Effects of ultraviolet radiation on corneal epithelial metabolism.

JUDITH FRIEND, AND RICHARD A. THOFT.

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The destructive effects of ultraviolet (UV) light on the cornea have long been attributed to damage to nucleic acids and proteins in the epithelium. Whether or not there are primary effects on the processes responsible for energy transformation has not been investigated, although such effects have been found in all other forms of corneal injury which have been studied to date. Therefore, in an attempt to investigate whether there is any alteration of the energy transformation pathway after injury believed to affect primarily structural proteins, some of the metabolites and enzymes responsible for energy transformation in the corneal epithelium have been measured after far-ultraviolet radiation (UV-B). These are correlated with evolution of the clinical changes, as well as with changes in corneal hydration.

Methods

Preparation of animals. Adult rabbits weighing 2 to 3 kg were exposed to the radiation of a 30-watt germicidal UV lamp at a distance of 10 cm from the anterior corneal surface for 15 min per eye. We immobilized the animals but avoided instrumentation or medication of the lids or cornea. The emission spectrum of the GE30T8 lamp source used is predominantly 257 nm, with lesser emissions at 310 nm and 360 nm (Fig. 1). The calculated exposure at 257 nm was 1.48 × 10^7 ergs (70 to 80 times the photokeratitis threshold), and exposure at all other wavelengths was below threshold. At 0, 4, 7, 24, 48 and 72 hr after exposure, rabbits were killed by an overdose of sodium pentobarbital, and tissue samples were prepared as described below.

Histological studies. Histological examination was performed at 24 hr after exposure. The whole excised cornea was fixed in 4% buffered glutaraldehyde. Scanning electron microscopy was performed by standard techniques on the epithelial

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References


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Key words: 5-fluorocytosine, fungal endophthalmitis, penetration, aqueous, vitreous, serum, pharmacokinetics, subconjunctival

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Model. Further in vivo evaluation of this regimen in animals and man seems warranted.

From the Department of Ophthalmology (Dr. Haft, Dr. Friedman) and the Department of Medicine, Division of Infectious Diseases (Dr. Walsh, Dr. Miller), Montefiore Hospital and Medical Center, Albert Einstein College of Medicine, and the Department of Pharmacy (Dr. Loron), Montefiore Hospital, Bronx, N. Y. This work was supported in part (Dr. Walsh) by a U. S. Public Health Service Training Grant T01 AI 0040-05 from the National Institute of Allergy and Infectious Diseases and by the National Eye Institute, grant RO1 EY 00613-05. Submitted for publication Jan. 11, 1978. Present address of Dr. Friedman: Department of Ophthalmology, Mt. Sinai School of Medicine, New York, N. Y. Reprint request: Dr. Michael H. Miller, Department of Medicine, Division of Infectious Diseases, Montefiore Hospital and Medical Center, 111 East 210th St., Bronx, N. Y. 10467.

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The destructive effects of ultraviolet (UV) light on the cornea have long been attributed to damage to nucleic acids and proteins in the epithelium. Whether or not there are primary effects on the processes responsible for energy transformation has not been investigated, although such effects have been found in all other forms of corneal injury which have been studied to date. Therefore, in an attempt to investigate whether there is any alteration of the energy transformation pathway after injury believed to affect primarily structural proteins, some of the metabolites and enzymes responsible for energy transformation in the corneal epithelium have been measured after far-ultraviolet radiation (UV-B). These are correlated with evolution of the clinical changes, as well as with changes in corneal hydration.

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Fig. 1. Germicidal lamp spectral distribution (for 1-watt input to lamp typical of G15T8, G25T8, G30T8, G8T5, and G4T4/1).

surface, with critical-point CO₂ drying and platinum coating. Transmission electron microscopy was performed on Epon-embedded sections of the cornea.

Preparation of tissue samples and biochemical studies. Epithelium was removed by scraping with a No. 15 blade and immediately frozen in liquid nitrogen, followed by lyophilization, weighing, and storage at —70°C until extraction. For adenosine triphosphate (ATP) analysis the corneal epithelium was frozen in situ with a stainless steel conformer cooled in liquid nitrogen, followed by lyophilization of the enucleated globe and scraping of the freeze-dried epithelium. Corneal epithelium and stromal hydrations were determined separately by weighing the tissue before and after lyophilization and are expressed as milligrams of H₂O per milligram dry weight.

Glycogen was measured after hydrolysis by the hexokinase method as described by Reim et al. Values are expressed in micromoles glucose units per gram dry weight tissue.

ATP was measured by the firefly luminescence test after perchloric acid extraction and is expressed as micromoles ATP per gram dry weight.

Epithelial enzyme activities were measured after glass-to-glass homogenization of the lyophilized tissue in phosphate buffer at pH 7.6 on ice, followed by repeated freeze-thawing. Glucose-6-phosphate dehydrogenase (G-6-PDH), 6-phosphogluconic dehydrogenase (6-PGDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), and aldolase (ALD) were measured by enzymatic optical tests according to the method of Bergmeyer. Phosphorylase (P-ase) measurement was by the enzymatic optical method of Jones and Wright. Activities were determined in International Units per gram of protein at 24°C. Protein determination was by the Lowry method. Statistical significance was determined by the Student’s t test.

Results. The radiation exposure invariably produced a clinical photokeratitis, first manifest at about 8 hr after irradiation as mild conjunctival hyperemia and fine granularity of the corneal epithelium. Keratitis was maximal at 24 to 48 hr, with stippling and erosion of the corneal surface but without sloughing of the epithelium. Mild subepithelial haze was discernible at 24 and 48 hr. By 72 hr the surface appeared lustrous, with almost complete disappearance of the subepithelial haze. Scanning and transmission electron microscopy showed that the pathologic changes were limited to the epithelium. Inflammatory cells were rarely seen.

Changes in corneal hydration as a function of time are illustrated in Table I. Progressive increase in hydration parallels the clinical keratitis,
Table I. Corneal hydration following ultraviolet irradiation

<table>
<thead>
<tr>
<th>Time after exposure (hr)</th>
<th>Epithelial hydration (mg H2O/mg dry wt.)</th>
<th>p value</th>
<th>Stromal hydration (mg H2O/mg dry wt.)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (6)</td>
<td>5.06 ± 0.92</td>
<td></td>
<td>3.51 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>4 (4)</td>
<td>5.30 ± 0.29</td>
<td>&gt;0.05</td>
<td>3.64 ± 0.04</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>7 (4)</td>
<td>5.52 ± 1.06</td>
<td>&gt;0.05</td>
<td>3.88 ± 0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>24 (6)</td>
<td>7.22 ± 3.43</td>
<td>&gt;0.05</td>
<td>4.17 ± 0.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>40 (3)</td>
<td>6.85 ± 0.44</td>
<td>&gt;0.05</td>
<td>4.67 ± 1.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>48 (4)</td>
<td>6.37 ± 2.30</td>
<td>&gt;0.05</td>
<td>4.69 ± 0.94</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate the sample size. The values shown are averages ± S.E.M. The p values given compare a value to the comparable control value by the Student t test. Stromal values were significant (p < 0.01). Epithelial hydration differences are not statistically significant (p > 0.05).

Table II. Glycogen content of epithelium after UV irradiation

<table>
<thead>
<tr>
<th>Time after exposure (hr)</th>
<th>Glycogen content (μmol glucose/gm dry wt.)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>260 ± 24 (4)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>222 ± 85 (4)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>24</td>
<td>163 ± 10 (11)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>48</td>
<td>273 ± 33 (4)</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>72</td>
<td>308 ± 17 (4)</td>
<td>&lt;0.025</td>
</tr>
</tbody>
</table>

Values are expressed as ±S.E.M., with the number of determinations in parentheses. The p values given compare the glycogen level to the control glycogen level, by the Student t test.

with maximal hydration of both epithelium and stroma at 24 hr although only the stromal values are statistically significant.

Changes in the epithelial glycogen are illustrated in Table II. Maximum reduction of glycogen occurred at 24 hr, but levels had returned to normal and above by 48 and 72 hr. Again, the maximum change appeared at times of peak photokeratitis. ATP levels were not affected.

The enzymatic activities measured were normal at 4, 7, 17, 24 and 48 hr. LDH activity showed a slight but insignificant decrease at 24 hr and G-6-PDH, 6-PGDH, MDH, ALD, and P-ase were comparable to control values at all times.

**Discussion.** Clinically and ultrastructurally, the damaging effect of UV irradiation in these experiments was confined to the anterior portion of the cornea, primarily to the epithelial layer. There were, however, significant changes in stromal hydration. Minimal changes are found at 4 hr, with a maximum hydration at 24 to 48 hr, commensurate with the maximal clinical effect. Although functional damage to the endothelium has not been ruled out as a cause for this swelling, the normal electron microscopic appearance of that layer, in addition to the anterior stromal location of the edema fluid, make entrance of the fluid from the anterior route most likely. The coincidence of maximal epithelial keratitis with maximal stromal swelling implicates increased leakage through the damaged surface layer as the mechanism responsible for increased stromal hydration. In experimental animals, epithelial damage or removal invariably results in marked stromal swelling, with hydration values even exceeding those reported here. There may have been an increase in epithelial hydration also, but the large variability of the epithelial values, reflecting our attempts to avoid manipulation of the epithelium prior to biochemical determinations, makes this uncertain.

The drop in epithelial glycogen content, although consistent with the fact that even minimal trauma can decrease glycogen stores, is unique in its time course. Glycogen in other instances decreases rapidly, whereas in the UV-damaged epithelium the fall is modest until 24 hr after irradiation, coincident with the clinical appearance of maximal keratitis.

Glycogen depletion can be caused by increased use of glucose and glycogen or by decreased glycogen synthesis. Net glycogen catabolism could occur if energy needs outstripped the available glucose flux. The slow decrease in glycogen over 24 hr may be a direct effect on glycogen synthesis, rather than an increased demand for energy seen after other forms of injury. In direct injury to the epithelium a rapid fall in glycogen is characteristic and parallels an increased epithelial hydration.
with probable increased rates of cellular cation exchange.

The curious phenomenon of the latent period between exposure and clinical manifestation of injury remains to be explained. The mobilization of epithelial cellular glycogen and increase in cellular hydration are also rather slow following UV exposure. This may indicate that the effects of the radiation on the pathways responsible for energy transformation are secondary to cellular structural and enzymatic protein changes. Possibly, as in other cells, damage to structural membranes occurs through peroxide formation, with subsequent lipid peroxidation and cellular permeability changes. The delay in onset could also be explained by an effect of UV on RNA and DNA, with delayed transcription and translation to protein synthesis or frank inactivation by nucleotide dimerization or crosslinkage.

Regardless of the molecular mechanism involved, the effects noted in experimental UV keratitis can be accounted for by changes observed in the epithelial layer. These differences in the time course of the response to UV injury and to other forms of epithelial stress imply that epithelial response to insult may differ depending on the form of stress applied.

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From the Department of Cornea Research, Eye Research Institute of Retina Foundation, and the Cornea Service, Massachusetts Eye and Ear Infirmary, Boston, Mass. This work was supported in part by Biomedical Research Support Grant 8R05527, Eye Research Core Services Support Grant P30 EY-01784, Research Fellowship No. ET-05132 (Dr. Foulks) and Research Grant EY-01830, all from the National Eye Institute, National Institutes of Health; and in part by the Massachusetts Lions Eye Research Fund, Inc. Submitted for publication Nov. 18, 1977. Reprint requests: Gary N. Foulks, M.D., Eye Research Institute of Retina Foundation, 20 Stanford St., Boston, Mass. 02114.

Key words: corneal epithelium, ultraviolet radiation, photokeratitis

REFERENCES


Increased saccadic latencies in amblyopic eyes. KENNETH J. CIUFFREDA,* ROBERT V. KENYON, AND LAWRENCE STARK.

Increased saccadic latencies were measured in the amblyopic eyes of subjects having amblyopia without strabismus, constant strabismus amblyopia, and intermittent strabismus. The subjects tracked a small, bright spot of light moving with random, horizontal step displacements of 0.25 to 8.5 degrees over the central retina. Normal saccadic latencies were generally found during monocular tracking with the nonamblyopic eye as well as during binocular tracking. Studies of eye-hand reaction time in amblyopic eyes have shown delays to occur over the central retina; our new finding establishes this for saccadic initiation. Normal trajectories found for all tracking speeds, normal saccadic latencies measured when the nonamblyopic eye was utilized for tracking, and synchronous movement of the eyes under all test conditions point to a sensory rather than motor basis underlying these delays. Our results are interpreted in terms of a processing delay in the sensory pathways leading from the central region of the amblyopic eye to those centers involved in saccadic initiation.

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