fistulas. Retinoids, on the other hand, differentially modulate fibroblast growth and contractile properties and therefore may aggravate undesirable wound-healing responses.

**Key words:** butyrate, proliferative vitreoretinopathy, retinoic acid, vitamin A, wound healing

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**References**


**Separate Mechanisms for Retinal Damage by Ultraviolet-A and Mid-Visible Light**

Laurence M. Rapp, Barbara L. Tolman, and Hardeep S. Dhindsa

Retinal damage by light has two distinct action spectra, one peaking in the ultraviolet-A (UVA) and the other in the mid-visible wavelengths (green light). Here we show in a single animal species, the Long Evans rat, that UVA and green light can produce histologically dissimilar types of damage. UVA light in particular produces severe retinal damage at low irradiation levels. Furthermore, the mechanism of damage by UVA light is different from that of green light as determined by their relative rhodopsin bleaching efficiencies. These results provide convincing evidence that different chromophores mediate damage by UVA and green light. By producing both classes of damage in a single species, a sound model is provided for further investigation into the different forms of photic retinopathy. Invest Ophthalmol Vis Sci 31:1186–1190, 1990.

Retinal phototoxicity is an incompletely understood phenomenon. One of the barriers to a better understanding of photic retinal damage has been the absence of a consistent action spectrum across the animal species that have been studied. The action spectrum for damage in the predominately rod retinas of rodents has been found to peak in the mid-visible wavelengths (green light). Ironically, green light also represents the wavelengths most efficient in bleaching the visual pigment, rhodopsin, and thus in stimulating vision. 1–3 Studies on the primate retina, in contrast, have shown that the action spectrum for photic damage peaks in the ultraviolet-A (UVA) wavelengths. 4 Few studies have examined the damaging capacity of UVA light in nonprimates.5–7 None of these has assayed rhodopsin bleaching efficacy of UVA light. However, vertebrate rhodopsin does have a small absorption peak in the UVA, in which rhodopsin is bleached with approximately one third the efficiency of its peak in the visible. 8 In order to determine if damage by both green and UVA light is mediated by rhodopsin absorption, we sought to produce these types of damage in a single animal species, the Long Evans rat, and subsequently to characterize the rhodopsin bleaching efficacy of each spectral band.

**Materials and Methods. Animals:** Pregnant Long Evans (pigmented) rats were purchased from Charles...
River Breeding Laboratories (Wilmington, MA). The female offspring of these animals were used for experimentation when they reached 50–70 days of age. All experimental procedures in this study conformed to the ARVO Resolution on the Use of Animals in Research.

Light exposure: Animals were dark-adapted for 12–14 hr and anesthetized by intraperitoneal injection of sodium pentobarbital (Abbott, North Chicago, IL) (50 mg/kg body weight). During light exposure, an isothermal heating pad was used to help maintain body temperature. The animal’s right eye was retracted with tape, and drops containing 1% atropine sulfate (Sigma, St. Louis, MO) for pupillary dilation and 0.5% proparacaine HCl (Akorn, Abita Springs, LA) for local anesthesia were applied to the cornea. A patch of black darkroom cloth was used to block any light from reaching the left (nonexposed) eye.

The light source used was a 1000-W xenon arc with a stabilized voltage supply. Neutral density filters were used to adjust light intensity, and an infrared filter was used to eliminate heat. The filters used for modifying the spectral output of the light were a Schott UG1 colored glass filter (UVA light; peak wavelength = 355 nm, 80-nm bandwidth at 50% peak) and an Oriel 57560 broadband interference filter (green light; peak wavelength = 500 nm, 70 nm bandwidth at 50% peak). The desired combination of filters was placed in a collimated beam originating from the light source. Light was transmitted to the retina through a fiber optic bundle positioned 2 mm from the corneal surface of the animal’s eye. Spectral irradiance of the light at the animal’s cornea was measured at 10-nm intervals in both the UVA and visible portion of the spectrum using a model 585 EE & G Spectroradiometer. These values were summed to obtain the overall irradiance.

Microscopy: Eyecups were fixed in 2.5% glutaraldehyde/1.0% formaldehyde, postfixed in 1.0% osmium tetroxide, and then processed for embedding in an Embed 812-Araldite 502 plastic mixture. Sections inclusive of the vertical meridian passing through the optic nerve were cut at 0.5 μm and stained with 1% toluidine blue. For morphometric assessment of outer nuclear layer (ONL) thickness, measurements were made at 0.25-mm intervals extending 3.0 mm on either side (superior and inferior) of the optic nerve. The measurements from all loci on the exposed (right) eye were averaged, and the change in ONL thickness was expressed as a percent of the nonexposed (left) eye. For electron microscopy, tissue was fixed and embedded as above, and thin sections (800–900 Å) stained with uranyl acetate/lead citrate were viewed on a Joel 100-CX electron microscope.

Rhodopsin: For measurement of the rhodopsin bleaching capacity of UVA and green light in vitro, rhodopsin was extracted from isolated retinas in the dark for 2 hr at room temperature with 1% octyl-β-glucopyranoside (Calbiochem, San Diego, CA). Rhodopsin solution with hydroxylamine (added to remove photoproducts) was then placed in quartz cuvettes and initial optical density (OD) measured spectrophotometrically. The solutions were exposed to either UVA or green light for intervals ranging from 10 sec to 40 min. The intensity of the bleaching lights was equal to that which caused threshold retinal damage (see Results for values). Spectrophotometric measurements were made (in darkness) after each period of light exposure. Complete bleaching of the rhodopsin solutions with high-intensity white light for 10 min was performed to obtain the baseline OD. Assay of control solutions, subjected only to ambient light and room temperature, indicated that no measurable bleaching occurred within 40 min.

The bleaching capacity of UVA and green light at their threshold intensity for damage also was examined in vivo by exposing eyes of anesthetized rats to these lights for 5 min. After exposure, the eyes were removed quickly and placed in ice-cold Tris buffer. The retinas then were isolated and extracted in 600 μl 1% octyl-β-glucopyranoside per retina. Rhodopsin concentration was calculated based on the absorbance difference (before and after complete bleaching) of extracts using a molar extinction coefficient of 42,000.

Results. Initial experimentation with 4-hr exposure to UVA light at the maximal intensity afforded by our light source (2800 μW/cm²) resulted in severe damage to all retinal layers. Figure 1 shows the post-exposure sequence of UVA phototoxicity. Damage at 1 day included edema throughout the retina, and pyknosis of both inner nuclear layer and ONL cells. The retinal pigment epithelium (RPE) was intact but showed melanin aggregation. By 2 days, many photoreceptor nuclei were missing and the ONL contained unusual debris. Outer segments were disorganized, but only to a mild degree. The RPE was severely attenuated. After 4 days, macrophages (or detached RPE cells) had invaded the area previously occupied by photoreceptor cells, and the RPE was missing. The orderly discrete layering of inner retinal cells was disrupted.

In order to quantitate the difference in retinal susceptibility to UVA and green light, photoreceptor cell degeneration was assessed by measuring ONL thickness in the central retina. Rats were exposed to a broad range of factorially related intensities of UVA and green light, beginning with those that produced no damage. Table 1 shows that a constant relation-
ship between exposure intensity and ONL thickness was obtained for both the UVA and green light. A 10% loss of ONL thickness, chosen as the criterion for threshold damage, was caused by 100 μW/cm² of UVA versus 5000 μW/cm² of green light, a 50-fold difference in intensity.

The threshold intensities for damage were used to bleach rhodopsin in vitro and in vivo. Figure 2 shows that in vitro the green light bleached essentially all of the rhodopsin in solution in less than 1 min. In contrast, UVA light bleached only about 50% of the rhodopsin in 40 min. In vivo, 5-min exposures to the green and UVA light resulted in rhodopsin levels (nanomoles/retina) of 0.08 ± 0.06 (5.9% control) and 1.27 ± 0.53 (98.6% control), respectively.

### Table 1. Percent decrease in ONL as a function of light intensity for green and UVA exposure

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Intensity (μW/cm²)</th>
<th>ONL thickness (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green</td>
<td>1000</td>
<td>98.0</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>5000</td>
<td>89.3</td>
</tr>
<tr>
<td></td>
<td>10,000</td>
<td>63.0</td>
</tr>
<tr>
<td>UVA</td>
<td>50</td>
<td>96.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>90.3</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>72.3</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>52.9</td>
</tr>
</tbody>
</table>

Fig. 2. First-order rate plots for the in vitro bleaching of rat rhodopsin by light intensities of 100 μW/cm² UVA (triangles) and 5000 μW/cm² green (squares). A 50% loss of optical density was observed within 12 sec of exposure to green light, whereas by 40 min UVA light still had not caused a 50% loss. This represents a factor of at least 200 to 1 for the rate of UVA to green light bleaching efficacy.
Figure 3 shows electron micrographs of rat retinas 1 day after exposure to 5000 μW/cm² of green light and 500 μW/cm² of UVA. Retinal damage by green light was characterized by severe derangement of the rod inner and outer segments, with relatively minor changes in the nuclei. The RPE appeared normal. In the UVA-exposed retinas, there was minimal disorganization of the outer segments, late stage pyknosis...
of all photoreceptor nuclei, and severe swelling and distortion of rod inner segment and RPE mitochondria.

Discussion. The findings of this study distinguish in the rat two classes of retinal damage caused by UVA and green light. Histologically, the characteristics of damage by UVA light were found to be distinct from those of green light. At early time points postexposure, changes in rod outer segments caused by UVA light were minimal in comparison to the marked damage caused by green light. The distortion of mitochondrial structure and pyknosis of nuclei were much more evident in UVA- than in green-light-exposed retinas. Also, damage to the inner retina was observed only in UVA-exposed eyes. Other investigators have documented these unique features of UVA light damage in species other than rats.1,4,5,9

The sensitivity of the rat retina to UVA light was demonstrated by the finding that threshold damage occurred with light intensities that were 50 times less than with green light. This finding is underscored by the fact that only a small fraction of the corneal level of UVA light reaches the retina, primarily because of lens absorption. Assuming that the rat lens has a spectral transmission similar to that of the human lens, less than 5% of UVA light incident upon the cornea is transmitted to the retina. In contrast, approximately 85% of the corneal irradiance of green light reaches the retina.10

Rhodopsin is believed to be the chromophore mediating visible light damage in the rat. The current data show a marked discrepancy in rhodopsin bleaching capacity for green and UVA light at intensities that have an equal degenerative effect on photoreceptor cells. Since the in vivo rhodopsin bleaching appeared to be minimal for UVA light, this is strong evidence that separate mechanisms are involved in the initiation of green and UVA light damage. Among the various candidates suggested as the chromophore mediating UVA retinal damage are, most notably, melanin, retinaldehyde, and mitochondrial enzymes such as the cytochromes and flavins.1,4,5,9,11

Our findings support the hypothesis that mitochondrial enzymes are the most likely mediators of UVA damage. These enzymes are ubiquitous to all retinal cells and therefore may explain the transretinal damage seen in this study. Moreover, at the ultrastructural level the most prominent finding is the severe swelling of the mitochondria, which is in accord

with the histologic appearance of UVA phototoxicity in primates.9 We currently are initiating research to identify biochemically the mitochondrial enzymes which may mediate UVA damage. We feel that a further understanding of retinal UVA phototoxicity is important, since this phototoxicity has been implicated in the etiology of macular degeneration.11

Key words: retina, phototoxicity, ultraviolet light, rhodopsin, Long Evans rats

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