Effects of UV-A Radiation on Lens Epithelial NaK-ATPase in Organ Culture
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PURPOSE. To investigate the mechanisms involved in the damage caused by UV-A irradiation at 365 nm on the eye lens.

METHODS. Bovine lenses obtained from animals 1 to 5 years of age were placed in specially designed organ culture chambers for preculture. Twenty-four hours later, the lenses were irradiated by 33 J/cm² UV-A at 365 nm. During irradiation, the lenses were oriented in the culture so that the anterior surface faced the incident UV-A radiation source. After irradiation, lens optical quality was monitored throughout the 8 days of the culture period, and lens samples were taken for analysis of NaK-ATPase activity.

RESULTS. Lens optics and NaK-ATPase activity were affected by irradiation of 33 J/cm². The effects on lens epithelial NaK-ATPase activity were stronger at the equators than at the center. The damage to the activity at the center was reversible, as the lens optically recovered from the UV-A damage.

CONCLUSIONS. Lens NaK-ATPase activity can recover from damage caused by UV-A at 365 nm. When the lenses received irradiation of 33 J/cm², NaK-ATPase activity recovered from the damage during the culture period only at the center and not at the equators of the epithelium. (Invest Ophthalmol Vis Sci. 1999;40:1616-1620)

The human eye filters out major radiation below 400 nm. The filtration is a two-step process: light below 320 nm (UV-B) is maximally absorbed by the cornea with some light transmitted by the cornea down to 290 nm, whereas light between 320 nm and 400 nm (UV-A) penetrates and is maximally absorbed by the lens. The human lens contains low-molecular-weight compounds that absorb maximally at approximately 365 nm. The amount of radiation that is absorbed determines the potential for damage to the absorbing tissue. Radiation that is not absorbed by a superficial tissue will be transmitted and can affect a deeper tissue. The function of the eye lens is to focus light on the retina, and consequently, the lens must remain transparent throughout life. Epidemiologic and clinical observations have indicated a link between sunlight exposure and cataract. There is substantial evidence that solar radiation can also damage the human retina if the eye is fixated on the sun. Exposure to sunlight may also be associated with an increased risk of macular degeneration. In geographic locations where the UV components of sunlight are more intense, dark brown cataracts occur at a higher frequency than in locations where UV components are weak. Cataracts occur more commonly in the elderly and are also associated with high levels of UV radiation and sunlight.

Very little is known about the mechanisms by which UV-A causes damage to the eye lens or the ability of the lens to recover from that damage. We have shown that UV-A radiation above 22.4 J/cm² has damaging effects on lens optics and epithelial enzymes. We measured the optical quality and the activities of the three enzymes, hexokinase, catalase, and glucose-6-phosphate dehydrogenase, after irradiation of 22.4 J/cm², 33.6 J/cm², and 44.8 J/cm². Full recovery of lens optical damage and activity of hexokinase, catalase and glucose-6-phosphate dehydrogenase in lens epithelium was observed after 8 days in culture after irradiation of 22.4 J/cm². After irradiation of 33.6 J/cm², there was only partial recovery of lens enzymes and optics. The ability of the lens to repair itself was absent after irradiation of 44.8 J/cm². In the present study we investigated the nature of the damage of 33 J/cm² UV-A irradiation at 365 nm to lens optics and lens epithelial NaK-ATPase in lenses of different ages. We analyzed the damage using a special lens organ culture system. This system enabled us to apply controlled amounts of radiation and to observe the damage and ability of the lens to recover in order to determine the mechanisms by which UV-A affects the eye lens.

METHODS

Organ Culture System

Bovine lenses were carefully excised from eyes obtained from animals 1 to 5 years old, 2 to 4 hours after enucleation. Each lens was placed in a specially designed culture chamber. The culture chamber consisted of two compartments connected by a round hole. The diameter of the hole was 1 mm smaller than the diameter of the lens. The lens sat between the two compartments, leaving a clear space filled with culture medium below and above the lens. Both lens surfaces were bathed in a total of 24 ml culture medium consisting of M199 with Earle’s salt, 8% fetal calf serum, and antibiotics (100 U/ml penicillin and 0.1 mg/ml streptomycin). The medium was replaced with fresh sterile medium every 24 hours. The lenses were incubated at 35°C.

The investigation was conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

UV-A Irradiation

The lenses were irradiated through the Pyrex glass culture container. Most of the column of medium above the lens was removed during UV exposure, leaving the lens with 5 ml medium, mainly under the lens, whereas a thin layer of medium, 1 mm thick, was left covering the upper surface of the lens. The lenses were oriented so that the anterior surface faced the incident UV-A radiation source. The 400-W UV lamp (VL-400L-365 nm; Vilber Lourmat, Cedex 2, France) provided radiation intensity of 8.5 mW/cm² at 365 nm. Through the
Pyrex cover, at the distance of the lens from the UV lamp, the radiation was 7.465 mW/cm² at 365 nm, measured by an IL 1700 radiometer (International Light Inc, Newburyport, MA). The lenses received 33 J/cm² of 365 nm energy when exposed to four irradiation steps of 8.25 J/cm² each. Between each of the irradiation steps there was a break of 20 minutes, to keep the temperature of the culture dish below 35°C.

**Optical Quality Monitoring**

After irradiation, lens optical quality was monitored throughout the culture period. Lens optical measurements were taken as previously described, using an automated scanning laser system that records both relative transmittance and focal length across the lens. The laser scanner consisted of a low-powered helium-neon laser mounted on a computer driven xy table with two video cameras and a video frame digitizer. The laser was programmed to scan across the lens in the axial direction in small steps (0.5 mm), while the video cameras transmitted the image of the refracted beam to the video digitizer. A custom software program determined the focal length and relative intensity of each refracted beam from the digitizer image. The optical center was first determined for each lens by finding the position of zero or minimum refraction for both the x and y directions, and then the program determined the focal lengths for 11 beam positions at equal step sizes on each side of the center. The system measured relative changes in transmittance of the lens by measuring the excitation state of a 512 x 512-pixel television screen matrix for each exiting (refracted) laser beam. However, scatter measurements have been difficult to interpret in comparison with focal measurements, and therefore, this study concentrated on focal length results.

**Preparation of Lens Samples and Enzyme Analysis**

The lens epithelium was dissected under a binocular stereomicroscope. Cuts were made along the equators, and the lens capsule and its adhering epithelium were removed from the entire lens. Care was taken that no fiber tissue remained attached to the epithelium. The tissue was immediately immersed in 500 μl Tris buffer (pH 7.4). The tissue was sonicated in an ultrasonic disintegrator (MSE 150-W; MSE Sanyo, Leicester, UK) at 50 W for 10 seconds. This procedure was performed twice. ATPase activities of the homogenates were measured according to Hightower and McCready.

The method showed activity of total Mg²⁺-dependent NaK-activated ATPase. Histochemical analysis of Mg²⁺-activated ATPase was performed according to Sheeham and Hrapchak.

**Protein Analysis**

Protein concentration of the homogenates was measured by the micromethod of Lowry et al.

**Statistical Analysis**

All results were analyzed using Student’s t-test. A change was defined as significant if the difference between the control and treated groups reached a level of significance of \( P < 0.05 \).

**RESULTS**

Bovine lenses aged 1 to 5 years were placed in organ culture for preincubation. Three hours later and again 24 hours later, lens optical measurements were taken, and only the optically good quality lenses were used for the experiment. The results of 206 lenses are presented. The lenses were separated into two age groups according to lens diameter: “young” (1 year old; 15-16 mm diameter) and “old” (4-5 years old; 17-18 mm diameter). In each group of 22 beams that passed through the lens at the time point of 120 hours in organ culture.

Optical quality of the lenses during the 192 hours of the experiment is shown in Figures 1 and 2. There is almost no change of the focal length variability in the control lenses, during the culture period. Young (15-16 mm diameter) lenses that received irradiation of 33 J/cm² UV-A show an increase in focal length variability (Fig. 1) after 120 hours in culture. Focal length variability (sharpness of focus) increased up to twofold. These changes returned to control levels 24 hours later. In old lenses of 17 to 18 mm diameter that received the same amount of irradiation (Fig. 2), the increase in focal length variability started 24 hours earlier, increased up to 3.5-fold at the time of 120 hours and returned to control levels at the seventh day of culture (168 hours). Focal length variability represents the variation in the focal lengths of the 22 beams that passed through the lens during each scan and is calculated as the SEM of the average of the 22 focal lengths. The variation indicates that opacity was most severe in some sectors of the lenses.

NaKATPase specific activity in lens epithelium is shown in Figure 3 for the 15- to 16-mm diameter lenses and Figure 4 for the 17- to 18-mm diameter lenses. ATPase control activity did
FIGURE 2. Effect of UV-A radiation of 33 J/cm² on the focal length variability of old (4–5 years, 17–18 mm diameter) lenses in organ culture compared with that in control lenses of the same diameter. Focal length variability represents the variation in the focal lengths of the 22 beams that passed through the lens during each scan and is calculated as the SEM of the average of the 22 focal lengths. Each point contains scans from six or more lenses. Ultraviolet-treated lenses were irradiated 24 hours after they were placed in culture with 33 J/cm² UV-A at 365 nm. P < 0.01 for points at 96 hours, 120 hours, and 144 hours in organ culture.

FIGURE 3. ATPase activity in control and 33 J/cm² UV-A-treated bovine lens epithelium of 15–16-mm diameter lenses, as a function of time in organ culture. Specific activity is shown as nanomoles pi/μg protein. (A) ATPase activity in total epithelium; (B) ATPase activity in epithelium center; and (C) ATPase activity in epithelium equators. *P < 0.05, statistically significant. The fresh lenses (0 time in organ culture) were damaged slightly owing to the stress conditions of transferring them from the live animal eye to the culture conditions. It takes some time for the lens to recover and for the enzymes to reach the maximum control levels in organ culture.

DISCUSSION

We have shown that UV-A radiation of 33 J/cm², in addition to its effects on lens optical quality, causes damage to lens epithelial NaK-ATPase activity. Our results show that the lenses had the ability to repair part of the damage. In another system of squirrel lenses in vitro UV-A radiation of 28.8 J/cm² to 72 J/cm² at 360 nm also diminished NaK-ATPase enzyme activities. Also, superficial opacities developed in dogfish lenses in vitro, because of near-UV exposure. In that research whole-mounts of lens epithelium exhibited breakdown of actin filaments in the basal region of the cells within 18 hours of UV exposure. Very little is known about the repair of UV-A-induced damage in mammalian cells. Sidjanin et al. measured cell survival as the induction and repair of DNA single-strand breaks in cultured rabbit lens epithelial cells after 18 J/cm² UV-A exposure. Their results indicate that UV-A can induce not change significantly with time in culture. ATPase activity at the center of the lens epithelium (Figs. 3B and 4B) is higher than that at the equators (Figs. 3C and 4C). The effects of the irradiation for the 15–16-mm lenses (Fig. 3) started at the center of the lens epithelium 96 hours after irradiation (120 hours in culture) and return to control levels 24 hours later (Fig. 3B). At the equators the irradiation effects started 72 hours after irradiation (Fig. 3C) and there was no return to control levels during the culture period. For the older lenses 17 to 18 mm in diameter (Fig. 4) the effect of the irradiation on ATPase activity started 24 hours earlier in both total epithelium (Fig. 4A) center and equators (Figs. 4B and 4C), and it took more time for the enzyme at the center to recover from the damage (Fig. 4B) than in the 15–16-mm lenses (Fig. 3B). The effects of the irradiation on lens epithelium is also shown by histochemical methods (Fig. 5). Figure 5A shows ATPase control activity at the center of 16-mm lens at five days of the culture period. Administration with the ATPase inhibitor ouabain (Fig. 5B) diminished the activity in the preparation. The effect of the irradiation at this stage is mainly by damaging the cells (swelling) and not the enzyme itself (Fig. 5C).
FIGURE 4. ATPase activity in control and 33-J/cm² UV-A-treated bovine lens epithelium of 17- to 18-mm diameter lenses, as a function of time in organ culture. Specific activity is expressed as nanomoles pi/microgram protein. (A) ATPase activity in total epithelium; (B) ATPase activity in epithelium center; and (C) ATPase activity in epithelium equators. *P < 0.05, statistically significant. The fresh lenses (0 time in organ culture) were damaged slightly owing to the stress conditions of transferring them from the live animal eye to the culture conditions. It takes some time for the lens to recover and for the enzymes to reach the maximum control levels in organ culture.

single-strand breaks in lens epithelial cells in culture, and the cells can repair most of it. Our study shows that UVA repair mechanisms exist in intact lenses under organ culture conditions, including repair of damaged optical quality and NaK-ATPase activity. The correlation between enzyme activities and lens optical quality is still not clear. At the center of lens epithelia, NaK-ATPase activity was reduced and recovered in full correlation with changes in optical quality. At the equators of the lens, UVA radiation reduced NaK-ATPase activities 24

FIGURE 5. Histochemical localization of ATPase activities in lens epithelium after 5 days of incubation in organ culture. (A) Control; (B) in the presence of the enzyme inhibitor, ouabain; (C) after 33 J/cm² UVA irradiation. Small arrow shows enzyme activity; large arrow shows cell swelling. Scale bar, 25 μm.
hours before optical quality was affected, and there was no recovery of enzyme activity during the culture period. There is no evidence yet how recovery of enzyme activity might be accomplished. It is still not clear whether it is a new synthesis or reactivation of damaged enzyme molecules.

Studies of NaK-ATPase have shown that multiple isoforms of both the α- and β-subunits exist. In the case of the α-subunit there are three known isoforms: α1, α2, and α3 enzyme with the α3 isofrom differing in its apparent affinity for sodium. The expression of multiple isoforms of the α- and β-subunits of NaK-ATPase in mammalian tissues gives rise to the complex molecular heterogeneity that characterizes the Na pump. Blanco et al. have stated that all catalytic activities of the NaK-ATPase have been ascribed to the α-subunit however, normal activity requires the presence of the β-subunit. They showed, using recombinant baculoviruses to infect insect cells, that the α-subunit, without the β-subunit, has catalytic activity. Blanco et al. also found that the accompanying β-subunit isofrom does not drastically affect the properties of the α3 polypeptide. The most pronounced kinetic differences in NaK-ATPase function are the result of variations in α isoform composition. The lens epithelium is thought to conduct Nαk transport for the entire lens cell mass. Lens fibers have a poor ion transport capacity. Moseley et al. showed that adult rat lens epithelium expresses more than one isoform of NaK-ATPase catalytic subunit, whereas only the α1 isoform can be detected in fiber cells. By western blot, NaK-ATPase α1, α2, and α3 polypeptides were observed as sharp bands at 100 kDa to 108 kDa. In fiber cells, only NaK-ATPase α1 immunoreactive polypeptide was detected. However, NaK-ATPase α-subunit mRNA was not found in adult lens fibers, suggesting that ongoing α1 synthesis is low.

Garner and Horwitz identified the NaK-ATPase isoforms present in mammalian lens. According to their findings, α1 is the main isoform in lens epithelial cells located toward the equator. The catalytic subunit of the central lens epithelium is α3 (the α2 isoform is located in the lens fiber cell membranes). According to this information, it is possible that the α1 isoform is more sensitive to UV-A radiation than the α3 isoform.

Concerning the effect of age on the lens, Liang stated that when browning products appear, the lens absorbs more light above 300 nm and becomes more susceptible to photo-oxidation from radiation of light at this wavelength. Consequently, the old lens is more susceptible to photo-oxidation by UV-A light than is the young lens. Pirie showed that sunlight initiates absorption of photons by tryptophan. This can form various photo products, including N-formylkynurenine, which can act as a sensitizer, absorbing additional photons leading to the destruction of various amino acids. This type of reaction can lead to enzyme inactivation. We still do not know whether the radiation causes oxidative damage to lens epithelial NaK-ATPase. In our study, younger lenses had the ability to recover better than older lenses; however, the effects of UV on the lens may be accumulative. In this case older lenses would be more sensitive to radiation because of prior damage.

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References