

Prevention of UV-Induced Damage to the Anterior Segment Using Class I UV-Absorbing Hydrogel Contact Lenses

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PURPOSE. To determine whether class I ultraviolet (UV) light-blocking contact lenses prevent UV-induced pathologic changes in a rabbit model.

METHODS. Twelve rabbits were assigned to 1 of 3 treatment groups ($n = 4$), as follows: senofilcon A (class I UV blocking) contact lenses; lotrafilcon A contact lenses (no reported UV blocking); no contact lens. The contralateral eye was patched without a contact lens. Animals received UV-B (1.667 J/cm²) exposure daily for 5 days. Postmortem tissue was examined as follows: in the cornea, the expression of matrix-metalloproteinases (MMPs) was evaluated by zymography, and apoptosis was evaluated by TUNEL and caspase-3 ELISA; ascorbate in the aqueous humor was evaluated by nuclear magnetic resonance spectroscopy; crystalline lens apoptosis was evaluated by TUNEL and caspase-3 ELISA.

RESULTS. Exposed corneas showed a significant increase in MMP-2 and -9, TUNEL-positive cells, and caspase-3 activity in the lotrafilcon A group compared with the senofilcon A group (all $P = 0.03$). A significant decrease in aqueous humor ascorbate was observed in the exposed lotrafilcon A lens-wearing group compared with the exposed senofilcon A lens-wearing group ($P = 0.03$). Exposed crystalline lenses had significantly increased caspase-3 activity in the lotrafilcon A group compared with the senofilcon A group ($P = 0.03$). Increased numbers of TUNEL-positive cells were noted in both the lotrafilcon A and the non-contact lens groups.

CONCLUSIONS. The authors show that senofilcon A class I UV-blocking contact lenses are capable of protecting the cornea, aqueous humor, and crystalline lens of rabbits from UV-induced pathologic changes. (*Invest Ophthalmol Vis Sci.* 2010; 51:172-178) DOI:10.1167/iops.09-3996

It is well established that both acute and chronic ultraviolet (UV) light exposure can lead to various ophthalmic abnormalities. The type and extent of damage from UV radiant energy is associated with the wavelength, duration, intensity, and size of the exposure. Exposure to UV-B and UV-A radiation (nearly all UV-C radiation is filtered by the atmosphere) is associated with photochemical damage to cellular systems, though some thermal damage from UV-A is possible as well. Typical abnormalities include ocular surface conditions such as

spheroidal degeneration, UV keratitis, pinguecula, and pterygium.¹ Similarly, both clinical (epidemiologic) and basic research have shown the exposure to UV-B radiation causes crystalline lens opacification, and cataract remains the most common cause of visual impairment throughout the world.^{2,3} Cataract extraction is one of the most common surgical procedures performed today because there are no effective medical therapies.^{3,4} Estimates of the increase in UV-associated cataract in the next 20 years are staggering given the current rates of ozone depletion.⁵ Even if the progression of cataracts is slowed, the reduction in morbidity associated with cataracts and the costs to society could be dramatically impacted.

Class II UV-absorbing polymers have been available in contact lenses for many years. Most recently, class I UV-absorbing silicone hydrogel polymers have been introduced and provide the highest level of UV protection. It is thought that these unique materials may provide protection relative to UV-induced ophthalmic exposure.⁶⁻¹² A hydrogel contact lens is in intimate contact with the ocular surface, potentially protecting the surface it covers in addition to the internal structures of the eye that are vulnerable to UV-induced damage. Thus, we hypothesize that use of class I UV-absorbing hydrogel polymers will prevent deleterious changes associated with UV exposure seen in the cornea, aqueous humor, and crystalline lens. We had three general aims to test this hypothesis: evaluate the impact of these hydrogel polymers on the upregulation of matrix metalloproteinase (MMP)-2 and -9 in the cornea and corneal apoptosis, evaluate the impact on aqueous humor ascorbic acid levels, and evaluate the impact in crystalline lens epithelial cells through DNA fragmentation in addition to examining the expression of cleaved caspase-3.

METHODS

Design and In Vivo Ultraviolet Radiation Exposure

The protocol was approved by the Institutional Animal Care and Use Committee and was conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. This was a prospective, no-masked clinical study that included 12 adult pigmented rabbits (weight range, 2.5-3.0 kg). The number of rabbits was selected based on the minimal number needed to show differences in the observed outcomes. Animals were premedicated with acepromazine (1 mg/kg)¹³ and were anesthetized intramuscularly with ketamine (50 mg/kg)¹³ before they were randomly assigned to 1 of 3 treatment groups ($n = 4$ per treatment group). The treatment groups were as follows: senofilcon A contact lenses (absorbs at least 99% of UV-B and 90% of UV-A; Acuvue Oasys, Johnson & Johnson, New Brunswick, NJ); lotrafilcon A contact lenses (minimal UV absorption; Focus Night and Day, CIBA Vision, Duluth, FA); no contact lens (but exposed to UV radiation). The contralateral eye of each rabbit was patched without contact lenses.

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All animals were exposed to 1.667 J/cm² UV-B (0.98 mW/cm²) daily for 5 days. UV was provided using broad-band lamps (Phillips FS-40; American Ultraviolet Company, Lebanon, IN) fitted with specialized filters (Kodacel; Eastman Kodak, Rochester, NY). Lamps emitted wavelengths between 280 and 400 nm, with a peak at 313 nm. According to the manufacturer, the emitted light contained approximately 60% UV-B and 40% UV-A, while UV-C was filtered. UV levels were measured using a radiometer (UVX; UVP Inc., Upland, CA). Each rabbit underwent slit lamp and photodocumentation on a daily basis. Corneas were evaluated with fluorescein and lissamine green daily to document corneal changes. The animals were allowed to recover from the consecutive UV radiation exposures for 2 days. On day 8, the rabbits were euthanized by an overdose of sodium pentobarbital.

Aqueous humor was removed from the anterior chamber using a 0.5-mL insulin syringe with a 28-gauge needle before enucleation. The eyes were enucleated and further dissected within 30 minutes of death. Corneas were excised and cut in half with a scalpel blade, ensuring that a central portion of the cornea was present in both tissue specimens. For each eye, half the cornea was snap-frozen using liquid nitrogen, and the other half was placed in 10% neutral-buffered formalin. The crystalline lens for each eye was dissected as follows: using Vannas scissors, the lens was cut into two halves, taking care to ensure that the central portion of the lens was present in each sample. Half the lens was snap-frozen, and the remaining half was placed in 10% neutral-buffered formalin. Frozen tissues were stored at -80°C until further processed.

Zymography

To evaluate MMP expression in the cornea, gelatin zymography was performed. Samples were homogenized at 4°C in 50 mM Tris-HCl, pH 7.4, including a protease inhibitor cocktail that did not contain EDTA (Calbiochem, San Diego, CA) and phenylmethylsulfonyl fluoride (Sigma-Aldrich, St. Louis, MO) to eliminate nonspecific protease activity. Supernatants from each tissue homogenate were collected after centrifugation at 12,000g, 4°C. Protein concentrations for each tissue sample were obtained by Bradford protein assay (Bio-Rad, Hercules, CA).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gelatin zymography was performed as previously described.¹⁴ Briefly, all protein extracts were adjusted to a concentration of 15 µg and were mixed with SDS-PAGE sample buffer. Samples were fractionated in an 8% polyacrylamide gel containing gelatin (0.5 mg/mL) by electrophoresis at 100 V for 90 minutes. Gels were run in duplicate. To ensure that equal amounts of protein were loaded in each lane, one gel was stained (Coomassie PhastGel Blue; Pharmacia Biotech, Uppsala, Sweden) for 2 hours and destained with 7% acetic acid. The other gel was soaked in 0.25% Triton X-100 for 30 minutes at room temperature to remove the SDS and then incubated in digestion buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 10 mM CaCl₂, 2 µM ZnSO₄, and 0.01% Brij-35) at 37°C overnight to allow substrate digestion. The gels were then stained with 0.1% staining solution (Coomassie PhastGel Blue; Pharmacia Biotech) for 2 hours, and destained with 7% acetic acid. Gelatinolytic activities produced clear bands of digested gelatin against a dark blue background of stained gelatin. Digested bands were compared with active-recombinant human MMP-2 and MMP-9 standards (Oncogene, San Diego, CA) included as positive controls and protein molecular weight markers (BioRad) run in tandem. Gels were imaged (ImageStation 4000MM; Eastman Kodak, Rochester, NY) and analyzed using Kodak imaging software to obtain densitometry readings of digested gelatin bands for semiquantitative analysis (expressed as arbitrary densitometry units).

Caspase-3 ELISA

Apoptosis was evaluated in both the cornea and the lens via two different experimental techniques. The first experiment performed to evaluate apoptosis was a caspase-3 ELISA. Caspase-3 activity was determined with a caspase-3 colorimetric assay kit (R&D Systems, Min-

neapolis, MN) according to the manufacturer's instructions. Tissue lysate was incubated with caspase-3 colorimetric substrate (DEVD-pNA), and the release chromophore pNA was read on a microplate reader (Infinite M200; Tecan, Durham, NC) at 405 nm (expressed as arbitrary densitometry units).

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Nick-End Labeling

The second experimental technique used to evaluate apoptosis in the cornea and crystalline lens was the TUNEL assay. TUNEL detection was performed on paraffin-embedded tissue slides with an apoptosis detection system (Promega, Madison, WI), according to the manufacturer's instructions. The slides were treated with terminal deoxynucleotidyl transferase and fluorescein-labeled dUTP to produce TUNEL-positive cells. Sections were also stained with propidium-iodide (Sigma-Aldrich) and imaged under a fluorescence microscope (Nikon 80i; Nikon Instruments, Melville, NY). Apoptosis was calculated as the number of TUNEL-positive nuclei per slide (cornea or lens). Two separate, non-consecutive slides per eye were examined from each animal; all positive cells were counted in each slide.

Nuclear Magnetic Resonance Spectroscopy

Levels of ascorbate were evaluated in the aqueous humor via NMR spectroscopy. Five hundred microliters of aqueous humor was aspirated from each eye and frozen. The samples were then lyophilized to reduce the water signal in the NMR analysis. The samples were further dissolved in a 500 µL solution of 0.25 mM sodium-3'-trimethylsilylpropionate-2,2,3,3-d₄ (TSP) in deuterium oxide (D₂O).

High-resolution NMR spectra were recorded on an NMR spectrometer (DRX800; Bruker Biospin GmbH, Rheinstetten, Germany) operating at 800.13 MHz for protons and equipped with a cryoprobe. [1H]-Spectra were recorded at 25°C. Water suppression was obtained with a 3-second presaturation pulse, followed by a 90° pulse angle for acquiring the one-dimensional proton spectra. One hundred twenty-eight transients recorded a spectral region of 11.1 kHz, and the free induction decays were collected with 32,000 points, giving an acquisition time of 1.46 seconds. The final repetition delay was 5 seconds. Chemical shift referencing in parts per million was performed relative to TSP at 0 ppm. Peak assignments were performed according to previous reports.¹⁵⁻¹⁹ Metabolites were quantified by relating their peak area with the added standard TSP, and the peak integrals were calculated using special software (TopSpin 1.3; Bruker Biospin).

Statistical Analysis

All outcome data are from 12 rabbits. As described, right eyes of each rabbit were assigned to 1 of 3 lens-wearing conditions (senofilcon A, lotrafilcon A, or no lens) in groups of four rabbits. The left eye of each rabbit was covered with a patch. Nonpatched and patched eyes of each rabbit were exposed simultaneously to UV radiation. Given the number of rabbits included in the study, only nonparametric statistical methods were used. Data analyses focused on three main comparisons. The first comparison of exposed right eyes wearing senofilcon A to corresponding eyes with a patch (within the same four rabbits) was made using the Wilcoxon signed rank test (the hypothesis was that there is no difference between the two eyes if the UV-blocking contact lens provides benefit). The second comparison of exposed right eyes wearing senofilcon A lenses to exposed right eyes wearing lotrafilcon A lenses (four different rabbits in each contact lens-wearing group) was made using the Wilcoxon rank sum test (the hypothesis was that there should be a difference between the two eyes on the outcomes if the UV-blocking contact lens provides benefit). The third comparison of exposed right eyes wearing lotrafilcon A to exposed right eyes wearing no contact lenses (again, four different rabbits in each group) was made using the Wilcoxon rank sum test (the hypothesis was that there should be no difference in the outcomes). For some of the outcomes comparing the senofilcon A lens and patched conditions (e.g., TUNEL [cornea and crystalline lens], MMP-2, and MMP-9), Wil-

coxon signed rank test statistics could not be computed because zero was the average response in both conditions across the rabbits.

RESULTS

Clinical Changes

Daily slit lamp examinations did not reveal any increase or change in staining pattern, as evaluated by fluorescein and lissamine green. One rabbit in each contact lens treatment group had fluorescein-positive corneas; however, the pattern and distribution of stain uptake were small and focal. There was a marked increase in conjunctival edema and hyperemia in all the exposed eyes. Corneal neovascularization and increased corneal edema were noted in all the exposed eyes (varying from mild to moderate) that received either no contact lens or the lotrafilcon A contact lens. Crystalline lens sutures became more prominent in the exposed eye of two rabbits; one rabbit was wearing a lotrafilcon A contact lens, and one rabbit was not wearing a contact lens.

Corneal Changes

Zymography showed basal expression of MMP-2 only in unexposed patched eyes and the senofilcon A lens-wearing eyes. There was no basal expression of MMP-9 in any of the corneas examined. There was a significant increase in both MMP-2 ($P = 0.03$) and MMP-9 ($P = 0.03$) expression in the lotrafilcon A lens-wearing eyes compared with the senofilcon A lens-wearing eyes (Figs. 1A, B). No significant differences were detected in MMP expression when no contact lens (but exposed) and lotrafilcon A lens-wearing conditions were compared. A representative zymography gel is shown in Figure 1C.

Outcomes associated with corneal apoptosis showed no statistical difference when the senofilcon A lens-wearing eyes were compared with the patched eyes for either TUNEL ($P = 0.87$) or caspase-3 activity ($P = 0.99$). As seen in Figures 2A and B, there was a significant increase in caspase-3 activity ($P = 0.03$) and in the number of TUNEL-positive cells ($P = 0.03$) when senofilcon A lens-wearing eyes were compared with lotrafilcon A lens-wearing eyes. Lastly, in the comparison of no contact lens (but exposed) and lotrafilcon A lens-wearing conditions, there were no differences in either number of TUNEL positive cells or caspase-3 activity.

Aqueous Humor Changes

Figure 3 demonstrates the amount of ascorbate present in the aqueous humor samples, as determined by NMR spectroscopy. There were no significant differences in ascorbate when the senofilcon A lens-wearing eyes were compared with the patched eyes. A significant decrease ($P = 0.03$) in ascorbate was observed in the senofilcon A lens-wearing eyes compared with lotrafilcon A lens-wearing eyes. Aqueous humor ascorbate levels were similar in the no contact lens (but exposed) and lotrafilcon A lens-wearing conditions (Fig. 3).

Crystalline Lens Changes

Outcomes associated with lenticular apoptosis showed that neither caspase-3 activity nor TUNEL-positive cells significantly differed between the senofilcon A lens-wearing eyes and the patched eyes (Figs. 4A, B). When senofilcon A lens-wearing eyes were compared with lotrafilcon A lens-wearing eyes, there was a statistical difference in caspase-3 activity ($P = 0.03$) but not in the TUNEL analysis ($P = 0.14$) (Figs. 4A, B). In the comparison between no contact lens (but exposed) and lotrafilcon A lens-wearing conditions, there was no difference in caspase-3 or TUNEL (Figs. 4A, B).

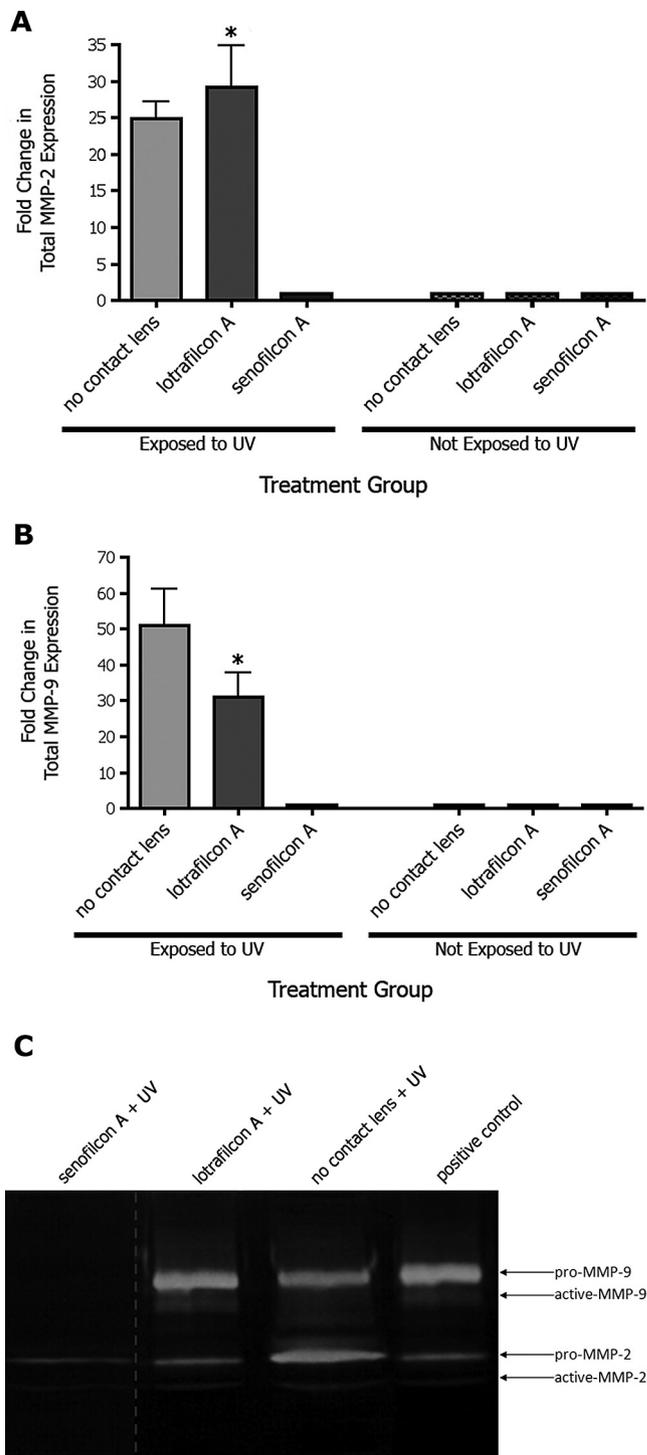


FIGURE 1. MMP expression in rabbit corneas after UV exposure. Densitometry readings from all samples were analyzed and normalized for loading control purposes. (A) In UV-exposed corneas, MMP-2 was significantly ($*P = 0.03$) increased in the lotrafilcon A group compared with the senofilcon A group. (B) MMP-9 was significantly ($*P = 0.03$) increased in the exposed corneas of those wearing lotrafilcon A contact lenses compared with those wearing senofilcon A contact lenses. No statistically significant differences were noted in either MMP-2 or MMP-9 when the lotrafilcon A lens-wearing group was compared with the non-lens-wearing control group. When the senofilcon A-wearing eyes were compared with the patched control eyes, no significant differences were found. (C) Representative zymography gel of UV-exposed corneas wearing either lotrafilcon A, senofilcon A, or no contact lens.

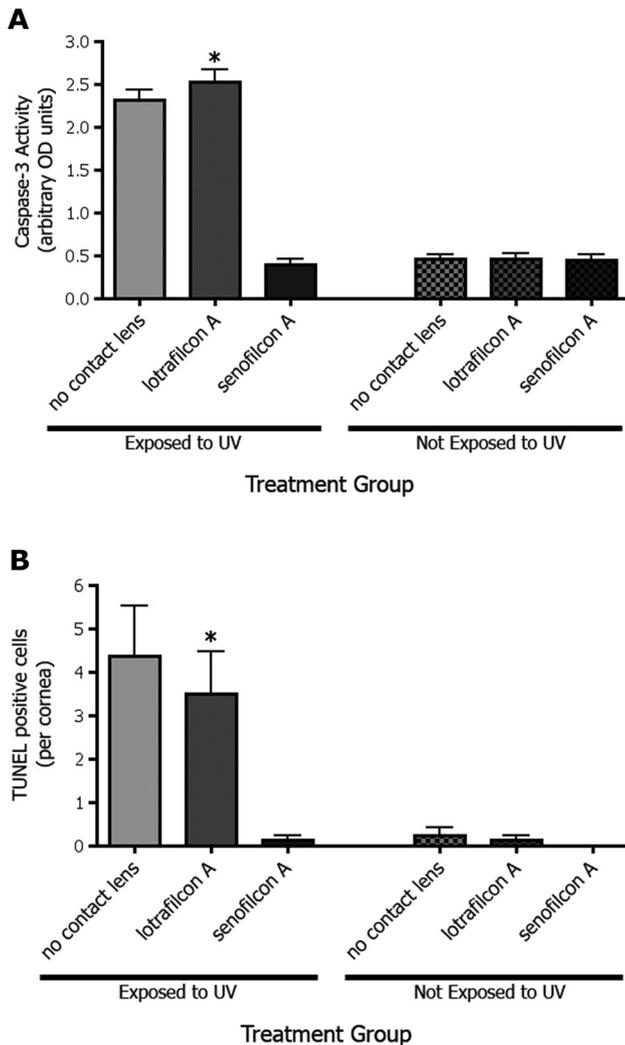


FIGURE 2. Apoptosis in rabbit corneas after UV exposure. (A) A statistically significant ($*P = 0.03$) increase in caspase-3 activity was observed in the exposed lotrafilcon A lens-wearing group compared with the exposed senofilcon A-wearing group. (B) The total number of TUNEL-positive cells was counted, and a significant ($*P = 0.03$) change was noted when the lotrafilcon A lens- and senofilcon A lens-wearing groups were compared. No significant differences were found in either caspase-3 activity or TUNEL-positive cells when the lotrafilcon A lens-wearing group was compared with the non-lens-wearing control group. When the senofilcon A group was compared with the unexposed patched control eyes, no significant differences were noted in either caspase-3 activity or TUNEL-positive cells.

DISCUSSION

Sunlight with a wavelength shorter than 295 nm (i.e., UV-C) is mainly absorbed by the ozone layer.²⁰ However, UV-B (290–320 nm) and UV-A (320–400 nm) can reach the eye. The human cornea absorbs 92% of UV-B at 300 nm, and the cutoff for rabbit corneal transmittance is 320 nm.^{21,22} The human crystalline lens has demonstrated 36% absorbance at 320 nm and 48% absorbance at 340 nm.^{20,23,24} UV-B radiation makes up only a small percentage of the solar spectrum but produces most of the damage to mammalian cells.^{20,24} UV-B has been shown to induce the production of MMPs by the corneal epithelium and stroma in dogs and humans.^{25,26} UV induction of MMPs is thought to contribute to the pathogenesis of keratitis in both species. In human corneas, MMP-9 is produced by cells in the basal layer of the epithelium directly adjacent to the

basement membrane.²⁷ Moreover, several studies indicate that MMP-9 can be produced by keratocytes of the superficial corneal stroma.^{28–30} Like MMP-9, MMP-2 is able to degrade the basement membrane.³¹ In the cornea, MMP-2 appears to be produced primarily by stromal fibroblasts, whereas corneal epithelial cells are reported to produce little of the enzyme.³² After UV radiation, human corneal fibroblasts produce MMP-2.²⁶ Based on our findings and those of other investigators, it seems likely that UV induction of MMP production and activation plays a role in initiating or maintaining enhanced proteolytic activity in the corneas of keratitis.^{24,25} Furthermore, inflammatory cells recruited to corneas affected with keratitis undoubtedly sustain and amplify MMP activity.

Gelatin zymography is the most common method for examining gelatinases in cells and media samples and can measure the total potential enzymatic activity in a sample.³³ Our zymographic analysis of the control patched rabbit corneas demonstrated low basal levels of MMP-2 expression and no detectable basal levels of MMP-9 expression. There was significant induction of both MMP-2 and -9 in both the lotrafilcon A group and the non-lens-wearing control group compared with the senofilcon A group (both $P = 0.03$). In contrast, there was no difference in MMP-2 ($P = 0.68$) or MMP-9 ($P = 0.48$) expression in the senofilcon A test eyes compared with the unexposed (patched) eyes. These data support the hypothesis that blocking UV exposure can decrease protease expression in the cornea. Although this study did not examine the expression of tissue inhibitors of MMPs, it should be noted that corneal disorders and diseases can occur when there is an imbalance between MMPs and their inhibitors, thereby causing pathologic degradation of stromal collagen and proteoglycans.

As apoptosis has emerged as an important regulator of development and homeostasis in tissues and disease processes, methods to quantify apoptosis and to distinguish it from necrosis have been developed. The TUNEL assay detects DNA strand breaks in situ in tissue sections. Given that much remains incompletely understood about the molecular pathways of programmed death, it is generally accepted that performing more than one basic protocol to confirm an observation of apoptotic cell death is necessary. The expression of activated caspase-3 is now seen as an alternative to TUNEL.

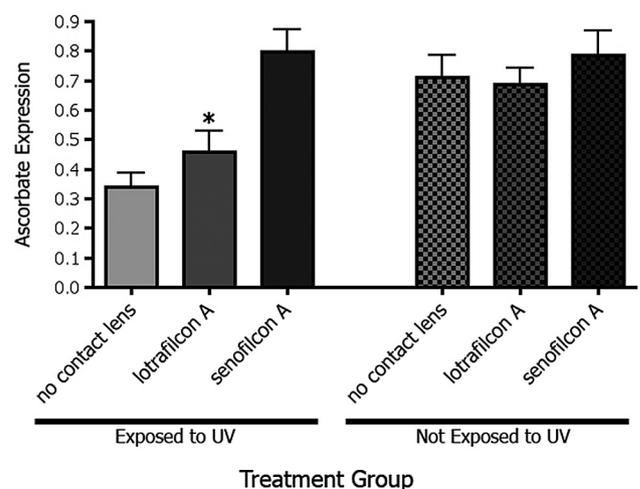


FIGURE 3. Aqueous humor ascorbate levels after UV exposure. A statistically significant ($*P = 0.03$) decrease in aqueous humor ascorbate was observed in the exposed lotrafilcon A lens-wearing group compared with the exposed senofilcon A lens-wearing group. There were no significant differences in ascorbate when the lotrafilcon A lens-wearing group was compared with the non-lens-wearing group or when the senofilcon A group was compared with the unexposed patched control eyes.

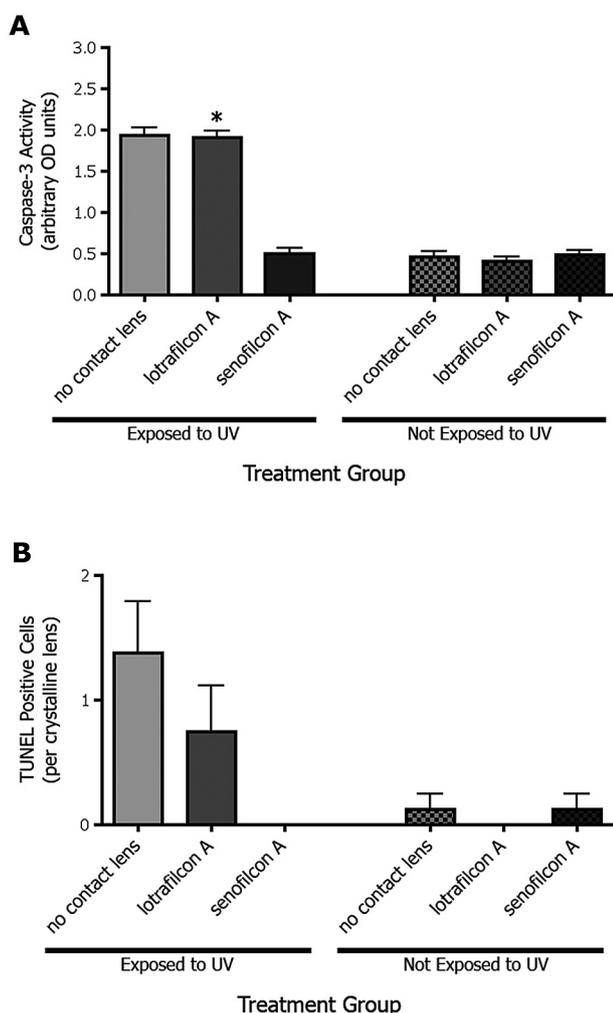


FIGURE 4. Apoptosis in the rabbit crystalline lens after UV exposure. **(A)** A statistically significant ($*P = 0.03$) increase in caspase-3 activity was observed in the exposed lotrafilcon A lens-wearing group compared with the exposed senofilcon A lens-wearing group. No significant differences were found in caspase-3 activity when the lotrafilcon A lens-wearing group was compared with the non-lens-wearing control group. **(B)** There were no significant differences between any of the treatment groups when the total number of TUNEL-positive cells was counted. When the senofilcon A group was compared with the unexposed patched control eyes, no significant differences were noted in either caspase-3 activity or TUNEL-positive cells.

It has been reported that UV-B focused on the center of the rabbit cornea can result in full-thickness corneal damage with apoptosis of corneal epithelium, keratocytes, and corneal endothelial cells.³⁴ Apoptosis of corneal epithelial cells, visualized by TUNEL staining, peaked 24 hours after UV exposure.³⁴ We have previously confirmed UV-induced corneal damage using mice,³⁵ and UV-induced cell damage and death in non-ocular cells such as skin keratinocytes is well documented.³⁶⁻³⁸ Cell death is generally classified as either apoptotic or necrotic. Low levels of external insults induce apoptosis, whereas higher levels typically induce necrosis.³⁹ This general trend has been shown to apply to both human corneal and lens epithelial cell lines exposed to UV-B.^{34,40} At low-dose UV exposure, cell death appears to be primarily apoptotic. At higher exposure levels, cell death shows both apoptotic and necrotic characteristics.^{34,40} In our study, the induction of cell death from UV exposure to the cornea was determined via caspase-3 activity and by use of the TUNEL assay. There was

induction of TUNEL-positive cells and caspase-3 activity in the non-contact lens control group and the lotrafilcon A group after irradiation. The use of senofilcon A contact lenses significantly decreased apoptosis in the cornea, as evidence by a reduction in TUNEL-positive cells and caspase-3 activity (both $P = 0.03$). It is likely that UV-induced cell death in our experiments primarily resulted from apoptosis, though some TUNEL-positive cell deaths might have been caused by necrosis and other forms of DNA damage such as single-strand breaks.

Based on previous experiments,⁴¹⁻⁴³ NMR spectroscopy was chosen as a suitable method to evaluate the levels of ascorbate in the aqueous humor. NMR spectroscopy is a fast and nondestructive technique that uses only small sample volumes. The composition of the aqueous humor is suggested to play a protective role in the pathogenesis of cataract, acting as a filter against UV-B radiation.⁴⁴ This filtering effect is related primarily to its high ascorbic acid concentration, operating as an antioxidant.^{15,45-47} Ascorbic acid is known to scavenge free radicals in the aqueous,⁴⁸ protect against UV-induced DNA damage to the lens,⁴⁹ and minimize UV radiation by absorption and by suppressing the fluorescence of radiation.^{46,47,50} Aqueous humor from patients with cataract has shown decreased levels of ascorbic acid compared with levels in patients without cataract.⁴⁸ It has been reported that a significant decrease in ascorbic acid concentration is observed in the aqueous humor after UV exposure,^{46-48,51} supporting the data observed in this study. We have shown that there was a significant decrease in ascorbic acid after UV exposure in the lotrafilcon A group and the non-lens-wearing control group ($P = 0.03$). However, disruption of the blood-aqueous barrier induced by UV-B radiation and resulting in a decrease of ascorbate level in the aqueous humor cannot be excluded. In the senofilcon A lens-wearing eyes, there were no significant changes ($P = 0.87$) in ascorbic acid levels (compared with the patched eyes), supporting the hypothesis that contact lenses with UV-absorbing polymers are able to prevent reductions in aqueous humor antioxidant levels, thus potentially protecting the crystalline lens from cataractous changes.

The crystalline lens readily absorbs UV-A (36% at 320 nm and 48% at 340 nm) and the remaining 2% of UV-B (at 300 nm) not absorbed by the cornea and aqueous humor.^{21,23} The wavelength range between 295 nm and 320 nm has been shown to be efficient in producing UV-induced cataracts in rabbits.^{23,52} The primary targets of UV-B are the lens epithelial cells (LECs), resulting in unstable free radicals causing molecular changes.⁵³⁻⁵⁵ These changes can include degradation or modification of lens proteins, increased DNA damage, and changes in cell survival.^{53,56} Epidemiologic studies have shown that cataracts are most common in the inferonasal portion of the lens^{55,57} and have correlated the risk of cataract formation with various sources of radiation.⁵⁸⁻⁶⁰ An analysis of data from Australia, China, Tibet, and the United States that controlled for age, sex, race, income, and medical practices found that the probability of cataract surgery increases by 3% for each degree south in latitude in the United States.⁵ By the year 2050, assuming 5% to 20% ozone depletion, there will be 167,000 to 830,000 more cases of cataract, causing an increase in cataract operations that will result in added costs of \$563 million to \$2.8 billion.^{61,62} UV light is an obvious oxidative stress, and eyes are more susceptible to UV damage with age. Levels of UV filtering by the crystalline lens decreases linearly with age, at a rate of 12% per decade.⁵¹ These filters become modified and then act as photosensitizers.^{63,64}

UV radiation can initiate deleterious changes in the LECs, including the disruption and modification of programmed cell death.^{65,66} Recent data suggest that LEC apoptosis is an initiating factor in noncongenital cataract formation.^{67,68} Dysregulation of LEC apoptosis is associated with opacification of the

rat lens and can be stimulated by UV-B radiation.⁶⁷⁻⁷⁰ Using human LEC cultures, research has demonstrated that UV-B can induce the formation of apoptotic bodies, resulting in cell death through specific activation of the mitogen-activated protein kinase pathway.⁵⁶ To characterize the mechanism by which UV-B induces apoptosis in human LECs, concentration- and time-dependent activation of caspase-3 has previously been determined.⁵⁶ This accords with the data obtained in the present study of increased caspase-3 activity in the lotrafilcon A group and the non-lens control group after UV exposure. In addition, we have shown that on exposure to UV irradiation, there was a significant decrease in caspase-3 activity in the senofilcon A group compared with the lotrafilcon A group ($P = 0.03$). Thus, the LECs were protected from apoptosis in the senofilcon A group. There was no significant decrease in the number of TUNEL-positive cells in the senofilcon A group compared with the non-contact lens group and the lotrafilcon A group, likely because of the low number of TUNEL-positive cells in all treatment groups. The dose of UV radiation used in this study was relatively low, and the exposure time was acute. It is possible that the LECs were not exposed to sufficient UV irradiation to result in significant changes in TUNEL-positive cell counts. Alternatively, a larger sample size of rabbits would likely have yielded more power to find the observed difference to be significant.

The daily dose of UV-B used in this study (1.667 J/cm²) approaches the reported threshold for developing permanent cataract after a single application on pigmented rabbit eyes.⁵² A previous study calculated the dose of solar UV-B radiation reaching the human cornea; Zigman⁷¹ reported average values of 0.105 J/cm² during a 1-hour exposure. Thus, the daily UV-B dose used in this study was roughly equivalent to exposing the human cornea to approximately 16 hours of sunlight. In addition, the UV-B dose used in this study was similar to previously published report that examined the effects of UV-B on the aqueous humor in rabbits.⁷² Pitts et al.⁷³ demonstrated damage to all cellular layers of the primate cornea after exposure to 0.08 J/cm² UV-B. Although the dose used in this study was substantially higher, loss of corneal epithelium was not noted in any of the treatment groups. Although corneal edema developed in exposed eyes assigned to either the lotrafilcon A treatment group or the no contact lens treatment group, neither changes in the overall thickness of the cornea nor loss of stromal tissue were examined in this study.

The experiments outlined in this study support our initial hypothesis that wearing UV-blocking contact lenses shows similar protective findings in the cornea and crystalline lens as observed in the patched (control) eyes. This is supported by data from previous studies demonstrating that UV-absorbing contact lenses can minimize UV-induced ocular damage.⁶⁻¹² In addition, lotrafilcon A lens-wearing eyes showed deleterious corneal and crystalline lens findings similar to those of the non-lens-wearing (exposed) eyes. Thus, eyes wearing senofilcon A lenses had UV protection compared with lotrafilcon A lens-wearing and non-lens-wearing (exposed) eyes.

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