation mechanism is not known, but it has been suggested that the prosthetic group of GR, flavine adenine dinucleotide (FAD), which is located in the neighborhood of a redox active disulfide, in the active center of the enzyme might be involved. Further studies would be necessary to understand these mechanisms, especially if one takes into account that it is generally accepted that UV-A radiations are absorbed by the cornea; besides, it has been recently shown that the *in vivo* effect of UV-A found in the intact

lens changes by homogenization of the lens,[28] indicating some differences between the *in vivo* conditions and the experimental ones.

Besides the enzymes directly involved in maintaining the normal redox status of the cell, glucose-6-phosphate dehydrogenase (G6PD) plays a key role in protection against reactive oxygen species. G6PD catalyzes the first reaction of the pentose phosphate pathway metabolizing 10–20% of the lens glucose.[29] It converts glucose-6-phosphate to 6-phosphoglucono-δ lactone by reducing $NADP^+$ to NADPH. A second molecule of NADPH is generated in the next step of the pathway, catalyzed by another enzyme, 6-phosphogluconate dehydrogenase (6PGD), as shown in the following reactions:

glucose-6-phosphate + $NADP^+$ G6PD 6
-phosphoglucono-δ lactone + NADPH + H^+

6-phosphoglucono-δ lactone + H_2O lactonase 6
-phosphogluconate + H^+

6-phosphogluconate + $NADP^+$ 6PGD ribulose 5
-phosphate + NADPH + CO_2

By these reactions, the cellular level of NADPH required for biosynthetic processes and protection against oxidative damage in the lens is sufficiently maintained. Under conditions of oxidative stress, the pentose phosphate pathway can increase in order to supply a higher amount of NADPH needed by the glutathione protective system[30]; a decreased efficiency of the pentose phosphate pathway can make the lens more vulnerable to oxidative injury (Table 2).

The specific activity of glucose-6-phosphate dehydrogenase declines with age in the whole rat lens,[11,31] although there are data showing that the decrease is not significant.[32] The decline in activity occurs sharply with age in some animals, for example, bovine lens, whereas in guinea pig lens the activity decreases slowly.[33] Interestingly, NADPH level in guinea pig lens is about 50-times higher than in other lenses.[34] In humans it has been reported that the average enzyme activity per normal lens is not age dependent[32]; however, the specific activity showed a tendency to decrease between the ages of 21 and 50 years, and the K_m values for G6P substrate increased correspondingly. The analysis of G6PD activity separately in cortex and nucleus shows that although synthesis of the enzyme continues throughout life at about the same rate, the activity falls in the nucleus.[32] It seems that with age, inactivated enzyme accumulates in nucleus and is not removed by various proteases present in lens cortex but absent from nucleus.[22]

The enzyme responsible for supplying the second NADPH molecule by the pentose phosphate pathway, 6-phosphogluconate dehydrogenase (6PGD), shows a lower activity in the older cortex than in the whole young lens, suggesting that unlike G6PD, its synthesis may decrease with age. Again, the specific activity is lower in the lens nucleus, indicating a slow inactivation of the enzyme in that region.[32]

Various metabolic changes have been found in cataract, and oxidative stress has been considered as a primary event in this process. Significant decreases in the activity of glucose-6-phosphate dehydrogenase, and of other glutathione-related enzymes (GR and GPx), as well as a decreased glutathione level were found in cataractous lenses.[35] However, at different stages of the disease progression or in certain types of cataract, the amplitude or the direction of changes in G6PD activity could be different. For example, decreased activity of G6PD, 6PGD, and GR occur at later stages on the way from early to mature cataract formation, suggesting

TABLE 2 **Changes of the Lens Glucose 6-Phosphate Dehydrogenase Activity**

Condition	Species	Changes	References
Age	Rat lenses	Significant age-related decrease	11
	Sheep, rat, human	Not significant change with age	32
Cataract	Human	Significant depletion in G6PD activity	32, 35
		lenticular G6PD activity decreases:	
Diabetes	Humans and rats with alloxan-induced diabetes	Steeply in humans gradually in rats	36
Drugs	Cultured bovine lenses	Severe, irreversible activity reduction	37
Irradiation	UV-B irradiated rats lens homogenate	Reduced specific activities of G6PD and 6PGD	38
G6PD deficiency	Human	Not significant differences	39
	Human male; human female	Undetectable G6PD activity; not significantly different	40 22

that they are a consequence of the lens injury and not a cause of it.[22]

In patients with anterior subcapsular cataract and in those with mixed cataract, the specific activity of lenticular G6PD was lower than in control matched for age.[41] G6PD activity appeared to increase in the cortical region of brunescent lens as compared with the yellow lens, but this may be due to decreased solubility of the protein.[4] GSH level decreased both in cortical and nuclear region without accumulation of GSSG. The results might suggest a stimulation of the pentose phosphate pathway under increased oxidative stress conditions, but it seems that the defense capacity of the glutathione redox system was overcome by the intensity of stress. Unlike G6PD, the activity of another important oxidant scavenger enzyme, GPx, decreased under the same conditions.[6] Of course other authors find the activity of G6PD lower in cataracts compared with normal.[32]

Diabetes-induced cataract has been studied for quite a long time, but the interest in changes to enzymes of glutathione metabolism in diabetes and the effect on the development of the mature cataract has increased recently. The activity of the enzyme glucose-6-phosphate dehydrogenase was found significantly diminished in diabetic patients.[36] Human cataractous lenses showed a sharp decrease in glucose-6-phosphate dehydrogenase activity, whereas the rat lenses with alloxan-induced diabetes showed a gradual decrease of this enzyme with the prolongation of diabetes (Table 2). There are various explanations regarding the connection between diabetes and oxidative stress, one of them being glycation. Glycation, the nonenzymatic reaction between a free amino group in proteins and a reducing sugar, slowly inactivates the enzymes, as demonstrated for G6PD and GR, among other enzymes.[26,42] Recently it has been shown that glutathione can be glycated.[43] GSH concentration in diabetic lens is significantly decreased and the glucose concentration much higher than normal, which may favor the formation of Amadori products of the different forms of glutathione, contributing to a decrease of glutathione level and to augmentation of oxidative stress present in diabetic lens. Glutathione can prevent glycation *in vitro* so lower levels would be part of a positive feedback system of loss of function.[44,45]

In both the aged and diabetic lens, advanced glycation end products (AGEs) formation of various molecules have been shown to be markedly elevated, indicating the involvement of the later Maillard compounds in cataract formation. Glycation of lens crystallins induces significant age-related alterations, leading to aggregation and covalent cross-linking, and finally to cataract formation.[46] A comparative study on blood and lens antioxidant status in normal, senile, and diabetic cataract patients found G6PD and GR activities in blood lower in both pathological groups than in the control and lower in diabetic than in senile cataracts, whereas in lens both enzymes show higher activity in diabetes than in senile cataract.[7] We suppose that the presence in the lens of the molecular chaperone α-crystallin could contribute to a partial protection of enzymes against inactivation. This is based on our previous data that demonstrated the specific protection by α-crystallin of G6PD and GR against glycation-induced inactivation.[27,42] α-Crystallin also protected G6PD against MDA, a common marker of lipid peroxidation,[47] and protected 6PGD against inactivation by carbamylation,[48] a modification that can damage enzymes and other proteins and may play a role in the complications in patients with chronic renal failure (CFR). These patients have an increased risk of cataract formation.[49]

In other circumstances, such as pharmaceutical damage induced by topical anesthetics (Alcaine, Fluress, and Fluoracaine), epithelial G6PD activity falls considerably and does not recover as the optical quality of the lens recovers.[37] UV-B radiation exerts oxidative damage on the lens, inhibits lens enzymes, and induces cataractogenesis. α-Crystallin prevented the loss of G6PD activity from the irradiated lens and protected the enzyme against inactivation.[50] In addition to protection by α-crystallin, we found that small organic stress molecules (SOS molecules, often called chemical chaperones) were able to protect G6PD against glycation and even promote its renaturation after inactivation both by glycation and by guanidine.[51]

Unlike the data concerning changes of G6PD in various forms of cataract, there are studies on G6PD deficiency, a genetic disease occurring mainly in Mediterranean countries. It seems there is a relationship between the erythrocyte G6PD deficiency and cataract,[22,40] but there are also data indicating that G6PD deficiency in general is not a factor in cataractogenesis.[39,52]

It is apparent that decreased G6PD may play a role in human cataract whether its decrease is caused by a genetic defect, by post-translational modification of the enzyme, or in some other way. Very recently we have

shown that some activity of GR can be revived by reducing agents and, at least for GR, by α-crystallin.[53] This revivial of activity lost during the slow progression of cataract shows a possible way to protect the enzymes against real *in vivo* insults.

CORNEA

The transparent and refractive properties of the mammalian cornea are essential for proper image formation on the retina. Simultaneously, cornea functions as a protective barrier for the eye. Because the corneal surface comes in direct contact with various kinds of environmental stress, multiple defense systems are required to reduce chemical and oxidative stress, to confer mechanical strength, and provide an immunologic surveillance system. In cornea, GSH plays an important role in maintaining normal hydration level, in protecting cellular membrane integrity, and degrading xenobiotic agents. When the cornea is under oxidative stress (e.g., in the presence of H_2O_2), a rapid turnover of endothelial GSH via glutathione reductase and the hexose monophosphate shunt is required. However, unlike the rat lens, the synthesis of glutathione in rat cornea forms a minor portion of the L-cysteine metabolic products.[54]

Under conditions of less stress, partially inhibited GR can supply the reduced need for GSH to counteract the oxidative stress.[55] At low aqueous humor concentrations of hydrogen peroxide, the GSH redox system seems to be more important than catalase in maintaining the integrity of the corneal endothelium, while catalase assumes greater importance at higher peroxide concentrations.[56] Various diseases or disorders affecting cornea are accompanied by alterations of the corneal redox state (Table 3). In rabbits with herpes simplex 1–induced keratitis, a significant loss of GSH and no increase in the oxidized glutathione has been found. Corneal GSH level was partially recovered, and the virus titer decreased after topical administration of GSH.[57]

TABLE 3 **Deficiencies of the Corneal Redox State**

Compound	Species	Condition	Reference
GSH	Rabbit	HSV-1–induced keratitis	57
GSH	Guinea pigs	Galactosemia	58
GR	Human	Keratoconus	59, 60
GR	Human	Other corneal diseases	60
GR	Human	Excimer laser Keratectomy	61
G6PDH	Pig	UV-A and UV-C light	62
G6PDH	Rabbit	Alkali-burnt cornea	63

Glycation has an important role in altering corneal biochemistry during diabetes and ageing. Corneal layers have been found glycated in diabetic patients, and AGEs detected, which correlate with morphological alterations in human cornea; age-related cross-linking occurs largely on the collagen component of the cornea (stroma and lamina).[46] The presence of an imbalance in the redox system under such circumstances has been proved by the decreased GSH level in galactose-fed guinea pigs, as well as a reduced level and cellular uptake of GSH by cornea with age.[58] Glutathione reductase, the enzyme responsible for maintaining glutathione in the reduced state (Fig. 1), and also involved in the control of corneal hydration,[55] plays an important role in protecting cornea against active oxygen species.[64] In a group of corneal diseases (herpetic keratitis, secondary endothelial decompensation, macula cornea), most of them deriving from inflammatory corneas, GR activity was significantly elevated as compared with normal corneas.[59] It has been supposed that GR activity may increase to combat active oxygen species produced by phagocytic keratocytes during the respiratory burst. Recent data support the hypothesis that keratoconus cornea undergoes oxidative stress and tissue degradation.[60] However, GR activity in keratoconus corneal epithelial extracts was not statistically significantly higher than in normal corneas.[60] Alteration of glutathione-related enzyme activities was found in the excimer laser keratectomy of cornea, a treatment that may initiate free-radical formation. Twenty-four hours after treatment, corneal glutathione reductase and glutathione peroxidase activities significantly decreased in a group receiving deep traditional photorefractive keratectomy (PRK).[61] It has been suggested that free radical–mediated complications may be reduced by using antioxidants.

Cornea has a high activity of the pentose phosphate pathway; glucose-6-phosphate dehydrogenase activity studied during corneal epithelial growth and cell differentiation increased up to 150-fold when corneal epithelial cells constituted a differentiated four- to five-layered epithelium.[65] G6PD activity may play an important role in corneal antioxidant defense against UV-induced oxidative stress. In fresh porcine corneas exposed to UV-A or a small dose of UV-C, G6PD activity was significantly enhanced, but large doses of UV-C appear to

damage the pentose phosphate pathway.[62] (Table 3). Under damaging conditions, such as alkali-burnt cornea, glucose-6-phosphate dehydrogenase activity was significantly decreased in epithelium and was absent in stroma.[63]

RETINA

The retina is a part of the central nervous system, and its metabolism is very complex; it is one of the most vascularized tissues in the body and has one of the highest oxidative metabolic rates per tissue weight. Factors such as high oxygen flux, light, and polyunsaturated fatty acids induce a high susceptibility of retina to oxidation. A complex defense mechanism to protect against oxidative stress has been described in retina. The major water-soluble antioxidant is glutathione (GSH), as in the rest of the eye. It functions primarily in the cytoplasm and mitochondria. The enzymes glutathione reductase (GR), involved in reducing the oxidized glutathione (GSSG) to GSH by NADPH-dependent pathway, and glucose-6-phosphate dehydrogenase, a key enzyme of the pentose phosphate pathway (Fig. 1), are present in rat retina, rat rod outer segments, bovine rod outer segments, and cultured human RPE cells.[12,66] The distribution of the glutathione antioxidant system in the different types of retinal cells is presented in Table 4. In addition to GSH, retina cells also contain water-soluble defense enzymes, lipid-soluble antioxidants, and melatonin, a pineal gland hormone, present in retina as well, which acts as an antioxidant and stimulates antioxidative enzymes.[73]

These systems protect retina against various types of stress, such as oxygen, light or other stimulators of the oxidative stress, but when their capacity for protection can be overcome by the intensity and length of the unfavorable conditions, changes of the tissue may occur. The great vulnerability of the retina to oxidative stress suggests that various retinal diseases involve oxidative damage of cells (Table 5).

The reduced enzymatic activity of glutathione reductase (GR) in diabetic rat retina, and of other glutathione-related enzymes, such as glutathione peroxidase, glutathione transferase, and of superoxide dismutase, as well as an increased lipid peroxidation,[74] indicate an increased level of the oxidative stress that may play an important role in the development of diabetic retinopathy. However, retinal glutathione level in the early stages of diabetes has been found similar to control, and no effect on the glutathione synthesizing enzymes, glutathione synthetase (GSHS) and gamma-glutamyl cysteine synthetase (GCS) (Fig. 1), has been detected in diabetes and experimental galactosaemia (Table 5). It has been reported that increased AGEs in diabetes could play an important role in retinal capillary cell apoptosis and that oxidative stress is involved in this process.[85] The role played by these compounds in the pathogenesis of diabetic retinopathy is not defined yet, although experimental data have demonstrated that AGEs may be responsible for retinal vascular lesions and that they are not only localized to vascular basement membranes (BMs) but also appear to accumulate in the retinal pericytes, inducing BM thickening, and cause breakdown of the inner blood-retinal barrier.[75]

There are some contradictory opinions concerning the role of oxidative stress in the etiology of age-related macular degeneration (ARMD). The hypothesis that ARMD may result from oxidative injury of the retinal pigment epithelium (RPE) is supported by pathological studies indicating that damage of the RPE is an early event in ARMD, by *in vitro* studies showing that the oxidant induces apoptosis of RPE cells and GSH protects

TABLE 4 **Distribution of GSH System in Retinal Cells**

Tissue	Species	Activity	Reference
Photoreceptor outer segments	Bovine	Active pentose phosphate pathway, NADPH production	67
	Rabbit and rats	Active glutathione-related enzymes	68
Muller glial cells	Rat retinal Muller cells (SV40 transformed cell line)	Glutamylcysteine synthetase (GCS) expressed under oxidative stress conditions	69
	Guinea pig	GSH synthesis	70
Retinal horizontal cells	Carp retina	GSH involved in the gap junctional intercellular communication	71
Retinal pigment epithelial cells	Cultured human cells	Intracellular GSH protects against oxidant-induced apoptosis	72

TABLE 5 **Retinal Diseases Involving Oxidative Damage of Cells**

Condition/disease	Change	Tissue	Species	Reference
Diabetic retinopathy	Decreased GSH level; GR activity decreased.	Retina	Streptozotocin-diabetic rats	74
	Decreased GR activity; no effect on GSHS and GCS	Retina	Diabetic and galactosemic rats	75, 76
Age	No age-related change in GR and G6PD activities.	Retina	Rats	77
	No effect on GR and G6PD specific activities	Macula	Normal human	78
ARMD	Inhibition of intracellular GSH synthesis	Retinal epithelial cells	Human	79
Glaucoma	Decreased GSH level and antioxidant enzymes activity	Retina	hyaluronic acid–induced glaucomatous rats	80
Iatrogenic retinopathy (indomethacin, tamoxifen, thioridazine, chloroquine)	Dose-dependent decreases in G6PD activity and GSH concentrations	Retina	Male albino rats	81
Photooxidative damage	GR	Neuroretina	Albino rats	82
	GSH	Retina	Mice	83
Vitrectomy	Increased GS activity; no change in GR level	Retinal tissue	Pigmented rabbits	84

ARMD, age-related macular degeneration.

them,[86] as well as by lower activity of blood GR in ARMD patients, compared with controls[87] (Table 5). Other results do not provide evidence for a relation between oxidative stress, estimated by the activity of antioxidant enzymes, and ARMD severity.[88] Although most experiments demonstrate the oxidative damage of RPE, the precise relation with ARMD progression remains still unclear. Perhaps the strongest evidence for a role of oxidation in ARMD is derived from the clinical trial of antioxidants against the disease. The Age-Related Eye Disease Study (AREDS) reported that pharmacological doses of antioxidants and zinc, taken for approximately 6 years, delayed the progression of intermediate ARMD to advanced ARMD.[89] Of course the negative result for this mixture in relation to cataract undermines the popular view that oxidation is a primary event in human cataract. The results of a clinical trial, AREDS, on the role of the antioxidants vitamins E, C, beta-carotene and zinc with copper in the development of cataracts and AMD, followed for 6.3 years, found no effect on cataract; the patients at high risk of developing advanced ARMD lowered their risk by 25%.[90]

Patients with glaucoma, similar to the patients with cataract, have compromised antioxidant defense systems.[91] In glaucoma, retinal ganglion cells die probably through an apoptotic process, which is known to involve free radicals.

The analysis of retinal oxidative damage in rats with glaucoma induced by the chronic injection of hyaluronic acid in the eye anterior chamber showed a significant decrease in total retinal activity of some antioxidant enzymes, in GSH level, and in the melatonin content, paralleled by a significantly increased lipid peroxidation.[80] However, more glutathione has been found in retinal Müller cells of the glaucomatous monkey than in normal cells.[76] Whether this high GSH level is due to an increase in extracellular glutamate and its cellular uptake, or is the result of glutamate-induced formation of reactive nitrogen and oxygen species, to protect both neurons and Müller cells against further oxidative damage it is not certain.[92]

The etiology of drug-induced retinopathy is largely unknown. The mechanisms proposed thus far are not unanimously accepted. Clinical and experimental data indicate that oxidative stress may contribute substantially to drug-induced retinal disease. The interaction of ocular redox systems with possible retinal toxins may stimulate retinal oxidative stress.[81]

Drugs that lead to retinal toxicity are pharmacologically and structurally diverse, yet most have one

characteristic in common: they all produce oxidative stress (Table 5) and the antioxidants offer some protection against injury.

Light impinging on the retina and pigment epithelium is a source of oxidative stress, which can induce compensatory upregulation of antioxidant enzyme activities,[93] and can be partially normalized by the effect of GSH and thioredoxin.[83] Some retinal cells (e.g., neurotrophin-dependent retinal ganglion cells; RGCs) may use the reactive oxygen species as part of the signaling process for cell death. Compared with other retinal cells, RGCs are remarkably resistant to cell death induced by superoxide anion, hydrogen peroxide, or hydroxyl radical.[94]

Some complications produced by oxidative stress in the retina may occur after vitrectomy. The enzymic activities of retinal glutathione synthetase (GSH-S) and glutathione reductase (GR) after vitrectomy can be improved by intraocular irrigation with saline solution.[81] A significantly increase in GSH-S activity, and a less significant increase of GR level have been reported. The mechanism of this recovery has not been explained yet, but a relation with *de novo* synthesis of GSH has been suggested.[89]

OTHER PARTS OF THE EYE

The components of the glutathione antioxidant system are well represented in uvea. Two forms of glutathione reductase have been found in the bovine ciliary body: the dimer and a large aggregate.[95] Glucose 6-phosphate dehydrogenase is also present in ciliary body, and a stimulation of the pentose phosphate pathway occurs under oxidative stress.[96] A recent study on the antioxidant enzymes in human iris showed by an immunogold labeling technique that both Cu/Zn SOD and acidic glutathione S-transferase were localized in all the iris cells.[97] These results, along with previous data suggesting that the highest glutathione peroxidase activity among the intraocular tissues examined occurs in rabbit iris and ciliary body,[98] indicate that an active enzyme system protects the iris against oxidation.

Studies on the calf trabecular meshwork (TM) showed the presence of glutathione, glutathione reductase, and glucose-6-phosphate dehydrogenase, indicating that TM is well supplied with the capacity to generate NADPH and use it to maintain GSH.[99,100] It has been supposed that GSH is able to protect TM against H_2O_2-induced oxidative damage, which would decrease the aqueous humor outflow.

Thus the same enzyme systems are present in all tissues of the eye to generate NADPH and to maintain GSH (Fig. 1). In normal circumstances, these systems suffice to protect the tissues against oxidation and other chemical modifications. In times of unusual stress or advanced age, a weak point in the system may fail leading to visual impairment. Investigation of the causes of failure and the weak points is at an early stage.

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