Additivity and repair of actinic retinal lesions. GARY A. GRIESS AND MICHAEL F. BLANKENSTEIN.

Extensive exposures to light of intensities insufficient to produce thermal damage can still result in retinal damage via nonthermal mechanisms. In this work, the additivity and the repair rate for this actinic damage were measured. Rhesus monkey retinas were exposed to 458 nm light from an argon-ion laser at a dose equivalent to half the threshold retinal irradiance. After prescribed time intervals, the retinal sites were re-exposed to determine the split-dose threshold. This threshold is related to the single-dose threshold through the additivity, which in turn is dependent on the time between exposures. The observed recovery of tissue could be fitted by a single exponential with a time constant of 4 days. This result is incorporated in an analytic expression for the cumulative effect of repeated doses.

Light can be toxic to the retina in at least two ways. Thermal damage is well documented and appears to be the primary damage mechanism for exposure durations between 10^-9 and 10 sec. For longer exposures, an apparent nonthermal mechanism exhibits characteristics quite different from that causing thermal damage. This mechanism is most effective at shorter wavelengths and has associated with it negligible temperature rise. It has been designated variously as nonthermal, photomechanical, or actinic damage. This report is concerned with the quantitative characterization of additivity and repair of actinic damage in primate retinas with funduscopically visible lesions used as the endpoint.

The literature concerning the cumulative nature of actinic damage is limited. Ham et al. reported that two 1000 sec exposures at 441.5 nm spaced 49 hr apart produced a lesion at one-half the 3000 sec threshold power. But they also said that four or more exposures at one-fourth threshold spaced 48 hr apart did not produce a lesion. Lawwill et al., using electrophysiological and histological endpoints, reported that 1 hr exposures at 514.5 nm for 4 consecutive days produced the same damage as a single 4 hr exposure. Sperling et al. compared lesions from a single 120 min exposure with those of daily intermittent exposures and found histologically different patterns. Recovery from superthreshold blue-light lesions has been histologically traced by Tso et al. and Ham et al. over a span of months, but repair of subthreshold damage has not been looked at.

Fig. 1 illustrates the concepts utilized in the split-dose technique. For an exposure of duration, t, there will be a threshold retinal irradiance, E0, which is the dosage at which there is 50% probability of damage (ED50). Actinic damage exhibits reciprocity. That is, for an exposure duration of 2t, the ED50 will be 0.5 E0. This may be considered the limiting case where two pulses of duration, t, are contiguous. If the two pulses are separated by an interval At, the magnitude of the second dose required to produce a lesion, E2, will depend on the additive contribution of the first dose. If the first dose is set at E0/2, the value of E2 can range from E0/2 for complete additivity to E0 for no additivity, as follows:

\[
E_2 = E_0 - AE_0/2
\]

where A is the additivity: 0 \leq A \leq 1. Solving for A gives the following:

\[
A = 2(1 - \frac{E_2}{E_0})
\]

The additivity will be some function of the time between exposures, At, depending on the repair of the tissue after the initial insult.

Methods and materials. A Spectra-Physics Model 171 argon-ion laser tuned to the 458 nm emission was used. A combination of a 2 mm aperture and a +8.0 diopter lens served to produce uniform 200 \(\mu\)m retinal images. The beam profile and divergence were determined by beam scans at the corneal plane and several other positions. The power at the position of the eye was measured with a Scientech 362 laser powermeter. The exposure intensity was varied through the use of calibrated neutral density filters. The experimental layout is shown in Fig. 2.

Experimental subjects were adult rhesus monkeys (Macaca mulatta) ranging in body weight from 3 to 6 kg. 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.
Fig. 1. Graphic representation of terms describing the split-dose technique. $E_0$, threshold irradiance for duration $t$; $E_0/2$, threshold for duration $2t$ (reciprocity); $E_2$, threshold irradiance of second dose after pre-exposure at 0.5 $E_0$.

Fig. 2. Experimental layout for retinal exposures. A, Argon-ion laser; B, electromechanical shutter; C, mirror; D, neutral-density filter; E, 2 mm aperture; F, +8.0 D lens; G, swingout mirror; H, fundus camera; I, subject eye or powermeter.

Prior to use, anesthesia was induced by intramuscular injection of a mixture of 2.2 mg/kg xylazine and 12.8 mg/kg ketamine hydrochloride. This anesthetic is preferred over sodium pentobarbital because it does not require intravenous administration and animals detoxify more rapidly. These considerations were important because of the necessity to expose and examine each animal on several successive days.

When exposures were made, eye movement was inhibited by retrobulbar injections of lidocaine. Eyes were held open with a stainless steel speculum and frequently irrigated with normal saline to prevent corneal drying. Extramacular sites...
Fig. 3. Log additivity vs. interval between doses. Linearity indicates that $A = e^{-\Delta t/T}$, where $1/T$ is the repair rate constant.

### Table I. Split-dose threshold, $E_2$, at 458 nm

<table>
<thead>
<tr>
<th>$\Delta t$ (hr)</th>
<th>$E_2^a$</th>
<th>UCL$^a$</th>
<th>LCL$^a$</th>
<th>$A^b$</th>
<th>$\ln A$</th>
<th>No. of sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.257</td>
<td>0.312</td>
<td>0.204</td>
<td>0.987</td>
<td>-0.013</td>
<td>64</td>
</tr>
<tr>
<td>24</td>
<td>0.277</td>
<td>0.324</td>
<td>0.233</td>
<td>0.907</td>
<td>-0.097</td>
<td>80</td>
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<tr>
<td>48</td>
<td>0.362</td>
<td>0.414</td>
<td>0.321</td>
<td>0.572</td>
<td>-0.559</td>
<td>65</td>
</tr>
<tr>
<td>72</td>
<td>0.507</td>
<td>0.507</td>
<td>0.318</td>
<td>0.442</td>
<td>-0.817</td>
<td>36</td>
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<tr>
<td>96</td>
<td>0.411</td>
<td>0.499</td>
<td>0.343</td>
<td>0.379</td>
<td>-0.970</td>
<td>47</td>
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<tr>
<td>144</td>
<td>0.449</td>
<td>0.541</td>
<td>0.370</td>
<td>0.229</td>
<td>-1.475</td>
<td>70</td>
</tr>
<tr>
<td>$\infty$</td>
<td>0.507$^c$</td>
<td>0.560</td>
<td>0.433</td>
<td>0</td>
<td>$-\infty$</td>
<td>65</td>
</tr>
</tbody>
</table>

UCL = upper confidence limit; LCL = lower confidence limit; $\ln A = \log_e A$.

$^a$W/cm$^2$ at retina, calculated for 200 $\mu$m image and ocular transmission 0.69.

$^b$Additivity, $A = 2(1 - E_2/E_0)$.

$^c$ED$_{50}$ for a single 100 sec exposure ($E_0$).

Initially, a threshold was determined for 100 sec exposures to determine the value of $E_0$ to be used. Then, in the split-dose experiments, each site was exposed for 100 sec at $E_0/2$, and the time of each exposure was noted. At a prescribed interval ($\Delta t$) after the first dose, a second 100 sec dose was applied to the same target site. The second doses were graduated in order to determine the split-dose ED$_{50}$ ($E_2$).

were chosen with venation used for landmarks. From 15 to 20 sites were exposed per eye. Eyes were examined for funduscopically visible lesions by two independent observers at 24 and 48 hr after the final exposures. The ED$_{50}$ threshold values and 95% confidence limits were determined by probit analysis. Dosages were calculated in terms of retinal irradiance, on the basis of a 200 $\mu$m spot size and ocular transmission of 69% at 458 nm.  

$^{1}$
Results. The single-dose thresholds for 100 and 200 sec exposures were 0.507 and 0.257 V/cm², respectively, so that reciprocity was clearly evident. The single-dose threshold compared favorably with the value of 0.52 W/cm² given by Ham et al.¹ for 100 sec exposures at 458 nm with a 500 μm retinal spot size. Thresholds were well below the dosages expected for thermal damage, since the calculated temperature rise was less than 1°C.

The nonthermal lesions appeared funduscopically similar to thermal lesions but had a slower rate of development. Generally, the lesions were not observed on the day of exposure, but by 24 hr after exposure they were funduscopically visible. Beyond 24 hr after exposure, there was little significant change in lesion appearance. At 11 weeks after exposure, the lesions had faded but were still discernible. In the split-dose experiments, none of the conditioning exposures at E₀/2 produced visible lesions by the time of the second exposure. After the second exposure, the temporal development of lesions was the same as that after single doses.

Table I presents the experimental thresholds (ED₅₀) along with 95% fiducial limits (upper confidence limit [UCL], lower confidence limit [LCL]) as determined by probit analysis. A and ln A determined from equation 2 are also given. The dependence in ln A (2[1 - E/E₀]) on Δt is shown by Fig. 3, where the linear fit is good (correlation coefficient 0.989). The exponential time constant, τ, may be determined from either the slope or the Δt intercept at ln A = -1. This value of τ turned out to be almost exactly 4 days.

Discussion. This work has shown that repeated subthreshold exposures to blue light produce cumulative retinal changes which are countered by an exponential repair process: A = exp (−Δt/τ). The 4-day time constant helps explain the split-dose findings of Ham et al.¹ and Lawwill et al.³ This empirical repair rate is slower by a factor of 2 than that observed for the corneal epithelium after UV insult.⁷

The damage and repair mechanisms are unidentified, but the dynamics can be described empirically by a rate equation:

\[ \frac{dT}{dt} = hI - kT \] (3)

where T represents the level of toxic products or extent of damage produced by the radiant intensity, I. The scalars h and k are spectral sensitivity and repair rate, respectively (k = 1/τ = 2.9 × 10⁻⁶ sec⁻¹).

The threshold for intermittent exposures is of practical concern for laser safety. This is most readily determined if it is assumed that each exposure is identical in intensity, I₀ and duration, t₀. If the time between exposures, Δt, is taken as being large compared to t₀, then equation 3 simplifies so that after the Nth exposure, T will be as follows:

\[ T = hI₀t₀(1 + e^{-kΔt} + e^{-2kΔt} + \ldots + e^{-NkΔt}) \] (4)

This may be written in closed form as follows:

\[ T = hI₀t₀\left(\frac{1 - e^{-NkΔt}}{1 - e^{-kΔt}}\right) \] (5)

At threshold, the toxin level is taken to be a critical value, Tc. Then, for intermittent exposures, the threshold value of each dose is as follows:

\[ I₀t₀ = \frac{T_c}{h}\left(\frac{1 - e^{-kΔt}}{1 - e^{-NkΔt}}\right) \] (6)

Therefore, for repetitive pulses, the single-dose threshold value, I₀/t₀, is reduced by the factor (1 - e⁻ᵏΔᵗ)/(1 - e⁻ᴺᵏΔᵗ) when exposures are repeated. This would account for both addition and repair of damage while maintaining a realistic margin of safety.

This work was conducted at the Laser Effects Branch of the USAF School of Aerospace Medicine, Brooks AFB, Texas, under Contract F33615-77-C-0615. Submitted for publication Dec. 22, 1980. Reprint requests: Gary A. Griess, Ph.D., Technology Incorporated, Life Science Division, P.O. Box 32644, San Antonio, Texas 78216.

Key words: retina, rhesus, eye, laser, photochemical, repair, split-dose

REFERENCES

Sixteen patients with osteogenesis imperfecta (OI) have undergone a thorough eye examination. These patients had statistically significantly lower ocular rigidity measurements than a group of normal volunteers matched on age, sex, and refractive error. In addition, the corneal diameter and length of the eyeball was smaller in OI patients than that in controls. Possible correlations of low ocular rigidity with biochemical changes in scleral collagen await further investigation.

Osteogenesis imperfecta (OI) is an inherited disease of bone affecting between 10,000 and 20,000 persons in the United States. OI is usually inherited as an autosomal dominant, but cases of sporadic or recessive inheritance have been reported. Given the variability in clinical and genetic expression, the term OI probably represents several clinically similar but genetically distinct disorders. Although blue sclera, along with fractures and deafness, have been identified as cardinal clinical features of OI, there has been poor correlation of the presence or absence of blue sclera with the other features of the disease. 2 This may be because of subjectivity in estimating the presence or absence of blue sclera as well as grading the color. A more quantitative assessment of the properties of sclera could be of value in determining the functional characteristics of this tissue in OI patients. The purpose of this presentation is to report the findings of a complete ocular evaluation of sixteen patients with OI as compared with age-, sex-, and refractive error–matched controls.

Materials and methods. Sixteen patients with OI ranging in age from 9 to 56 years underwent a complete ocular examination, including measurement of corneal diameter, corneal thickness centrally, anterior chamber depth, horizontal and vertical keratometry, pupil size, palpebral fissure size, length of globe, ocular rigidity, refractive error, and scleral color. Table I presents the techniques used for each of these measurements. These patients represented the broad clinical and genetic spectrum of OI. Each had a lifelong history of multiple fractures, compatible radiologic findings, and no chemical abnormality indicative of another cause for the brittle bone disease. Skeletal deformities, scoliosis, and hemias were associated findings. A control group consisted of 16 normal volunteers matched on age, sex, and refractive error to the patients with OI. All ages matched within 2 years. This control group also had the complete eye examination. For each variable, first the right eye then the left eye of each person was measured. Statistical analysis involved the paired t test and calculation of the intraclass correlation coefficient. 3

Results. Because each patient was matched to a control, the paired t test provided a sensitive procedure for identifying differences between the patient group and the control group. For each variable, a value for each person was determined by averaging the two eyes. The appropriateness of this averaging was investigated by the intraclass correlation coefficient, a measure of relatedness of the two eyes, which accounts for the variation in measurements between persons. An intraclass correlation coefficient close to 1 indicated similar measurements between eyes.

Table I

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Technique</th>
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<tbody>
<tr>
<td>Corneal diameter</td>
<td>Millimeter ruler</td>
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<tr>
<td>Central corneal thickness</td>
<td>Millimeter ruler at ambient light</td>
</tr>
<tr>
<td>Anterior chamber depth</td>
<td>Hagg-Streit pachymeter</td>
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<td>Horizontal and vertical</td>
<td>American Optical keratometry</td>
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<tr>
<td>keratometry</td>
<td></td>
</tr>
<tr>
<td>Pupil size</td>
<td>Millimeter ruler in primary position</td>
</tr>
<tr>
<td>Palpebral fissure size</td>
<td>Millimeter ruler at ambient light</td>
</tr>
<tr>
<td>Length of globe</td>
<td>A-scan ultrasonography</td>
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<td>Ocular rigidity</td>
<td>Indentation technique</td>
</tr>
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<td>REFRACTIVE ERROR</td>
<td>Retinoscopy and manifest refraction</td>
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<td>SCLEERAL COLOR</td>
<td>Masked grading of external photographs</td>
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