A Class I (Senofilcon A) Soft Contact Lens Prevents UVB-Induced Ocular Effects, Including Cataract, in the Rabbit In Vivo

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PURPOSE. UVB radiation from sunlight is known to be a risk factor for human cataract. The purpose in this study was to investigate the ability of a class I UV-blocking soft contact lens to protect against UVB-induced effects on the ocular tissues of the rabbit in vivo.

METHODS. Eyes of rabbits were exposed to UVB light for 30 minutes (270–360 nm, peak at 310 nm, 1.7 mW/cm² on the cornea). Eyes were irradiated in the presence of either a UV-blocking senofilcon A contact lens, a minimally UV-blocking lotrafilcon A contact lens, or no contact lens at all. Effects on the cornea and lens were evaluated at various times after exposure.

RESULTS. Eyes irradiated with no contact lens protection showed corneal epithelial cell loss plus lens epithelial cell swelling, vacuole formation, and DNA single-strand breaks, as well as lens anterior subcapsular opacification. The senofilcon A lens protected nearly completely against the UVB-induced effects, whereas the lotrafilcon A lens showed no protection.

CONCLUSIONS. The results indicate that use of a senofilcon A contact lens is beneficial in protecting ocular tissues of the rabbit against the harmful effects of UVB light, including photokeratitis and cataract. (*Invest Ophthalmol Vis Sci.* 2011;52:3667–3675) DOI:10.1167/iops.10-6885

Maturity-onset cataract continues to be a major global health problem, accounting for nearly one half of the world's blindness.^{1,2} By 2020, population expansion and increased life expectancy may double the number of people (20 million) who are blind because of cataract. In the United States alone, 20 million people older than 40 years have cataract in either eye, and this number may rise to 30 million by 2020.³ The only treatment available at this time for cataract is surgery,⁴ and fortunately we live in an era in which this sight-restoring operation has become highly effective and safe, when performed by a skilled, well-trained surgeon. However, such surgery is costly and not equally available in all regions of the world. The 1.8 million cataract operations performed each year in the United States consume two thirds of the total Medicare budget for vision, over \$4 billion.^{5–7} In addition to diminishing the suffering and costs associated with cataract, there are other

reasons for trying to delay the onset of this age-related disease for as long as possible. Besides its role of focusing light, the natural lens performs other important functions, including filtering both ultraviolet and blue light, thereby protecting the retina from possible light-induced damage^{8,9} (exposure of the retina to blue light is greater after cataract surgery than at any other point in a patient's lifetime¹⁰). Also, by means of mitochondrial respiration occurring in its epithelial layer, the lens maintains levels of oxygen in the aqueous humor at normal, low levels, possibly preventing the onset of glaucoma.^{11–13} As with all surgeries, cataract surgery carries with it possible complications, which can include globe perforation, spikes in intraocular pressure, dislocated lens fragments, retinal detachment, and endophthalmitis,^{14–17} and according to recent reports, cases of postcataract surgery endophthalmitis may be on the increase.^{18,19} Vitreoretinal complications after cataract surgery are known to occur at a higher incidence in the developing world²⁰ and in patients with vascular diseases such as diabetes mellitus.²¹ Despite a reduction in the occurrence of posterior capsular opacification (PCO) after cataract surgery, this frequent complication has not been completely eradicated, and the only treatment for PCO, Nd:YAG laser capsulotomy, adds additional possible complications, as well as an increased financial burden on the health care system.^{22,23} A further incentive for trying to delay the onset of cataract is the suggestion that cataract surgery may increase the risk or progression of age-related macular degeneration.^{24,25}

It is generally accepted that long-term exposure of humans to sunlight increases the risk for maturity-onset cataract.^{26–28} It has been recognized for some time that the closer one lives to the equator, the higher the incidence of cataract.²⁹ A number of epidemiologic studies have linked the UVB radiation (290–315 nm wavelength) present in sunlight to the formation of lens cortical opacities,^{30–32} and these types of cataracts are expected to increase significantly with the observed thinning of the stratospheric ozone layer.³³

The human cornea absorbs essentially all incident light below 300 nm wavelength, as well as much of the UVB radiation between 300 and 315 nm, such that less than 2% of the total ultraviolet radiation reaching the lens epithelium is UVB,^{34,35} and most of this is absorbed in the first few layers of lens cells.^{36,37} Absorption of UV light by the ocular surface is strongly linked with the onset of several acute or degenerative diseases including photokeratitis (snowblindness), climate droplet keratopathy and pterygium.^{28,38} Even the relatively small proportion of UVB radiation that strikes the lens epithelium is capable of damaging this tissue, causing loss of homeostatic control of ions, which presumably initiates cortical cataract in the aging human.³⁹ Numerous studies with experimental animals have documented the ability of UVB light to induce lens anterior subcapsular and cortical opacities, in addition to causing significant corneal damage,^{40–44} although it should be noted that these studies were short-term and involved UVB doses to ani-

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mals that may have had different corneal UV transmission properties than the human.

UV-blocking contact lenses may offer an effective means of preventing or delaying UVB-induced damage to the ocular surface, as well as UVB-induced maturity-onset cataract. Class II UV-blocking contact lenses have been available for many years⁴⁵; however, class I silicone hydrogel lenses composed of UV-blocking materials that absorb 99% of incident UVB radiation and 90% of UVA have been introduced only recently.⁴⁶ To our knowledge, there has been only one study that has tested the ability of class I contact lenses to protect against UV-induced damage to the eye,⁴⁷ and that investigation did not focus primarily on the possible prevention of UV-induced cataract. The purpose of the current work was to use an experimental rabbit in vivo model to evaluate the efficacy of a class I UV-blocking contact lens in protecting against UVB-induced damage to the cornea and lens, including UVB-induced cataract.

METHODS

Rabbits (New Zealand White, 2.0–2.5 kg) were obtained from Kuiper Rabbit Ranch (Indianapolis, IN). All studies conformed to the ARVO Statement for Use of Animals in Ophthalmic and Vision Research and were approved by the Oakland University Animal Care and Use Committee. Euthanatization of the rabbits was conducted by first anesthetizing the animals with xylazine and ketamine HCl, followed by injection of an overdose of pentobarbital sodium.

UV Irradiation

Eyes of rabbits were exposed to UVB irradiation by the method of Andley et al.⁴² Before irradiation, the animals were tranquilized with an intramuscular injection of xylazine (20 mg/kg) and ketamine HCl (5 mg/kg), and the eyes were fully dilated with 1% tropicamide (Mydril; Ocusoft, Inc., Richmond, TX). During irradiation, the rabbits were confined in an adjustable retaining cage that protected most of the animal, except the head from the UV light. One eye of each animal was exposed to UV radiation from a bank of UV lamps (UBL FS20T 12/UVB; National Biological Corp., Twinsburgh, OH), at a distance of 18 cm. Contralateral control eyes were patched. UV levels were determined with a radiometer (UVX Digital; San Gabriel, CA) equipped with either a UVA sensor at 365 nm (model UVX-36) or a UVB sensor at 312-nm (model UVX-31). The maximum intensity of light from the UV lamps was at a wavelength of 310 nm, but the lamps also emitted UVA light plus a small amount of UVC (the wavelength spectral distribution was 270–360 nm). Use of a UVC filter (0–53, 2-mm-thick; Corning Co-Star, Corning, NY) was found to produce results identical with those without the filter. The UVB irradiance on the corneas of the animals was 1.7 mW/cm², which is approximately 34 times the maximum exposure of the human cornea to UVB contained in sunlight.³⁴ The eyes were irradiated for 30 minutes to produce a total fluence of 3 J/cm².

The eyes were exposed to UV radiation in the presence of either a senofilcon A contact lens (Acuvue Oasys; Vistakon, Division of Johnson & Johnson Vision Care, Inc., Jacksonville, FL), which absorbs 99% of incident UVB and 90% of UVA,⁴⁶ a lotrafilcon A contact lens (Focus Night and Day; CIBA Vision, Duluth, GA), which absorbs 30% of incident UVB and 15% of UVA⁴⁶ or no contact lens at all. UV transmittance spectra for Acuvue Oasys and Focus Night and Day contact lenses are shown in Figure 1. Contact lenses were applied to the corneas of tranquilized rabbits. A drop of saline was added to the contact lens (to prevent air being trapped between the lens and the cornea) and, using the tip of the index finger, the lens was applied to the cornea.

Evaluation of UVB-Induced Corneal Effects

Corneas were stained in vivo with 0.25% sodium fluorescein (Fluorox; Altaire Pharmaceuticals, Inc., Aquebogue, NY) and flushed with sterile

FIGURE 1. UV transmittance spectra for an Acuvue Oasys senofilcon A contact lens (—) and a Focus Night and Day lotrafilcon A contact lens (---). Adapted with permission from Moore L, Ferreira JT. Ultraviolet (UV) transmittance characteristics of daily disposable and silicone hydrogel contact lenses. *Cont Lens Anterior Eye*. 2006;29:115–122. © Elsevier.

phosphate-buffered saline (PBS). The eyes of the animals were examined with a slit lamp photo microscope (Carl Zeiss Meditec, Dublin, CA) using blue light illumination (485-nm excitation, 530-nm emission). Areas of the cornea with damaged or missing epithelia showed as green, while areas with intact epithelia were not stained by the fluorescein. Effects were documented by a digital camera (D40; Nikon, Tokyo, Japan) attached to the slit lamp. Loss of corneal epithelia was also evaluated in enucleated eyes 15 hours after UVB irradiation. The eyes were fixed with 4% formalin and embedded in wax. Sections were stained with hematoxylin and eosin, and photographs were taken under light microscopy.

Lens Photography

UVB-induced damage to the lens was evaluated with a slit lamp biomicroscope (Carl Zeiss Meditec) after induction of full mydriasis with 1% tropicamide. Results were documented by photography. In addition, enucleated eyes were photographed under a dissecting microscope after removal of the cornea and iris. This method allowed visualization of the whole lens, including areas of the lens that were protected from UV irradiation by the iris.

Evaluation of UVB-Induced Lens Epithelial Cell Death

The anterior capsule/epithelium of an isolated lens was carefully removed with capsular scissors under a dissecting microscope. A flat mount was made on a slide with the epithelium facing up. The specimen was stained with a live/dead cell viability staining kit (Invitrogen, Chicago, IL), and examined under a fluorescence microscope (Nikon). The number of live and dead cells (dead cells stained as red) present in the central region of the epithelium was determined with commercial image-analysis software (Scion Corp., Frederick, MD).

Transmission Electron Microscopy

The morphology of the central lens epithelium and anterior cortex was analyzed by transmission electron microscopy. Lenses were treated with OsO₄, rinsed with cacodylate buffer (pH 7.4), and fixed overnight in 2.5% glutaraldehyde. The lenses were rinsed again with cacodylate buffer and treated with OsO₄ in the cold (4°C). After they were rinsed once more with cacodylate buffer, the lenses were dehydrated in graded ethanol and embedded in Epon 812 media. Ultrathin sections

FIGURE 2. Fluorescein staining of rabbit corneal epithelium, 15 hours after a 30-minute in vivo exposure of the eye to UVB radiation (1.7 mW/cm^2), with and without contact lens protection. Eyes were examined and photographed using slit lamp biomicroscopy with a blue filter. Green stain indicates UVB-induced loss of corneal epithelium. (A) Normal. (B) No contact lens. (C) Senofilcon A lens. (D) Lotrafilcon A lens. Representative results for three experiments (three animals) for each condition.

were cut from the central epithelium. Sections were stained with uranyl acetate and lead citrate, observed with a transmission electron microscope (model 400; Phillips, Eindhoven, The Netherlands), and photographed as described previously.⁴⁸

Lens Epithelial DNA Damage

DNA single-strand breaks were assessed with a modification of the single-cell gel electrophoresis method of Singh et al.,⁴⁹ as described elsewhere by us.^{50,51} The anterior central capsule plus epithelium of the rabbit lens were carefully dissected under a microscope, placed in a test tube containing 0.5 mL of trypsin/EDTA, and incubated for 5 minutes at 37°C . The tube was vortexed to release epithelial cells from the capsule, which was removed before the cells were pelleted by centrifugation. The epithelial cells were thoroughly mixed with 0.8% low gel-point agarose in phosphate-buffered saline (PBS) and cast on a sandwiched micro gel on a frosted glass slide. The cell protein was dissolved in pH 10 lysing buffer (1% sarkosyl, 2.5 M NaCl, 100 mM EDTA, 10 mM Tris-HCl, and 1% triton X-100) in the dark at 4°C for 1 hour. After lysis, the gel was soaked in 300 mM NaOH and 1 mM EDTA running buffer for 20 minutes to unwind the nuclear DNA. Electrophoresis was performed for 20 minutes at 17 V in a submarine gel tank. The gel was neutralized with 0.4 M Tris-HCl buffer (pH 7.5) for 5 minutes, stained with 1 drop of 20 $\mu\text{g/mL}$ ethidium bromide, and covered with a coverslip. The gel was photographed with a fluorescence microscope with a g filter (Y2E/C filter, 540–580 nm excitation, 600–660 nm emission; Nikon). The migration of DNA of 30 representative single cells for each experiment was analyzed from the photographs by measuring from the margin of the cell nucleus to the tip of migrating fragmented DNA.

Biochemical Analyses

Anterior aqueous humor (50 μL) was withdrawn from the eyes of rabbits by using a syringe and 30-g needle after anesthetization of the corneas with a 0.5% solution of tetracaine (Pontocaine; Breon Laboratories, New York, NY). Levels of ascorbic acid in the aqueous were determined by using the method of Omaye et al.⁵² Concentrations of reduced and oxidized glutathione (GSH and GSSG) in isolated central lens capsule/epithelia were determined by using the Tietz method.^{53,54} Before analysis, each capsule or epithelium was stored in 200 μL 50 mM EDTA at -80°C and then sonicated in the same solution.

RESULTS

Eyes of rabbits were irradiated with UVB light (1.7 mW/cm^2) for 30 minutes (3 J/cm^2), with and without contact lens protection, and the corneas stained with fluorescein after 15 hours. Severe loss of corneal epithelial cells was observed in eyes irradiated with no contact lens protection (Fig. 2B), compared with the normal eye (Fig. 2A). The senofilcon A lens provided complete protection against UVB-induced loss of corneal epithelium (Fig. 2C), whereas the lotrafilcon A lens provided no protection (Fig. 2D compared to Fig. 2B). Histologic examination of the corneas showed nearly complete exfoliation of the epithelium in the central region of the cornea after irradiation with no contact lens (Fig. 3B). Again, the senofilcon A lens provided complete protection (Fig. 3C), whereas the lotrafilcon A lens offered no protection (Fig. 3D). Irradiation in the absence of a contact lens also produced swelling and haziness of the corneal epithelium, 15 hours later (results not shown). Aqueous flare, anterior chamber fibrinous exudates and iris hyperemia were also observed 15 hours after UVB exposure, but without appearance of hypopyon (results not shown). All these effects were prevented by the senofilcon A lens, but not by the lotrafilcon A lens.

Slit lamp examination of rabbit eyes 48 hours after a 30-minute exposure to UVB radiation showed extensive lens opacification in eyes with no contact lens protection (Fig. 4B). Typical of UVB-induced cataract, most of the opacification was located either within the lens epithelial layer or just underneath the epithelium in the superficial cortex. The senofilcon A lens protected completely against UVB-induced lens opacification (Fig. 4C); whereas, the lotrafilcon A lens showed no protection (Fig. 4D). Similar results were observed when the cornea and iris of eyes of irradiated euthanized animals were removed and examined under a dissecting microscope with coaxial illumination (Fig. 5). Opacification was observed in the region of the lens not protected from UVB light by the iris (Fig. 5B). Opacification was prevented by the senofilcon A lens (Fig. 5C), but not by the lotrafilcon A lens (Fig. 5D).

UVB-induced damage of the rabbit lens epithelium was assessed in greater detail with the use of transmission electron

FIGURE 3. Light micrographs of the central region of the rabbit corneal epithelium, 15 hours after a 30-minute in vivo exposure of the eye to UVB radiation (1.7 mW/cm^2), with and without contact lens protection. (A) Normal. (B) No contact lens. (C) Senofilcon A lens. (D) Lotrafilcon A lens. Representative results for three experiments (three animals) for each condition. Note the nearly complete exfoliation of the epithelium in (B) and (D); epithelial cell loss was not as severe in the corneal periphery (data not shown). Magnification, $\times 100$.

FIGURE 4. Slit lamp photographs of rabbit eyes, 48 hours after a 30-minute in vivo exposure to UVB radiation (1.7 mW/cm^2), with and without contact lens protection. (A) Normal. (B) No contact lens. (C) Senofilcon A lens. (D) Lotrafilcon A lens. Representative results for three experiments (three animals) for each condition.

microscopy, 48 hours after a 30-minute exposure (Fig. 6). Severe damage was observed to the central lens epithelium of eyes not containing a contact lens, including cell swelling, vacuole formation, nuclear fragmentation, and chromatic condensation (Fig. 6B). The senofilcon A lens completely protected against the damage (Fig. 6C), whereas the lotrafilcon A lens showed no protection (Fig. 6D).

UVB-induced cell death in the central region of the lens epithelium was assessed with the use of a live/dead cell viability staining kit. Figure 7B shows an increase in the number of dead epithelial cells (red staining) present in the lens of a rabbit eye irradiated with no contact lens, 48 hours after a 30-minute irradiation (Fig. 7B compared to Fig. 7A). The senofilcon A lens protected nearly completely against UVB-induced cell death (Fig. 7C compared to Fig. 7A). The lotrafilcon A lens offered no protection (Fig. 7D compared

FIGURE 6. Transmission electron micrographs of rabbit lens epithelium (epi) and fibers (fibers), 48 hours after a 30-minute in vivo exposure to UVB radiation (1.7 mW/cm^2), with or without contact lens protection. (A) Normal. (B) No contact lens. (C) Senofilcon A lens. (D) Lotrafilcon A lens. Representative results for three experiments (three animals) for each condition. Note the lens epithelial cell damage in (B) and (D): cell swelling, vacuole formation (black arrows), nuclear fragmentation (white arrows) and chromatic condensation (arrowheads). Magnification: (A) $\times 7100$; (B, C) $\times 4400$; (D) $\times 5600$.

to Fig. 7B). Quantification of the results by counting live and dead cells produced the same result (Table 1A). There was no significant difference ($P > 0.1$) between the percentage of dead cells present in the lens epithelia of eyes irradiated with a senofilcon A lens ($2.2\% \pm 0.5\%$) compared with lens

FIGURE 5. Retroillumination photographs of rabbit eyes, 48 hours after a 30-minute in vivo exposure to UVB radiation (1.7 mW/cm^2), with and without contact lens protection. The animals were euthanized, and the cornea and iris removed from enucleated eyes before being photographed. (A) Normal. (B) No contact lens. (C) Senofilcon A lens. (D) Lotrafilcon A lens. Representative results for three experiments (three animals) for each condition.

FIGURE 7. Live and dead cells present in the central region of the lens epithelium of rabbit eyes, 48 hours after a 30-minute in vivo exposure to UVB radiation (1.7 mW/cm^2), with and without contact lens protection. Each lens capsule plus epithelium was removed, a flat mount prepared, and the slide stained for live (nucleus stained green) and dead (nucleus stained red) cells. (A) Normal. (B) No contact lens. (C) Senofilcon A lens. (D) Lotrafilcon A lens. Representative results for three to four experiments (three to four animals) for each condition. Quantification of the data is shown in Table 1A.

TABLE 1. Effect of UV Radiation In Vivo on Rabbit Lens Epithelial Cell Viability and Extent of DNA Single-Strand Breaks, with and without Contact Lens Protection

	Normal	UV, No Contact Lens	UV Senofilcon A Lens	UV Lotrafilcon A Lens
A. Dead lens epithelial cells (% of total cells)	1.5 ± 0.2	7.2 ± 1.7	2.2 ± 0.5*†	8.3 ± 3.3‡§
B. DNA migration (arbitrary units)	0 ± 0	8 ± 1	0.5 ± 0.1	6.5 ± 1.5¶

A. Analysis was conducted as described in the legend to Figure 7, 48 hours after a 30-minute in vivo exposure to UVB radiation (1.7 mW/cm²). Live and dead cells in the visual fields for each condition were counted for three to four experiments (three to four animals for each condition). The mean number of total cells for all the experiments was 1534 ± 108. Results are expressed as the mean ± SD.

B. The study was performed as described in the legend to Figure 8, immediately after a 30-minute in vivo exposure to UVB radiation (1.7 mW/cm²). Relative migration of damaged DNA (an indication of the extent of DNA single-strand breaks) for 30 cells is expressed as the mean ± SD. for three experiments (three animals for each condition).

* $P < 0.01$ vs. no contact lens.

† $P > 0.1$ vs. normal.

‡ $P > 0.1$ vs. no contact lens.

§ $P < 0.05$ vs. normal.

|| $P < 0.001$ vs. no contact lens.

¶ $P > 0.1$ vs. no contact lens.

epithelia of normal eyes (1.5% ± 0.2%). Also, there was no significant difference between the percentage of dead cells present in lenses of eyes irradiated without a contact lens (7.2% ± 1.7%) compared to those irradiated with a lotrafilcon A lens (8.3% ± 3.3%).

Since UVB-induced damage to lens epithelial DNA has been implicated in the formation of human cataract,^{55,56} we evaluated damage to DNA in the UVB-exposed rabbit lens epithelium. Substantial DNA single-strand break damage was observed in eyes containing no contact lens (Fig. 8B), compared to controls (Fig. 8A). The senofilcon A lens appeared to protect nearly completely against the DNA damage (Fig. 8C); however, the lotrafilcon A lens showed little apparent protection (Fig. 8D). The DNA damage results are quantified

in Table 1B. Although the senofilcon A lens showed significant protection against UVB-induced lens epithelial DNA damage ($P < 0.001$), there was no significant difference between damage observed with no contact lens compared to that with the wearing of a lotrafilcon A lens ($P > 0.1$).

Ascorbic acid in the aqueous humor is known to be an important protector against UVB-induced DNA damage in the lens epithelium.⁵⁷ For this reason, we measured levels of ascorbate in the aqueous fluid of rabbit eyes immediately after exposure to UVB radiation, and found a slight but significant decrease (6.5%; $P < 0.05$) compared with the patched contralateral control eye (Table 2A). Since the ratio of reduced to oxidized glutathione (GSH/GSSG) is a sensitive indicator of the level of oxidative stress in a tissue,⁵⁸ we measured levels of GSH and GSSG in central lens capsule epithelia immediately after exposure of rabbit eyes to UVB radiation in vivo. Surprisingly, no difference was seen in the percentage of GSSG found in the lens epithelia of UVB-exposed eyes, compared with that in the lens epithelia of patched contralateral control eyes; in both cases, the level of GSSG was approximately 2% (Table 2B). Since we observed only a slight effect of UVB light on the level of ascorbate in the aqueous humor and no effect on the level of GSSG in the lens epithelia, these parameters were not investigated further with the use of contact lenses.

FIGURE 8. Photomicrographs of DNA contained in the central region of the lens epithelium of rabbit eyes, immediately after a 30-minute in vivo exposure to UVB radiation (1.7 mW/cm²), with and without contact lens protection. The direction of electrophoresis is from right to left; note that damaged DNA migrates faster in the electric field than normal DNA. Background staining is artifact caused by ethidium bromide collecting on the ground glass slide. (A) Normal. (B) No contact lens. (C) Senofilcon A lens. (D) Lotrafilcon A lens. Representative results for three experiments (three animals) for each condition. (D, inset, arrows) Examples of the margin of the cell nucleus (right arrow) and the tip of DNA (left arrow) used to measure DNA migration. Quantification of the data is shown in Table 1B.

TABLE 2. Effect of UV Radiation of the Rabbit Eye In Vivo on the Level of Ascorbic Acid in Aqueous Humor and Percentage of Oxidized Glutathione in the Central Lens Epithelium*

	Control	UV
A. Ascorbate in aqueous humor, mM	1.23 ± 0.06 (4)	1.15 ± 0.06 (4)
		$P < 0.05$
B. GSSG in the central lens epithelium, %	2.2 ± 0.9 (6)	1.09 ± 0.6 (4)
		$P > 0.1$

Results are expressed as the mean ± SD. The number of experiments (number of animals) is shown in parentheses.

* Analyses were conducted immediately after a 30-minute in vivo exposure to UVB radiation (1.7 mW/cm²). The patched contralateral eye served as the control.

DISCUSSION

In this study, a senofilcon A contact lens provided nearly complete protection against UVB-induced damage to rabbit corneal epithelium (Figs. 2, 3), despite use of a relatively high dose of UVB radiation. The 1.7 mW/cm^2 irradiance used was 34 times the maximum irradiance of UVB light contained in sunlight striking the human cornea (assuming that 17% of ambient UVB impinges on the cornea).³⁴ Based on the maximum amount of UVB reaching the human cornea from sunlight, 0.187 J/cm^2 in 1 hour,³⁴ the UVB dose of 3 J/cm^2 was equivalent to exposing the cornea to 16 hours of sunlight condensed into 30 minutes. This exposure was 55 times the 0.055 J/cm^2 threshold for UVB-induced damage to the rabbit cornea, observed at a wavelength of 310 nm.⁴³

Earlier studies have demonstrated the harmful effects of UVB light on the cornea,^{28,42,43,47} particularly with regard to the most common acute effect, photokeratitis.⁵⁹ Previous work has linked UVB-induced corneal cell death with mechanisms of oxidation involving the generation of reactive oxygen species, resulting in apoptosis.^{60,61} A study using UVB irradiation conditions similar to those in the current work showed losses of antioxidants in the rabbit cornea of nearly 100% for reduced glutathione and 75% for ascorbic acid, 1 day after exposure.⁶² The UV action spectra for the rabbit cornea is about the same as that for the human, with maximum sensitivity occurring at 270 to 288 nm, and one-tenth maximum sensitivity being seen at 310 nm.^{59,63} The corneal epithelium is the first line of UVB filtering defense for the rest of the eye.^{64,65} At the peak wavelength used in this study, 312 nm, the cornea absorbs 75% to 85% of incident UVB light,^{40,66} and much of this is absorbed by the corneal epithelium.⁶⁷ UVB is known to accelerate the loss of corneal epithelial cells,⁶⁸ as was observed in the current work. The nearly complete exfoliation of rabbit corneal epithelium observed 15 hours after the UVB dose (Fig. 3B), was also reported by Pitts et al.⁴³ at a wavelength of 295 nm.

Since the senofilcon A lens absorbs 99% of incident UVB light, it was able to completely protect the corneal epithelium from damage (Figs. 2C, 3C), whereas the lotrafilcon A lens, which exhibits only minimal UVB absorption, offered no protection (Figs. 2D, 3D). The senofilcon A lens comprises a modern class I UV-blocking silicone hydrogel polymer that is oxygen permeable. Several earlier studies using older style class II UV-blocking lenses with lower oxygen permeability also used rabbit/UVB models to document contact lens protection against corneal damage.^{45,69–71} A recent investigation showed that the senofilcon A lens was able to protect against UVB-induced corneal apoptosis in the rabbit.⁴⁷

In addition to protecting the rabbit cornea against a high dose of UVB, the senofilcon A contact lens also protected the crystalline lens (Figs. 4–8). If we assume that absorption of the incident 1.7 mW/cm^2 of UVB light by the cornea was 98%,³⁴ then the irradiance reaching the lens epithelium, 0.034 mW/cm^2 , would have been 34 times the maximum irradiance of UVB contained in sunlight striking the human lens.³⁴ The dose of UVB received by the lens, 0.06 J/cm^2 , was comparable to an exposure of 16 hours of sunlight condensed into 30 minutes.³⁴ This UVB radiation would have consisted primarily of 295- to 315-nm light, and 100% of it would have been absorbed by the lens,⁴⁰ mainly by the epithelium.^{36,37} The UVB dose to the cornea in this study, 3 J/cm^2 , was four times the 0.75 J/cm^2 threshold dose for UVB-induced damage to the rabbit lens, observed at a wavelength of 310 nm,⁴³ which compares to 55 times the threshold dose for the cornea, as mentioned above. The peak wavelength of the UV action spectrum for cataract in the rabbit ranges from 297 to 300 nm,^{39,43} even though very little of this light is able to pass through the cornea to reach the lens. The senofilcon A contact lens, but not the lotrafilcon A

lens, was able to absorb these highly damaging wavelengths of light and prevent UVB-induced lens effects.

The finding that acute UVB exposure produced primarily epithelial and anterior subcapsular opacities in rabbit lenses (Figs. 4–6) is in agreement with the results of several earlier *in vivo* investigations involving rabbits,^{40,42,43} rats,^{41,44} and mice.^{72,73} In contrast, Jose⁷⁴ showed that chronic long-term exposure of mice to low levels of UVB light generated posterior lens opacities, which she posited were caused by UVB-induced DNA damage and concomitant division and differentiation failures, resulting in aberrant migration of cells to the posterior pole, a situation analogous to x-ray cataract. Epithelial vacuoles observed in the present study at four times the UVB threshold (Fig. 6B) were also observed by Pitts et al.⁴³ at two times the UVB threshold and were caused presumably by UVB-induced effects on lens membrane function, including altered cation transport and increased permeability.⁷⁵ The UVB radiation damaged most of the various types of lens epithelial organelles (Fig. 6B), resulting in epithelial cell death (Fig. 7B, Table 1A). It is not surprising that the lens epithelium was severely affected in this study by an acute dose of UVB, since this single layer of cells is rich in UVB-absorbing targets, including crystallins, enzymes, membranes, and DNA.⁷⁵ At the peak UVB wavelengths that induce cataract, 297 to 300 nm, almost all absorption of light by proteins would be due to the amino acid residue tryptophan,⁷⁶ which is present in high concentration in lens epithelial proteins, compared with the number of UVB photons striking the lens surface.³⁹ It is known that irradiation of lens proteins with UVB light produces substantial photooxidation of tryptophan, as well as generation of damaging active species of oxygen, including superoxide anion and H_2O_2 .⁷⁷ H_2O_2 at higher than physiological levels is toxic for the lens epithelium⁷⁸ and most likely contributed to the observed UVB-induced cell death (Fig. 7B, Table 1A). Photo-oxidation of tryptophan is also known to generate damaging singlet oxygen, which has been proposed to play a role in human cataract.^{77,79} Other UV absorbers in the rabbit lens include flavins and pyridine nucleotides (particularly NADH^{80}), as well as some heme proteins, such as catalase⁸¹ and mitochondrial cytochromes.⁸² The rabbit lens also contains micromolar levels of kynurenine and 3-hydroxykynurenine,⁸³ compounds that absorb both UVA and UVB light.

Cell nuclei of the UVB-irradiated rabbit lens epithelium were highly affected by the radiation (Fig. 6B), resulting in formation of DNA single-strand breaks (Fig. 8B, Table 1B). Such breaks form transiently after exposure of cells to UVB, as a result of excision repair of radiation-induced pyrimidine dimers.⁸⁴ Formation of UVB-induced cyclobutane pyrimidine dimers and pyrimidine-pyrimidine^{6–4} photoproducts has been demonstrated in cultured human lens epithelial cells using a dose comparable to that estimated to reach the lens epithelium in the present *in vivo* study (0.06 J/cm^2 , see above).⁸⁵ DNA single-strand breaks occurring in UVB-exposed bovine lens epithelial cells reached a maximum number 30 minutes after the treatment.⁸⁶ In the present study, the time between exposure of the rabbit to UVB and analysis of DNA single-strand breaks in the isolated lens epithelium was also approximately 30 minutes. In a related previous study, DNA damage as indicated by unscheduled DNA synthesis was detected in human donor lenses exposed to UVB at wavelengths $>295 \text{ nm}$, either directly or while the lens was still present in the intact donor eye.⁸⁷

Even though DNA absorbs light maximally at a wavelength of sunlight that does not reach the lens epithelium, 260 nm, this study has shown that UVB can damage lens epithelial DNA *in vivo* (Fig. 8B, Table 1B). It is known that much of the biological damage caused by solar irradiation is due to absorption of light at or above 300 nm, and DNA can absorb weakly

at these higher wavelengths.⁸⁸ Thus, even though the cornea absorbs most light below 300 nm, lens epithelial DNA can be damaged in situ by sunlight.⁵⁵ The action spectra below 313 nm for induction of DNA single-strand breaks in cultured human cells, and for UVB-induced death of cultured rabbit lens epithelial cells, have been reported to coincide closely with the spectrum for nucleic acid absorption.^{89,90} However, UVB light can also cause indirect oxidative damage to cellular DNA,⁹¹ and it is possible that UVB-induced generation of H₂O₂ via photo-oxidation of tryptophan, as described above, may have contributed to the DNA single-strand break formation observed in this study. Indeed, H₂O₂ has been shown to induce DNA single-strand breaks in cultured lens epithelial cells via a Fenton reaction mechanism.⁹²

The DNA damage observed in this study in rabbit lens epithelium after in vivo exposure to UVB light has also been reported for human lens epithelial fragments collected during cataract surgery,^{56,93} suggesting that certain types of human maturity-onset cataract may be linked with prior DNA damage, as hypothesized earlier.⁵⁵ Kleiman and Spector,⁵⁶ using the same technique for detecting DNA single-strand breaks as used in the current investigation, showed a significantly higher proportion of DNA single-strand breaks in 50% of the cataractous lens epithelial fragments analyzed, compared to clear donor lens controls. Similarly, Worgul et al.⁹³ found a large number of micronucleated cells, indicative of prior genotoxic damage, in human cataractous lens epithelial fragments. A more recent investigation reported a possible association between polymorphisms of a certain DNA repair enzyme gene and the formation of human maturity-onset cortical cataract.⁹⁴

To our knowledge, the present study is the first to use an animal model to demonstrate protection by a UV-blocking contact lens against UVB-induced cataract (Figs. 4C, 5C), and UVB-induced damage to the lens epithelium (Figs. 6C, 7C, 8C; Table 1). However, a previous investigation showed that a senofilcon A lens could block UVB-induced lens apoptosis in the rabbit.⁴⁷

The 30-minute UVB exposure produced only a slight decrease (6.5%) in the level of ascorbic acid in the aqueous humor of the rabbit, immediately after the dose (Table 2A). Rabbit aqueous humor has been shown to absorb a substantial amount of 300-nm light in vitro, and most of this is due to the presence of ascorbate.⁹⁵ In addition, it is known that ascorbic acid is photooxidized by UVB light to form dehydroascorbate, with protection being afforded against photooxidation by reduced glutathione.⁹⁶ It is not surprising, however, that we saw only a slight decrease in ascorbate in our study, despite the high dose of UVB that was given. Aqueous humor in the rabbit is known to turn over rapidly, 6% per minute in the posterior chamber and 1.2% per minute in the anterior chamber⁹⁷ and, in addition, the fluid contains approximately 0.02 mM reduced glutathione,⁹⁸ which would reduce dehydroascorbate as it was being formed. Other researchers have observed nearly 50% losses of aqueous humor ascorbate in the rabbit, 1 to 2 days after exposure to a level of UVB similar to that used in this study.^{47,99} These large decreases were most likely the result of delayed UVB-induced effects on ascorbate pumping mechanisms existing in the ciliary process since after 1 to 2 days, all the original aqueous humor would have been replaced many times over. Effects of UVB on ascorbate pumping in the ciliary body may indeed be relevant in cases of chronic exposure of humans to UVB radiation.

No increase in the level of oxidized glutathione (GSSG) was observed in the lens epithelium immediately after UVB exposure (Table 2B), despite the fact that substantial death of these cells occurred 2 days later (Table 1A). Hightower and McCready¹⁰⁰ also found no increase in GSSG level in the epithelia of cultured rabbit lenses after a cataractogenic UVB dose; a

decreased level of epithelial GSH was detected hours after the in vitro UVB exposure (but not immediately after), and this was presumably due to leakage of the tripeptide into the culture medium. Other investigators have also failed to detect an accumulation of GSSG in lenses of rabbits and rats 1 day and 1 week, respectively, after a cataractogenic UVB dose, despite coincident large losses of GSH.^{101,102} It is possible that even a strong dose of UVB light does not result in oxidation of GSH in the lens epithelium cytoplasm. It may be that most of the absorption of UVB light by lens epithelia is accomplished by the organelles, particularly the cell nuclei. Treatment of cultured lens epithelial cells with a level of hyperbaric oxygen sufficient to induce DNA single-strand breaks and a complete inhibition of growth also did not affect levels of GSH and GSSG,¹⁰³ and treatment of cultured lenses with hyperbaric oxygen produced loss of GSH and accumulation of GSSG in lens fibers, but not in the epithelium.¹⁰⁴ Surprisingly, even completely opaque human cataracts, containing very little GSH in the cortex and nucleus, and most of that oxidized, possessed normal epithelial levels of GSH, with no GSSG.⁹⁸

In summary, a rabbit model was used to investigate UVB-induced effects on the cornea and lens. A class I UV-blocking senofilcon A contact lens was found to prevent nearly all UVB-induced effects on the ocular tissues of the rabbit, including corneal and lens epithelial damage and cataract. A minimally UV-blocking lotrafilcon A contact lens had no significant protective effect. It is concluded that the use of a senofilcon A contact lens is beneficial for protecting ocular tissues of the rabbit against harmful effects of UVB light, including photokeratitis and cataract.

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