Visible Retinal Lesions From Ultrashort Laser Pulses in the Primate Eye

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Purpose. To evaluate the effects of ultrashort laser pulses of visible wavelengths on the retinas of rhesus monkey eyes and to perform threshold measurements for minimum visible lesions (MVLs) at pulsewidths from nanoseconds to femtoseconds.

Methods. Single laser pulses at visible wavelengths were placed within the macular area of live rhesus monkey eyes at varying pulse energies at five pulsewidths (4 ns, 60 ps, 3 ps, 600 fs, and 90 fs). The number of visible lesions was determined after 1 hour and 24 hours post-exposure, and a probit analysis was performed for the dosage, causing 50% probability for damage (ED50) as well as the 95% fiducial intervals for ED50. Fluorescein angiography (FA) was performed, and hemorrhagic lesions were recorded as they became visible.

Results. The ED50 threshold doses at the 1-hour reading, calculated from the measured data, decreased from 1.5 μJ at 4 ns to 0.60 μJ at 600 fs, but it increased to 1.18 μJ at 90 fs. At the 24-hour reading, the ED50 calculated doses decreased from 0.90 μJ at 4 ns down to 0.26 μJ at 600 fs, but it increased to 0.43 μJ at 90 fs. Fluorescein angiography visible lesion ED50 values were all higher than MVL values, showing that FA was not as sensitive in determining damage levels.

Conclusions. Laser pulses for pulsewidths between 4 ns and 90 fs are capable of producing visible lesions in monkey eyes with energies less than 1 μJ. Fluorescein angiography is not as sensitive in determining threshold levels as visually observing the retina through a fundus camera. Invest Ophthalmol Vis Sci. 1995;36:879-888.

Our goal in this study was to evaluate retinal damage thresholds for single pulsewidths in the rhesus monkey fundus. Further, our goal was to acquire urgently needed data to assess potential human retinal hazards that could be applied to new national laser safety standards for subnanosecond laser systems operating in the visible and near-infrared spectral regions. We have determined the threshold dosages for visible lesions for pulsewidths of 90 and 600 femtoseconds (fs) and 3 picoseconds (ps) at 580 nanometers (nm) and for pulsewidths of 60 ps and 4 nanoseconds (ns) at 532 nm. These subnanosecond laser ocular tissue interaction studies are critical in identifying hazards to the human eye and in considering future clinical applications in laser surgery. Such studies also provide new insight into the biologic information of intense electromagnetic fields.

The National Laser Safety Standard, ANSI Z136.1-1993,1 defines a maximum permissible exposure for the retina from visible and near infrared laser radiation. This standard applies to pulse durations down to 1 ns; it is based on retinal injury studies conducted on primate eyes for continuous wave and pulsed laser systems with pulsewidths greater than 2 ns. The few retinal studies that have been reported for pulsewidths less than 1 ns in the rhesus monkey eyes2-6 were almost all for picosecond pulsewidths. Two studies have been reported for femtosecond pulses in rabbits. In one study, Birngruber et al7 measured the 50% probability for damage (ED50) visible threshold dosage in chin-
chilla gray rabbit eyes for 80-fs pulsewidths at 625 nm. In the other study, Toth et al. reported damage thresholds for 90-fs pulsewidths at 580 nm in the Dutch-belted rabbit.

This study uses rhesus monkey eyes to determine the ED$_{50}$ threshold doses necessary to create visible lesions in the macular region for various pulsewidths from 4 ns down to 90 fs. In our work, we define a “visible lesion” as a visible change in the fundus readily seen by at least two observers. Both observers must agree that the change in the fundus is actually a lesion before that exposure site is counted as a positive event. A minimum visible lesion (MVL), then, is defined as a change in the fundus due to laser insult just minimally visible by the two observers, either ophthalmoscopically or from photographs of fluorescein angiography (FA).

In an earlier study, we determined the ED$_{50}$ MVL dosages for dutch-belted rabbit eyes$^9$-10 from 5 ns down to 90 fs; herein, we present our findings for the rhesus monkey eyes.$^9$ For the present primate study, we found a notably lower threshold for MVLs compared with our rabbit studies. These results are important in providing eye safety information because laser systems capable of producing subnanosecond pulses are in widespread use throughout the research, medical, and military communities. The low energy required for intraretinal hemorrhages was identified, although subretinal hemorrhages were similarly difficult to create (or more so than in the rabbit). These studies demonstrate that the rabbit model cannot be extrapolated to predict human ocular injury.

**METHODS**

**Experimental Systems**

The ultrashort pulse laser system shown in Figure 1 produces a range of pulsewidths, wavelengths, and energy levels with relative ease of reconfiguration. It produces single pulses with an adjustable pulse repetition rate between single pulses and 10 pulses per second (pps). All pulses generated can have energies greater than 100 microjoules ($\mu$J). This system consists of a dye laser pumped by a mode-locked (82 MHz) pulse-compressed, frequency-doubled Neodymium:yttrium-aluminum-garnet (Nd:YAG) laser. The dye laser output is amplified by a three-stage pulse dye amplifier, which is pumped by a seeded, frequency-doubled Nd:YAG regenerative amplifier. Pulsewidths are measured by an INRAD Slow Scan Autocorrelator. The pulses from the pulse dye amplifier can also be compressed to achieve below 100-fs pulses by chirping the pulses before amplifying and rephasing the spectrally broadened pulse in time, thereby giving rise to the compressed pulse afterward. The pulse dye amplifier pulses range between 3 ps and 90 fs at 580 nm. Pulses of 60 ps and 4 ns at 532 nm are generated by the seeded Nd:YAG regenerative amplifier or the Nd:YAG Q-switched laser.

The incident laser beam was apertured to provide a uniform spatial profile with a beam diameter of 2.5 mm for delivery to the corneal surface. Single pulses were delivered to monkey eyes by deflecting the beam off a glass beamsplitter (Fig. 1) mounted on a Zeiss (Oberkochen, Germany) fundus camera; the beam-splitter path was adjusted such that the deflected beam was collinear with the optical axis of the fundus camera. These 580-nm beamsplitters have antireflective coatings that minimize second-surface reflections and prevent double-pulse generation. For suprathreshold experiments at 90 fs, the incident beam aperture was opened to 5 mm and the glass beamsplitter was replaced by a front surface mirror. The laser beam path length from aperture to incident corneal surface was 1 m. The beam divergence was ~0.5 milliradian. The unamplified 580 nm mode-locked beam at 82 MHz from the pulse dye amplifier was used to align retinal exposure sites. The output of the dye laser, 300-fs pulsewidths with 82 MHz at 580 nm and 20 to 30 mW, was shuttered between 100 and 200 msec and used for producing retinal marker lesions.

The monkey cornea was positioned approximately 1 cm in front of the beamsplitter, with the retina in the focal plane of the fundus camera. The single pulse was split by the 580-nm beamsplitter so that the reflected pulse could be sent to the eye while the transmitted pulse could be measured and its energy value recorded for each exposure to the eye. The reflected–transmitted (R–T) ratio was measured at the beginning and end of each session to ensure that its value did not change. Energies and ratios were measured by a joulemeter–ratiometer (Moletron [Portland, OR] JD2000 or an OM4001) with one detector (Moletron J3-09 or J4-09) at the eye position calibrated against a second detector intersecting the fraction of the beam being transmitted. Throughout this article, “laser energy delivered” means the energy delivered to the corneal surface as described and without any contact lens or other device to control the image size delivered to the retina within the eye.

**In Vivo Model**

Mature rhesus monkeys, each weighing from 2.2 to 6.9 kg, were maintained under standard laboratory conditions (12 hours light–12 hours dark). Rhesus monkeys were screened before exposure to ensure that no eye was more than 0.5 D from being emmetropic. All procedures were performed during the light cycle. The treatment and procedures used in this study conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, as well
In Vivo Preparation

Rhesus monkeys were chemically restrained using 10 mg/kg ketamine hydrochloride (HCl) intramuscularly. Once restrained, 0.16 mg atropine sulfate was administered subcutaneously. Two drops each of proparacaine HC1 0.5%, phenylephrine HCl 2.5%, and tropicamide 1% were administered to both eyes. Under ketamine restraint, the monkey had intravenous catheters placed for administration of warmed lactated Ringer's solution (10 ml/kg per hour flow rate) and for administration of propofol. An initial induction dose of propofol (5 mg/kg) was administered to effect. The state of anesthesia was maintained in the monkey using 0.2 to 0.5 mg/kg per hour of propofol through a syringe pump. The monkey was intubated with a cuffed endotracheal tube. A peribulbar injection of 2% lidocaine was administered to reduce extraocular muscular movement. The monkey was securely restrained in a prone position on an adjustable stage for the fundus photography, laser exposure, and FA. Before FA, 0.6 ml of 10% fluorescein (Alcon Laboratories, Fort Worth, TX) was administered intravenously. The monkey's blood pressure and pulse were continuously monitored throughout the experimental protocol. The monkey's normal body temperature was maintained by the use of circulating hot water blankets.

Baseline fundus photography was performed before laser exposures. The eyelids were held open with a wire lid speculum, and the cornea was moistened throughout the procedures with 0.9% saline solution. The retina was viewed with a modified fundus camera through a glass beamsplitter. All macular exposures (15 to 30) were delivered to each eye, without any contact lens, in a rectangular grid pattern in the macular region of the fundus. Visible marker lesions (created by shuttered exposures of the dye laser output at 82 MHz) marked the exposure grid in columns and rows. To aid in localizing the exposure sites, an L-shaped grid pattern of marker lesions was placed around the edge of the macular region before the MVL exposures, as shown in Figure 2. As seen within the grid pattern, there are small whitish spots just visible to the naked eye that are representative of MVLs used in the data base to determine ED<sub>50</sub> values. These laser exposures were delivered at 90 fs and 580 nm, with energy variations between 0.1 and 10 µJ per pulse, and were placed within the grid pattern, centered over the macular region for left and right eyes of our subjects.

Suprathreshold lesions (greater than 10 µJ) were
placed extramacularly and away from the threshold grid so that a wider scattered pattern would avert overlap of lesions. At least two examiners evaluated all eyes at 1 hour and 24 hours after exposure. Visible lesions at a given exposure site were reported only if the two examiners identified a lesion. Fundus photography and FA were performed at 1 hour and 24 hours after exposure. Fundus photographs of ophthalmoscopically visible lesions and fluorescein angiograms were evaluated for lesion presentation. Two eyes were exposed with a grid of nine shots for each pulsewidth and enucleated after 1 hour and 24 hours after exposure for histopathologic evaluations. The results of the histopathology study of the lesions will be published in a later treatise.

Fundus photography (including fluorescein angiography) and observations of lesion formation by the researchers was performed by monocular viewing through the Zeiss fundus camera optical system. Thus, the optics were not changes between fundus viewing and photography, and viewing and photography were performed interchangeably. Photographs for FA were taken immediately before the dye injection and were continued at intervals of a few seconds until 5 minutes had elapsed, providing a sequence of photographs for the development of fluorescein leakage. After fluorescein injection and angiography, in (most) animals, lesions were also assessed for fluorescence by viewing through the camera system with excitation and barrier filter in place. However, fluorescein leakage for the smaller lesions could not be identified by this method, and it was not used for this article.

**Statistical Analysis**

The Probit Procedure was used to estimate the ED$_{50}$ dose for creating an MVL in the retina for 4-ns, 3-ps, 600-fs, and 90-fs pulsewidths and to estimate the 95% confidence intervals for the ED$_{50}$s. Enough data were taken to ensure that the fiducial limits were reasonably narrow. The probit procedure was used for the ophthalmoscopically visible lesion data at 1 hour and 24 hours and for the data from the fluorescein angiograms. Table 1 includes the 1- and 24-hour estimated doses for ED$_{50}$ thresholds, along with the slope of the probit curve for the 24-hour reading. Also included are the number of subjects, number of eyes, and total exposures for each pulsewidth delivered.

**RESULTS**

**Visible Lesion Thresholds**

For the pulsewidths generated at 532 nm (4 ns and 60 ps), not all the lesions developed during the first hour, and exposures at 60 ps took longer to develop than did the 4-ns exposures. The number of lesions observed increased by 25% at 4 ns and by 32% at 60 ps between the 1- and 24-hour readings after exposure. Consequently, the calculated 24-hour ED$_{50}$ threshold dosages had to be reduced considerably for both pulsewidths, as listed in Table 1. This table includes the 1- and 24-hour estimated doses for ED$_{50}$ thresholds, with the 95% fiducial intervals along with the slope of the probit curve for the 24-hour reading. The slope is calculated using the ED$_{85}$ and ED$_{50}$ points to obtain the values listed at each pulsewidth. Also included are the number of subjects, number of eyes, and total exposures for each pulsewidth. The retinal response to minimal exposures was consistently a pale gray to white lesion increasing in whiteness and in size as energy increased in all exposures. The range of
energies (0.03 to 6.6 μJ) for both pulsewidths was placed macularly. For the 4-ns study, two eyes from two different monkeys were used for a total of 50 exposures in the macular region. At 60 ps, five eyes from five different monkeys were exposed, with 88 total exposures in the macula.

For the pulsewidths evaluated at 580 nm (3 ps, 600 fs, and 90 fs), the delay in the appearance of a visible lesion to minimal retinal laser exposures depended on the pulse energy and on the pulsewidth. The retinal response to these exposures was consistently a pale gray to white lesion increasing in whiteness and in size as energy increased, as before. At 3 ps, threshold retinal lesions were visible almost immediately; 98% were visible after only 1 hour. The range of energies (0.45 to 1.43 μJ) producing a visible lesion did not change between the 1- and 24-hour evaluations.

For 600-fs pulsewidths, the time required for a lesion to appear increased significantly; only half were visible after 10 minutes. From the 1-hour reading to the 24-hour reading, we observed an increase of 30% in the number of visible lesions at a given exposure level. The MVL ED_{50} threshold dosage calculated for 24 hours was less than half the value calculated for 1 hour. Also, the range of pulse energies from minimum-to-maximum (minimum lesion to maximum no lesion) decreased significantly from the 1-hour reading to the 24-hour reading (0.22 to 3.0 μJ to 0.17 to 0.45 μJ).

At 90-fs pulsewidths, the delay in appearance of visible lesions past the 1-hour reading was even more pronounced, and the calculated ED_{50} values were larger than the 600-fs values for the 1-hour and the 24-hour calculations. Again, the ED_{50} dosage calculated at 24 hours was less than half the value at 1 hour, showing that a large number of lesions (28) developed between 1 hour and 24 hours. In fact, a 48% increase in the number of visible lesions (58 to 86) occurred at the 24-hour reading. The range of energies from minimum-to-maximum did not change significantly during the 24-hour postexposure examinations (0.16 to 1.8 μJ to 0.10 to 1.4 μJ). Above 1.4 μJ, all energies delivered showed visible lesion development. Out of 122 data points taken at 90 fs within the macula, 94 exposures were within the energy range of 0.1 to 1.4 μJ, and 49 lesions developed within 24 hours. For 3 ps, four monkeys and four eyes were used for a total of 68 exposures. For 600 fs and 90 fs, six monkeys were used for each pulsewidth with six eyes exposed with 112 exposures and seven eyes exposed with 122 exposures, respectively, as listed in Table 1.

In our studies, FA appeared to be much less sensitive in identifying retinal lesions than in determining the lesions ophthalmoscopically. This insensitivity occurred across all readings for our pulsewidths, wavelengths, and observation times. At 3 ps, the fluorescein angiography visible lesion (FAVL) ED_{50} value dropped from 2.8 μJ at the 1-hour reading to 1.3 μJ at the 24-hour reading. However, the FAVL ED_{50} values were much higher than the ophthalmoscopically determined MVLs. For the 600-fs pulses, the FAVL ED_{50} value dropped from 3.7 to 1.5 μJ after 24 hours. These values are six times higher than the MVLs read ophthalmoscopically. In each case, the 24-hour reading was lower than the 1-hour reading. This was not the case with 90 fs, where the 24-hour calculated FAVL ED_{50} thresholds were more than 1.6 times the value at 1 hour and more than 6.7 times the MVL ED_{50} value. Using FA as an endpoint, the trend of more lesions showing up after 24 hours dramatically reversed at 90 fs, and many of the lesions counted after 1 hour simply disappeared after the 24-hour FA evaluation. Although the number of funduscopically visible lesions increased from 58 to 86 after 24-hour exposure, the number of lesions

### Table 1. Minimum Visible Lesions Threshold: ED_{50} for Rhesus Monkeys at the 95% Confidence Level

<table>
<thead>
<tr>
<th>Pulsewidth</th>
<th>1-Hour Reading ED_{50} (μJ)</th>
<th>24-Hour Reading ED_{50} (μJ)</th>
<th>Slope of Probit Curve</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 ns</td>
<td>1.5 (0.75–8.93)</td>
<td>0.9 (0.60–1.35)</td>
<td>2.68</td>
</tr>
<tr>
<td></td>
<td>2 subjects, 2 eyes, 50 exposures</td>
<td>60 ps</td>
<td>0.66 (0.46–1.05)</td>
</tr>
<tr>
<td></td>
<td>5 subjects, 5 eyes, 88 exposures</td>
<td>3 ps</td>
<td>0.68 (0.40–0.91)</td>
</tr>
<tr>
<td></td>
<td>4 subjects, 4 eyes, 68 exposures</td>
<td>600 fs</td>
<td>0.60 (0.43–0.84)</td>
</tr>
<tr>
<td></td>
<td>5 subjects, 6 eyes, 112 exposures</td>
<td>90 fs</td>
<td>1.18 (0.83–2.09)</td>
</tr>
<tr>
<td></td>
<td>5 subjects, 7 eyes, 122 exposures</td>
<td>90 fs</td>
<td>1.18 (0.83–2.09)</td>
</tr>
</tbody>
</table>

Fiducial limits are in parentheses.
ns = nanoseconds; ps = picoseconds; fs = femtoseconds.
TABLE 2. Fluorescein Angiogram Minimum Visible Lesion Threshold: FAVL ED<sub>50</sub> for the Rhesus Monkey Compared to MVL

<table>
<thead>
<tr>
<th>Pulsewidth</th>
<th>FAVL Reading (μJ)</th>
<th>MVL Reading (μJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 ns</td>
<td>FAVL *</td>
<td>1.8 (1.2-3.7)</td>
</tr>
<tr>
<td>532 nm</td>
<td>FAVL 1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>60 ps</td>
<td>FAVL 1.9 (1.1-10.1)</td>
<td>1.5 (0.98-4.4)</td>
</tr>
<tr>
<td>532 nm</td>
<td>MVL 0.66</td>
<td>0.43</td>
</tr>
<tr>
<td>3 ps</td>
<td>FAVL 2.8 (2.1-4.7)</td>
<td>1.3 (1.0-1.6)</td>
</tr>
<tr>
<td>580 mm</td>
<td>MVL 0.68</td>
<td>0.58</td>
</tr>
<tr>
<td>600 fs</td>
<td>FAVL 3.7 (2.5-6.3)</td>
<td>1.5 (1.2-2.0)</td>
</tr>
<tr>
<td>580 nm</td>
<td>MVL 0.60</td>
<td>0.26</td>
</tr>
<tr>
<td>90 fs</td>
<td>FAVL 1.9 (1.2-5.1)</td>
<td>2.9 (1.6-13.6)</td>
</tr>
<tr>
<td>580 nm</td>
<td>MVL 1.18</td>
<td>0.43</td>
</tr>
</tbody>
</table>

* Data not at 95% confidence level.

FAVL = fluorescein angiography visible lesion; MVL = minimum visible lesion; ns = nanosecond; ps = picosecond; fs = femtosecond.

showing up on FA decreased from 43 at 1 hour to 25 after 24 hours. The FA data are given in Table 2, as are the MVL ED<sub>50</sub> values for comparison.

Hemorrhagic Lesion Threshold

In the rhesus monkey, laser exposures at 90 fs were directed to the macula for lesions created with energies under 10 μJ. All laser exposures exceeding this value were directed away from the macula, most of which were directed nasally to the optic disk, and a few were directed outside the temporal arcades. Of the 122 exposures (0.01 to 9.3 μJ) delivered to the macula at 90 fs, seven hemorrhagic lesions were produced with 0.83 to 4.8 μJ energy (2 to 11 times MVL ED<sub>50</sub> of 0.43 μJ). While delivering energy within the macular grid pattern, the laser infrequently intersected the network of retinal microvessels. Macular hemorrhagic lesions were seen only when the exposure site coincided with a small blood vessel. The area of the hemorrhages was approximately 50 to 250 μm in diameter (estimated relative to the optic nerve and vessel size in photographs), and the hemorrhages were either thin with lacy margins or very slightly thickened with smooth round margins. The hemorrhage location appeared to be intraretinal for several lesions; however, without stereo imaging, it was difficult to differentiate small intraretinal versus subretinal hemorrhages. With FA, the blood from the macular hemorrhages blocked fluorescence from the underlying retinal vessel and the choroid. At one of the sites, there was late fluorescein leakage from the margin of the hemorrhage. Although many hemorrhages appeared almost immediately, three hemorrhages in one eye, not visible immediately after laser exposure, developed within 1 hour and increased in size over the next 24 hours. These same hemorrhagic lesions were not visible orthophoscopically 29 days after laser exposure. All data for hemorrhagic versus nonhemorrhagic lesions are summarized in Table 3 for all exposures and pulsewidths.

Of 21 suprathreshold energy exposures (14 to 105 μJ) at 90 fs and 580 nm placed outside the macula, five hemorrhagic lesions were produced by energies ranging from 38 to 105 μJ. One of these lesions demonstrated a very faint (<50 μm) red lesion ringed by a white chorioretinal lesion. Three lesions were probably subretinal hemorrhages (although intraretinal blood leakage from retinal vasculature is possible) of approximately 50 to 100 μm diameter with a rim of white chorioretinal thickening. One laser site (81 μJ) over a retinal venule demonstrated an immediate retinal hemorrhage that enlarged over 24 hours. With FA, the injured retinal vessel demonstrated leakage within the area of blocked fluorescence from the hemorrhage.

Sixteen suprathreshold lesions (14 to 82 μJ) were nonhemorrhagic even though the laser energy for several of these was delivered directly to overlying retinal blood vessels. The lesions were white, and, as with rabbit lesions, their size increased as pulse energy increased.

For the 600-fs pulses, 112 exposures were placed within the macula, and all had energies between 0.02 and 15.5 μJ. Seven of 14 exposures with energies ranging from 3.6 to 15.5 μJ produced hemorrhagic lesions in the macular region. Three exposures with energies of 2.1 μJ were placed nasally to the optic disk, and all three produced hemorrhages. All lesions produced at this pulsewidth appeared to be in intraretinal vessels; none were thought to be choroidal hemorrhages. Similar results were found at 3-ps pulses, where six hemorrhagic lesions were produced in the macula by energies ranging from 1.6 to 11.4 μJ; all were intraretinal hemorrhagic lesions. All hemorrhagic lesions produced in the macula were counted as positive lesions in the MVL data pool because they were always visible funduscopically.

At the 4-nsec pulsewidth, there were no hemorrhages produced for pulse energies ranging up to 5 μJ within the macular area. At 60-ps pulsewidths, there was only one hemorrhage produced with 6.6 μJ intraretinally; no attempt was made to produce hemorrhages with suprathreshold doses. Of the 88 total exposures for 60 ps and 532 nm, only one pulse had an energy greater than 4.1 μJ, and it produced a hemorrhage. All pulses within the range of energies used (0.03 to 6.6 μJ) for both pulsewidths were placed within the macular area; no attempt was made to create hemorrhages within or outside the macular area with suprathreshold energies.
Visible Retinal Lesions From Ultrashort Laser Pulses

TABLE 3. Hemorrhagic Lesions Produced in Rhesus Monkey Eyes for All Pulsewidths and Pulse Energies

<table>
<thead>
<tr>
<th>Pulsewidth</th>
<th>Energy Delivered to Cornea (µJ)</th>
<th>Hemorrhagic Lesions</th>
<th>Nonhemorrhagic Lesions</th>
<th>Total Lesions</th>
<th>Total Exposures</th>
</tr>
</thead>
<tbody>
<tr>
<td>90 fs</td>
<td>0.01-9.3 extramacular(-14-105)</td>
<td>7</td>
<td>63</td>
<td>70</td>
<td>122</td>
</tr>
<tr>
<td>600 fs</td>
<td>0.02-15.5 extramacular-(2)</td>
<td>5</td>
<td>17</td>
<td>22</td>
<td>(22)</td>
</tr>
<tr>
<td>3 ps</td>
<td>0.03-11.4</td>
<td>6</td>
<td>41</td>
<td>47</td>
<td>68</td>
</tr>
<tr>
<td>60 ps</td>
<td>0.03-0.6</td>
<td>1</td>
<td>49</td>
<td>50</td>
<td>88</td>
</tr>
<tr>
<td>4 ns</td>
<td>0.09-5.0</td>
<td>0</td>
<td>25</td>
<td>25</td>
<td>50</td>
</tr>
</tbody>
</table>

DISCUSSION

We have determined the MVL thresholds for laser pulsewidths from 4 ns down to 90 fs. As listed in Table 1, all ED90 values are below 1.0 µJ, with the exception of the 1-hour readings at 4 ns and 90 fs. In assessing the implications of retinal laser damage observed in this study, we consider biologic and laser variables that impact the damage thresholds measured. The biologic variables that affect ED90 include species, ocular anatomy, retinal lesion location, retinal vasculature, pigmentation of the retinal pigment epithelium, and choroid. This study is the first to report lesions in primate eyes for pulsewidths from 4 ns down to 90 fs; it is directly comparable to all other data reported for the primate eyes down to 6 ps, including the data from which the ANSI Z136.1-1993 standard is derived.

The laser variables that impact the determination of retinal damage thresholds include wavelength, pulse duration or pulsewidth, beam diameter incident upon the cornea, beam profile, retinal spot size, beam divergence, and optics used in the pulse delivery. One benefit from our study is the evaluation of a wide range of laser pulsewidths using the same species and delivery system. The change in MVL thresholds identified in our study for the five pulsewidths and two wavelengths suggests that the calculated ED90 thresholds depend not only on pulsewidth but on the wavelength as well, as expected.

For the two pulsewidths at 532 nm, 4 ns, and 60 ps, the number of lesions observed between the 1-hour reading and the 24-hour reading increased between 25% and 32%, which had the effect of lowering the MVL ED90 threshold doses calculated by probit analyses. At 4 ns, the threshold dose decreased from 1.5 to 0.9 µJ after 24 hours, whereas the value at 60 ps decreased from 0.66 to 0.43 µJ after 24 hours. The slope of the probit curve increased from 2.68 to 3.03 when the pulsewidth was reduced from 4 ns to 60 ps, whereas the ED90 decreased from 0.9 to 0.43 µJ, respectively. However, these slopes are larger than those reported by Lund and Beatrice (1.58 for the slope of the regression line defined as ED84/ED50) for doubled Nd:YAG pulses at 140-ns pulsewidths.

Ophthalmoscopically, the time interval for development of retinal MVLs for the 580-nm wavelength increased significantly for 600 and 90 fs as with 4 ns and 60 ps, but this was not true for the 3 ps. For 600 fs, there were 21 exposures between 0.17 and 3.0 µJ, out of a total of 112 exposures, that required more than 1 hour to develop. These delayed lesions reduced the MVL ED90 threshold dosage calculations from 0.60 µJ (1 hour) to 0.26 µJ (24 hours), with reduced fiducial limits as well. At 90 fs, there was a 48% increase in the number of visible lesions after 24 hours compared to the 1-hour reading. For 90 fs, there was a total of 34 exposures, with dosages ranging between 0.10 and 2.0 µJ that required longer than 1 hour to develop into visible lesions. These additional lesions reduced the calculated MVL ED90 threshold values from 1.18 µJ (1 hour) to 0.43 µJ (24 hours); there was a similar reduction in the fiducial limits.

The slope of the probit curve at 3 ps was almost identical to that at 4 ns, but then it changed greatly as the pulsewidth was reduced down to 90 fs. The value at 600 fs was more than 150% (4.11) the value at 3 ps, but then it dropped to 60% of the 3 ps value at 90 fs. We attribute this large swing up and down to the stochastic effects of nonlinear propagation and self-focusing within the eye at these shortest pulsewidths. Rockwell et al. measured the nonlinear index of refraction in vitreous humor, and they developed a simplified model to predict the self-focusing effects for light propagating in the eye. Their model predicts a critical peak power below which the focused image collapses (beam collapse) into a filamentary propagating beam, as predicted by Powell et al. When we compare the peak power in our pulses for the MVL ED90 value in Table 1, we find that the value at 600 fs is just below the critical peak power of 500 kW, and the value at 90 fs is an order of magnitude above the critical power. Thus, we expect that self-
focusing effects without beam collapse at 600 fs should lower the threshold MVL ED\(_{50}\) values because of a smaller retinal image size. Also, it is possible for the beam to collapse at 90 fs; this collapse could cause nonlinear effects to occur within the vitreous or retinal layers. Laser-induced breakdown could occur anterior to the retina and produce a shock wave causing mechanical damage to the neural layer. This type of damage may prevent leakage and thus would not show up in fluorescein angiography, which would increase the calculated threshold dose. Nonlinear effects from beam collapse could also prevent some of the energy in the pulse from reaching the retina and increase the MVL ED\(_{50}\) threshold dose above those for longer pulsewidths, whose peak power levels are much lower than the critical power.

In search of a damage model that would fit our data, several models were considered; some were rejected outright because they could not adequately describe our findings. As an example, when we consider the thermal model usually ascribed to damage from longer pulsewidths, that is, greater than 1 ns, we calculated the temperature rise for all pulsewidths below 1 ns and found the \(\Delta T\) to be 14°C or less. These calculations are based on an image diameter of 30 \(\mu\)m and a pigmented epithelium 10 \(\mu\)m thick, with all the energy reaching the retina being absorbed in this layer. Thus, we reject the thermal model to describe our damage thresholds because the temperature-time history is not even close to being adequate to cause damage.

Photochemical damage processes, as discussed by other researchers\(^5\) for the picosecond pulsewidths, appear to be a possible damage mechanism because of the latency of the development of lesions. In all cases, our threshold dose at 24 hours was lower than at 1 hour, which suggests either photochemical damage or possibly mechanical damage due to acoustic or shock waves. Our histopathologic results will, when they become available, help us to describe better the damage mechanisms.

Another damage mechanism that we cannot reject, especially for the femtosecond pulses, is the possibility of direct membrane effects resulting from the intense electric fields associated with these pulses.\(^5\) The peak power going into the eye at the ED\(_{50}\) threshold at 90 fs was 5 MW, and thus the peak irradiance at the retina was well up into the GW/cm\(^2\) (gigawatts) range. With such extremely high retinal irradiances, retinal cell damage that would agree with the latency of the observed injury would be most likely to occur.

In determining ED\(_{50}\) threshold levels for rhesus monkeys, our FA studies did not show the sensitivity that our direct ophthalmoscopic examinations did. With probit calculations for the 1- and 24-hour readings for the five pulsewidths studied, there were seven instances when the fiducial confidence intervals did not overlap, and only two in which they did. The exceptions were 90-fs thresholds at the 1-hour reading (2.09 \(\mu\)J MVL versus 1.2 \(\mu\)J FAVL) and the 24-hour reading at 4 ns (1.35 \(\mu\)J MVL versus 1.2 \(\mu\)J FAVL). However, the FAVL ED\(_{50}\) decreased between the 1- and 24-hour readings for all pulsewidths with the exception of 90 fs, for which it increased by almost 60%.

Thus, many of the FA lesions visible at 1 hour after exposure at 90 fs disappeared and were not visible after 24 hours. These findings contrast with other FA studies, including our own, in Dutch-belted rabbits\(^9\) in which at 5 ps, 500 fs, and 90 fs the MVL-to-FAVL ratios for 1 hour after exposure were 1.2, 2.8, and 3.7, respectively, showing greater sensitivity in FAs. The same procedure was used in determining our FAs for the rhesus monkey in this study. With observation, and with analysis of fundus photographs and fluorescein angiograms, we found fluorescein angiography to be unreliable in identifying the small, barely above threshold, lesions at short pulsewidths. The choroidal pattern of fluorescence could not be differentiated from the minimally fluorescing <30 \(\mu\)m lesions in these cases. Also, we enlarged our fluorescein angiograms photographically by 5 to 1 in comparison to the strip photographs and found no significant change in our ability to read lesions.

Birngruber et al\(^7\) noted fluorescein angiography to be more sensitive than observation of minimal short pulse laser lesions; however, they artificially maintained a constant lesion size of 50 \(\mu\)m in a rabbit eye. In addition, their fluorescein ED\(_{50}\) was 0.75 \(\mu\)J, whereas our visible lesion threshold (MVL ED\(_{50}\)) was only 0.43 \(\mu\)J, a little more than half their value for fluorescein and less than one tenth their visible lesion threshold of 4.5 \(\mu\)J. This difference can be accounted for by the difference in image size as well as the different species, that is, primate versus rabbit.

Borland et al\(^7\), with 15-ns and 40-ns lesions, noted a similar problem with fluorescein angiography of small laser lesions. As reported in 1978, the granular appearance of the fluorescein "was of the same order as the small size lesions: 10–30 microns," and "small image lesions were extremely difficult to identify at just suprathreshold exposure levels." When they plotted the regression lines of the probit curves, they identified a reduction in statistical reliability with FA because of the confusion between threshold lesions of small image size and the background grain of the choroidal flush for minimal image size exposures. In our study, not only do we have minimal size lesions in which we would expect a reduction of fluorescein reliability for the same reasons mentioned above, but we also have shorter pulse lesions that should produce less thermal area of damage. As Borland’s group observed, thermal damage with disruption of zona occludentes and cell walls was associated with FA-positive
lesions. A histopathologic study of our ultrashort pulse lesions will provide additional insight into our findings in the future.

CONCLUSION

Our data for the rhesus monkey can be compared with other published data as included in the data base used to establish the ANSI Z136.1-1993 standard, shown in Figure 3. The only known data points for rhesus monkeys for pulsewidths < 1 ns at visible wavelengths are also shown in Figure 3 (Goldman et al. and Bruckner and Taboada). Because Goldman et al. did not perform a probit study, our data are more directly comparable to the Bruckner and Taboada datum point at the 6-ps pulsewidth. At 4 ns and below, our ED$_{50}$ thresholds for the 24-hour readings (within the circles) for rhesus monkeys show a slight downward trend until 90 fs is reached; then the estimated value becomes larger. This abrupt change in slope may be due to nonlinear effects such as self-focusing and/or beam collapse from the high peak powers at 90 fs. The solid black line shown in Figure 3 represents the ANSI retinal maximum permissible exposure for pulsewidths down to 1 ns, which is 0.5 $\mu$J/cm$^2$. Thus, 0.2 $\mu$J at the cornea for a pulsewidth of 1 ns is considered safe; however, one cannot extrapolate this safe level to pulsewidths below 1 ns because our data include nine visible lesions out of a total of 54 exposures at or below 0.2 $\mu$J for pulsewidths below 1 ps (90 fs and 600 fs). However, below 1 ns, ANSI recommends a constant irradiance for decreasing pulsewidths; therefore, at 100 fs, the safe limit would only be 20 $\mu$J, or more than four orders of magnitude below our MVL-ED$_{50}$. It is obvious from Figure 3 that our MVL ED$_{50}$ thresholds are an order of magnitude or more below those in the data bank for pulsewidths longer than 1 ns and do not decrease with pulsewidth to an appreciable extent. New interim standards must be set for picosecond and femtosecond laser pulsewidths because laser systems that produce tens of millijoules per pulse below 100-fs pulsewidths are now commercially available. These pulses can have energies as much as five orders of magnitude greater than necessary to create visible lesions in the eye.

Key Words

femtosecond laser, fluorescein angiography, laser injury, monkey, retina, retinal hemorrhage, visible lesion

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References


