In Vitro Inhibition of Lens Epithelial Cell Growth by Continuous Wave Nd:YAG Laser

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Bovine lens epithelial cells were suspended in MEM medium and subjected to continuous wave, low power, pulsed neodymium:yttrium-aluminum-garnet (Nd:YAG) laser irradiation. The temperature of each suspension was maintained at 36 °C. Laser applications ranged from 1 to 10 watts and from 100 to 2000 seconds, but the total dose to each of the epithelial cell suspension was 2000 J. Six to thirty-nine percent of the cells were dead immediately after irradiation. Surviving cells, cultured for 15 days, showed decreased attachment and failed to grow. These preliminary results suggest that the Nd:YAG laser may be used during cataract surgery to prevent subsequent lens epithelial cell proliferation and the resulting vision reduction and glare. Invest Ophthalmol Vis Sci 30:714–716, 1989

During the last half-decade, the acceptance of posterior chamber lenses as the most reliable type of intraocular lens has led to modifications in techniques for extracapsular cataract extraction. The most frequent complication of extracapsular cataract extraction, the late opacification of the lens capsule, arises from two distinct causes: fibrosis of the capsule and proliferation of lens epithelial cells on the capsule. The latter is more common and has been found in 32–50% of cases after 2–5 years of follow-up. Dense cell proliferation results in low visual acuity due to decreased transmissible light. Slight proliferation, on the other hand, causes glare due to light scattering.

Prevention of the late proliferation of lens epithelial cells relies on intraoperative removal or deactivation of these cells on the capsule. Techniques proposed to date for this purpose (mechanical scraping, vacuum cleaning, wet-field coagulation, cryocoagulation, application of mitotic inhibitors and ultrasonic cleaning) have shown limited efficacy.

In addition to the thermal effect, it has recently been shown that laser light has a direct damaging effect on some cancer cells even at a low temperature, which does not cause any denaturation and photo-coagulation. Since laser treatment seems to be a promising method for destroying residual lens epithelial cells, we studied the effects of continuous-wave, low-power, pulsed neodymium:yttrium-aluminum-garnet (Nd:YAG) laser action on the growth and seeding efficiency of cultured bovine lens epithelial cells.

Materials and Methods

Lenses were removed from bovine eyes immediately after enucleation; 0.3% dispase II reagent (Sanko Pure Chemicals Co., Ltd., Tokyo, Japan), was used to remove lens epithelial cells from the capsules. Isolated lens epithelial cells were briefly rinsed and suspended in sterilized MEM medium containing 8% fetal bovine serum (FBS). One milliliter of suspended cell solution was transferred to each vial tube.

For this experiment, delivery of stable, low-power Nd:YAG laser was essential for accurate dose control. A SLT Contact laser (Surgical Laser Technologies, Japan Co., Ltd., Tokyo), which has excellent power stability, was therefore selected for delivery of the Nd:YAG laser beam. Different power levels with different irradiation times were applied to obtain a constant dose of 2000 J. To eliminate any thermal effect of the laser beam on the suspended cell solutions, the vials were maintained in an incubator at a constant temperature of 36 ± 0.1 °C. However, in this experimental system, intracellular thermal effect was not clarified. Furthermore, in order to maintain wide and low-power density exposure, the laser beam, passing through optical quartz fiber, was diffused upon delivery by a SLT Frosted Probe (Surgical Laser Technologies, Japan Co., Ltd., Tokyo) (Fig. 1) placed in the vials. The power density at the surface of the probe...
was less than 0.5 W/cm², for maximum power of 10 W, calculated from the total area of the probe and input power.

After laser treatment, a small part of cell suspension was stained with crystal violet (Methylrosanilinium chloride; WAKO Pure Chemicals Co., Ltd., Osaka, Japan) and counted on a hemacytometer in order to determine cell viability. It was reported that the nuclei of the living cells are stained with crystal violet.9

Next the growth and adhesion of the surviving lens epithelial cells after laser application were studied. The cells in each cell suspension were transferred into 24-well Falcon dishes (Becton Dickinson, Oxnard, CA). The cells numbers that was originally plated to each well ranged from 8.0 to 14.3 × 10⁴ with an average 11.7 ± 1.7 × 10⁴. The cells were cultured in MEM medium containing 8% FBS at 36°C for at least 15 days in a humidified atmosphere of 95% air and 5% CO₂. Cells were counted 1, 4, 7 and 15 days after inoculation, as described above, and the growth rate was calculated. The number of cells adhering to the culture dishes 24 hr after inoculation was calculated. Control cell suspensions were not exposed to laser irradiation.

The animals used in this study were treated according to the ARVO Resolution on the Use of Animals in Research.

**Results**

Table 1 shows the number of surviving cells measured immediately after Nd:YAG laser irradiation. Compared with unirradiated controls, some irradiated cells survived with the stain method. It was shown that from 6 to 40% of the cells were killed immediately following the laser irradiation.

Results for laser treated and control cells cultured for 15 days are indicated in Table 2. Both cell growth rates and rates of cell adhesion were decreased by Nd:YAG laser irradiation. These results indicated not only a decreased growth rate but also a disorder of cell adhesion.

**Discussion**

The current communication presents in vitro evidence that lens epithelial cell proliferation may be prevented by direct application of continuous-wave Nd:YAG laser irradiation. Since our experimental system ruled out any specific laser effects, the results obtained may indicate specific photopathy, that is, a cell-deactivating effect, produced by the laser. We did not, however, evaluate the relationship between the effects of irradiation and the power and application time. This point should be studied in detail in a clinical situation. Moreover, in the current experimental system, the surviving cells may have been in the Go stage where cells are not prepared for DNA replication in the cell cycle.10 In vitro investigation of the effects of IV collagen or growth factors must be carried out with cells in a more native situation. In vivo investigation using experimental animals is also necessary.

The current study was suggested by treatment of different cancers with photoradiation therapy,11 laser photodynamic therapy (PDT), and laser hyperthermia.7,8,11 Usually, in this kind of treatment several lasers are used in combination with photosensitizers. Pheophorbide-A (ph-A) is considered a promising

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**Table 1. Effects of laser treatment on lens cell viability**

<table>
<thead>
<tr>
<th>No.</th>
<th>Exposure</th>
<th>% Viability, mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Nonirradiation</td>
<td>100 ± 0</td>
</tr>
<tr>
<td>2</td>
<td>1 W/1000 sec</td>
<td>68 ± 4</td>
</tr>
<tr>
<td>3</td>
<td>1 W/2000 sec</td>
<td>70 ± 6</td>
</tr>
<tr>
<td>4</td>
<td>5 W/200 sec</td>
<td>94 ± 10</td>
</tr>
<tr>
<td>5</td>
<td>5 W/400 sec</td>
<td>60 ± 3</td>
</tr>
<tr>
<td>6</td>
<td>10 W/100 sec</td>
<td>61 ± 7</td>
</tr>
<tr>
<td>7</td>
<td>10 W/200 sec</td>
<td>71 ± 7</td>
</tr>
</tbody>
</table>

Conditions of the laser exposure of control and samples from 1 to 6 are indicated, and the number of surviving cells is indicated with % viability that was counted with stain method and with a hemacytometer.
photosensitizer for use with a continuous wave Nd:YAG laser.\textsuperscript{8,11,12} Mashiko and associates, studying the absorption and fluorescence spectrum of ph-A dissolved in blood subjected to continuous-wave Nd:YAG laser irradiation, found that the laser-induced excitation of ph-A was due to a two-photon absorption process.\textsuperscript{12} Fujishima and associates have already used Nd:YAG laser and ph-A in PDT and laser hyperthermia treatments on experimental brain tumor, but results were inconclusive.\textsuperscript{11} Obviously, if this kind of treatment is to be used for prevention of secondary proliferation of lens epithelial cells, ph-A must be checked for ocular toxicity and for selective absorption by the lens epithelial cells.

Use of the correct type of laser device is particularly important in treatment of lens epithelial cells located in the posterior chamber, a relatively difficult area for surgical observation and maneuvers. Recently, a new contact probe with continuous-wave Nd:YAG laser was developed for the purpose of laser-induced local interstitial hyperthermia.\textsuperscript{7} The new probe allows a wider angle of irradiation and diffusion of lower-power laser energy (less than 5 W). Continuous monitoring with temperature sensors can be used with the probe so that the desired temperature in a known volume of tissue can be maintained, even over a prolonged period (eg, 20–40 min).

At present, we feel that the Nd:YAG laser deserves further testing as a possible tool for inhibiting lens epithelial cell proliferation following cataract surgery, thereby preventing postoperative glare and vision reduction.

Key words: continuous wave pulsed Nd:YAG laser, lens epithelial cell, proliferation, laser hyperthermia, cell culture

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### References


### Table 2. Growth and adhesion rate of lens epithelial cell after laser application

<table>
<thead>
<tr>
<th>Cell number, ×10^6 cells/well, mean ± SD</th>
<th>Control (n = 3)</th>
<th>1 day</th>
<th>4 days</th>
<th>7 days</th>
<th>15 days</th>
<th>Growth rate (%) during T = 15 days, mean ± SD</th>
<th>Adhesion rate (%), mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Originally plated</td>
<td>13.4 ± 0.7</td>
<td>10.8 ± 0.5</td>
<td>7.1 ± 0.6</td>
<td>7.1 ± 0.6</td>
<td>10.8 ± 0.6</td>
<td>17.9 ± 0.3</td>
<td>165.7 ± 8.2</td>
</tr>
<tr>
<td>1 (n = 3)</td>
<td>12.0 ± 1.2</td>
<td>8.0 ± 0.5</td>
<td>4.8 ± 0.3</td>
<td>4.5 ± 0.1</td>
<td>1.2 ± 0.3</td>
<td>7.2 ± 0.4</td>
<td>14.7 ± 3.8</td>
</tr>
<tr>
<td>2 (n = 3)</td>
<td>10.0 ± 0.2</td>
<td>5.1 ± 0.1</td>
<td>3.5 ± 0.5</td>
<td>2.5 ± 0.3</td>
<td>1.9 ± 0.3</td>
<td>7.6 ± 0.1</td>
<td>38.0 ± 4.6</td>
</tr>
<tr>
<td>3 (n = 3)</td>
<td>8.6 ± 0.5</td>
<td>3.5 ± 0.1</td>
<td>3.5 ± 0.7</td>
<td>3.7 ± 0.4</td>
<td>1.4 ± 0.3</td>
<td>7.8 ± 0.1</td>
<td>39.0 ± 6.5</td>
</tr>
<tr>
<td>4 (n = 3)</td>
<td>13.3 ± 0.6</td>
<td>3.5 ± 0.3</td>
<td>3.6 ± 0.4</td>
<td>2.5 ± 0.4</td>
<td>1.4 ± 0.4</td>
<td>7.9 ± 0.1</td>
<td>39.7 ± 5.3</td>
</tr>
<tr>
<td>5 (n = 3)</td>
<td>10.5 ± 0.5</td>
<td>2.9 ± 0.3</td>
<td>3.6 ± 0.2</td>
<td>2.6 ± 0.3</td>
<td>1.6 ± 0.2</td>
<td>7.5 ± 0.1</td>
<td>55.3 ± 6.9</td>
</tr>
<tr>
<td>6 (n = 3)</td>
<td>9.6 ± 0.3</td>
<td>3.2 ± 0.5</td>
<td>3.3 ± 0.2</td>
<td>2.7 ± 0.2</td>
<td>2.6 ± 0.1</td>
<td>8.3 ± 0.3</td>
<td>33.5 ± 13.3</td>
</tr>
</tbody>
</table>

Growth rate means the rate between cell number of 15th day and those of 1st day in each sample. Adhesion rate means the rate between cell numbers of the 1st day and the originally plated cell number.

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