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Photodamage to Calf Lenses In Vitro by Excimer Laser Radiation at 308, 337, and 350 nm
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Calf lenses in vitro were irradiated using an excimer laser at wavelengths of 308, 337, and 350 nm for times ranging from 10 minutes to 5 hours. The laser power was 2.0 watts (W) at 308 nm, 0.2 W at 337 nm, and 2.0 W at 350 nm. During irradiations, the visible light transmission (632.8 nm) of the lenses was measured and found to be decreased markedly with 308- or 337-nm irradiation. No change in visible light transmission was observed with irradiation at 350 nm. Irradiated lenses were also compared with dark control lenses by photographic record. Lenses exposed to 308-nm ultraviolet (UV) radiation for 10-30 minutes showed significant yellow-brown pigmentation and colorless opacification compared with dark controls. Lenses exposed to 337-nm UV light showed primarily colorless opacity with little pigment production. Lenses exposed to 350-nm radiation for up to 1 hour were visibly indistinguishable from dark controls. After photolysis, the lenses were separated into water-soluble and insoluble fractions and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Compared with dark controls, UV-exposed lenses (308 or 337 nm) showed loss of 20-30-kilodalton (kD) material and production of higher molecular weight material at 40–60 kD and >100 kD. There was no evidence of such changes after 350-nm exposure. The data gave the following order for the degree of photodamage: 308 nm < 337 nm > 350 nm. An action spectrum for lens damage is presented. Invest Ophthalmol Vis Sci 31:2180–2184, 1990

The concept that exposure of human and animal lenses to ultraviolet (UV) wavelengths of sunlight is a factor in the formation of cataracts has existed for many years.1-5 Since wavelengths of light shorter than about 295–300 nm are totally absorbed by the cornea, only longer wavelengths are relevant to the in vivo lens.

Lens damage can be initiated either by 280–310-nm UV radiation which is absorbed directly by the lens proteins, primarily by tryptophan residues,6-8 or by longer wavelengths of UV or even visible light absorbed by photosensitizers, such as riboflavin9-11. In the current studies we did not add exogenous photosensitizers.

When human lenses in vitro and solutions of extracted crystallins were irradiated for 48 hr with near-UV light in the presence of tryptophan, their absorption of visible light at 440 nm increased, and they became more intensely yellow-brown.12

Past experiments in other laboratories measured long-term (months) effects of UV radiation in vivo.13,14 The main advantage of long-term experiments is that they permit use of low radiation levels, which are similar to the levels encountered in the ambient environment. A problem with long-term irradiation studies is the necessity of animal maintenance or extended lens incubation, with the possibility of lens damage from sources other than UV exposure.

The excimer laser provides a high average intensity of UV radiation. We used the excimer laser to induce lens opacity and pigmentation over short periods of time, convenient for laboratory experiments, e.g., times on the order of 10–100 min. The experiments were done at three different wavelengths in the UV range, but with constant total energy exposure to compare quantitatively the degree of lens damage at these wavelengths and to produce a photographic record of the appearance of lenses irradiated at three different UV wavelengths. One earlier paper15 reported the effect of excimer laser radiation on calf lenses at 308 nm. Another considered the effect of nitrogen laser radiation at 337 nm on rat lenses.10 The current work extends these studies to three UV wavelengths, all using the same laser source to irradiate calf lenses. Since the UV flux was monitored at each wavelength, we were able to construct an action spectrum for UV-induced calf-lens photodamage.

An advantage of our short-term laser irradiation experiments is that there was no in vitro lens deterioration due to bacterial growth or other causes. On the other hand, it is important to recognize that high-intensity laser UV may cause some effects which are different than those associated with low-intensity UV from incoherent sources.
Materials and Methods

Eyes from 3-6-month-old calves were obtained from a local slaughterhouse within 1 hr of death. The lenses were removed a few hours afterward and were used immediately or kept at -4° C until needed. Lenses were placed in quartz cells and irradiated with the excimer laser under either dry atmospheric conditions or immersed in Ringer's solution. No difference was found in the results for dry or Ringer's irradiations.

The Lumonics (Model EX-520) (Lumonics, Inc., Ontario, Canada) excimer laser was operated at an average power of 2.0 watts (W) at a repetition rate of 50 hertz (Hz) at 308 nm, 0.2 W at a repetition rate of 100 Hz at 337 nm, and 2.0 W at a repetition rate of 50 Hz at 350 nm. The beam dimensions exiting the aperture of the laser were 8 mm X 20 mm at 308 nm, 10 mm X 20 mm at 337 nm, and 6 mm X 20 mm at 350 mm. The beam was focused to a 5 mm X 7-mm spot at the sample position by using a 50-mm focal-length quartz lens. The UV-laser power output was checked during irradiation with a Scientech 365 power and energy meter (Scientech, Inc., Boulder, CO). The irradiation times at 308 and 350 nm were 60 min or less and 5 hr or less at 337 nm. The visible light (632.8 nm) transmission of the lenses was monitored, using the apparatus shown in Figure 1. The UV beam from the excimer laser, and visible light from a 5-mW (Spectra Physics Model 105-1) (Mountain View, CA) helium-neon laser, were made colinear by using a quartz plate as a beam splitter. After both beams passed through the sample, the UV light was prevented from entering the photomultiplier tube by a Corning filter, CS 2-61 (Corning, NY). The transmitted visible light was detected by the phototube continuously during UV irradiation. (In separate experiments, the He–Ne laser output was shown to be constant to within ± 2% over 10 hr, and thus we were able to normalize the visible light transmission data to constant intensity.) The output from the phototube in Figure 1 was detected by a Metrabyte DAS-16F interface (Taunton, MA) which digitized the photovoltage signal for processing by Labtech Notebook software (Laboratory Technologies, Inc., Wilmington, MA) in an International Business Machines PS/2 computer system (Armonk, NY). Thus, the raw transmission data could be converted into relative transmission or relative optical density (OD), with the reference OD = 0 for dark controls.

The possibility of sample heating due to absorption of laser radiation was checked. The temperature rise in 1.0 ml of aqueous medium surrounding a calf lens irradiated for 30 min at 308 nm was measured with a mercury-in-glass thermometer and found to be less than 1° C.

After irradiation, the anterior one third of the lens (irradiated face) was dissected away by slicing the lens perpendicular to the optic axis. This procedure yielded about 0.25 g of material and allowed us to standardize the amount taken from each lens. This irradiated lens material was homogenized in 0.01 M Tris buffer at pH 7.4 (2 ml of buffer per lens) and centrifuged at 15,000 rpm for 30 min. A 20-μl sample of the supernatant was combined with 60 μl of sample buffer, and 2 μl was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 15% polyacrylamide slab gels, stained with Coomassie blue. The sample buffer was prepared from: 1.8 ml of distilled water, 1.5 ml of 0.5 M
Tris HCl, 1.2 ml of glycerol, 2.4 ml of 10% SDS, 0.6 ml of mercaptoethanol, and 0.3 ml of 0.05% (w/v) bromophenol blue. The water insoluble fraction from centrifugation was washed twice with Tris buffer and then dissolved in 1 ml of sample buffer. A sample of 20 μl of this was combined with 40 μl of sample buffer and then analyzed by SDS-PAGE in the same manner as the supernatant.

The UV absorption spectra of whole calf lenses were measured as follows. A lens was inserted into a 1.0-cm square quartz cell. The light entered through the anterior lens face and traversed a path 1.0 cm in length. An empty cell was the reference.

**Results**

Plots of the relative ODs at 632.8 nm versus time of calf lens exposure to 308-, 337-, and 350-nm radiation are shown in Figure 2. The relative OD increased from 0 at time zero to about 0.45 after the 30-min irradiation at 308 nm. Figure 2 also shows that the
relative OD increased from 0 initially to 0.11 after the 4-hr exposure to 337 nm. However, there was less than 0.01 change in OD for lenses irradiated at 350 nm for 30 min. Even lenses irradiated for up to 1 hr at 350 nm showed no significant change in OD.

Changes in lens transparency could also be seen from photographs comparing dark control with UV-irradiated lenses. As seen in Figure 3, lenses irradiated at 308 nm for 30 min had yellow pigmentation and significant opacity at the irradiated lens surface. Small opacification with only a slight yellow color was seen for lenses irradiated at 337 nm for 5 hr (Fig. 4). No changes were seen for lenses irradiated at 350 nm for 1 hr (Fig. 5). These irradiation times correspond to a constant total exposure at each wavelength of 3.2 kilojoules of UV radiation energy.

Irradiated calf lenses were analyzed by SDS-PAGE after separation into water-soluble and insoluble fractions. The insoluble fraction was found to increase by about 30%, relative to the soluble fraction, after UV exposure. Figure 6 shows the effect on the water-soluble and insoluble fractions of calf lenses with 308-nm photolysis for 10 min. Dimers and higher molecular weight material (some of which did not enter the gels) was generated by UV irradiation, indicating formation of interpoly peptide crosslinks. These changes were most evident in the insoluble fraction.

The SDS-PAGE gels of water-soluble and insoluble fractions of calf-lens dark controls and lenses after 5-hr exposure to 337 nm radiation were also obtained. These gels revealed reduced amounts of 20–30 kilodalton (kD) material, new bands at 45–60 kD, and higher molecular weight material (> 100 kD) similar to the 308-nm exposed lenses.

Discussion

The UV-absorption spectra of calf lenses showed that they absorbed most strongly at 308 nm, with much less absorption at 337 nm and 350 nm (Fig. 7). This is consistent with the fact that the observed radiation damage was in the order: 308 nm > 337 nm > 350 nm (Fig. 8). A closer comparison of the data in Figures 7 and 8 suggests that much of the “absorption” at wavelengths greater than 350 nm in Figure 7 may be due to light scattering, rather than true chromophore absorption, since no photodamage was seen at 350 nm in Figure 8.

The following UV-induced lens damage was found: decreased visible light transmission, increased visible light scattering, polypeptide-crosslinking, and fragmentation of lens polypeptides. These changes were similar to those detected by others in aging and cataractous human lenses. Pirie and Walrant and Santus showed that the first photooxy-
dition product of tryptophan, N-formylkynurenine (NFK), could serve as a photosensitizer for further tryptophan oxidation by UV light. Tryptophan was then found to be photolyzed by UV-light exposure to yield many pigmented and fluorescent compounds, not yet identified. Some of these may bind to human and animal lens proteins in vitro, thereby altering their physical and chemical properties. Such changes in tryptophan residues in lens proteins would result in increased pigmentation of the lens and photosensitivity at longer wavelengths, e.g., 337 nm, as seen in the current work.

The SDS-PAGE profiles of lenses irradiated at 308 and 337 nm showed that the lens proteins underwent changes, including polypeptide photocrosslinking to form dimers and higher molecular weight material. Such changes have been previously observed in UV-irradiated lenses and lens proteins.

Lens damage at 308 nm was confined to the anterior face of the lens. This face was irradiated directly by the UV light and hence absorbed much more radiation than the other lens regions. Thus we could see yellow pigmentation and opacity at the lens surface but not in the lens interior. At 337 nm the radiation caused opacification but not pigmentation. This differs from the 308-nm result and could indicate a change in photolysis mechanism. The shorter wavelength is probably absorbed by tryptophan residues in the lens proteins, but the longer wavelength is beyond the normal tryptophan-absorption region and could be absorbed by some other chromophore (e.g., NFK) resulting in different photochemistry. Since the 337-nm radiation could penetrate more deeply into the lens, damage was seen to extend farther into the lens interior than at 308 nm.

The action spectrum of normal rat lens photodamage was measured previously by Borkman et al. They observed that the photochemical action in the rat lens was very large at 308 nm but less than 10% of the 308-nm value at 350 nm. This is consistent with our finding of little or no action in calf lenses irradiated at 350 nm (Fig. 8).

In conclusion, some of the macromolecular changes that occur in proteins of human lenses with aging and cataracts seem to be modeled by exposing whole calf lenses to near-UV laser radiation.

Key words: lens, UV, photodamage, opacity, light scattering

References