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Oxidative Responses Induced by Pharmacologic Vitreolysis and/or Long-term Hyperoxia Treatment in Rat Lenses

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Abstract

Purpose—The aim of the study was to investigate the protective effects of intact vitreous gel on the lens after pharmacologic vitreolysis and hyperoxia exposure in rats *in vivo*.

Methods—Eyes of Sprague-Dawley rats were induced to posterior vitreous detachment (PVD) by pharmacologic vitreolysis, and the rats with and without PVD were treated with hyperoxia 3 h per day for 5 months. Lens transparency was monitored by a slit-lamp biomicroscope. A series of biochemical measurements were made in extracts of the lens cortex and nucleus. Ascorbate levels were measured in the aqueous and vitreous humors.

Results—No significant differences in lens transparency or morphology were observed in all groups, and no significant biochemical changes were observed in the cortex or nucleus of lenses of the PVD group. In the lens nucleus, the values of water-soluble protein concentration in PVD + hyperoxia group were lower than that of the PVD group. The levels of water-soluble proteins, glutathione (GSH) and ascorbate decreased in the hyperoxia group with an intact vitreous body. Vitreolysis enhanced the effect of hyperoxia, decreasing soluble protein, GSH and ascorbate below the levels seen in eyes with vitreolysis alone. The levels of antioxidants and soluble proteins were lower in the lens nucleus, and the effects of vitreolysis plus hyperoxia were more significant in the nucleus. Hyperoxia and hyperoxia plus vitreolysis reduced catalase activity and increased oxidized GSH to a greater extent in the lens cortex, although these treatments increased protein-GSH mixed disulfides in both regions. Long-term hyperoxia also lowered ascorbate levels in the vitreous and aqueous humors, an effect that was enhanced by vitreolysis.

Conclusions—Exposure to excess molecular oxygen produces significant oxidative damage to the lens, especially the lens nucleus. These effects were enhanced by pharmacologic vitreolysis, indicating that intact vitreous gel protects the lens from oxidative damage.

Keywords

Cataract; hyperoxia; oxidative stress; pharmacologic vitreolysis; posterior vitreous detachment

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DECLARATION OF INTEREST

The authors report no conflicts of interest.

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INTRODUCTION

Age-related cataract remains the major cause of blindness worldwide with greater prevalence in developing countries.^{1,2} In most parts of the world, nuclear cataracts are the most common type. Fortunately, the pathogenic mechanisms leading to nuclear cataract are becoming clearer. Oxidative stress resulting from exposure to excess molecular oxygen appears to be an important factor contributing to nuclear opacification.^{3,4} Evidence for the importance of exposure to excess oxygen in promoting age-related nuclear cataract first came from studies of cataract after long-term hyperbaric oxygen (HBO) therapy. Patients who underwent therapeutic HBO treatment for more than one year all showed a myopic shift and most developed nuclear cataracts or substantially increased nuclear opacification. The myopic shift is caused by an increase in the refractive power of the lens, presumably due to increased hardening of the cytoplasm in the lens nucleus. A myopic shift is also an early indication of incipient nuclear sclerotic cataract. Several subsequent studies of patients undergoing HBO therapy confirmed the occurrence of a myopic shift, even after relatively short-term HBO exposure.^{6,7} Pre-cataractous changes in experimental animals demonstrated the detrimental effects of HBO treatment at the biochemical level. These included increased insolubilization of cytoplasmic proteins, loss of reduced glutathione (GSH), formation of GSH disulfides, oxidation of membrane lipids and degradation of cytoskeletal proteins.⁸⁻¹⁰ These studies provide a direct link between excessive oxygen exposure and nuclear cataract.

Low levels of oxygen are maintained around the lens by the gel nature of the vitreous body and by the reaction of ascorbate with oxygen.¹¹⁻¹³ Several clinical studies have shown that vitrectomy, age-related vitreous liquefaction and high myopia, a risk factor for vitreous degeneration, increase the risk of nuclear cataract formation.¹⁴⁻¹⁸ Recent studies in animals showed that enzymatic liquefaction of vitreous humor combined with a posterior vitreous detachment (PVD) elevated the levels of oxygen in the mid-vitreous chamber and resulted in higher levels of oxygen reaching the lens nucleus.^{19,20} These studies suggested that loss of the structure of vitreous gel might increase fluid circulation within the vitreous chamber, permitting more oxygen to reach the lens after diffusing out of the retinal vasculature.^{16,20,21}

Research on the function of the vitreous gel has been impeded because, unlike humans, animal models do not develop nuclear opacities after vitrectomy or vitreous liquefaction. Our previous studies on vitrectomy in animals and hyperoxia-induced lens damage *in vitro* showed that vitrectomy or exposure to hyperoxia *in vitro* were associated with a decreased activity of anti-oxidative enzymes and an increase in oxidative damage to lens proteins.^{22,23} The antioxidant N-acetylcysteine slowed or prevented damage caused by exposure to elevated oxygen, suggesting that exposure to oxygen increased reactive oxygen species (ROS) within the lens cells. To extend these studies and develop models to test whether interventions that reduce exposure to oxygen protect against nuclear cataracts, we examined the effect of vitrectomy and breathing elevated levels of oxygen at normal pressure (hyperoxia) and whether the liquefaction of the vitreous in rats would increase lens oxidative damage.

Enzymatic destruction of the vitreous gel (vitrealysis) is being tested in patients as a potentially less invasive means of performing vitrectomy. Based on previous studies of enzyme-induced PVD in animals,²⁰ we performed an intravitreal injection of hyaluronidase and plasmin to induce vitreous liquefaction and a PVD in rats. Scanning electron microscopy (SEM) confirmed the efficacy of this treatment. We then examined biochemical changes in the lens cortex and nucleus, aqueous humor and vitreous fluid in animals receiving vitrealysis alone, hyperoxia alone, or a combination of vitrealysis and hyperoxia.

MATERIALS AND METHODS

Materials

Hyaluronidase and plasmin were purchased from Sigma Chemical Company (H-3506, Sigma, St. Louis, MO) and Calbiochem Chemical Company (527621, Calbiochem, Billerica, MA), respectively. Enzyme quantification kits were obtained from Jiancheng Biology Company (Nanjing, China) and protein quantification kits were obtained from Biosynthesis Biotechnology Company (Beijing, China). Mouse anti-GSH antibody and goat anti-mouse IgG antibody were purchased from Millipore Chemical Company (Millipore, Billerica, MA) and Santa Cruz Chemical Company (Santa Cruz, CA), respectively. All other chemicals and solvents were obtained from local suppliers.

Experimental Animals

Eight-week-old male Sprague-Dawley rats, weighing 200–220 g, were provided by the Animal Laboratory of the Fourth Military Medical University (Xi'an, China). All procedures conformed to the Institutional Animals Ethics Committee and were in full compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

All eyes were examined by slit-lamp with dilated pupils before intravitreal injection. Those with any eye defects were excluded. Sixty rats were randomly divided into four groups and right eyes of each group were treated as experimental eyes. The control group received a single 10 ml balanced salt solution (BSS) intravitreal injection. The PVD group was injected with 5 U hyaluronidase+0.5 U plasmin, total volume 10 μ l, into the vitreous cavity to achieve vitreous liquefaction and complete PVD. The hyperoxia group had a single dose of 10 μ l BSS intravitreal injection and then was exposed to oxygen (80–85%) 3 h per day for 5 months. Animals in the PVD+hyperoxia group were injected with 5 U hyaluronidase+0.5 U plasmin and then were treated with hyperoxia 3 h per day for 5 months (see details below).

Intravitreal Injection of Enzymes

The animals were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg). The pupils were dilated with 1% tropicamide. The animals received intravitreal injection by using a 30-gauge needle under visualization by an ophthalmic microscope. Intravitreal location of the injected eye was checked through the dilated pupil. No hemorrhage, retinal detachment, cataract or other complication was observed after intravitreal injection.

Hyperoxia Treatment

Fifteen rats were exposed to hyperoxia in a sealed 80 $\times$ 80 $\times$ 40 cm polymethyl methacrylate (PMMA) box with an air inlet and an air outlet. 100% O₂ flowed into the box from the air inlet (5 L/min), and the concentration of oxygen was gradually raised to 85% over a 40 min period and then maintained at 80–85% oxygen for 3 h daily. Soda lime (Beijing, China) was placed inside the PMMA box to absorb CO₂.

The pressure in the chamber was kept at 1.0 absolute atmosphere. The temperature inside the box was maintained at 22–25°C. During the treatment, oxygen concentration inside the sealed chamber was monitored by an oxygen meter (CY-12C; Hangzhou, China).

Slit-lamp Examination and Cataract Classification

After injection, the pupils of the rats were dilated and lenses examined weekly using a slit-lamp biomicroscope without anesthesia (Haag-Streit BQ 900 model, Koeniz, Switzerland).

Experiment on Lens

After 5 months of treatment, the animals were sacrificed by an overdose of sodium pentobarbital and enucleated. Right eyes were removed for morphological and biochemical evaluations. Eyes were transferred to 0.9% neutral normal saline and the lenses were removed through a posterior approach. Lenses were frozen immediately in crushed dry ice and divided into lens cortex (plus epithelium) and nucleus with microforceps using an ophthalmic microscope. The nucleus averaged 36.9 mg wet weight, accounting for approximately 67% of the total lens weight; the remainder of the lens (cortex plus epithelium) averaged 17.8mg wet weight and accounted for approximately 33% of the total weight. The samples were placed into pre-weighed Eppendorf tubes and maintained at -80°C until further analysis.

Lens Preparation

All cortical and nuclear fractions were ground in 0.9% ice cold neutral normal saline (1:19), and homogenized by a handheld homogenizer for 15 min, and then centrifuged (4272 *g*, 10 min) in Eppendorf tubes. All the biochemical parameters were analyzed in the soluble fraction of the lens homogenates.

Water-soluble Protein Determination

The clear supernatant was used for water-soluble protein determination, which was determined by the Coomassie brilliant blue method with a protein assay kit from Jiancheng Company (Nanjing, China).

GSH Determination

Following centrifugation (4272 *g*, 10 min) in 0.05ml of 10% trichloroacetic acid (TCA), the content of free sulfhydryl groups in each lens cortex or nucleus was determined by colorimetric analysis with 5-5'-dithiobis-(2-nitrobenzoic acid) (DTNB), according to the method of Harding. Since most of the free sulfhydryl groups in the lens are accounted for by GSH, "GSH" is used in place of "free sulfhydryl groups."

GSH Disulfide Determination

The concentration of GSSG in each lens cortex or nucleus was measured by the DTNB method, following the centrifugation (4272*g*, 10 min) in 0.15 ml of 10% TCA with a GSSG assay kit from Jiancheng Company (Nanjing, China). The kit used GSH reductase to reduce GSSG in the presence of NADPH, and then the released GSH reacts with DTNB, a chromogen which can be measured at 405 nm. The vial was incubated at 25°C and was detected spectrophotometrically at 405 nm.

Protein-bound GSH Determination

Each lens cortex or nucleus was homogenized in RIPA buffer (800 μl of lysis buffer for one cortex; 500 μl for one nucleus) (Sigma Biotechnology) with proteinase inhibitor (100:1) on ice and then centrifuged at 15 866 *g* for 10 min twice in Eppendorf tubes at 4°C . The clear supernatants were used for PSSG Western blot. Protein concentrations were determined with the BCA protein assay kit (Beijing, China). Proteins ($\sim 50 \mu\text{g}$ per lane) were first separated on a 12% SDS-polyacrylamide gel and then transferred onto PVDF membranes by electrophoresis for 100 min (100 V). After transfer, membranes were incubated with blocking solution (5% milk in TBST) at room temperature for 1 h and then incubated with mouse anti-GSH primary antibody (1:600 dilution; Millipore Biotechnology) overnight at 4°C . The membranes were then incubated with goat anti-mouse IgG secondary antibody (1:12000 dilution; Santa Cruz Biotechnology) at room temperature for 1 h. Labeled protein was visualized by enhanced chemiluminescence detection (Millipore Biotechnology)

(exposure time was 180 s) and visualized and quantified using the Bio-Rad imaging system (Bio-Rad Laboratories (Shanghai) Co., Ltd., Shanghai, China). The results were normalized to the level of β -actin in each sample. The analysis was repeated five times and the means and S.E. of the relative intensity were calculated.

Assay of Catalase Activity

Catalase (CAT) activity was assayed by spectrophotometric recording of the cleavage of H_2O_2 at 240nm as described by Beers and Sizer.²⁴ The reaction mixture contained 0.023M H_2O_2 in 0.05M phosphate buffer (pH 7.0). One unit of enzyme activity was defined as 1mM of H_2O_2 cleaved per minute at 37°C. As H_2O_2 was added externally, the amount was far in excess of that of GSH. For this reason, GSH peroxidase activity (for which GSH is an obligatory substrate) was assumed to be negligible compared to CAT (whose only substrate is H_2O_2). The final volume of each enzyme reaction was 3 ml of substrate and 20 μl supernatant of lens homogenates. Enzyme activity was expressed as units per mg of protein, and one unit of CAT activity represented 1 mmol H_2O_2 decomposed per min.

Ascorbate Determination

Approximately 15 μl aqueous humor or 10 μl vitreous were carefully removed with a 30 gauge needle immediately after the animals were sacrificed and prior to the opening of the bulb of the eye. The aqueous humor or vitreous obtained from two rats in each group was regarded as one sample for analysis. The concentration of ascorbate in the lens homogenates or vitreous and aqueous humor was determined with 2,2'-dipyridyl and ferric chloride at 525nm as described by Okamura,²⁵ following centrifugation (4882 *g*, 15 min) in 15 μl of 10% TCA.

Statistical Analysis

Results are expressed as mean values $\pm$ standard error (SE). The Tukey test in one-way analysis of variance was used for testing statistical significance among groups (the significances >0.1 in the test of homogeneity of variances). Statistical analyses were processed by the SPSS Version 13.0 (SPSS Inc., Chicago, IL). Statistical significance was considered to be $p<0.05$.

RESULTS

Clinical Examination

No hemorrhage, retinal detachment, cataract or other complication was observed after intravitreal injection of BSS or enzymes. Two rats (one each from the PVD + hyperoxia group and the hyperoxia group) died after 45 and 89 days of hyperoxia treatment. Pathological examination confirmed that the rats died from damage to the lungs (data not shown). Slit-lamp examination detected no significant morphological changes or evidence of lens opacification after 5 months of treatment.

Scanning Electron Microscopy

Intravitreal injection of 5 U hyaluronidase and 0.5 U plasmin induced complete PVD. SEM was performed 7 d after injection of BSS or enzymes. It revealed a dense network of vitreous fibrils adhering the retinal surface, indicating that injection of BSS did not produce PVD (Figure 1A). In contrast, SEM analysis showed that intravitreal injection of these enzymes produced a smooth retinal surface, suggesting both vitreous liquefaction and PVD (Figure 1B).

Concentration of Water-soluble Protein

Induced PVD or hyperoxia alone did not significantly reduce the water-soluble protein concentration in the lens cortex 5 months after treatment. However, in the lens nucleus, the values of water-soluble protein concentration in percent of control were 0.96 ± 0.04 and 0.88 ± 0.04 in PVD group and PVD + hyperoxia group, respectively, which had significant difference ($p = 0.027$) (Table 1). In agreement with these values, the ratio of water-insoluble protein to water-soluble protein increased with the extent of oxidative stress, particularly in the lens nucleus (data not shown).

Concentration of Reduced GSH

In the control lenses, GSH levels were more than three times higher in the cortex than in the nucleus. GSH levels in the cortex were not significantly altered by any of the treatments. Vitreolysis did not significantly reduce GSH levels in the nucleus ($p > 0.05$, Table 2). However, rats exposed to hyperoxia alone showed a significant decrease of the nuclear GSH concentration ($p = 0.011$) and PVD+hyperoxia resulted in a further decreased level of nuclear GSH ($p = 0.001$) compared with the control group. Nuclear GSH was also lower in the PVD+hyperoxia group compared with the PVD group ($p = 0.046$).

Concentration of Oxidized GSH (GSSG)

In all groups, the level of GSSG was more than four times lower in the lens nucleus than in the cortex (Table 3) and none of the treatments significantly affected GSSG levels in the nucleus. Vitreolysis had no significant effect on GSSG levels in lens cortex. However, GSSG content in the cortex increased significantly in the hyperoxia group and the PVD + hyperoxia group ($p = 0.034$ and $p = 0.012$, respectively).

Concentration of Protein-GSH Mixed Disulfide (PSSG)

A representative western blot showing the PSSG content in the cortex and the nucleus of lenses is shown in Figure 2. After normalizing the data to the intensity of the β -actin bands, there was no significant change in PSSG levels in the lens cortex in any of the treatment groups. However, the PSSG level significantly increased in the nucleus of lenses following hyperoxia exposure or hyperoxia in combination with PVD.

The Activity of CAT

CAT activity was significantly higher in the lens cortex than in the nucleus (Figure 3). Although PVD treatment decreased the CAT activity by 14%, this difference was not statistically significant. However, CAT activity was significantly lower in the lens cortex after hyperoxia treatment ($p = 0.041$) or after PVD+hyperoxia ($p = 0.015$, Figure 3A). Pharmacologic vitreolysis, hyperoxia exposure or both did not significantly alter the activity of CAT in the lens nucleus (Figure 3B).

Concentration of Ascorbate in Lens

Ascorbate levels were significantly lower in the lens nucleus than in the cortex. None of the treatments significantly affected ascorbate levels in the cortex. However, hyperoxia and PVD+hyperoxia significantly decreased the ascorbate concentration in the lens nucleus ($p = 0.041$ and $p = 0.012$, respectively; Table 4, compared to control).

Concentration of Ascorbate in Vitreous and Aqueous Fluids

Eyes that were exposed to hyperoxia or PVD+hyperoxia showed a significant decrease in the ascorbate level of the vitreous fluid compared with the controls ($p = 0.037$ and $p = 0.002$, respectively; Table 4). A similar decline in the ascorbate concentration in the aqueous

humor was seen after hyperoxia treatment or PVD+hyperoxia ($p = 0.043$ and $p = 0.020$; Table 4). Although ascorbate levels were lower in the vitreous and aqueous humors of eyes treated with PVD+hyperoxia than those with hyperoxia alone, these differences did not reach statistical significance.

DISCUSSION

Under normal physiologic conditions, the lens exists in a low oxygen environment.^{3,26,27} However, the oxygen concentration around the lens may increase after age-related liquefaction of the vitreous body or vitrectomy surgery. Vitreous degeneration or destruction may permit more oxygen to reach the lens from the retinal circulation, which may lead to excess production of ROS and cause lens injury.^{20,26,28,29} In the past, clinical findings have led some laboratories to investigate the impact of vitreous gel on the oxygen level and transparency of the lens in experimental animals.^{13,22,30} In the present study, we investigated the protective effects of intact vitreous gel on lens following pharmacologic vitreolysis and hyperoxia treatment, as reflected by intracellular anti-oxidative agents, such as reduced GSH, ascorbate and CAT. We also investigated changes associated with oxidative damage, including the level of water-soluble protein, oxidized GSH (GSSG) and protein-GSH mixed disulfide (PSSG) in this *in vivo* animal study. These results were consistent with a role for the intact vitreous body in protecting the lens against oxidative damage arising from exposure to excess oxygen.

Oxidative effects on lens proteins have been linked with the formation of human age-related cataracts, particularly nuclear cataract.^{25,31} Results from this study showed that treatment with pharmacologic vitreolysis alone did not have significant effect on the water-soluble protein level of the young rat lens. However, animals exposed to hyperoxia had a significant loss of water-soluble protein in the lens nucleus, but not the cortex, suggesting that the reduction of water-soluble protein was the result of the high oxidative load. The nucleus has a lower capability in antioxidant defense than the cortex, thus the proteins in the centre of the lens are more prone to oxidative insult. These data are consistent with previous studies on HBO exposure, which significantly decreased the levels of water-soluble protein and cytoskeletal protein in the lens nucleus.³² In addition, treatment of rats with pharmacologic vitreolysis combined with hyperoxia accelerated the loss of water-soluble proteins both in the lens nucleus and cortex. The current results also supported the view that the intact vitreous gel protects the lens against oxidative stress by minimizing the circulation of fluid in the vitreous cavity. This would be expected to reduce exposure of the lens to oxygen diffusing from the superficial retinal vasculature.^{13,16,20} On the other hand, young rats were used in the study, and thus the lenses would have high antioxidant activity, compared to lenses of older animals. Older patients are much more likely to develop HBO-induced and vitrectomy-induced nuclear cataracts compared to younger patients.^{5,13}

GSH metabolism plays an important role in the protection of lens against oxidative stress. GSH is an essential antioxidant that protects against free radical-mediated lens injury and protects the protein thiol groups of the lens from oxidative damage.^{12,33} Several studies have suggested that loss of reduced GSH from the nuclear region of the lens causes lens protein disulfide formation, which precedes age-related cataract formation.^{12,34,35} From the present results, treatment with pharmacologic vitreolysis alone had little effect on GSH and GSSG contents in the cortex and nucleus of the lens. However, the level of GSH in the lens nucleus was reduced by pharmacologic vitreolysis when combined with hyperoxia treatment (Table 2). These results are in accordance with those observed from the lens of guinea pigs, which showed a dramatic decrease of lens nuclear GSH comparing to that of the lens cortex following HBO treatment.^{32,36} Hyperoxia could deplete GSH, resulting in relatively low level of GSH in the nucleus of the lens. Combining with the low activity of the GSH redox

cycle in this region makes the nucleus especially vulnerable to oxidative stress. GSSG concentrations increased more rapidly in the cortex of rat lens than in the nucleus after hyperoxia and PVD+hyperoxia treatment. It was likely that oxygen levels in this model are higher in the lens periphery than in the center, which diffuse into the cortical region and oxidize GSH to form GSSG. This result, which has been reported previously, reflects the relatively low ability of the lens nucleus to regenerate GSH from GSSG as a result of low activities of key antioxidant enzymes.⁸

The generation of protein-linked GSH (PSSG) is also believed to represent an early cataractous state.³⁷ The present data demonstrated that the relative concentration of PSSG in the rat lens nucleus was higher than in the cortex. Although hyperoxia treatment significantly depleted GSH in the cortex, and not in the nucleus, hyperoxia increased PSSG formation only in the nucleus. These results are in accord with the report that when GSH is oxidized to GSSG, the GSSG may then react with proteins to form mixed disulfides.³⁸ It seems possible that the higher concentration of GSH and, perhaps, the greater exchange of GSH for PSSG more effectively reduced the deleterious effects of PSSG formation in the cortex than in the nucleus, as had also been demonstrated with use of an in vivo HBO model.³² These results also supported the report that nearly 80% of PSSG is located in the cortical region of the rat lens.³⁷

When lenses are exposed to high oxygen environment, lens enzyme superoxide dismutase converts superoxide radicals to hydrogen peroxide, which is one of the molecules responsible for oxidative injury to the lens. CAT protects against relatively high concentrations of peroxide.³⁹ We showed previously that the activity of CAT in the lens decreased after vitrectomy and that treatment with the antioxidant, N-acetylcysteine prevented the decrease in CAT activity.²² In the present study, a dramatic reduction of CAT activity was observed in the lens cortex in the hyperoxia treatment groups (Figure 3). This may be due to the higher CAT levels in the cortex+epithelium and the fact that the epithelium is the first site of oxygen reaction in the lens.^{9,40}

High ascorbate levels in the ocular tissues are thought to protect against the harmful effects of ambient oxidation reactions involving oxygen and its radicals.⁴¹ The ascorbate in the ocular fluids is actively transported across the ciliary epithelium from the plasma into the posterior chamber where it is presumed to diffuse into the vitreous.⁴² Recent research reported that human vitreous gel contains a high concentration of ascorbate that reacts directly with oxygen, providing another level of protection against oxygen exposure to the lens.¹³ Our data did not show any differences in ascorbate levels of the ocular tissues between groups following pharmacologic vitreolysis treatment and normal control. However, the content of ascorbate in the lens nucleus, vitreous and aqueous humors was significantly reduced after pharmacologic vitreolysis combined with hyperoxia treatment. The results suggested that a high level of oxygen could produce oxygen free radical products, resulting in decreased ascorbate concentration in lens nucleus and the ocular fluids, which made ocular tissues more susceptible to oxidative damage. It should be noted that our ascorbate measurements were higher in the aqueous and vitreous than those reported by DiMattio.⁴³ We cannot be sure about the source of these differences, although they may be due to differences in the ages of the animal used or the different ascorbate assays employed in the two studies.

Intraocular oxygen is mostly derived by diffusion across the retinal vasculature and cornea, as well as from the iris vasculature.²⁹ The lens epithelial cells, which have a higher concentration of mitochondria, consume more oxygen than the fiber cells at the posterior of the lens.⁴⁴ This means that protecting the posterior of the lens from exposure to excess oxygen is an important aspect of ocular physiology. The oxygen level around the posterior

of the lens is largely regulated by the vitreous gel and the ascorbate within the vitreous. Recent studies showed that a modest increase in the concentration of inspired oxygen could lead to a substantial increase in the partial pressure of oxygen at the posterior of the lens.^{20,26,29} The results of the present study showed that increased inspired oxygen reduced the GSH content, ascorbate content and CAT activity in the lens, providing evidence that exposure to increased levels of molecular oxygen may overwhelm the antioxidant defenses of the lens.

The effects of hyperoxia treatment were generally most severe in the lens nucleus. This is consistent with the lower levels of antioxidant protection in the nucleus and agrees with the observation that nuclear opacities occur after HBO treatment in humans and animals^{5,32} and after vitreous degeneration or vitrectomy in humans.^{16,26}

Although hyperoxia had a more significant effect on the antioxidant defenses of the eye and lens than vitreolysis alone, vitreolysis tended to increase the harmful effects of hyperoxia. The relatively small contribution of vitreolysis may be due to the small volume of vitreous in the rat eye and the short distance between the retina and the posterior surface of the lens. Our ongoing studies on rabbit eyes⁴⁵ provide clearer evidence of the role of the vitreous gel in protecting the lens from damage by oxygen.

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FIGURE 1.

Scanning electron micrographs ($\times 3000$) of the vitreoretinal interface of rat eyes, one week after intravitreal injection of hyaluronidase/plasmin or BSS. (A) Control (BSS): a dense network of vitreous cortex adhering to the retinal surface. (B) Hyaluronidase/plasmin: a smooth retinal surface is present, free of cortical vitreous, indicating successful induction of a PVD.

FIGURE 2.

Effect of hyperoxia or hyperoxia + vitreolysis on GSH-protein mixed disulfide formation in the lens 5 months later. The western blot shows typical results obtained in five separate experiments. Results are expressed as mean $\pm$ S.E. ($n = 3$). Lanes 1–4: cortex; Lanes 5–8: nucleus. 1 - control group; 2 - PVD group; 3 - hyperoxia group; 4 - PVD + hyperoxia group; 5 - control group; 6 - PVD group; 7 - hyperoxia group; 8 - PVD + hyperoxia group. The graphs show the amount of PSSG relative to the β -actin level in the cortex plus epithelium or the nucleus. Relative PSSG values in the cortex plus epithelium were not significantly affected by any of the treatments ($n = 5$). Treatment with hyperoxia or hyperoxia + vitreolysis significantly increased PSSG levels in the nucleus, when compared to controls or

eyes with PVD alone (Control versus PVD + hyperoxia: $p = 0.004$; PVD versus PVD + hyperoxia: $p = 0.006$). Due to lower levels of p-actin in the nucleus, only three blots of nuclear proteins were suitable for quantification.

FIGURE 3.

Effects of PVD and hyperoxia treatment on the activity of CAT in the lens cortex (plus epithelium) (A) and nucleus (B) 5 months later. The data is expressed as mean $\pm$ S.E. ($n = 6$).

* $p < 0.05$ compared to control group.

TABLE 1

Levels of water-soluble protein in the lens cortex (plus epithelium) and nucleus 5 months after PVD and hyperoxia treatment (percent of control).

	PVD	Hyperoxia	PVD+hyperoxia
Lens cortex	0.98±0.03	0.96±0.03	0.95±0.05
Nucleus	0.96±0.04	0.91±0.08	0.88±0.04 *

The data are expressed as mean ± S.E. ($n = 6$).

*
 $p < 0.05$ compared to hyperoxia group.

TABLE 2

Levels of GSH in the lens cortex (plus epithelium) and nucleus 5 months after PVD and hyperoxia treatment (mg GSH/g protein).

	Control	PVD	Hyperoxia	PVD+hyperoxia
Lens cortex	13.10±0.10	12.91±0.10	12.85±0.10	12.70±0.10
Nucleus	3.65±0.11	3.40±0.09	3.18±0.10 *	3.02±0.07 **, ***

The data are expressed as mean ± S.E. ($n = 6$).

*
 $p < 0.05$ compared to control group.

**
 $p < 0.01$ compared to control group.

 $p < 0.05$ compared to PVD group.

TABLE 3

Levels of GSSG in the lens cortex (plus epithelium) and nucleus 5 months after PVD and hyperoxia treatment (mg GSSG/g protein).

	Control	PVD	Hyperoxia	PVD+hyperoxia
Lens cortex	1.92±0.09	2.10±0.08	2.28±0.08 *	2.34±0.09 *
Nucleus	0.38±0.05	0.38±0.02	0.39±0.02	0.40±0.04

The data are expressed as mean ± S.E. ($n = 6$).

* $p < 0.05$ compared to control group.

TABLE 4

Levels of ascorbate in the lens cortex (plus epithelium), nucleus, vitreous fluid and aqueous humor 5 months after PVD and hyperoxia treatment (mmol/L).

	Control	PVD	Hyperoxia	PVD+hyperoxia
Lens cortex	0.332±0.005	0.324±0.004	0.321±0.003	0.318±0.001
Nucleus	0.078±0.003	0.072±0.003	0.065±0.003 *	0.063±0.004 *
Vitreous fluid	0.508±0.013	0.489±0.015	0.458±0.009 *	0.437±0.009 **,***
Aqueous humor	0.312±0.014	0.288±0.013	0.252±0.015 *	0.245±0.015 *

The data are expressed as mean ± S.E. (*n* = 6).

* *p*<0.05 compared to control group.

** *p*<0.01 compared to control group.

*** *p*<0.05 compared to PVD group.