Ultraviolet Radiation Effects on the Corneal Epithelium

By

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Since military troops are involved in extensive outdoor activities with chronic exposure to solar radiation, and since ultraviolet radiation (UVR) lasers may play a role in the future military environment, a thorough understanding of UVR damage mechanisms would be crucial to the development of intervention and treatment modalities. The present research was directed at quantifying possible alterations in corneal epithelial metabolic activity secondary to in vivo exposure to UVR in the rabbit.

A 5,000 watt Hg-Xe arc lamp served as the UVR source. The radiant exposures were kept constant at 0.05 J·cm⁻² for all UVR wavelengths used (290, 300, 310, and 360 nm). Wavelength isolation was accomplished with a double monochromator providing a 6 nm full bandpass. The four experimental wavelengths were chosen based on an interest in maintaining an environmental relevance, since 290 nm UVR and above can be found at the earth's Continued
surface. Micropolarographic measurement of corneal oxygen uptake rates served as an in vivo index of UVR-induced effects on oxidative metabolism. Microfluorometric analyses of key epithelial energy metabolites (glucose, glycogen, ATP, and PCr) were used as an in vitro index of UVR-induced effects on overall metabolic activity. A paired difference analysis of the oxygen uptake rate data demonstrated a decrease in relative corneal oxidative metabolic activity that was wavelength-dependent. These same experimental UVR exposure conditions served to significantly increase epithelial glucose and glycogen concentrations. Although the epithelial ATP concentrations were unchanged, the epithelial PCr concentrations (a high energy phosphate bond reservoir) decreased as a result of UVR exposure.

These data demonstrate a decrease in corneal epithelial oxidative metabolic activity as a result of UVR exposure, and infer an adverse effect on glycolytic metabolism, as well. It is suggested that immediate UVR-induced metabolic inhibitory effects can be responsible for the pattern of epithelial cell loss seen in photokeratitis.
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Introduction

Ultraviolet radiation (UVR) has been implicated within public health arenas as a potential stimulus for degenerative ocular changes since the time of the ancient Greeks. Recent speculation concerning changes in the nature of the atmospheric ozone layer has led to increased interest in adverse effects of UVR exposure. The advent of UVR lasers have further spurred research interest concerning the determination of possible mechanisms of action, since the development of a full spectrum of applications is dependent upon a thorough understanding of operant mechanisms.

The ideal tissue for investigating the in vivo effects of UVR is the corneal epithelium. As the most anterior tissue layer of the eye, the corneal epithelium is subject to a direct interaction with incident radiation. The tissue is uncomplicated by spurious absorbers, thermal effects, or pigmentary photochemistry. In addition, its avascularity and accessability enable response gathering uninfluenced by circulatory system factors remotely external to the tissue. Finally, the corneal epithelium is exposed to UVR on a daily basis, so exposure studies are not subjecting the tissue to a completely unnatural condition.

Many investigations into the effects of UVR on the corneal epithelium have concentrated on morphological evaluations utilizing the biomicroscope, the light microscope, and/or the electron microscope (Verhoeff and Bell, 1916; Cogan and Kinsey, 1946; Pitts and Tredici, 1971; Ringvold, 1980 and 1983). Such studies have provided detailed information concerning the delayed structural changes characteristic of UVR damage that occur 4 to 12 hours after exposure. As a result of this histological detection delay, information concerning either immediate or functional effects of UVR cannot be probed by such methods.

Millodot and Earlam (1984), seeking to evaluate this damage-delay phenomenon, revealed the presence of a period of decreased corneal sensitivity immediately following exposure to UVR. Their finding appears to signify an immediate effect of UVR upon the sensory neurons subserving the corneal epithelium. If such is the case, and knowing that these axons appear deep within the

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2 Xenophan's treatise "Anabasis" discusses the condition of "snowblindness."
basal cell layer of the corneal epithelium and within the anterior stroma, it would be reasonable to assume that there might also be an immediate effect of UVR on the corneal epithelium itself. Therefore, the purpose of this study was to evaluate the possible immediate effect of exposure to UVR on the metabolism of the corneal epithelium in the rabbit.

Selection of the exposure wavelengths (290, 300, 310, and 360 nm) was based on an interest in maintaining an environmental relevance. An additional factor was the intention of creating a distinctive span of effects by maintaining a constant level of radiant exposure at 0.05 J·cm² with varied wavelength challenges. Measurement of the corneal oxygen uptake rate served as an in vivo assessment of epithelial metabolic activity, while micro-fluorometric metabolite analyses served as an in vitro index of epithelial metabolic activity.

Materials and methods

Experimental animals

Healthy, adult, Dutch-belted, pigmented rabbits were used as the experimental animals. The animals were housed in NIH-approved quarters under controlled, normal lighting conditions. The animals were maintained, and the experiments were conducted in accordance with procedures outlined in the "Guide for Laboratory Animals Facilities and Care" of the National Academy of Sciences-National Research Council. Anesthesia was maintained throughout the course of the experiment with intramuscular injections of Ketalar (10 mg/kg) and Rompun (5 mg/kg). The UVR-exposed animals were sacrificed by cervical dislocation 2 minutes after exposure was discontinued. The eyes were then immediately removed and immersed in liquid nitrogen in order to prevent significant change in metabolite levels. Control animals, after a mock-exposure period under the same level of anesthesia as the experimental animals, underwent the same tissue preparation procedures.

Oxygen electrode

The micropolarographic oxygen probe consisted of a platinum cathode (25-um diameter) and a silver anode embedded in a plastic carrier. A potassium chloride (KCl) solution served as an electrolyte bridge between the cathode and the anode. An oxygen-permeable polyethylene membrane, 25 um thick, effectively sealed the entire electrode-KCl assembly into one operating unit. The micropolarographic system was similar to that used by Benjamin and Hill (1986).
In general terms, when a voltage is imposed across the KCl bridge from the anode to the cathode, oxygen present at the sensor tip undergoes an electrolytic reduction at the platinum cathode, with the reverse reaction occurring at the anode. The current generated across the cathode/anode gap is directly proportional to the oxygen concentration of the solution when the system is properly calibrated. The generated current is amplified by a blood gas analyzer and recorded vs time on a physiograph.

Calibration procedure

The probe was calibrated by first placing it in an air-saturated saline solution of 155 mm Hg partial pressure of oxygen. The probe was then placed in a nitrogen-saturated saline solution of 0 mm Hg partial pressure of oxygen. The time required for the recorded oxygen tension to then drop from 140 mm Hg to 40 mm Hg provided the time constant of the micropolarographic system. The experimental procedure involved applying the probe to the anterior surface of the corneal epithelium of the living, anesthetized rabbit. The sensor, when applied to the eye, provided a limited reservoir of oxygen for the underlying tissue. The average rate of oxygen depletion from the sensor reservoir, between recordings of 140 and 40 mm Hg, and after correction for the time constant, became the measure of corneal oxygen uptake rate, which in turn represented a relative measure of the aerobic requirement of the corneal epithelium. Since the aerobic metabolic rate of a tissue directly reflects mitochondrial function (Graymore, 1969), a micropolarographic oxygen electrode, when used to record the rate of oxygen uptake by the anterior surface of the intact cornea, would primarily serve as a relative index of corneal mitochondrial activity.

In vivo micropolarographic application

The eyes of 32 rabbits were exposed in vivo to specific radiant exposure levels of UVR. There were 8 rabbits used in the 290 nm experimental group, 12 rabbits used in the 300 nm experimental group, 7 rabbits used in the 310 nm experimental group, and 5 rabbits used in the 360 nm experimental group. Prior to UVR exposure, five baseline oxygen uptake rates were determined for each eye using a micropolarographic oxygen sensor that was attached to a pH/blood gas monitor in the fashion of Benjamin and Hill (1986). Oxygen uptake rates again were determined 2 minutes post-UVR exposure, enabling the experimenter to compare the change in oxygen uptake rate as a result of the UVR exposure. The uptake rate values were subjected to a paired-difference analysis. A two-way analysis of variance (ANOVA) was performed to examine both within and between experimental groups, and to determine the presence or absence of an overall effect of UVR on the measured oxygen uptake rates.
In vitro processing procedure

The rabbit eyes were transferred from the liquid nitrogen container into a -80°C freezer for storage until tissue processing could be accomplished. The cornea was removed from the globe by dissection under -30°C conditions in a wedeen-type cryostat. The isolated cornea was cut into halves, which were mounted on a sectioning button by immersion in a dry ice-cooled hexane solution. The corneal button-mount then was transferred to a cryostatic microtome where tissue sectioning was performed. The resulting central cornea cross sections were approximately 20 micrometers (µm) in thickness. The sectioned tissue samples were placed in a metal tissue holder, covered with glass slides, and inserted into a vacuum tube. The tube was placed in a -20°C freezer and attached to a vacuum pump. The tissue then was freeze-dried for a 24 hour period. After the freeze-drying process was completed the tissue was kept at -20°C until assayed.

Samples needed for assay were thawed under vacuum for 1 hour to prevent condensation-stimulated enzyme action. The different layers of the cornea were defined clearly which permitted easy isolation of the corneal epithelium under a 3X binocular dissecting microscope. Tissue size was determined by dry weight, rather than by tissue section dimensions, which permitted the analysis of very small and irregularly shaped specimens. The tissue samples immediately were weighed on a quartz fiber fishpole balance possessing µg sensitivity. After weighing and recovery, the samples were placed in an oil well rack for specific metabolite assay.

Underlying principles

The cycling system contains several enzymes which catalyze specific interrelated reactions yielding a "net reaction." A byproduct of this multi-step reaction is reduced nicotinamide adenine dinucleotide phosphate (NADPH), which fluoresces light of 460 nm wavelength when excited with UVR of 340 nm wavelength. By measuring the amount of this reduced pyridine nucleotide fluorescence, the original concentration of the assayed metabolite can be inferred by calculation (Lowry and Passonneau, 1972). Appropriate blanks and standards were employed to monitor the reliability of the assays. Individual enzymatic cycling and incubating techniques permitted isolation of the specific metabolite being analyzed.

Source measurement

Source calibration and radiometric quantification followed the procedures described by Pitts, Cullen, and Hacker (1977). The UVR source was a 5,000 watt xenon-mercury (Xe-Hg) high pressure arc lamp, powered by a 10 kilowatt (kw) direct current
power supply regulated to ± 0.5 percent and capable of delivering from 0 to 80 amps at 25 to 65 volts to the arc electrodes. The lamp housing was cooled by a double-blower system. The radiation from the source was focused at a monochromator entrance slit by the housing optics. A 10 cm quartz-enclosed water chamber was placed between the focusing lenses and the monochromator in order to remove infrared radiation. The desired UVR waveband was obtained with a double grating monochromator with gratings blazed at 300 nm and grooved with 1,180 grooves/mm, allowing approximately a 5 nm bandpass. The linear dispersion equated to a value of 0.82 nm/mm. Entrance, intermediate, and exit slits were set to pass a nominal full bandpass of 6.6 nm. The system was aligned with a helium-neon laser and the wavelength counter was calibrated with a mercury source. Exposure durations were set with an electronic shutter controlled by a preset counter. The preset counter allowed exposure durations of any desired length with millisecond accuracy.

The exit optical beam was focused by a quartz lens with a beam size of 1.6 cm by 1.8 cm at the plane of the experimental animal's cornea. A 16-junction thermopile, traceable to a National Bureau of Standards (NBS) standard source, was used to characterize the spectral irradiance of the UVR source. When taking the spectral irradiance readings, the thermopile was placed in the same position relative to the monochromator exit port as the rabbit's cornea was to be situated during UVR exposure. The irradiance \( E_e \), in watts per square centimeter \((W \cdot cm^{-2})\), incident on the thermopile was determined by the following relationship:

\[
E_e = kV_t. 
\]

The value "k" represents the thermopile calibration constant in microwatts per square centimeter per microvolt \((\mu W \cdot cm^{-2} \cdot \mu V^{-1})\), while the value "V_t" represents the average (mean) of three thermopile-voltmeter readings in microvolts \((\mu V)\). The calibration constant for the thermopile used in this experiment was 5.131 \( \mu W \cdot cm^{-2} \cdot \mu V^{-1} \). The radiant exposure \( H \), in Joules per square centimeter \((J \cdot cm^{-2})\), was calculated by the formula:

\[
H = E_e t. 
\]

The value "t" is simply time in seconds; it should be kept in mind that a Joule is a watt-second. Therefore, for a given irradiance \( E_e \), the exposure duration "t" can be varied to obtain different values of radiant exposure \( H \) as desired. Conversely, a radiant exposure can be kept constant, even though wavelength irradiance may differ, by varying the time of exposure. This means of output characterization and source calibration was estimated to have a ±/10 percent accuracy.
The variation of "t" in order to obtain a constant "H", in the context of the wavelengths used in this experiment, creates an outcome that is somewhat dependent upon the validity of the principle of reciprocity (i.e., the biological effects or endpoints are independent of exposure time and irradiance). Corneal effects of a krypton-ion laser, with simultaneous output at 350.7 and 356.4 nm (3:1 ratio), illustrates that the product of threshold intensity and the pulsewidth is a constant; the thresholds for multi-pulse exposures have been shown to be in agreement with those for single-pulse exposures (Zuclich and Connolly, 1976). A similar corneal damage pattern can be elicited from helium-cadmium laser data at 325 nm (Ebbers and Sears, 1975). Based on the literature, it would not be unreasonable to assume that reciprocity holds for all four UVR wavelengths utilized in this experiment.

Results

Introductory data (Figures 1 and 2) have been previously published (Lattimore, 1989a; Lattimore, 1989b), and currently are used as a basis for detailed analyses. Figures 3 and 4 isolate oxygen uptake as a function of corneal epithelial PCr, ATP, glucose, and glycogen concentrations. In the current unit format these data are not easily compared in any fashion other than in a general graphical relationship. Within this general framework there is very close correlation, implying a direct relationship between UVR-altered oxygen uptake rate and certain metabolite changes. ATP was found to be stable across all changes in oxygen uptake associated with UVR exposure (r = 0.07). Glucose and glycogen were found to accumulate as a function of UVR-altered oxygen uptake (r = 0.97 and 0.98, respectively); PCr depletion also correlated well with changes in UVR-altered oxygen uptake rates (r = 0.84).
Figure 1  By plotting the UVR-altered corneal oxygen uptake rate data as a function of wavelength, and by making separate datasets for each radiant exposure, a "family" of plots is obtained. A two-way analysis of variance demonstrated an overall significant between-groups difference (p < 0.0001), as well as revealed an interactive effect between wavelength and dose (p < 0.005). Unexposed eyes exhibited no significant change in corneal oxygen uptake rates over the course of the experiment.
Figure 2  The bar-chart illustrates various epithelial metabolite concentrations as a function of the wavelength of UVR exposure. An analysis of variance of the data demonstrated a highly significant overall, between groups effect (p<0.0001) for glucose, glycogen, and PCR. An analysis of variance of ATP data failed to demonstrate a significant effect (p>0.65), illustrating an apparent ATP-sparing process.
Figure 3  Plotted decreases in the oxygen uptake rate as a function of PCr and ATP, illustrate ATP-sparing at the apparent expense of PCr.
Figure 4  Plotted decreases in the oxygen uptake rate correlate highly with accumulations of glucose and glycogen. However, plotted units are not directly mathematically comparable. Therefore, the established relationship between oxygen and glucose should, for the moment, be considered a qualitative relationship only.
Discussion

The corneal epithelium is known to conduct both aerobic and anaerobic metabolic activity concurrently (Kinoshita and Masurat, 1959). According to some estimates, the rabbit corneal epithelium routinely consumes up to 85 percent of available glucose in anaerobic channels, with the remaining 15 percent used via aerobic channels (Riley, 1969). When anaerobic conditions are artificially imposed upon a cornea (i.e., by the application of a thick contact lens), the tissue response has been portrayed to be increased anaerobic activity, inferred by the depletion of epithelial glycogen stores (Uniacke and Hill, 1972). Yet, under the UVR exposure conditions of this experiment, decreased oxygen utilization was evidenced with a contradictory accumulation of both glucose and glycogen, rather than the expected depletion. This paradox led to the examination of oxygen uptake changes as a dependent variable of key metabolites.

The results indicate: a decrease in oxygen consumption, an apparent decrease in glucose utilization, a stabilization of ATP, and a decrement in PCr. The manifested close relationship between the research variables point toward an alteration of oxidative or mitochondrial activity resulting from UVR exposure. In addition, paradoxical glucose and glycogen accumulations suggest a secondary underlying effect on the anaerobic or glycolytic chain. A global enzyme inactivation can be excluded because glycogen and ATP storing are mediated enzymatically. Therefore, it can be concluded that observed UVR effects are the result of more than one damage mechanism. However, this close correlation stems from a superficial view; translation of oxygen data to units more directly comparable could provide a greater insight into operant mechanisms of UVR damage.

By plotting oxygen uptake changes as a function of exposure duration (Figure 5), one can examine slope differences in wavelength effects. The 360 nm data possess a distinctly different slope than the other wavelengths. Since 360 nm oxygen and PCr levels are not significantly affected by UVR exposure, it is likely that 360 nm effects of UVR are not centered upon the mitochondria, but elsewhere in the metabolic chain. However, this analysis does not differentiate relative contributions of mitochondrial and anaerobic effects secondary to 290, 300, and 310 nm exposures. In an attempt to accomplish such a differentiation, oxygen data was mathematically integrated over the 0.05 J/cm² radiant exposure period. It was not necessary to do this for metabolite data, because metabolite data represent a natural integration over the total exposure.
Figure 5  Comparison of the oxygen-exposure time slope data highlight 360 nm effects to be independent of other wavelength mechanisms of action. The combined absence of oxygen effects accompanied by glucose accumulation suggest the presence of a damage mechanism isolated within the anaerobic stages of metabolism.
Since oxygen data are theoretically translatable from mm Hg O₂/sec to ul/cm², and metabolite data can be translated from nmol/ug to nmol/cm², a directly comparable unit correlation may be obtained. This method of data presentation (Figure 6) highlights the relative relationships between mitochondrial and anaerobic metabolism, with UVR wavelength. Short wavelength UVR (i.e., 290 nm and possibly shorter) are shown to possess predominantly an adverse mitochondrial effect on the corneal epithelium, since PCr dramatically falls off compared to the other wavelength exposures, and total glucose accumulations are much less than a standard, linear model would predict. As the exposure wavelength increases, total glucose accumulation and PCr depletion conform to a linear representation when compared to equivalent oxygen decrements. Therefore, it can be concluded that observed UVR effects on the corneal epithelium are the result of more than one damage mechanism. UVR exposures at or near 360 nm will produce effects predominantly by way of disruption of anaerobic/glycolytic metabolic pathways. UVR exposures at and possibly below 290 nm will produce effects predominantly by way of disruption of aerobic/mitochondrial metabolic pathways. UVR exposures at intermediate wavelengths will produce compound effects on the corneal epithelium involving both damage mechanisms.

Summary

Corneal epithelial metabolism is affected adversely by a dual mechanism of UVR damage. This duality differentially presents itself dependent upon exposure wavelength. While considerable overlap is possible, clearly short wavelength UVR is predominantly toxic to the mitochondrial system, while longer wavelength UVR predominantly affects the anaerobic metabolic pathways. Dual damage mechanisms, with overlapping action spectra could complicate the development of intervention and treatment modalities. Specific enzymatic analyses will be necessary to fully elicit wavelength specificities and potential treatment options.
Figure 6  Total glucose and PCr concentrations for 290 nm exposures are clearly outside any potential linear relationship that might be suggested by 300, 310, and 360 nm data. This dramatic dropoff in PCr, combined with the nonlinear accumulation of glucose found in 290 nm exposures suggest the presence of a damage mechanism isolated at the level of mitochondrial function.
References


