In-vitro retinal model reveals a sharp transition between laser damage mechanisms

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Abstract. We use laser damage thresholds in an in-vitro retinal model, and computational simulations to examine the laser exposure durations at which damage transitions from photothermal to photochemical at 413 nm. Our results indicate a dramatic shift in 1-h damage thresholds between exposure durations of 60 and 100 s. The trend in our in-vitro results is similar to a trend found in a recent study where retinal lesions were assessed 1-h post laser exposure in the rhesus eye. Our data suggest that nonthermal mechanisms did not significantly contribute to cell death, even for exposures of 60 s. Knowledge of the transition point, and lack of concurrent thermal and nonthermal damage processes, are significant for those wishing to devise a comprehensive computational damage model.

Keywords: laser-induced damage; cells; thermal effects; nonthermal effects.

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In this study, we use a combination of new in-vitro damage data and computational simulations of both temperature rise and damage thresholds at 413 nm to address the shift to photochemical damage processes in more detail. Except for the following changes, laser exposures were as described previously. Retinal pigment epithelial (RPE) cells (about 160 melanosomes/cell) were exposed to 413-nm laser irradiation in 48-well plates containing 0.1-mL complete Hank’s balanced salt solution. Cells were exposed to a 0.3-mm-diam flat-top beam (via an 88-mm focal length lens) in an environmentally controlled enclosure (Fig. 2) that ensured consistent temperature (35 to 37 °C) and relative humidity (60 to 70%). Estimated dose for 50% lethality (ED50) values were calculated using the Probit method, where Probit slopes represent the first derivative with respect to dose at a probability of 0.5. Systematic uncertainty (15%) in our irradiance values was calculated as previously described.

To simulate the experimental exposures, we used a laser-tissue damage program developed by our group. The model uses a laser propagation model (geometric optics assumed) to compute a source term (implementing Beer’s law of linear absorption) for a thermal heat solver. The heat solver accounts for the multiple layers comprising the system (buffer, cells, and plastic well plate) and air/surface boundary conditions to predict temperature rise at the 7-μm-thick cell layer. These
temperatures were input into an Arrhenius rate equation that was numerically integrated to determine damage outcomes. The two Arrhenius rate parameters ($A = 3.1 \times 10^{99} \text{s}^{-1}$; $E_a = 6.28 \times 10^5 \text{J/mol}^{-1}$) used in the damage integral ($\Omega$) calculation were those reported by Welch and Polhamus. A numerical search algorithm was used to determine the threshold irradiance that solved for an $\Omega$ value of 1 at the center of the beam.

The 1-h ED$_{50}$ irradiances for the current in-vitro exposures are given in Table 1. Consistent environmental conditions during exposures led to low variance (fiducial limits) about the threshold values. Notice that, as expected, the irradiance requirement for damage was reduced by extending the duration of the laser exposure, although there was no significant change between the 40- and 60-s thresholds. Table 1 also shows that the threshold value for the 200-s exposure was exactly half the 100-s threshold, defining irradiance reciprocity (nonthermal damage) for the 0.3-mm beam at 36 °C in the in-vitro retinal system.

An HTAP analysis of the in-vitro results [Fig. 1(b)] shows a trend similar to the 1-h assessments of Ref. 4 at 441.6 nm [Fig. 1(a)]. Power functions describing the in-vitro and in-vivo data had nearly identical exponents (0.76 and 0.78, respectively), but the in-vitro curve was shifted to lower threshold values by a factor of about 6.5, presumably due to differences in melanosome density as discussed previously. Although difficult to see in Fig. 1(b), the threshold data points for the 0.1- and 1.0-s exposures are near the origin. Notice the inverse relationship between irradiance and radiant exposure, such that the 0.1-s exposure required the greatest irradiance and the least radiant exposure [Fig. 1(b)] for generating threshold damage. However, as seen in Fig. 1, this trend is dramatically broken when the damage mechanism shifted to nonthermal, as indicated by irradiance reciprocity. This necessarily means that there was a significant thermal component in the death process for exposures of 1 min. The correlation coefficient for the power function describing the 0.1- to 60-s data [see Fig. 1(b)] would suggest a similar degree of thermal component over this entire range of exposure durations.

As a means of understanding the thermal component of the in-vitro damage thresholds, we simulated irradiance threshold values ($\Omega = 1$) for exposure durations of 0.1 to 200 s and

### Table 1

<table>
<thead>
<tr>
<th>Exposure Duration (s)</th>
<th>Number of samples</th>
<th>$ED_{50}$ (W cm$^{-2}$)</th>
<th>Lower FL*</th>
<th>Upper FL*</th>
<th>Probit slope</th>
</tr>
</thead>
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<tr>
<td>0.1</td>
<td>94</td>
<td>1.57</td>
<td>1.45</td>
<td>1.69</td>
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<tr>
<td>1.0</td>
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<td>28.7</td>
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<td>31.6</td>
<td>45.2</td>
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<td>8.2</td>
<td>10.8</td>
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</tr>
<tr>
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<td>24</td>
<td>4.7</td>
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<td>24</td>
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</table>
plotted the radiant exposure results alongside the in-vitro data [Fig. 1(b)]. The simulated curve fits fairly well with the in-vitro data, falling within the 95% confidence intervals for all but the 40-s data point. The deviation in simulated and in-vitro HTAP curves suggests that mixed damage mechanisms may exist for exposures of 40 to 60 s. However, we understand that our choice in values for the Arrhenius rate parameters, the fact that one of these parameters (frequency factor A) has a slight dependence on temperature, and our 15% systematic uncertainty (Table 1), are all factors that could lead to the observed disparity.

Looking at the irradiance thresholds in Table 1, we see no difference between the 40- and 60-s exposures, which means the continuation of radiant exposure thresholds along the power curve in the HTAP is directly proportional to the increase in exposure duration. However, this is not necessarily unexpected, because threshold irradiance (and temperature) is proportional to the inverse of log time under the Arrhenius damage model, which predicts a convergence of irradiance (and temperatures) at longer exposure durations. A similar scenario appears to play out in the rhesus eye. The tabulated retinal 1-h threshold irradiance values for the 5- to 16-s exposures reported in Ref. 4 were all three statistically identical as well.

Previous authors have used a peak temperature rise of 9 to 10 °C to signify a minimum requirement for damage by thermal means. As expected, simulated peak temperature rises (Fig. 3) for the 100- and 200-s in-vitro threshold values were low (3.8 and 1.9 °C, respectively). Figure 3 also shows that simulated peak temperature rises of greater than 10 °C were calculated for the in-vitro ED50 irradiance values for exposures of 60 s and shorter, which implies that sufficient heat was generated to produce death by thermal mechanisms. When we looked at the individual damage outcome data (damage versus no damage) for the 60-s exposures, we found that the lowest irradiance (23.3 W cm⁻²) that caused damage corresponded to a (simulated) temperature rise of 9.3 °C. Again, this indicated that all the 60-S exposure damage outcomes had sufficient temperature rises to cause damage by thermal means. However, this does not exclude the possibility of nonthermal (photo-oxidation) events occurring concurrently with this elevation of temperature. The lack of an intermediate temperature rise for the 40- and 60-s ED50 irradiance values, such as 5 to 9 °C, suggests that if concurrent mechanisms did exist, they were neither additive nor synergistic to the overall damage rate process.

Finally, we were interested in the expected temperature rise of cells receiving the 40- and 60-s reciprocity irradiance doses. On extrapolating (r⁻¹) from the 100- and 200-s irradiances, we simulated peak temperatures of 9.4 and 6.3 °C for the extrapolated irradiances corresponding to 40- and 60-s exposures, respectively. This suggests that if there did exist additive or synergistic effects from thermal and nonthermal damage mechanisms, it would be manifested in the data for 60-s exposures.

In conclusion, the in-vitro retinal model showed transitions in the damage mechanism for 1-h thresholds similar to those found previously in an in-vivo model. In the in-vitro model, the transition from photothermal to nonthermal damage was sudden, occurring somewhere between exposure durations of 60 and 100 s. Additional data in this exposure range are being collected to characterize this transition. The current data cannot rule out the possibility of photochemical oxidation occurring during damaging 60-s exposures at 413 nm. We are currently conducting experiments to address this issue. However, we believe that nonthermal processes do not contribute to cell death at 1-h postexposure.

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