Glutathione Peroxidase-1 Deficiency Leads to Increased Nuclear Light Scattering, Membrane Damage, and Cataract Formation in Gene-Knockout Mice

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PURPOSE. Previous in vitro studies with transgenic and gene-knockout mice have shown that lenses with elevated levels of glutathione peroxidase (GPX)-1 activity are able to resist the cytotoxic effect of H2O2 compared with normal lenses and lenses from GPX-1–deficient animals. The purpose of this study was to investigate the functional role of this enzyme in antioxidant mechanisms of lens in vivo by comparing lens changes of gene-knockout mice with age-matched control animals.

METHODS. In vivo lens changes were monitored by slit lamp biomicroscopy, and enucleated lenses were examined under a stereomicroscope in gene-knockout animals and age-matched control animals ranging in age from 3 weeks to 18 months. Transmission (TEM) and confocal microscopy were performed on different regions of lenses after the mice were killed at various times.

RESULTS. Slt lamp images showed an increase in nuclear light scattering (NLS) in gene-knockout mice compared with control animals. TEM revealed changes in the nucleus as early as 3 weeks of age by the appearance of waviness of fiber membranes. With increasing age, there was greater distortion of fiber membranes and distension of interfiber space at the apex of fiber cells compared with control mice. The changes in nuclear fiber membranes were even more dramatic, as observed by confocal microscopy, which was performed on thicker sections. In contrast to the changes in the lens nucleus, the morphology of the epithelium and superficial cortex remained unchanged in knockout animals during the same experimental period, consistent with slit lamp observations. Stereo microscopy of ex vivo lenses demonstrated a significant increase in opacification in gene-knockout mice relative to control animals of the same age. This effect became evident in mice aged 5 to 9.9 months and persisted thereafter in older animals, resulting in mature cataracts after 15 months.

CONCLUSIONS. The results demonstrate the critical role of GPX-1 in antioxidant defense mechanisms of the lens nucleus. The increased NLS appears to be associated with damage to fiber membranes in the nucleus, which is particularly susceptible to oxidative challenge because of the deficiency of GPX-1. It is suggested that the lens membrane changes in the knockout animals may be due to the formation of lipid peroxides, which serve as substrates for GPX-1. Cataract development in gene-knockout mice appeared to progress from focal opacities, apparent at an earlier age, to lamellar cataracts between 6 and 10 months, and finally to complete opacification in animals older than 15 months. This is the first reported phenotype in GPX-1–knockout mice. (Invest Ophthalmol Vis Sci. 2001;42:3247–3255)

Oxidative damage resulting from free radicals and/or H2O2 has been considered a major factor in the development of age-related cataracts.1–5 The oxidative modification of numerous cellular constituents such as proteins, membrane sulfhydryls, reduced glutathione (GSH) levels, cytoskeletal elements, and lipids are thought to result in lens opacification and nuclear cataracts. The nucleus of the lens is particularly susceptible to oxidative insult, because of the relative absence of antioxidant mechanisms.4,5

Aqueous humor of many species, including the human, has been shown to contain significant levels of H2O21,6–8 which may be formed from ascorbic acid present in the aqueous humor by light-catalyzed reactions.6,8 This constant oxidative challenge to the lens, if not counteracted, could lead to severe effects on many critical cell functions.3,5,10 It is now well established that the lens is endowed with defense mechanisms against H2O2-induced damage that may occur at the physiological levels of the oxidant present in the aqueous humor. The key enzymes that participate in the removal of peroxide are catalase and enzymes of the glutathione redox cycle, including glutathione reductase (GR) and glutathione peroxidase (GPX).11–16 Although the relative contribution of each of these enzymes in the detoxification of H2O2 is not known, they work in tandem. At low concentrations of H2O2, the glutathione redox cycle is the primary means of protection against H2O2-induced damage.16,17 The redox cycle is also responsible for maintaining high levels of GSH in the lens.18 Catalase, on the other hand, appears to be more important for the decomposition of H2O2 at higher concentrations.19,20

The direct relationship of GR to peroxide detoxification in the lens has been established by a number of in vitro studies.5,21–25 Exposure of cultured rabbit lenses or their epithelial cells to H2O2 after inhibition of GR has been shown to affect
cation transport systems, lens hydration, and membrane blebbing. Until recently, it has not been possible to evaluate the direct role of GPX, the second enzyme of the redox cycle, in protection against peroxide-induced damage because there were no specific inhibitors and because H$_2$O$_2$ is a substrate for both GPX and catalase. Also, in contrast to catalase, GPX uses substrates other than H$_2$O$_2$ and is capable of reducing cellular hydroperoxides. The organohydroperoxides also serve as substrates for the metal-catalyzed Fenton reaction to generate highly reactive hydroxyl radicals.

Cellular GPX (glutathione H$_2$O$_2$ oxidoreductase, EC 1.11.1.9, GPX-1) is one of four known Se-dependent GPXs. The other three forms are GPX-2 (gastrointestinal GPX), GPX-3 (plasma or extracellular GPX), and GPX-4 (phospholipid hydroperoxide GPX). The knockout model used in the present study has been shown to produce almost complete loss of GPX-1 activity in various tissues but has no effect on activities of GPX-3 and GPX-4. Transgenic mice in which GPX-1 is either overexpressed or made deficient through gene knock-out comprise a useful model to investigate the direct role of this enzyme against H$_2$O$_2$-induced oxidation. Spector et al. noted that variation of GPX-1 activity has only a minimal influence on a number of biochemical parameters, such as changes in protein thiols, thymidine incorporation, and choline transport when lenses were exposed to 120 to 500 $\mu$M H$_2$O$_2$. Using morphologic techniques, Reddy et al. have observed that lenses of transgenic mice with fivefold higher activities of GPX-1 afford a greater protection against the cytotoxic effects of 25 $\mu$M H$_2$O$_2$ than those in normal and gene-knockout animals in which the enzyme is barely detectable. Similarly, the lens epithelia of gene-knockout animals show much greater DNA damage than GPX-1-rich lenses when exposed to the same level of H$_2$O$_2$.

In view of these in vitro effects of H$_2$O$_2$ on lenses of transgenic and gene-knockout animals, it was considered possible that phenotypic changes in the lenses of knockout animals might occur in vivo. However, in contrast to the in vitro effect of H$_2$O$_2$ in GPX-1-deficient lenses, the ultrastructure of the epithelium and superficial cortex of the lenses in situ appeared normal; also, no cataracts were observed when the animals were killed at 3 weeks of age. Failure to note any changes in lens ultrastructure in situ may suggest that the endogenous level of peroxide in the aqueous humor of these animals may be lower than that used in studies in vitro or that the antioxidant mechanisms in the lens epithelium and cortex may be sufficient to withstand the oxidative challenge under in vivo conditions.

Because the animals in our earlier studies were killed at an early age (3 weeks), it is conceivable that any phenotypic changes in the lens due to the deficiency of GPX-1 may be expressed at a later stage of development. The objective of this investigation was therefore to observe lens changes with slit lamp biomicroscopy and stereomicroscopic examination of enucleated lenses ex vivo over a period of 18 months in gene-knockout animals compared with age-matched control mice. Simultaneously, transmission and confocal microscopy was performed on lenses of some of these animals killed at different ages. The results showed an increase in nuclear light scattering (NLS) of lenses of gene-knockout mice with deficiency of GPX-1 compared with age-matched control animals. This increase in NLS correlated with membrane changes in the lens nucleus that were also progressive as a function of age. The phenotypic nuclear membrane changes and increased NLS may be due to the accumulation of lipid peroxides and other organohydroperoxides that are known to serve as substrates for this enzyme. Thus, GPX-1, in addition to its participation in the detoxification of H$_2$O$_2$, may prevent the accumulation of organohydroperoxides or serve as a repair enzyme by continually removing them from the nuclear fiber membranes. Furthermore, lens opacities in gene-knockout mice appeared progressive with increasing age relative to those in control mice, leading to mature cataracts in animals older than 15 months.

**Materials and Methods**

**Knockout and Control Animals**

The methods for establishing gene-knockout animals have been previously described. Briefly, the mouse GPX-1 gene was disrupted by insertion of a neomycin resistance gene cassette derived from plasma pPNT32 into the EcoRI site located in exon 2. Chimeric mice were obtained by microinjecting homologous recombinant embryonic stem cells into C57BL/6 blastocysts, as described by Bradley. Germine transmission was obtained from four lines of chimeric mice. To minimize genetic variations and maximize the production of experimental animals, homozygous knockout mice and wild-type mice were initially derived from breeding two heterozygous knockout mice. Littermate homozygous knockout or wild-type mice were intercrossed to generate the experimental animals used in this study. All animals used were maintained and treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**GPX Activity**

The enzyme was assayed biochemically according to Paglia and Valentine, as modified by Lawrence et al. Each lens was separately homogenized in 0.5 ml sample buffer (pH 7.0) and centrifuged at 9000g for 15 minutes and the supernatant used for the enzyme assay. A unit of GPX activity was equivalent to the amount of enzyme per milliliter required to catalyze the oxidation of 2 mmol/min GSH with 10 mM GSH and 1 mM tertiary butyl hydroperoxide in a system coupled to GR in which 1 mmol/min reduced nicotinamide adenine dinucleotide phosphate (NADPH) was used. The extinction coefficient of NADPH was used. The expression of GPX activity was expressed as units per milliliter. The extinction coefficient of NADPH was used.

**Tissue Processing**

For morphologic studies, lenses were extracted from isolated mouse eyes through the posterior approach and the cortex and epithelium were fixed in 2.5% (wt/vol) glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4). They were dehydrated in an ascending series of ethanol concentrations and embedded in Epox 812. For fixation of the nuclear region, lenses were first fixed overnight in a 1:1 concentration of 2.5% (vol/vol) glutaraldehyde and 2% (vol/vol) paraformaldehyde prepared in 0.1 M cacodylate buffer (pH 7.4). After fixation, the cortex was removed by dissection under a microscope, and the nucleus was cut into small pieces to obtain the desired regions and fixed in the aforementioned fixative for 1 week. Tissue pieces were rinsed overnight in 6% sucrose in 0.1 M cacodylate buffer (pH 7.4). After dehydration in an ascending series of ethanol concentrations at 4°C, they were infiltrated with embedding medium (Lowicryl Polysciences Inc., Warrington, PA) at 4°C and polymerized under UV light overnight.

**Slit Lamp Biomicroscopy**

Transparency of lenses of control and experimental mice was assayed by two authors who are ophthalmologists (L-RL, AJ), using a slit-lamp photomicroscope (Carl Zeiss, Oberkochen, Germany) or a handheld slit lamp. The animals were anesthetized by an intramuscular injection of a mixture of xylazine (7 mg/kg body weight) and ketamine (35 mg/kg body weight) followed by induction of full mydriasis with tropicamide (1%) and phentolamine (1%). The mice were placed on a heating pad before slit lamp examination to prevent formation of cold cataracts, and the slit lamp observations were recorded.

**Lens Opaclification by Ex Vivo Examination**

For ex vivo examination of lens opacity, the animals were injected with a lethal dose of thiopental through the peritoneum. After cessa-
tion of breathing, the thorax was opened and the mice were perfused through the heart, first with heparinized isotonic saline solution until the blood was flushed out. The perfusion was continued with 60 ml of 4% paraformaldehyde (wt/vol) solution for approximately 10 to 15 minutes. The eyes were then enucleated. A sagittal incision was made with a fine scalpel so that the tissues (which were used for other morphologic studies) were minimally disturbed. The lenses were carefully dissected under a stereomicroscope and the nature and degree of opacification recorded. Lens opacities were classified as follows: 0, clear lens; 1, single focal opacity; cat II, a lamellar ring opacity between the cortex and nucleus; and cat III, total opacity or a mature cataract. These studies were performed at the Ophthalmology and Eye Hospital in Erlangen. The observations were made in a masked fashion by two independent observers, and no electron microscopy was performed on any of the lenses. The ex vivo observations were first confirmed in 18 animals in which the lenses were examined by an ophthalmologist (AJ) using a handheld slit lamp (Carl Zeiss). The findings in vivo and ex vivo were essentially similar. In view of this, lens opacities in the rest of the animals (74 knockout and 59 control mice) were classified from ex vivo observations by stereomicroscopy.

**Statistical Analysis**

The percentages showing opacification in knockout mice and control animals of the same age range were compared using the Pearson χ² or Fisher exact test.

**Transmission Electron Microscopy**

Thin sections were cut with a diamond knife, stained with 5% uranyl acetate and Reynolds lead solution, and examined under a transmission electron microscope (model LEM 2000; International Scientific Instruments, Milpitas, CA.).

**Confocal Microscopy**

The same embedded blocks (Lowicryl Kit; Polysciences Inc.) used for TEM were also used for confocal microscopy. Thick sections (6–12 μm) were cut using glass knives on a microtome (Reichert Ultracut S; Leica, Heidelberg, Germany) with the step advance set at 2 to 5 mm and the feed set at 2.5 mm at a speed between 2 and 5 mm/sec. By varying the step advance, it was possible to cut thicker sections. Approximately 5 to 10 sections in distilled water from each block were dried onto acid-cleaned glass microscope slides. Sections were viewed before 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) labeling to identify any possible autofluorescent structures.

A stock solution of the membrane probe DilC₁₄ or Dil (Molecular Probes, Eugene, OR) was prepared by adding 100 ml 100% ethanol to 100 mg solid Dil. The stock solution was aliquoted and stored at 20°C in brown vials until use. Plasma membranes of lens fiber cells were stained by adding a 1:100 dilution of stock Dil in 100% ethanol directly to tissue sections, followed by incubation in the dark for 10 minutes at room temperature. The Dil solution was removed, and the sections washed several times with 100% ethanol. The sections were then stored at 4°C for 24 hours before being viewed on an inverted scanning confocal microscope (model 410). equipped with an Aviovert-100 inverted microscope, an argon-krypton 488/568/667 laser, Plan-NeoFluor ×63 numerical aperture, 1.4 oil immersion objective, an FEI-88/568 Dicroic beam splitter, a KP 600 line selection filter, an LP 590 emission filter, and the software package LSM ver. 3.91; Zeiss, Thornwood, NY). Individual optical sections and z-series were taken with the pinhole set at 10 (full width at half-maximum [FWHM], 0.501 μm) to 16 (FWHM, 0.64 μm). Digital image files in tagged information format (TIFF) were imported into image management software (Photoshop ver. 3.0; Adobe Systems, Mountain View, CA) for labeling, image sizing, and brightness and contrast adjustments before printing on a dye-sublimation printer (Spectra Star DSX; Talley, Kent, WA). This method has the advantage in that thicker sections and larger areas of the nucleus, than those results in vivo.

**Results**

Biochemical assay of GPX in the lenses of GPX-1 gene-knockout mice showed that the enzyme was either absent or could not be detected; the enzyme activity of the control lenses was 13.5 ± 1.3 U/g wet weight. Despite the near absence of the enzyme in gene-knockout animals, the animals appeared completely normal. There were no differences in the growth rates or final body weights of the wild-type and gene-knockout mice. Also, during the 1-year period of observation, no cataracts were seen with the naked eye. It has been reported that homozygous male and female knockout mice grow normally, are apparently healthy for 20 months, and are also fertile. In the same study, histologic examination using light microscopy showed no abnormalities in many tissues of the knockout animals, including brain, heart, lung, kidney, liver, and spleen.

**Slit Lamp Biomicroscopy**

Lens changes were observed in normal and GPX-1–deficient mice with either a handheld slit lamp or a photograph slit lamp. In one series of experiments, slit lamp examination was performed in the knockout mice and age-matched control animals at 3 weeks, 2 months, 6 months, and 1 year of age (three animals in each group at each time period). At 12 months of age, an additional four knockout and age-matched control animals were examined. A greater NLS was observed in the lenses of knockout animals compared with age-matched control animals after 2 months of age and appeared to increase in older animals, compared with younger ones. Even in the normal (wild-type) animals, there was a slight increase in NLS as a function of age. Whereas the NLS in the 1-year-old knockout lens was not much greater than that of 6 months, the size of nucleus showing light scatter appeared larger than that of the younger animals.

In a much larger series of animals in which the observations were made with a handheld slit lamp and the isolated lenses examined under a stereomicroscope, there was clear evidence of complete lens opacification after 15 months of age (described later under ex vivo examination).

**Morphologic Changes**

To examine the changes in lens morphology, after slit lamp examination, the animals were killed and lenses were processed for electron microscopy, as described in the Materials and Methods section. TEM was performed in different regions of the lenses after the animals were killed. Figure 1 shows a set of representative TEM micrographs of the central epithelium and the superficial cortex of control and gene knockout lenses in mice 3 weeks, 2 months, 6 months, and 12 months of age. It is apparent that the morphology of both the epithelium and the cortical fibers in the two groups was similar. Also, no differences in the ultrastructure of the epithelium and cortex in the equatorial region or the posterior cortex were found in the control and experimental lenses of both groups at any age (data not shown).

We then examined the nuclear region of the knockout lenses with confocal and TEM microscopy in comparison with that of age-matched control animals. In contrast to the absence of morphologic changes in the epithelium and superficial cortex, the nuclear fiber membranes in the lenses of the knockout mice showed distinct changes, compared with age-matched control animals. Figure 2 shows representative confocal images performed on 400- to 600-μm-thick sections from different age groups of knockout animals in comparison with age-matched control animals. Although this method has the advantage in that thicker sections and larger areas of the nucleus, than those...
seen in TEM, can be visualized, the resolution is limited. The confocal optical sections (0.5 μm thick) show lipid-stained DiI binding to fiber cells cut in cross section from the nucleus. The white areas delineate the plasma membranes of the fiber cells.

Although the confocal images of 3-week-old lens nuclei from knockout mice were similar to those from normal animals of the same age, the confocal images of knockout lenses after 2 months of age showed dramatic changes compared with control lenses (compare Figs. 2D, 2F, and 2H with 2C, 2E, and 2G). Also, there were great variations in cross-sectional dimensions. They showed fiber cell enlargement, membrane blebbing, globulization, and formation of vesicles (Figs. 2D, 2F, and 2H). In the 6-month and 12-month-old knockout animals, there was a decreasing gradient of morphologic change. The more superficial the fiber cell, the more the region appeared similar to the corresponding normal mouse lens (data not shown). It may be noted that with increasing age in normal lenses, there was an enlargement of fiber space in some areas (Fig. 2E, 2G) compared with the younger lenses (Figs. 2A, 2C), which may be characteristic of normal aging process.

The same embedded blocks used for confocal microscopy were also examined at the ultrastructural level with TEM. In marked contrast to the absence of morphologic changes in the epithelium and superficial cortex, the nuclear fiber membranes in the lenses of the knockout mice showed distinct changes, compared with age-matched control animals beginning at 3-weeks-old and increasing progressively with age (Fig. 3). At 3 weeks of age, the nuclear fiber membrane structure in the knockout lenses (Fig. 3B) had a subtle wavy appearance compared with that of the age-matched control animals. At 2 months of age, the lens fiber membranes in the knockout animals showed increased regular undulation (Fig. 3D) whereas at 6 months the nuclear fiber membranes exhibited distortion and distension of interfiber spaces, as well as membrane blebbing, globularization, and fiber enlargement at the apex of the fibers (Fig. 3F). The corresponding control lenses, on the contrary, showed only slight membrane outpouching.

**Figure 1.** TEM micrographs of lens sections of control and GPX-1 gene-knockout mice. Left: Sections from the central epithelium and cortex of control lenses. Right: similar regions of lenses from gene-knockout animals: (A, B) 3 weeks old, (C, D) 2 months old, (E, F) 6 months old, (G, H) 12 months old.

**Figure 2.** Confocal images of lens sections from the deep nucleus of GPX-1 gene-knockout mice (right) and age-matched control animals (left): (A, B) 3 weeks old, (C, D) 2 months old, (E, F) 6 months old, and (G, H) 12 months old.
In 1-year-old knockout lenses the fiber membranes in some areas were partially disintegrated (Fig. 3H), and portions of the membranes appeared to have pinched off and internalized in the fiber cell in some areas. The inset in Figure 3H shows the internalization of the fiber membranes into the cell cytoplasm. The ultrastructure of the 12-month-old control membranes, although similar to that in 6-month-old control animals (Fig. 3E), appeared to have a greater number of outpouchings that were not confined to the apex of the fiber cells. Occasionally, the fiber membranes in the older control lenses showed interfibrillar space (Fig. 3E, arrows). These changes in fiber membranes are similar to those reported in hyperbaric oxygen-induced nuclear lens opacities and in immature human nuclear cataracts.

Although the sections of nuclear fibers were cut from the same blocks for both TEM and confocal microscopy, no membrane distortions are seen by confocal microscopy. However, the dimensions of the nuclear fiber cells in the control TEM and the control confocal images are of similar dimensions. This indicates that similar areas of the lens nucleus were imaged. In Figure 3H, the entire cell cross-sectional dimension can only be seen for the smallest cells. If lower TEM magnifications were used to show larger cell cross-sectional dimensions, then the fine ultrastructural changes would not be seen. The reason that the membrane invaginations do not appear in the confocal images is because these structures are less than 0.2 μm in amplitude.

**Cataract Formation as Observed by Ex Vivo Examination of Enucleated Lenses**

Table 1 shows data for lens opacities seen in individual gene knockouts and wild-type control animals as a function of age.
OPACIFICATION OF THE LENS WAS BILATERAL ALTHOUGH THE SEVERITY IN THE TWO LENSES WAS NOT ALWAYS THE SAME. BEFORE 15 MONTHS, NO CONTROL MICE SHOWED LENS OPACIFICATION (EITHER LAMELLAR OR COMPLETE OPACITIES). IN 10-TO-15-MONTH-OLD ANIMALS, 94% (17/18) OF THE LENSES FROM KNOCKOUT MICE SHOWED SOME DEGREE OF OPACITY VERSUS 0% (0/6) IN CONTROL MICE. IN 17% THERE WAS A MATURE CATARACT (TOTAL OPACITY), IN 44% A LAMELLAR CATARACT, AND IN 33% ONLY FOCAL OPACITIES. IN KNOCKOUT ANIMALS OLDER THAN 15 MONTHS, 71% (27/38) OF THE LENSES HAD MATURE CATARACTS AND 24% (9/38) LAMELLAR CATARACTS, BUT NONE OF THE ANIMALS SHOWED ONLY FOCAL OPACITIES. EVEN IN THIS AGE GROUP, ONE ANIMAL HAD CLEAR LENSES. ALSO, CATARACT FORMATION WAS OBSERVED IN ONE WILD-TYPE ANIMAL OLDER THAN 15 MONTHS, ALTHOUGH MATURE CATARACT WAS FOUND IN ONLY 1 OF 28 LENSES AND 15 LENSES WERE COMPLETELY CLEAR.

Lens opacification ratings (where opacification is defined as lamellar or total) in knockouts and control mice by age groups were compared with the Fisher exact test or Pearson χ² test, as summarized in Figure 4. The percentages of lenses showing opacification were significantly higher in knockout mice relative to control mice for months 5 to 9.9 (P < 0.0001), 10 to 15 (P = 0.007), and more than 15 (P < 0.0001). Thus, despite the variability in the degree of lens opacification, there is no doubt that knockout mice demonstrated a significant increase in opacification relative to the control of the same age. This effect became evident in mice aged 5 to 9.9 months and persisted thereafter in older animals.

**DISCUSSION**

In this investigation, GPX-1 played an important role in protection against oxidative stress in the lens, because the deficiency or near-absence of this enzyme in gene-knockout mice led to increased NLS, which appeared to be related to damage to nuclear fiber membranes. These initial changes in NLS led to late-onset cataracts between 5 and 10 months of age. This is the first report of a phenotypic change in knockout mice that appeared to grow normally, without any observed disease in many tissues that have been previously examined in these animals.27,31 Although the underlying mechanisms by which GPX-1 deficiency in knockout mice results in cataractogenesis remain to be elucidated, this enzyme plays a critical role in protection of the lens against oxidative stress.

The lens, whose function is to transmit light, must constantly cope with highly reactive oxygen radicals derived from light-catalyzed reactions in the ocular media. The defense against harmful effects of these free radicals is achieved by endogenous antioxidant compounds and enzymes present in the lens. The most important reducing compounds in the lens are GSH, which is continually regenerated by a number of metabolic reactions and ascorbic acid.5,18 In addition to superoxide dismutase (SOD), which protects against the damaging effect of the superoxide radical, the other key enzymes that give protection against H₂O₂ are GR, GPX, and catalase. Although the individual contributions of each of these enzymes in the detoxification of H₂O₂ are not established, all three enzymes may act cooperatively. Because GPX exhibits a lower Km for H₂O₂ than does catalase,12,14,17,19,37 and is capable of detoxification of hydroperoxides,55 it is generally thought to play a primary role in minimizing cellular oxidative damage under physiological conditions.12,16,20 At the low levels of H₂O₂ normally present in the aqueous humor, the principal mechanism for the removal of H₂O₂ is the glutathione redox cycle, whereas at a higher concentration of H₂O₂ the cells use catalase for its decomposition.

Until recently, the assessment of the contribution of these enzymes to cellular antioxidant mechanisms has made use of specific inhibitors of the enzymes10,38 or inhibition of GPX activity by Se depletion39,40 but the interpretation of the data has been difficult, because Se deficiency is known to affect other Se-containing enzymes.41,42 Furthermore, Se deficiency does not lead to complete inhibition of GPX.45 The present study has focused on the role of GPX-1 deficiency (the cellular form of GPX) in oxidative stress in the lens and cataract formation. The GPX-1-knockout mouse that was first described by Ho et al.31 provides a unique model for assessing the function of this enzyme in antioxidant defense in the lens, because other antioxidant enzymes such as SOD, catalase, glutathione-S-transferase and other forms of GPX are unaf-
affected in the various tissues examined.27 Despite the virtual absence of GPX-1 activity in the lens and many other tissues, the animals were apparently healthy and grew normally. Haliwell44 expresses the view that GPX-1 is not essential for everyday life, at least in mice, because the system can adapt and survive without it. Despite the absence of histopathologic changes in several tissues of the GPX-1-deficient animals,31 other studies point to the importance of this enzyme in protection against oxidative stress. It has been observed that GPX-1–knockout animals are susceptible to acute diquat-induced oxidative stress in vivo.45 There was 100% mortality in GPX-1–knockout mice within 2 hours after intraperitoneal injection of 24 mg/kg body weight, whereas the wild-type control animals survived for nearly 3 days. The biochemical basis for this protection has been ascribed to the ability of GPX-1 to maintain the redox status in mice under oxidative stress and protect against paraquat-induced destruction of lipids and proteins in vivo.46

In contrast to the absence of any histopathologic changes in other tissues,7 the lenses of the GPX-1–knockout animals showed increased NLS, characteristic of early cataractous changes. The progressive increase in lens NLS in the knockout animals as a function of age is apparently related to the observed ultrastructural changes in the fibers deep in the nucleus. The morphologic changes in the lens nuclear fiber membranes of the experimental animals were observed as early as 3 weeks of age and progressively increased compared with age-matched control animals. At the end of 6 and 12 months of age, there was extensive damage to the fiber membranes including partial disintegration and internalization of these membranes into the cytoplasm (Fig. 3). Thus, the deficiency or absence of GPX-1 resulted in membrane damage to the lens fibers in the deep nucleus with an associated increase in NLS. Surprisingly, the morphology of the epithelium and superficial cortex in the anterior, posterior, and equatorial regions of the knockout lenses remained normal. This is consistent with slit lamp examination in which the lens cortex remained transparent. Clearly, in these animals, the nucleus is more vulnerable to oxidative stress in the absence of the enzyme GPX-1. It is likely that the high concentrations of GSH and NADPH present in the epithelium and lens cortex of many species46–47 serve as chemical antioxidants. In addition, catalase present in the lens epithelium but not in the nucleus48 may have been able to compensate for the absent GPX-1 in the detoxification of H2O2. The absence of effect of the deficiency of GPX-1 in the lens epithelium and cortex is similar to findings in many other tissues where the histopathology appears normal. The morphologic changes noted in the lens nuclear fibers may be due to formation of lipid peroxides, which are known to affect membrane fluidity in many tissues.49–53 This hypothesis remains to be tested and is a subject for future investigation.

Whereas our results clearly demonstrated that deficiency or absence of GPX-1 in the lens leads to phenotypic changes in the lens nucleus and late-onset cataracts, the nature of the oxidant or oxidants involved in the observed membrane damage is not known. Because GPX specifically acts on H2O2 and lipid hydroperoxides and there were increased NLS and membrane changes in the nucleus, some naturally occurring oxidants, such as molecular oxygen and H2O2, or reactive species derived from these oxidants, must reach or be generated within the inner nucleus of the knockout lens. Otherwise, no damage to the nuclear fibers would be expected. It has been assumed that there is little free oxygen in the lens, although there is no general agreement on this assumption. Recent studies of animals exposed to hyperbaric oxygen suggest that some oxygen or its derivatives must diffuse into the inner nucleus, because this treatment increased the oxidation of proteins, thiols, and lipids in the lens nucleus.54,55 It is also likely that a small amount of H2O2 reaches the nucleus, causing the accumulation of lipid peroxides in the membranes. This initial formation of hydroperoxides, which cannot be detoxified in the knockout lenses due to the deficiency of GPX-1, could amplify lipid peroxidation. It is known that lipid peroxides can serve as substrates for the iron-mediated (Fe2+) Fenton reaction26 to generate highly reactive hydroxyl radicals that could lead to further membrane damage. However, additional studies are needed to clarify the nature of the lipid peroxides that accumulate in the lens nuclear fiber membranes in the absence of GPX-1 and to delineate the mechanism of membrane damage in the lens nucleus. It may be noted that even in the control lenses, there was some increased NLS as a function of age and there was evidence for enlargement of some fiber cells at 12 months of age (Fig. 2G), suggesting that oxidation in the nuclear fibers may be associated with the normal aging process, eventually leading to age-related onset of cataracts.

The results of the present study support the view that GPX-1 functions as the principal mechanism by which the lens nucleus is protected against oxidative damage by H2O2 and/or other free radicals generated in the tissue, and a near absence of this enzyme in gene-knockout animals leads to cataract formation. In an earlier study, we reported that lenses of transgenic animals, which had a fivefold higher activity of GPX-1, were able to resist the cytotoxic effects of H2O2 in the lens epithelium or cortex, compared with normal or gene-knockout animals.50 This is in accord with the findings of Sandstrom et al.56 who reported that a selenite-induced increase in GPX protects human cells from H2O2-induced DNA damage. Mirault et al.57 have also reported that overexpression of GPX by gene transfer increases the resistance of human breast cells to DNA damage induced by oxidative challenge. This was also found to be the case when the human lens cell line (SRA-01/04) was transfected with plasmid containing cDNA for GPX-1. In these cells the GPX-1 was four times higher than in the cells transfected with vector alone (control), and the DNA strand breaks induced by H2O2 were significantly lower compared with the control (Reddy VN, Lin L-R, unpublished observations, 1999).

In contrast to their appearance in our previous in vitro experiments, the morphology of the lens epithelium and cortex of gene-knockout mice in situ appeared normal, as seen by TEM, suggesting that the concentration of H2O2 in the aqueous humor of these animals may be lower than that used in the in vitro studies. The absence of morphologic changes in lens epithelium and cortex of GPX-1 gene-knockout animals as observed by light microscopy has also been reported in a recent paper by Spector et al.58 However, these investigators did not observe any lens opacities, and their findings are not in accord with those in the present study. The reason for the contradictory findings is not clear. Their studies58 largely focused on morphology of the anterior and posterior poles plus the bow region, and in those areas their results agree with ours. Their studies do not indicate the use of slit lamp or stereo microscopic examination as was the method of observation in the present study. Also, scanning electron microscopy (SEM) was used to examine the deep cortical and nuclear regions of 12-month-old lenses. Because SEM can reveal only the changes in cell surface rather than those in membrane structure, one would not expect to find the membrane changes seen with TEM. From their studies, it is also not clear how many animals were used at 12 months of age. Our study, in which a larger number of animals were used, has demonstrated that at 10 to 15 months of age 11 of 18 lenses (61%) had lamellar or total opacities. In animals older than 15 months, 71% (27/38) had mature cataracts (Table 1).
In conclusion, the present study has demonstrated the critical role of GPX-1 in protection against oxidative damage in the lens nucleus. The virtual absence of the enzyme in the gene-knockout mice resulted in increased lens NLS, which correlated with damage to the fiber cell membranes in the lens nucleus leading to late-onset cataracts. This is the only phenotypic change observed thus far in GPX-1-deficient animals. The mouse experimental model used in this investigation provides a unique system to study the functional role of this enzyme in antioxidant defense mechanisms in the lens and age-related onset of cataract, because other antioxidant enzymes such as GR, catalase, SOD, glutathione-S-transferase, and other forms of GPX appear to be unaffected in various tissues of these animals.  

References


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