Retinal damage from long-term exposure to laser radiation

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The maculae of rhesus monkeys were exposed to an argon-ion laser operated in the TEM₀₀continuous wave mode at a wavelength of 514.5 nm. Both ophthalmoscopic and histopathologic evaluations of exposure sites were obtained. Threshold (ED₀) values were obtained for 0.5, 5, 30, 120, and 1,000 sec. exposure times. Presence of minimum visible lesions was assessed ophthalmoscopically at both 1 hour and 24 hours after exposure. With increasing exposure times, a 24 hr. lesion-appearance criterion resulted in ED₀ values too low to be consistent with a thermal damage mechanism. In contrast, exposure to neodymium laser radiation at a 1,060 nm. wavelength for 120 sec. produced only ED₀ values consistent with those associated with thermal injury. These results suggest that the damage mechanisms for long-duration exposures to visible light may involve photochemical processes initiated by the interaction of visible light with the retinal photopigments.

Key words: argon laser, neodymium laser, retinal lesions, long-term continuous exposure, retinal damage threshold, pigment epithelium, photoreceptor outer segments, rhesus monkeys.

Retinal damage from long-term exposure to visible radiation has been evaluated in many investigations. Such studies have used widely varying light sources, including diffuse lighting surrounding caged rats, direct illumination of the retina with the light of an indirect ophthalmoscope, and retinal exposure to an argon laser. In these studies, possible mechanisms of injury have been examined, and the extent and type of retinal damage following exposure have been evaluated histologically. Experiments evaluating ocular damage from long-term exposure show retinal damage at exposure levels too low to be explained in terms of a thermal damage mechanism and indicate that a spectral dependence is associated with the lesion production. Evidence also suggested that the concentration of retinol (vitamin A) in the affected area is a factor in the injury process.

The study reported here was designed with two objectives. The first was to extend the ocular threshold-damage curve for exposure to visible radiation to 1,000 sec. to determine if the damage-producing power levels were too low to be consistent with a purely thermal damage mechanism.

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1970 and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources-National Research Council.
To this end, threshold values for retinal lesions were determined for 0.5, 5, 30, 120, and 1,000 sec. exposures.

The second objective was to emphasize the thermal mechanism of damage by making 120 sec. exposures with a neodymium laser at 1,060 nm. wavelength. This wavelength, when absorbed at the retina, can produce a temperature rise but is inefficient in bleaching photopigments; thus lesions produced by these exposures should stem primarily from thermal changes. The results of these exposures were compared to those obtained from exposures to a 514.5 nm. wavelength, which is readily absorbed by the photopigments.

Histological evaluation of the retinas exposed to 120 sec. argon laser radiation (514.5 nm.) was obtained so that the damaged retinal structures could be studied.

**Apparatus**

Two lasers were used in these experiments. In one experiment a control laser, Model 900, argon-ion laser was used to make the 0.5, 5, 30, 120, and 1,000 sec. continuous wave (CW) exposures at a 514.5 nm. wavelength. A Model 1000 Nd-YAG laser (Chromatix, Mountain View, Calif.), also operating in a CW mode, was used for the 120 sec. 1,060 nm. exposures.

The beam from the argon laser had a TEM₀₀₀ distribution, the beam diameter measured at the corneal plane was approximately 2.6 mm. at the 1/e² points, and the full-angle divergence was determined to be 0.3 mrad.

A schematic diagram of the exposure apparatus used for the argon exposures is shown in Fig. 1. Exposures were controlled with an electronic shutter by either of two methods. For 0.5 and 5 sec. exposures, a Gerbrands digital millisecond timer was used; for the longer exposures, a Beckman preset counter.

The desired power level for each exposure was controlled with an inconel-coated, neutral-density filter wheel. To determine the power level for each of the 30, 120, and 1,000 sec. exposures, approximately 10 percent of the beam was reflected from a pellicle beam splitter to an SGD-444 photodiode; and the signal from the photodiode was input to a Model 3439A digital voltmeter (Hewlett-Packard Co., Palo Alto, Calif.). For the 0.5 and 5 sec. exposures, the signal from the photodiode was input into an Model 580 radiometer (EG&G, Inc., Salem, Mass.). The monitoring instrument used for a particular exposure condition was cross-calibrated daily with a thermopile positioned (Eppley Laboratory, Inc., Newport, R. I.) at the corneal plane. The thermopile output voltage was measured with a Model 150A microvolt ammeter (Keithley Instruments, Inc., Cleveland, Ohio).

The subject (rhesus monkey) was placed in a mount that permitted adjustment in 6 degrees of motion. A Zeiss fundus camera was used to view the ocular fundus of the subject.

The rectal temperature of the monkey was monitored with a Model 73A telemetherometer, (Yellow Springs Instrument Co., Yellow Springs, Ohio).

The apparatus used for the neodymium exposures is similar to that described for the 120
sec. argon exposures, with only slight modification. A lens was introduced to compensate for the chromatic aberration of the eye at the 1,060 nm wavelength. Also a Scientech, Inc. (Boulder, Colo.) powermeter was substituted for the Eppley thermopile as the primary power detector.

Analysis of the neodymium laser beam indicated a TEM\(_{00}\) distribution with a 2 mrad, full-angle divergence and a 3 mm. beam diameter (1/e\(^2\) points) at the corneal plane.

**Procedure**

Pre-exposure preparation of the subjects included a tranquilizing injection of ketamine HCl (10 mg./kg. of body weight). Anesthesia was induced with sodium pentobarbital (25 mg./kg. of body weight) via an intravenous catheter inserted in the saphenous vein. Dilation of the pupils and cycloplegia was obtained with tropicamide (1 percent) and atropine. Eye movements were restricted with retrobulbar injection of lidocaine. A lid retractor was used to keep the lids open during alignment and exposure. Animals with refractive error greater than 0.50 D. in any meridian were not used in these studies.

To maintain corneal clarity, the cornea was moistened with saline solution during exposure. A gauze sponge was attached to the lid retractor to absorb excess saline solution so that pooling along the lower lid would not affect focusing of the beam.

All exposures were made in the macula, and to assist in locating the exposure sites precisely for both visual assessment and histological evaluation, suprathreshold marker lesions were placed along the bottom and temporal edge of the macula. These lesions served as fiducial marks to align the vertical and horizontal crosshairs of the fundus camera for exposure placement. In addition, the continued alignment of the crosshairs with the marker lesions after exposure served as a check on eye movement. Except for the 120 sec. exposure, 16 exposures of varying power were made in each macula. Only twelve 120 sec. exposures were made because of the increased exposure time. All exposures were made at a body temperature of 99° ± 2° F. (55° ± 1.1° C.).

The laser beam was aligned coaxially with the crosshairs of the fundus camera, and each exposure site was selected by placing these crosshairs at the desired retinal location. To change the location of the exposure site, the animal was moved relative to the laser beam, with care taken to ensure that the beam always passed through the central area of the cornea. The divergence of the laser beam was not altered for any exposure; thus all lesions were produced by minimal image exposures.

The experimental procedure was changed slight-

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**Table I. ED\(_{50}\) values for all exposure conditions**

<table>
<thead>
<tr>
<th>Exposure time (sec.)</th>
<th>Number of eyes</th>
<th>ED(_{50}) power (mW.) at cornea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 hr.</td>
<td>24 hr.</td>
</tr>
<tr>
<td><strong>Argon</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>16</td>
<td>9.4</td>
</tr>
<tr>
<td>5.0</td>
<td>20</td>
<td>6.4</td>
</tr>
<tr>
<td>30.0</td>
<td>7</td>
<td>5.4</td>
</tr>
<tr>
<td>120.0</td>
<td>21</td>
<td>4.4</td>
</tr>
<tr>
<td>1200.0</td>
<td>10</td>
<td>1.8</td>
</tr>
<tr>
<td>1800.0</td>
<td>10</td>
<td>0.12</td>
</tr>
<tr>
<td><strong>Neodymium</strong></td>
<td>120</td>
<td>27.6</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>26.1</td>
</tr>
</tbody>
</table>
Fig. 2. Vertical section through center of lesion, 3 hr. after exposure. The vacuolated area at the pigment epithelium-outer segment interface is approximately 0.012 by 0.016 mm. (hematoxylin and paragon; ×735.)

sions produced by very low power levels, a study was done to assess both lesion development and retinal structures affected. This was accomplished by making 120 sec. exposures at a power level of 0.7 mW. at 514.5 nm. in the same macula at intervals over a 24 hr. period and sacrificing the animal at the end of the experiment.

Results

All the data, except those obtained from the 1,000 sec. exposures, were analyzed by determining a damage threshold (ED_{50}) for each individual eye with the use of the probit technique. For the 1,000 sec. exposures, an up-and-down method was used. The mean of the individual eye thresholds was then calculated to determine an ED_{50} for the exposure duration under study. The ED_{50} is the dose for a 50 percent probability of a retinal exposure producing a lesion visible in a specific time.

The threshold data for all exposure conditions are summarized and listed in Table I. The 1 hr. and 24 hr. ED_{50} values obtained for argon exposures were evaluated statistically and found to be significantly different for the 5, 30, 120, and 1,000 sec. exposures. No significant changes were observed at 24 hours for the 0.5 sec. argon or the 120 sec. neodymium exposures.

The ophthalmoscopic appearances of near-threshold lesions for all exposure conditions were similar. They were small, whitish-grey, discrete lesions which could be located quite readily at specific locations within the grid pattern established by the marker lesions.

Histopathological inspection of the exposure sites showed that the lesions produced by the 0.7 mW., 120 sec. exposures at 514.5 nm. exhibit several stages of development. At 3 hours after exposure (Fig. 2) there is a definite vacuole in the outer segment layer at the interspace of the pigment epithelial layer. Unfortunately, it is difficult to completely assess the condition of the underlying pigment epithelial cells because of artefacts within this layer. In addition to the vacuolization, there are distortion and loss of morphology within the outer segment layer. There appears to be a sharp border between affected and unaffected cells.

Fig. 3 depicts lesion development at 17 hours after exposure. The lesion is char-
acterized by a swollen epithelial cell and loss of pigment granules in the apices of that cell and some of its neighbors.

The final stage of our evaluation occurred at 24 hours after exposure and is shown in Fig. 4. The center of the exposure site shows a pigment epithelial cell separated from the pigment epithelial layer. The nucleus of the cell is shrunken and appears degenerated, and again there is vacuolization and numerous pigment granules within the cell. The neighboring pig-
Discussion

To assist in analyzing threshold data, a damage curve for short exposures to visible radiation was extended (Fig. 5, solid line) to include long exposures. The ED<sub>50</sub> for 514.5 nm radiation obtained in this study with the use of a 1 hr. lesion-appearance criterion agrees reasonably well with this curve. Values obtained at the 0.5 and 5 sec. exposures are consistent with other threshold values obtained at or near these exposure times. Lappin<sup>10</sup> found 9 and 5.5 mW thresholds for 0.5 and 7 sec. exposures to a HeNe laser; Hemstreet,<sup>18</sup> 6.89 mW threshold for a 0.5 sec. exposure to an argon laser; and Davis and Mautner,<sup>15</sup> 8 mW threshold for 10 sec. exposures to a HeNe laser. All used rhesus monkeys as subjects and a 1 hr. lesion-appearance criterion.

However, when the data obtained from a 24 hr. lesion-appearance criterion are included in the threshold damage curve (Fig. 5, dashed line), the difference in observed ED<sub>50</sub> increases markedly with exposure time.

A separate study was performed to assess the lesion development following the 120 sec. argon exposures. Two eyes were examined with the fundus camera every 3 hr. during a 24 hr. period following exposures to a range of powers. Lesions gradually became visible over an 18 hr. period, with the order of appearance directly related to the exposure level. For the remaining 6 hr. of observation, the lesions seen did not increase in number, but they became more distinct and better defined.

The argon ED<sub>50</sub> data obtained for long exposures with a 1 hr. lesion-appearance criterion appear to support a thermal damage mechanism. However, the change in the slope that occurs with a 24 hr. criterion suggests the presence of another damage mechanism, and it has been hypothesized that the damage mechanism producing lesions is associated with extreme photopigment bleaching.<sup>11</sup>

The lesions illustrated in Figs. 3 to 5 were produced by 0.7 mW<sub>o</sub>, a power level that deviates markedly from a thermal damage prediction. They do not develop acutely but rather appear over a 24 hr. period, with the initial stage of development involving changes in the outer segments. Although the artefacts within the
pigment epithelium in Fig. 2 make it difficult to assess immediate effects in the pigment epithelium, evaluation of other exposure sites at later stages of development show that the pigment epithelial cells undergo a slow process of degeneration subsequent to outer segment involvement.

If photopigment bleaching by the incident radiation is a significant factor in producing the retinal lesions after relatively long exposures, it seemed reasonable that exposure to 1,060 nm. radiation, which reacts minimally with photopigments, would be inefficient in producing the same type of damage. To test this, exposures were made for 120 sec. to a 1,060 nm. wavelength. The 1 hr. ED$_{50}$ of 27.6 mW.
obtained for these exposures agrees well with an extrapolation of ED_{50} values for shorter exposures of the same wavelength (Fig. 6). The data used to construct the curve shown in this figure were obtained by different investigators for macula exposure to neodymium laser radiation, using minimum spot sizes and a 1 hr. lesion-appearance criterion. Evaluation of the exposure sites at 24 hr. after exposure showed no increase in the number of lesions.

Damage from short exposures to neodymium radiation has been interpreted as the result of increased temperature at the exposed site. The results of this study appear to be consistent with a thermal injury mechanism for 120 sec. exposures to neodymium radiation—as anticipated. In contrast, 120 sec. exposures to argon radiation produced lesions at power levels which departed significantly from that associated with thermal injury. Fig. 7 compares the macula lesion threshold curve obtained for neodymium exposures with that obtained for the argon wavelength.

A theoretical examination of the effect of temperature in producing damage in long-term exposures was made by calculating the temperature history in the center of the exposure site at the surface of the pigment epithelium for an ED_{50} exposure. These temperature values were obtained with a thermal model presently in use at the USAF School of Aerospace Medicine. Fig. 8 indicates that the temperature histories predicted from the 1 hr. criterion for the 120 sec. ED_{50} are similar for argon and neodymium. However, a significantly lower temperature was calculated from the 24 hr. criterion for the 120 sec. ED_{50} for argon. A temperature history for a 120 sec. exposure to neodymium, similar to that shown for the 0.54 mW. argon ED_{50} exposure, would require an exposure power level of approximately 3.6 mW. No neo-
dymium exposures were made at this power level; but neodymium exposures were made at 5 mW., and they produced no visible lesions—even after 24 hours. The relatively small temperature rise calculated from the 24 hr. criterion for the argon 120 sec. ED₅₀ and the fact that lesions were not observed following infrared (1,060 nm.) exposures at power levels that theoretically should produce approximately the same temperature increase tend to support the conclusion that small temperature rises for extended periods are not alone responsible for damage.

The results of this study support the view that the lower tolerances for long-duration exposures to visible light derive from photochemical processes, perhaps thermally moderated, initiated by the interaction of visible light with the photopigments.

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REFERENCES