Ultraviolet Radiation–Induced Cataract in Mice: The Effect of Age and the Potential Biochemical Mechanism

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Purpose. To study the effect of age on the morphologic and biochemical alterations induced by in vivo exposure of ultraviolet radiation (UV).

Methods. Young and old C57BL/6 mice were exposed to broadband UVB+UVA and euthanized after 2 days. Another batch of UV-exposed young mice was monitored for changes after 1, 2, 4, and 8 days. Age-matched nonexposed mice served as controls. Lens changes were documented in vivo by slit-lamp biomicroscopy and dark field microscopy photographs ex vivo. Lens homogenates were analyzed for glutathione (GSH) level, and the activities of thioredoxin (Trx), thioltransferase (TTase), and glyceraldehyde-3-phosphate dehydrogenase (G3PD). Glutathionylated lens proteins (PSSGs) were detected by immunoblotting using GSH antibody. Western blot analysis was also done for the expression levels of TTase and Trx.

Results. Both age groups developed epithelial and superficial anterior subcapsular cataract at 2 days postexposure. The lens GSH level and G3PD activity were decreased, and PSSGs were elevated in both age groups, but more prominent in the older mice. TTase and Trx activity and protein expression were elevated only in the young mice. Interestingly, lens TTase and Trx in the young mice showed a transient increase, peaking at 2 days after UV exposure and returning to baseline at day 8, corroborated by lens transparency.

Conclusions. The lenses of old mice were more susceptible to UV radiation–induced cataract. The upregulated TTase and Trx likely provided oxidation damage repair in the young mice. (*Invest Ophthalmol Vis Sci.* 2012;53:7276–7285) DOI: 10.1167/iovs.12-10482

The solar ultraviolet (UV) radiation that reaches the Earth consists of two components: UVA (315–400 nm) and UVB (280–315 nm). Several experimental[1–5] and epidemiologic investigations[6–8] and case studies[9,10] have shown a correlation between cataract development and exposure to UV radiation. UVB is particularly relevant to cataract development, since the energy of UVB is substantially absorbed within the lens.[11–13] The wavelength range shown to be most harmful for the lens is located around 300 nm.[14,15] The strong energy in the UV light can directly cause a DNA lesion in the lens by inducing thymine dimer formation.[16] UV can damage the lens by generation of reactive oxygen species (ROS)[17] that indirectly induce oxidative damage to DNA,[16,18–20] by disturbing cell proliferation in the lens epithelium,[21] by altering kinetic properties of enzymes in the energy metabolism,[22] by increasing insoluble and decreasing soluble protein,[17,23] and by disturbing the sodium potassium balance and thereby the water balance in the lens.[24,25]

The lens uses its high level of glutathione (GSH) along with several effective oxidation defense enzyme systems to eliminate oxidants.[19,26] It is known that there are two recently elucidated repair systems in the lens to reduce oxidized proteins/enzymes and to maintain redox homeostasis.[27] The GSH-dependent thioltransferase (TTase) system specifically dethiolates glutathionylated proteins or protein-S-S-GSH (PSSG), whereas the NADPH-dependent thioredoxin (Trx) system mainly reduces protein–protein disulfides (PSSPs), thus maintaining the cellular thiol/disulfide homeostasis.

TTase, also known as glutaredoxin (Grx), is an 11.8-kDa heat-stable cytosolic protein present ubiquitously in prokaryotes and eukaryotes.[28] It contains a conserved CXXC sequence at the active site, which makes the protein extremely resistant to oxidation. It catalyzes specifically the reduction of proteins that are thiolated by GSH (PSSG). The reduction of PSSG is carried out via GSH, which is oxidized to GSSG and recycled to GSH via the recycling system of NAPDH and glutathione reductase (GR).[27] TTase is also known to be a multifunctional enzyme with a role in many biochemical processes, including protein dethiolation,[29] reduction of ribonucleotide reductase,[28,30] reactivation of key glycolytic and oxidation defense enzymes,[31] reducing oxidized ascorbate with its dehydroascorbate reductase activity,[32,33] and participation in the regulation of signal transduction.[34,35]

Trx is a small 12-kDa protein, with two highly conserved vicinal cysteine residues (WCGPC) at the active site that can reduce protein–protein disulfides.[36] Oxidized Trx is then reduced by thioredoxin reductase (TR) with electrons donated from NADPH. The Trx/TR system plays an important role in the redox regulation of multiple intracellular processes, including DNA synthesis, cell proliferation, differentiation, anti-apoptosis, and protein/enzyme reduction and reactivation.[29,36–39]

Cataract formation is associated with many biochemical changes, and various oxidant-induced cataracts in animal models are often associated with loss in free GSH and modification of lens protein thiols into GSH-bound proteins (PSSGs) or protein-S-S-protein (PSSP).[19,26,27] The relevance of PSSG as a good marker for protein thiol oxidation in the lens is

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further established with the findings that aging and the degree of opacity in human old or cataractous lenses are well corroborated with the loss in free GSH and the accumulation of PSSG.[40,41]

Although UV radiation has been used successfully in the past for inducing light scattering or opacity in the animal lenses in vivo or in vitro, and the threshold of UVB for oxidative damage in lens epithelial cells or whole lens have been established,[42] little is known about the biochemical effects of animal aging in UV cataractogenesis, and the nature of the oxidation damage on the lens protein thiols or the role of thiol oxidation damage repair systems in lenses exposed to UV radiation. Considering the strong indications of solar UV radiation as a risk factor in age-related cataract, it is important to understand if aging compromises the protection mechanisms relevant to UV exposure. The objective of the present study, therefore, is to compare the age effect on the lens tolerance to UV exposure, and the effectiveness of the intrinsic thiol oxidation damage repair systems during recovery after the exposure. We used the C57BL/6 mouse as a model and found that old mice were more vulnerable to UV stress with more lens opacity, GSH loss, and PSSG accumulation than were young mice. The young mice showed a robust transient upregulation of Trx and TTase during postexposure recovery. The transient change of Trx and TTase corroborated with the transient accumulation of PSSG and loss in lens transparency, indicating a potential mechanism where both thiol oxidation damage repair systems may provide an efficient protection against UV exposure to maintain lens transparency in the young mice.

Materials and Methods

Materials

Glutathione (GSH), 5′,5′-dithiobis (2-nitrobenzoic acid) (DTNB), NADPH, hydroxy ethyl disulfides (HEDS), and ATP were all from Sigma Chemical Co. (St. Louis, MO). Bicinchoninic acid (BCA) protein assay reagent and chemiluminescent substrate were from Pierce (Rockford, IL). The specific antibody for glutathione was purchased from ViroGen Co. (Watertown, MA) and the antibody for beta-actin was made by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). TTase and Trx antibodies were purchased from Abcam (Cambridge, MA). All other chemicals and reagents were of analytical grade.

Experimental Animals

In total fifty-six 1-month-old and ten 16-month-old C57BL/6J pigmented mice, half male and half female, were used. The young mice were obtained from Harlan Laboratories (Haslett, MI), whereas the older mice were raised in house for aging studies, and were kept in a germ-free facility with up to five mice per cage and fed with regular rodent chow and water without restriction. The use of animals for this study was approved by the Institutional Animal Care and Use Committee, and the mice were maintained and treated according to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

UV Exposure

A custom-made UV system was used for this study. The UV source consisted of two parallel-unfiltered fluorescent tubes in a fixture with reflector (XX-15MR 302 nm; broadband UVB peaking at the 313 nm mercury line and having a right tail into the UVA, also including mercury peaks in the UVA and blue light; the spectrum graph is available at the producer website; UVP, Inc., San Gabriel, CA). The energy output of the lamp was measured to be 1.6 mW/cm^2 using a UVX digital radiometer with a UVX-31 302-nm sensor (UVP, Inc.). Since the meter was factory calibrated with a line source, its readings were adjusted with a correction factor for the XX-15MR broadband UV. Using the UVP Inc–provided factor of 1.43, the corrected UV radiation dose was 20.6 kJ/m^2. After being anesthetized by intraperitoneal injection of a mixture of 90 mg/kg of ketamine and 10 mg/kg of xylazine, mice of both control and experimental groups received mydriatic eyedrops in both eyes (0.5% tropicamide and 0.5% phenylephrine hydrochloride; Santen Pharmaceutical Co., Ltd., Osaka, Japan). In the first experiment eight 1-month-old and five 16-month-old mice were exposed during 15 minutes to the UV radiation. Equal numbers of age-matched unexposed mice were used as controls. All mice were euthanized after 2 days and the lenses were extracted for assays. In the second experiment, forty 1-month-old mice were exposed to the same UV procedure but they were euthanized in groups of eight mice at 1, 2, 4, and 8 days after the UV exposure. Eight unexposed mice were used as controls (0 time). For some assays, the epithelial layer was removed and pooled, whereas in others the whole lens was used either individually or pooled for subsequent analyses.

Morphologic Examination

The anterior segment was checked prior to UV exposure by slit-lamp examination (66Vision Co., Suzhou, China). Morphologic changes in the cornea and lens were monitored by slit-lamp microscopy at different time points after exposure. However, since UV-induced corneal cloudiness interfered with the slip-lamp lens examination, the lenses were enucleated and further examined and photographed under dissecting microscope using dark field illumination to record all the morphologic changes.

GSH Assay

Whole individual lens or six pooled epithelial layers were homogenized in 0.5 mL of pH 7.5, 1 mM Tris-HCl buffer. A portion of the homogenate was removed and mixed with an equal volume of 20% trichloroacetic acid (final 10% TCA) and the mixture was centrifuged at 1000*g* for 15 minutes at 4°C. The supernatant was used for GSH quantification based on Ellman's method.[43]

Enzyme Assays

Glyceraldehyde 3-phosphate dehydrogenase (G3PD) activity was assayed according to the method of Bergmeyer et al.[44] Whole lens was homogenized, centrifuged (1000*g*, 15 minutes at 4°C), and an aliquot of the supernatant was mixed with assay buffer (200 mM triethanolamine, pH 7.6) containing 1 mM ATP, 1 mM EDTA, 2 mM $MgSO_4$, 0.2 mM NADPH, 15 U/mL 3-phosphoglyceric phosphokinase, and 6 mM 3-phosphoglyceric acid, and the reaction was carried out at 25°C. The decrease in absorbance at 340 nm was monitored for 5 minutes and used to determine G3PD activity. TTase activity was determined with HEDS as substrate, following the method of Raghavachari and Lou.[45] The activity of Trx was determined by a previously described method,[46,47] which is based on the insulin reduction ability of Trx in the presence of NADPH and TR.

Protein Determination

Protein concentration was determined by the bicinchoninic acid (BCA) method with bovine serum albumin as the standard.[48]

Western Blot Analysis

Lens samples, prepared from individual lens or six pooled lens epithelial layers, with equal amounts of total protein, were first separated by 12% SDS-PAGE and then transferred to a polyvinylidene difluoride membrane (GE Healthcare, Broomfield, CO) to detect GSH conjugated protein, beta-actin, TTase, or Trx using specific anti-glutathione, anti-beta-actin, anti-G3PD, anti-TTase, and anti-Trx anti-

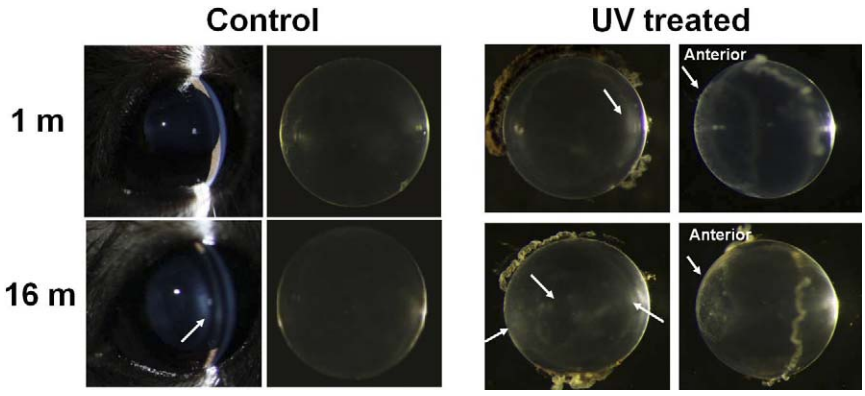

Figure 1. Formation of anterior subcapsular cataract in the lenses of 1- and 16-month-old mice after UV exposure. Eight 1-month-old mice and five 16-month-old mice were treated with UV radiation for 15 minutes and the lenses were extracted at the end of 48 hours. Equal numbers of 1- and 16-month-old untreated mice were used as the controls. A representative control and UV-treated lens from both ages are shown. For the controls, the slit-lamp images (×12 magnifications) are on the *left* and the dark field illumination macroscopic photos (×25 magnifications) of the extracted lenses are on the *right*. For the UV-treated group, the dissected lenses with a front view of the anterior are on the *left* and the side view of the anterior are on the *right*. An *arrow* indicates opacity.

bodies, respectively. The immunoblot was analyzed and visualized with an imaging system (Versadoc 5000 MP Imaging System; Bio-Rad, Hercules, CA).

Statistical Analysis

Single independent pairwise comparisons were performed using Student's *t*-test. Multiple comparisons toward a control group were performed with Dunnett's test. $P < 0.05$ was considered statistically significant.

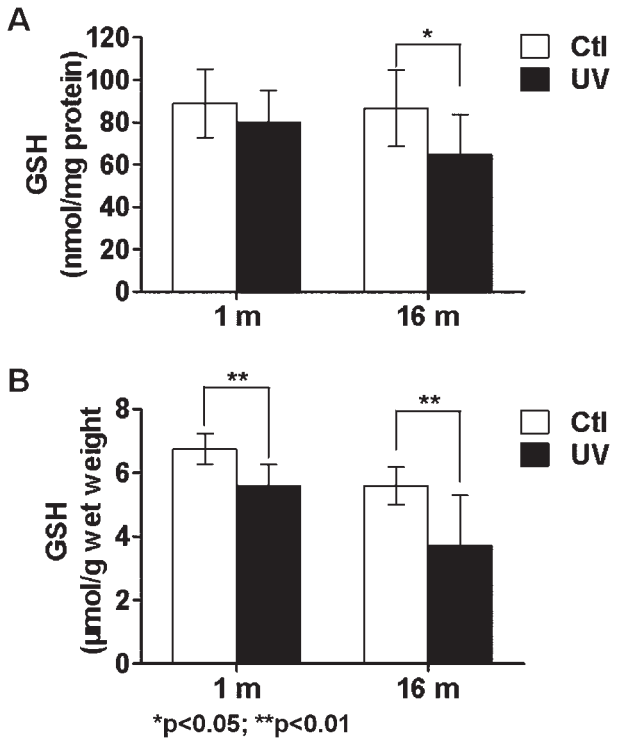

Figure 2. The age effect on UV-induced GSH loss in young and old mouse lenses. Lens samples from Figure 1 were used for GSH analyses. (**A**) Changes in the GSH pool observed in six pooled lens epithelial layer. (**B**) Changes in the GSH pool observed in the whole lens. The data are expressed as mean ± SD, $n = 4$ with three separate measurements. $^{*}P < 0.05$, $^{**}P < 0.01$.

Results

UV-Induced Morphologic Changes in Young and Old Lenses

Two age groups of mice (1 and 16 months old) were evaluated for cataract phenotype by biomicroscopy. Slit-lamp examination was only done to all animals before the experiment because UV exposure caused extensive corneal cloudiness, which hindered slit-lamp examination. The young mouse control lenses were clear (Fig. 1, top panel) but cortical opacity occurred in some of the old animal lenses (Fig. 1, bottom panel; see arrow), an indication of age-associated cataract development in the mice. Morphologic examination of the extracted lenses confirmed the slit-lamp observations. All UV-exposed young lenses developed opacity mainly at the anterior pole, whereas the older UV exposed lenses showed more overall opacity throughout the entire lens (Fig. 1; see arrows).

UV-Induced GSH Loss in Young and Old Lenses

Because GSH is known to be the first defense system against oxidative stress, the level of reduced GSH was measured in the whole lens or pooled epithelial layers from 1- and 16-month-old animals with and without UV exposure. As shown in Figure 2A, the GSH pool levels are similar in the epithelial layers of young and old mice controls. UV exposure suppressed 10% GSH in the younger tissue and 25% in the older tissue. For the whole lens, the untreated control showed 20% loss in the GSH pool simply from aging. With UV treatment, there was 34% GSH loss in the older lenses compared with that of the control. Less GSH loss (17%) was found in the younger lenses under the same experimental conditions (see Fig. 2B).

G3PD Activity in Young and Old Lenses after UV Exposure

G3PD activity was assessed in this study, given that this enzyme is essential for ATP production in the lens but is also very sensitive to oxidative stress.[31] Therefore, G3PD is a good indicator for UV-induced oxidative stress in exposed mouse lenses. G3PD activity (Fig. 3A) and protein level (Fig. 3B) were remarkably suppressed with aging. However, after UV exposure, the old age group showed a greater loss of G3PD activity (34%) than that of the 1-month-old mice (15% loss) in

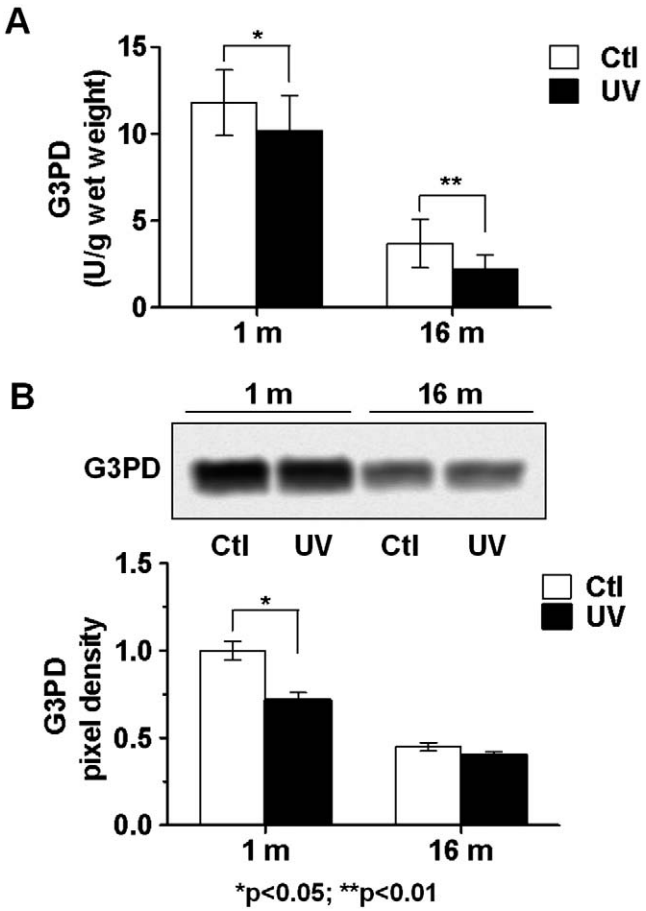

Figure 3. Changes in lens glyceraldehyde 3-phosphate dehydrogenase (G3PD) activity and protein expression in young and old mice after UV exposure. Whole lens homogenates prepared in the study described in Figure 1 were used for the comparison of G3PD activity and protein levels in young and old mice before and after UV radiation. (**A**) G3PD activity in the whole lens. (**B**) A representative Western blot analysis of individual lens homogenate using specific anti-G3PD antibodies. Pixel densities for all G3PD positive bands in comparison with the 1-m control lens, normalized to 1.0, are shown in the bar graph. Data are expressed as mean ± SD with three separate analyses. $^{*}P < 0.05$, $^{**}P < 0.01$.

comparison with the respective untreated controls (Fig. 3A). Additionally, the Western blot analysis (Fig. 3B) indicated that although the protein level of G3PD was reduced extensively with age, the protein expression was similar with or without UV exposure, indicating that the lowered G3PD activity found in the exposed groups was mostly due to UV inactivation.

Formation of Glutathionylated Proteins in Young and Old Lenses after UV Exposure

Upon oxidative stress, lens proteins became glutathionylated by forming protein-S-S-glutathione (PSSG) conjugates. Western blot analysis was done using specific anti-GSH antibody to examine the level of total PSSG in samples prepared from whole lens or pooled epithelial layers (6 layers) with and without UV exposure. The epithelial layers in young control lens showed a heavy band between 37 and 50 kDa and a weak band around 20 kDa. After UV exposure both bands increased in intensity plus additional bands appeared between 50 and 150 kDa (Fig. 4A). The PSSGs in the epithelial layers of the older age group showed a similar pattern to that of the young ones except there was an extra band appearing at the high molecular weight region (150 kDa). Upon UV exposure, all the PSSG positive bands were enhanced over the untreated control (Fig. 4A). Figure 4B summarizes the total level of PSSGs, in which high elevation in the older lens group can be seen after UV exposure.

When the whole lens proteins were analyzed, the GSH-conjugated proteins predominantly presented at 42 kDa, and minor bands between 20 and 25 kDa and 15 and 17 kDa (Fig. 4B). Compared with those of young mice, the lens proteins from the older mice showed more intensity in all the PSSG bands after exposure to UV, with additional PSSG positive bands appearing above 100 kDa. Further analysis indicated that the heavy band at 42 kDa contained beta-actin when beta-actin immunoprecipitation was performed (data not shown). Figure 4D summarizes the total PSSGs in the whole lens, in which a young lens has negligible PSSG increase. However, the older lens showed more PSSGs than the young ones, and a 20% increase was found after the UV exposure.

Differential Effect of UV Exposure on TTase and Trx in Young and Old Mouse Lenses

Since the lens proteins can be oxidized to protein-GSH mixed disulfides, protein–protein disulfides or high molecular weight aggregates from oxidative stress,[26] we examined the two oxidation damage repair enzymes for protein thiols, that is, TTase and Trx, in lens tissues with and without UV exposure. As shown in Figure 5A, TTase activity in the UV-exposed young lenses was 23% higher than that of untreated controls, but the activity remained the same in the older lenses following treatment. However, the untreated older lenses contained less than half of the activity as that of the young lenses (Fig. 5A). Similarly, Trx activity in the young lenses was enhanced 40% over the control by UV exposure, from 80 to 120 mU/g wet weight (Fig. 5B). This activation was not observed in the old lens. However, the Trx activity appeared to be higher in the old lenses (Fig. 5B).

Western blot analysis of the same lens samples indicated that UV exposure strongly upregulated both TTase and Trx protein expressions in the young lenses, whereas in the older lenses, there was no change in TTase but a slight elevation in Trx (Fig. 5C). This upregulation result was consistent with the findings of elevated enzyme activity shown in Figures 5A and 5B. Figure 5D depicts the comparative TTase-positive and Trx-positive immunoblots in Figure 5C.

Transient Morphologic Changes in Young Mouse Lenses during Recovery after UV Exposure

Because the acute UV study showed a unique resistance to UV radiation–induced GSH loss, G3PD inactivation, and PSSG formation in the lenses from younger animals, we conducted a second UV radiation study using only young mice (1 month old) to evaluate the mechanism of such resistance by monitoring the changes in several time points in an 8-day recovery study. We could conduct this study using 40 young mice because they were readily available from commercial sources, whereas it was not feasible to acquire old mice commercially nor from our current supply in house. In this 8-day UV study, all morphologic changes were documented with microscopic photographs of the extracted lenses using dark field illumination.

As shown in Figure 6, a transient opacification of the lenses is observed during the 8-day post-UV exposure. The lenses showed visible haziness in the epithelial and superficial anterior cortical regions on day 1, with increased severity and extension to the equator by day 2. However, the opacities

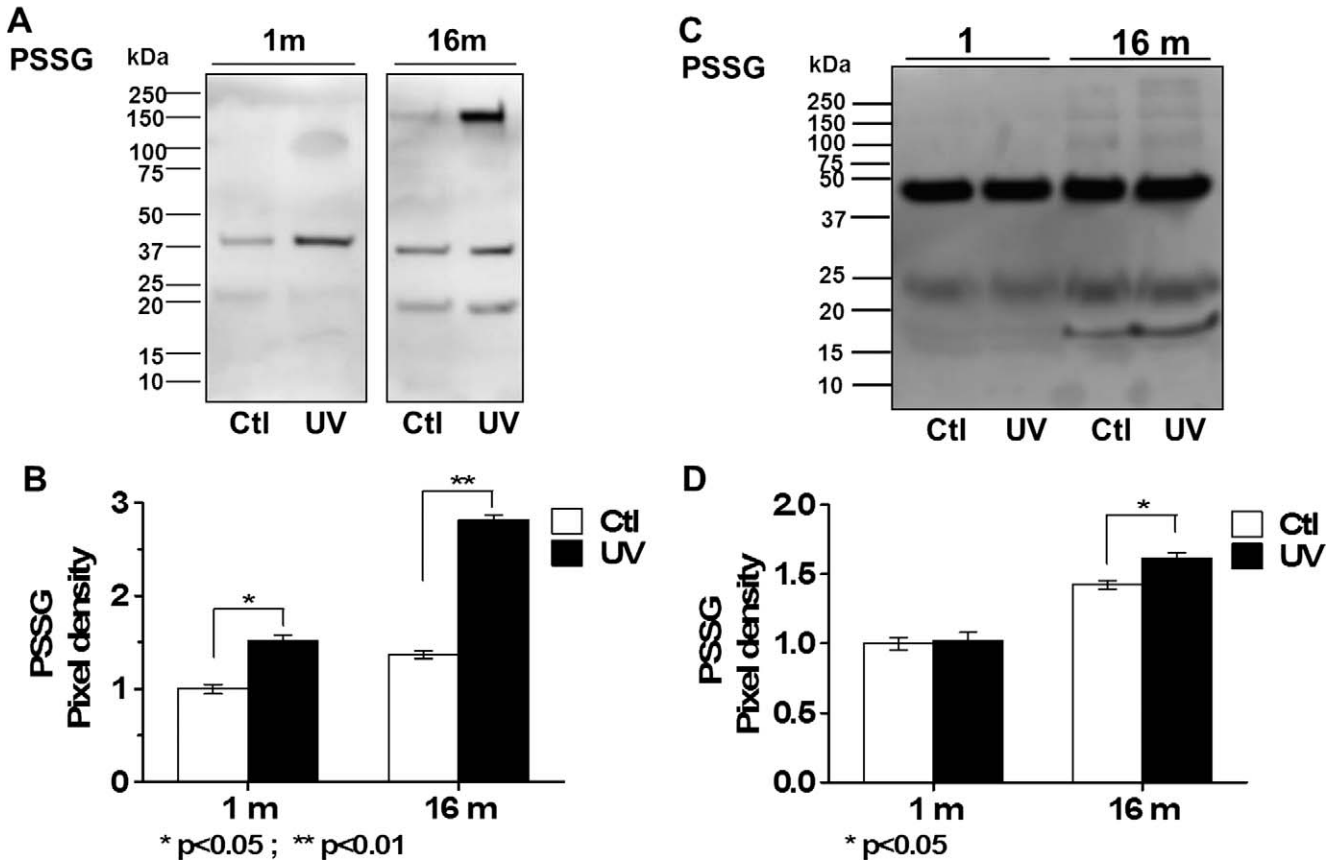

FIGURE 4. Detection of PSSGs in young and old mouse lenses after UV exposure. Whole lens homogenates prepared in Figure 1 were analyzed by Western blot for the level of glutathionylated proteins using anti-GSH antibodies. A representative immunoblot is shown. (**A**) Comparison of PSSG in the lens epithelial layer (pooled six layers). (**B**) Pixel density of all PSSGs (between 20 and 150 kDa) in (**A**), and in comparison with the value of 1-m lens control (normalized to 1.0). (**C**) PSSG in the whole lens homogenate. (**D**) Pixel density of all PSSGs (15–46 kDa) in (**C**), and in comparison with the value of 1-m lens control (normalized to 1.0). Eighty μg of protein was loaded per lane, and equal loading was confirmed by probing with β-actin antibody. The data are expressed as mean ± SD with $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$.

diminished with time, and only smaller patches remained at these regions by day 4 and nearly all disappeared by day 8 (Fig. 6).

Effect of Recovery Time on GSH Level and G3PD Activity in Young Mouse Lenses after UV Exposure

Figure 7A shows the GSH concentration in the lenses of young mice at various time points after UV exposure. Compared with the untreated control lenses (0 time), the GSH levels in UV-treated lenses dropped gradually, with an accelerated depletion started at 2 days after the UV exposure. GSH decreased 40% by the fourth day and remained at the same level until day 8. On the other hand, the G3PD activity in these young lenses showed a transient inactivation. It decreased 14% during the first day after UV exposure, remained at the same level by the second day, and then gradually recovered to the activity of untreated lenses (0 time) by the fourth day and even slightly increased above the control by the eighth day (Fig. 7B).

Transient Accumulation of PSSG in Young Lenses during Recovery after UV Exposure

Lens samples from untreated control animals (0 time group) and post UV-exposure day 1, 2, 4, and 8 day were analyzed for glutathionylated proteins with Western blot analysis using anti-GSH antibodies. Actin was used as protein loading control. As shown in Figure 8A, the pattern of the major PSSG positive proteins is similar to the findings in Figure 4C. These PSSG bands increased in intensity from day 0 to days 1 and 2 and then gradually decreased at day 4 and day 8. At 2 days postexposure when the lens showed the most opacity, lens proteins also displayed high molecular weight PSSGs near 200 kDa, an indication of protein aggregation. These high molecular weight PSSGs were dissipated to the basal level when the recovery period continued into days 4 and 8.

Actin was examined to ensure equal gel loading. The pixel density of the total PSSG at various time points in comparison with the 0 time control is summarized in Figure 8B.

Transient Activation and Upregulation of TTase and Trx in Young Lenses during Recovery after UV Exposure

During the 8-day post-UV–exposure recovery period, the lenses showed a transient activation in TTase (Fig. 9A) and Trx (Fig. 9B). The activity increase was initiated at day 1 and then peaked at day 2, after which it dropped back gradually to baseline by the end of day 8. The peak increase in activity over baseline was significant for both enzymes, with 20% elevation in TTase and 85% in Trx over the respective control (0 time).

The expression levels of both TTase and Trx were also evaluated by immunoblotting with respective specific antibodies. As shown in Figure 9C, TTase was transiently upregulated within 1–2 days and gradually dropped back to baseline level at

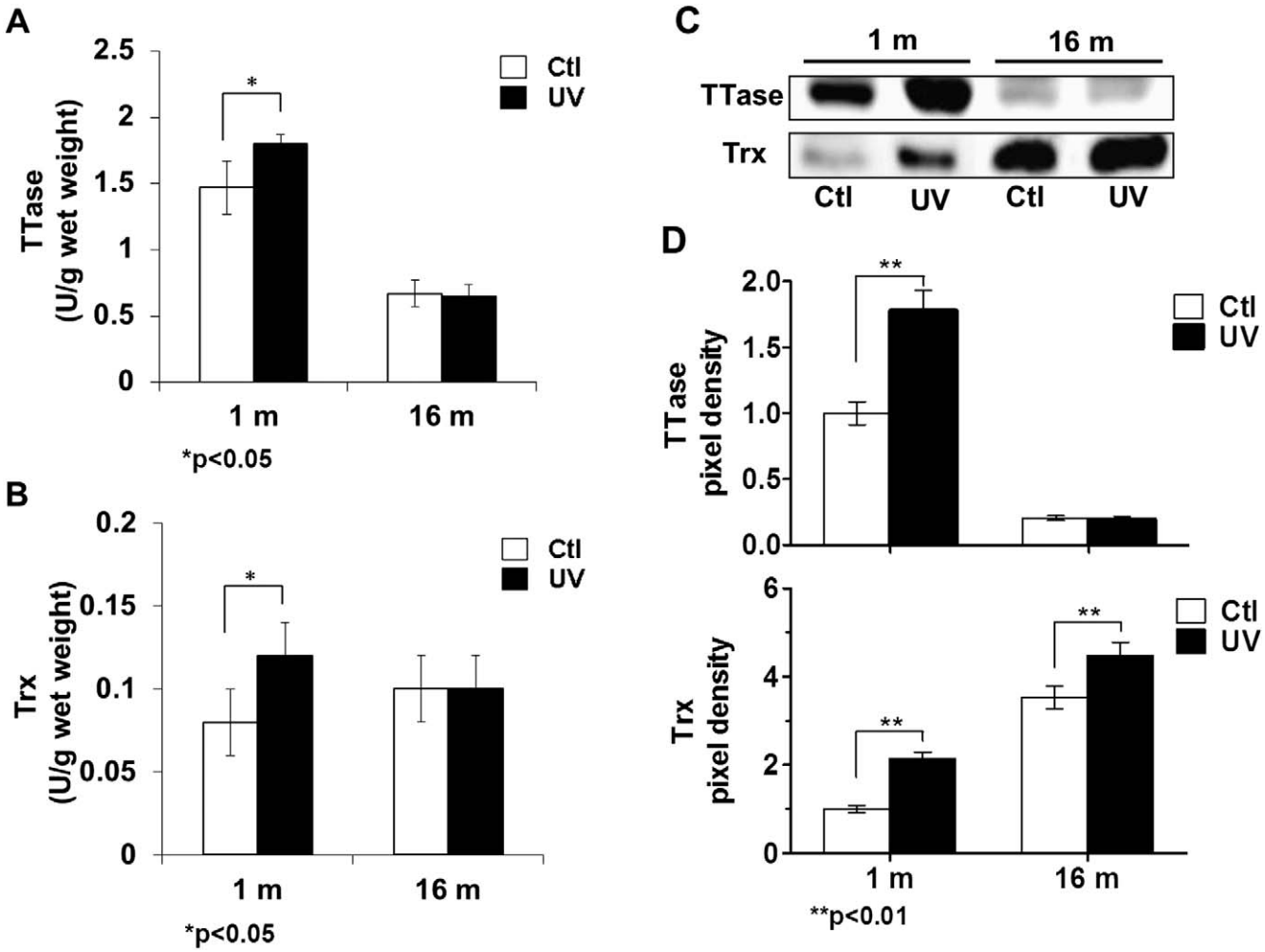

Figure 5. Effect of UV radiation on the activity and protein expression of TTase and Trx in young and old mouse lenses. Whole lens homogenates prepared in Figure 1 were analyzed for TTase, Trx enzyme activities, and protein expressions. (**A**) TTase activity in U/g wet weight. (**B**) Trx activity in U/g wet weight. (**C**) A representative Western blot analysis of the lens homogenate for the expression levels of TTase and Trx using specific anti-TTase and anti-Trx antibodies, respectively. (**D**) Relative pixel density of TTase and Trx expression in (**C**), in comparison with the value of 1-m lens control (normalized to 1.0). Data are expressed as mean ± SD, $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$.

day 4 and day 8. Similar results were found in Trx expression (Fig. 9C), in which the transient increase was extensive at day 1 and day 2, and abruptly normalized by days 4 and 8. The relative pixel density of TTase and Trx protein bands during the course of 8 days (Fig. 9C) is summarized in Figure 9D. Beta-actin was probed to ensure equal protein loading for the Western blot analysis.

Discussion

We have demonstrated in this study that pigmented mice subjected to in vivo UV irradiation (15 minutes at 20.6 kJ/m^2) can form anterior subcapsular cataract within 2 days, similar to the findings reported in young mice (6 weeks) with the same background used by Meyer et al.[49] and in young albino rats

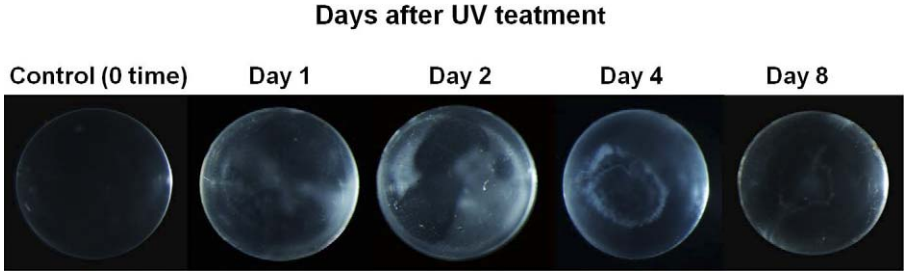

Figure 6. Morphologic changes of young mouse lenses during 1–8 days recovery period after in vivo exposure to UV. Forty 1-month-old mice were divided into 5 groups with 8 in each. The first 8 mice were controls (0 time) without UV exposure, whereas the others were exposed to UV radiation for 15 minutes and then euthanized at 1, 2, 4, and 8 days postexposure for lens morphologic and biochemical analyses. The dark field illumination macroscopic photos (×25 magnification) are from representative lenses extracted from each time point.

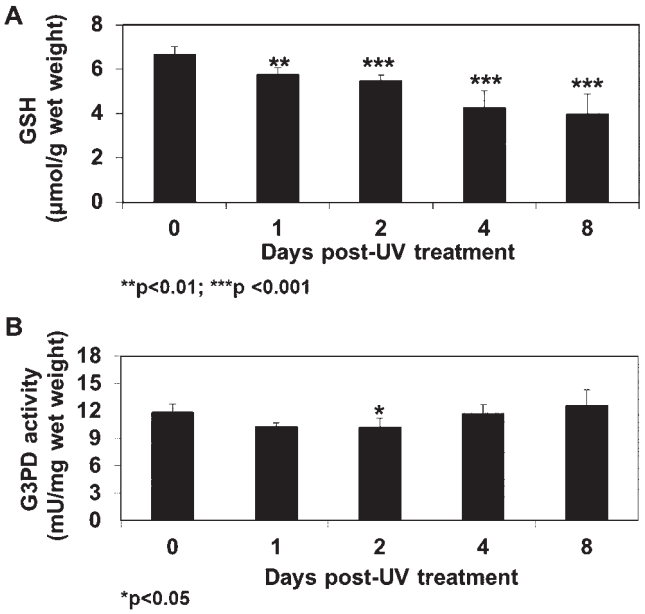

FIGURE 7. Changes in GSH level and G3PD activity during the 8-day post-UV treatment: a comparison of untreated (0 time), 1, 2, 4, and 8 days post-UV treatment. Lens samples from Figure 6 were used for GSH and G3PD analyses. (**A**) GSH level. (**B**) G3PD activity. Each time point consists of 8 mice at 1 month of age. The data are expressed as mean ± SD, $n = 8$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. the 0 time control (untreated lens).

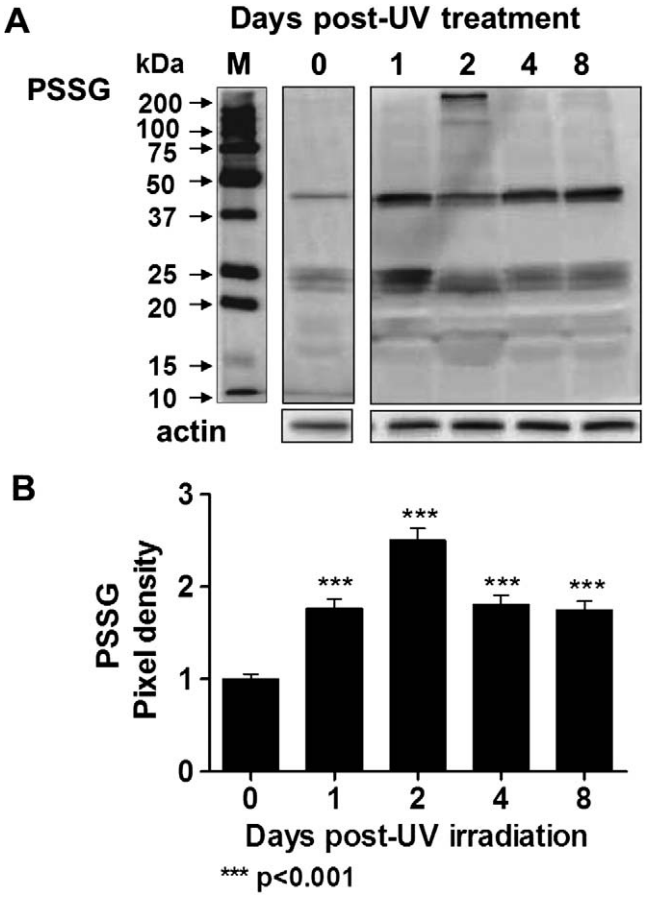

FIGURE 8. PSSG levels in the lenses of 0–8 days post-UV–treated 1-month-old mice. Lens samples from Figure 6 were used for PSSG analysis. (**A**) Western blot analysis was performed using lens homogenates from 1-, 2-, 4-, and 8-day recovery periods after UV treatment and in comparison with the lenses of untreated (0 time) animals. Samples (80 μg) were separated under nonreducing conditions on 12% SDS-PAGE. The representative WB shown was probed with anti-GSH antibody. Equal loading is shown with anti-actin antibody. (**B**) Pixel density of total PSSG bands were normalized to the beta-actin band and compared with the 0 time control value (normalized to 1.0). Data are expressed as mean ± SD, $n = 3$; $^{***}P < 0.001$.

used by Michael et al.[50] and Ayala et al.[51] Even though we used 2- or 3-fold higher UV dosage as the above-cited authors, the onset of the lens morphologic changes began approximately the same time (24 hours). The initial UV damage appears to be in the epithelial cell layer, given that the side view of the UV-exposed lenses in Figure 1 indicates a very visible contrast from the control lenses. This epithelial damage is in agreement with the findings of apoptosis-associated p53 elevation[51] and DNA damage-associated TUNEL-positive cells[50] in lenses of young rodents after brief UV exposure.

It is interesting that the age of an animal appears to play a very important role in the susceptibility of UV-induced cataract. Two previous studies explicitly investigated the age effect in UV cataractogenesis in the albino rat, using degree of cataract as the endpoint, and they showed a low tolerance to UV exposure in 3- and 6-week old rats, compared with older rats (52 weeks).[42,52] This contrasts to our current results on pigmented mouse where the macroscopic lens changes seemed to be more prominent in the old mice. The reason for this discrepancy is unclear, but likely the difference in the UV spectrum, the animal species, and the pigmentation of the animal may all play some roles. Interestingly, it has been shown recently that there are inherent differences in UV tolerance in lenses from albino versus pigmented rats.[53]

Besides more and widespread morphologic changes in the older lenses, all the biochemical markers indicated that the UV exposure we used caused more damage in older animals than that in the young ones. For instance, GSH depletion either in the epithelium or in the whole lens was much less in the young animals than that of the older ones. UV-induced PSSG formation or G3PD inactivation was more exaggerated in the older lens. Young animals likely have a high metabolic activity for synthesis and repair, and the transcriptional and translational functions are intact. Therefore, UV damage that occurs can be quickly repaired and the cellular or physiologic functions can be easily restored. Because lens transparency depends on the structural proteins to be in a reduced state to allow proper refractive index and light transmittance, oxidation of protein thiols can cause extensive protein–protein aggregation, and lens oxidative stress-induced GSSG can conjugate with protein thiols to form PSSG; moreover, if these glutathionylated proteins are not reduced in time, the glutathionylation-associated destabilization and conformational protein changes can induce PSSP and even high molecular weight aggregates.[27]

The TTase and Trx systems are known to dissociate PSSG and PSSP, respectively. Both enzymes have a property in responding to oxidative stress by quickly upregulating their gene expressions.[31,54–56] Our current study demonstrated that this property was weakened or dysfunctional in the old animals, at least in the acute study (Fig. 5), indicating a decreased ability to protect or repair damages induced from UV exposure. In particular, the inactivated G3PD by glutathionylation can be restored by TTase dethiolation,[31] which was displayed in the young lenses but appeared to be compromised in the older lenses (Figs. 3, 7B). The lens depends on ATP for various critical functions, including the active transport of

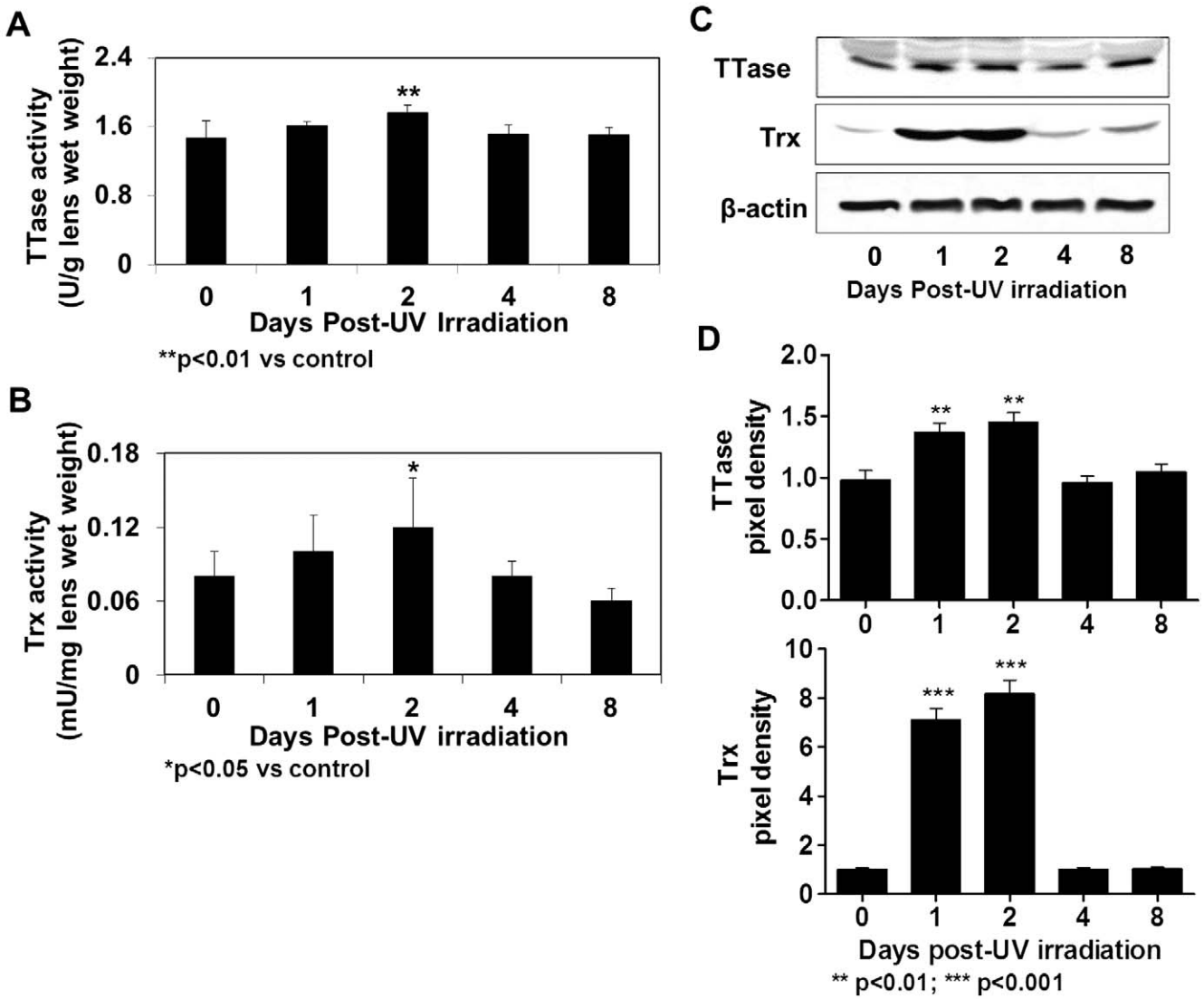

Figure 9. The enzyme activity and protein expression of TTase and Trx in lenses during the 8-day recovery period after UV exposure. Lens homogenates from untreated (0 time), 1, 2, 4, and 8 days were used for the analysis. (**A**) TTase activity. (**B**) Trx activity. Data are expressed as mean ± SD with $n = 8$. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. 0 time control (untreated). (**C**) Western blot of TTase and Trx with beta-actin for loading control. (**D**) Pixel density of TTase and Trx positive bands in comparison with the untreated control (normalized to 1.0). Data are expressed as mean ± SD with $n = 3$. $^{**}P < 0.01$, $^{***}P < 0.001$.

nutrients from aqueous humor. Loss in G3PD activity can diminish ATP production that can directly affect lens transparency. Our data showed that although aging alone induced both decreased G3PD expression and decreased catalytic activity (Figs. 3A, 3B), the results suggest that UV exposure suppressed only enzyme activity but not protein expression. Thus, even though we could not conduct a parallel 8-day recovery study on both young and old mice, we predict that the old mice may not have a recovery as speedy as that of the young mice. Certainly such speculation needs to be confirmed when a large supply of 16-month or older mice become available in the future.

Of all the glutathionylation or PSSG formation in the whole lens tissue, a consistent pattern in affected proteins is evident (Figs. 4C, 8). One major PSSG band is in the region of 20-25 kDa, where the lens crystallin proteins, such as alpha and beta-crystallins are located. Glutathionylation of crystallin proteins after UV radiation is to be expected and may contribute to lens opacity. Another PSSG band in the 15- to 20-kDa range is as yet to be identified as a glutathionylated protein species. The third major PSSG band appeared to be beta-actin based on our immunoprecipitation study (data not shown). Similar results were observed in a previous study in which a heavy PSSG band was formed at 42 kDa in H_2O_2-exposed mouse lens epithelial cells isolated from a wild-type mouse, but much more in cells isolated from a Grx2-null mouse.[57] Beta-actin is an important cytoskeletal protein; its alteration may affect lens structure and functions.

The recovery study with young lenses provided additional information on how well the morphologic change and recovery can be corroborated with the biochemical alteration and normalization. In particular, the transient subcapsular and supercortical damage by UV exposure was gradually enhanced and peaked after 2 days, and slowly diminished in 4 to 8 days. The loss in GSH was not reversible, but the extent of loss was never below the critical level of 40%, a level that is found (Lou, unpublished results, 2005) adequate for GSH to provide cellular function and lens protection.[58] Furthermore, the UV

exposure induced a smaller loss of GSH in the epithelial layer than that in the whole lens (Figs. 2A, 2B) since the recycling of GSH pool (GSSG to GSH) is most active in the epithelial layer.[58] The high level of GSH in these cells can help to restore UV-induced morphologic and biochemical damages. In the UV recovery study, adverse changes in G3PD and PSSG were also transient and coincided with the brief and strong upregulation and activation of TTase and Trx. These results explain why the UV-induced opacity was a transient change during the experimental period. Similar findings have been reported in the H_2O_2-induced transient damage in human lens epithelial cells,[31] in which a bolus amount of H_2O_2 caused a transient accumulation in PSSG and loss of G3PD activity with concomitant upregulation of TTase in the cells. UV-induced damage in liver cells also showed upregulation of TTase.[59] It was reported recently that UVB radiation induced more light scattering in the lens of TTase null mouse, compared with that of the wild-type control.[49] These findings strongly suggest that TTase is essential in protecting young lens against UV damage. Based on the acute studies (Figs. 2–5), the old animals might be less capable of protecting protein thiols from oxidative damage than the young mice. Further studies are needed to demonstrate this shortcoming in a long-term observation.

In conclusion, we report here a new finding that the aging lens from the pigmented mouse is very vulnerable to UV exposure, resulting in extensive metabolic and structural damage. In contrast, young lenses were resistant to UV stress, and were capable of repairing damage and restoring lens clarity. The mechanism for the differential age effect in our acute study is likely the protection by the thiol damage repair enzymes thioltransferase and thioredoxin, which are upregulated during the initial insult of UV exposure. This results in the timely repair of the ATP-generating G3PD enzyme as well as other oxidized lens proteins/enzymes. Restoration of these protein sulfhydryls may protect the lens from further pathology and may prevent lens opacification.

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References

1. Valenzeno DP. Photomodification of biological membranes with emphasis on singlet oxygen mechanisms. *Photochem Photobiol*. 1987;46:147–160.
2. Torriglia A, Zigman S. The effect of near-UV light on Na-K-ATPase of the rat lens. *Curr Eye Res*. 1998;7:539–548.
3. Tung WH, Chylack LT Jr, Andley UP. Lens hexokinase deactivation by near-UV radiation. *Curr Eye Res*. 1988;7:257–263.
4. Schmidt J, Schmidt C, Kojima M, Hockwin O. Biochemical and morphological changes in rat lenses after long term UV B irradiation. *Ophthalmic Res*. 1992;24:317–325.
5. Soderberg PG. Mass alteration in the lens exposure to radiation in the 300 nm wavelength region. *Acta Ophthalmol*. 1989;67:633–644.
6. Zigman S, Datiles M, Torczynski E. Sunlight and human cataracts. *Invest Ophthalmol Vis Sci*. 1979;18:462–467.
7. Taylor HR. The environment and the lens. *Br J Ophthalmol*. 1980;64:303–310.
8. Weale RA. The age variation of "senile"cataract in various parts of the world. *Br J Ophthalmol*. 1982;66:31–34.
9. Lerman S. Human ultraviolet radiation cataracts. *Ophthalmic Res*. 1980;12:303–314.
10. Baum J, Pitts DG. Posterior subcapsular cataract following intense ultraviolet radiation exposure: a case report. *Eye*. 1997;11:661–662.
11. Taylor HM, West SK, Rosenthal FS, et al. Effect of ultraviolet radiation on cataract formation. *N Engl J Med*. 1988;319:1429–1433.
12. Hightower KR. A review of the evidence that ultraviolet irradiation is a risk factor in cataractogenesis. *Doc Ophthalmol*. 1994–1995;88:205–220.
13. Löfgren S, Söderberg PG. Lens lactate dehydrogenase inactivation after UV-B irradiation: an in vivo measure of UVR-B penetration. *Invest Ophthalmol Vis Sci*. 2001;42:1833–1836.
14. Pitts DG, Glenn A. Fry Award Lecture—1977. The ocular effects of ultraviolet radiation. *Am J Optom Physiol Opt*. 1978;55:19–35.
15. Merriam JC, Löfgren S, Michael R, et al. An action spectrum for UV-B radiation and the rat lens. *Invest Ophthalmol Vis Sci*. 2000;41:2642–2647.
16. Kleiman NJ, Wang RR, Spector A. Ultraviolet light induced DNA damage and repair in bovine lens epithelial cells. *Curr Eye Res*. 1990;9:1185–1193.
17. Cejkova L, Lojda Z. Histochemical study on xanthine oxidase activity in the normal rabbit cornea and lens and after repeated irradiation of the eye with UVB rays. *Acta Histochem*. 1996;98:45–52.
18. Soderberg PG, Philipson BT, Lindstrom B. Unscheduled DNA synthesis in lens epithelium after in vivo exposure to UV radiation in the 300 nm wavelength region. *Acta Ophthalmol (Copenh)*. 1986;64:162–168.
19. Spector A. Oxidative stress-induced cataract: mechanism of action. *FASEB J*. 1995;9:1173–1182.
20. Spector A. Oxidative stress and disease. *J Ocul Pharmacol Ther*. 2000;16:193–201.
21. Li WC, Spector A. Lens epithelial cell apoptosis is an early event in the development of UVB-induced cataract. *Free Radic Biol Med*. 1996;20:301–311.
22. Andley UP, Song Z, Mitchell DL. DNA repair and survival in human lens epithelial cells with extended lifespan. *Curr Eye Res*. 1999;18:224–230.
23. Zigman S, Griess G, Yulo T, Schultz J. Ocular protein alterations by near UV light. *Exp Eye Res*. 1973;15:201–208.
24. Torriglia A, Zigman S. The effect of near-UV light on Na-K-ATPase of the rat lens. *Curr Eye Res*. 1988;7:539–548.
25. Soderberg PG. Na and K in the lens after exposure to radiation in the 300 nm wavelength region. *J Photochem Photobiol B*. 1991;8:279–294.
26. Augusteyn RC. Protein modification in cataract: possible oxidative mechanisms. In: Duncan G, ed. *Mechanisms of Cataract Formation in the Human Lens*. New York: Academic Press; 1981:72–115.
27. Lou MF. Redox regulation in the lens. *Prog Retin Eye Res*. 2003;22:657–682.
28. Holmgren A. Glutathione-dependent synthesis of deoxyribonucleotides: characterization of the enzymatic mechanism of Escherichia coli glutaredoxin. *J Biol Chem*. 1979;254:3672–3678.
29. Yoshitake S, Nanri H, Fernando MR, Minakami S. Possible differences in the regenerative roles played by thioltransferase and thioredoxin for oxidatively damaged proteins. *J Biochem (Tokyo)*. 1994;116:42–46.
30. Hoog JO, Holmgren A, D'Silva C, Douglas KT, Seddon AP. Glutathione derivatives as inhibitors of glutaredoxin and ribonucleotide reductase from Escherichia coli. *FEBS Lett*. 1982;38:59–61.
31. Xing KY, Lou MF. Effect of H_2O_2 on human lens epithelial cells and the possible mechanism for oxidative damage repair by thioltransferase. *Exp Eye Res*. 2002;74:113–122.

32. Wells WW, Wu DP, Yang YF, Rocque PA. Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity. *J Biol Chem*. 1990; 265:15361–15364.
33. Fernando MR, Satake M, Monnier V, Lou MF. Thioltransferase mediated ascorbate recycling in human lens epithelial cells. *Invest Ophthalmol Vis Sci*. 2004;45:230–237.
34. Stark DW, Chock PB, Mieyal JJ. Glutathione-thiyl radical scavenging and transferase properties of human glutaredoxin (thioltransferase): potential role in redox signal transduction. *J Biol Chem*. 2003;278:14607–14613.
35. Xing K-Y, Raza A, Lou MF. Low molecular weight protein tyrosine phosphatase (LMW-PTP) and its possible physiological functions in the eye lens. *Biochem Biophys Acta*. 2007; 1774:545–555.
36. Holmgren A. Enzymatic reduction-oxidation of protein disulfides by thioredoxin. *Methods Enzymol*. 1984;107:295–300.
37. Holmgren A, Lu J. Thioredoxin and thioredoxin reductase: current research with special reference to human disease. *Biochem Biophys Res Commun*. 2010;396:120–124.
38. Mochizuki M, Kwon Y-W, Yodoi J, Masutani H. Thioredoxin regulates cell cycle via the ERK1/2-cyclin D1 pathway. *Antioxid Redox Signal*. 2009;11:2957–2971.
39. Andoh T, Chock PB, Chiueh CC. The roles of thioredoxin in protection against oxidative stress-induced apoptosis in SH-SY5Y cells. *J Biol Chem*. 2002;277:9655–9660.
40. Lou MF, Dickerson JE Jr. Protein-thiol mixed disulfide in human lens. *Exp Eye Res*. 1992;55:889–896.
41. Lou MF, Dickerson JE Jr, Wolfe JK, Tung W, Chylack L Jr. Correlation of protein-thiol mixed disulfide level with the opacity and brunescence in human lens. *Exp Eye Res*. 1999; 68:547–552.
42. Dong X-Q, Ayala M, Löfgren S, Soderberg PG. Ultraviolet radiation-induced cataract: age and maximum acceptable dose. *Invest Ophthalmol Vis Sci*. 2003;44:1150–1154.
43. Ellman GL. A colormetric method for determining low concentration of mercaptans. *Arch Biochem Biophys*. 1958; 74:443–450.
44. Bergmeyer HU, Gawehn K, Grassl M. *Methods of Enzymatic Analysis*. 2nd ed. Weinheim, Germany: Verlag Chenie; 1974: 466–467.
45. Raghavachari N, Lou MF. Evidence for the presence of thioltransferase in the lens. *Exp Eye Res*. 1996;63:433–441.
46. Yegorova S, Liu A, Lou MF. Human lens thioredoxin: molecular cloning and functional characterization. *Invest Ophthalmol Vis Sci*. 2003;44:3263–3271.
47. Holmgren A, Bjornstedt M. Thioredoxin and thioredoxin reductase. *Methods Enzymol*. 1995;252:199–208.
48. Smith PK, Krohn RI, Hermanson GT, et al. Measurement of protein using bicinchoninic acid. *Anal Biochem*. 1985;150: 76–85.
49. Meyer LM, Löfgren S, Ho Y-S, et al. Absence of glutaredoxin1 (Grx1) increases lens susceptibility to oxidative stress induced by UVR-B. *Exp Eye Res*. 2009;89:833–839.
50. Michael R, Vrensen GFJM, Marle JV, Gan L, Soderberg PG. Apoptosis in the rat lens after in vivo threshold dose ultraviolet irradiation. *Invest Ophthalmol Vis Sci*. 1998;39: 2681–2687.
51. Ayala M, Strid H, Jacobsson U, Soderberg PG. P53 expression and apoptosis in the lens after ultraviolet radiation exposure. *Invest Ophthalmol Vis Sci*. 2007;48:4187–4191.
52. Löfgren S, Michael R, Soderberg PG. Impact of age and sex on ultraviolet radiation cataract in the lens. *Invest Ophthalmol Vis Sci*. 2003;44:1629–1633.
53. Löfgren S. Lenses from Brown-Norway pigmented rats are more tolerant to in vitro ultraviolet irradiation than lenses from Fischer-334 albino rats. *Acta Ophthalmol*. 2012;90:179–183.
54. Krysan K, Lou MF. Human thioltransferase (TTase) gene is controlled by AP-1 and mediated through redox signaling. *Invest Ophthalmol Vis Sci*. 2003;43:1876–1883.
55. Yegorova S, Liu A-M, Lou MF. Cloning, expression and characterization of human lens thioredoxin. *Invest Ophthalmol Vis Sci*. 2003;44:3263–3271.
56. Moon S, Fernando MR, Lou MF. Induction of thioltransferase and thioredoxin/thioredoxin reductase systems in cultured pig lenses under oxidative stress. *Invest Ophthalmol Vis Sci*. 2005;46:3783–3789.
57. Wu H-L, Lin L-R, Giblin F, Ho Y-S, Lou MF. Glutaredoxin 2 knockout increases sensitivity to oxidative stress in mouse lens epithelial cells. *Free Radic Biol Med*. 2011;51:2108–2117.
58. Giblin FJ. Glutathione: a vital lens antioxidant. *J Ocul Pharmacol Ther*. 2000;16:121–135.
59. Rosen JE, Chen D, Prahalad AK, Spratt TE, Shluter G, Williams GM. A fluoroquinolone antibiotic with a methoxy group at the 8 position yields reduced generation of 8-oxo-7, 8-dihydro-2′-deoxyguanosine after ultraviolet-A irradiation. *Toxicol Appl Pharmacol*. 1997;145:381–387.