Long-Term Effects of Q-Switched Ruby Laser on Monkey Anterior Chamber Angle

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In an attempt to clarify whether pulsed lasers might be able to cause permanent fistulas from the anterior chamber to the interior of the canal of Schlemm, slightly suprathreshold, low energy, small diameter Q-switched ruby laser pulses were applied to the trabecular meshwork of nine eyes of six rhesus monkeys. Clinical examinations during the next 2 months disclosed no adverse effect on the cornea, iris, lens, or retina. There was transient mild inflammation in five eyes. Intraocular pressure was not changed significantly; the facility determined by perfusion of the anterior chambers at 2 months was normal. Light microscopy and scanning electron microscopy show localized trabecular lesions; some are slightly indented, but there is no persistent penetration to Schlemm’s canal. Endothelial cells, confluent with those of the cornea, cover the inner (anterior chamber) surface of the lesions. Cross-sections through the center of a lesion show that trabecular meshwork and Schlemm’s canal have been obliterated by the treatment and healing; these changes are similar to those previously seen after argon laser monkey trabecular treatment. In the untreated areas, between pulsed laser application sites, trabecular meshwork and Schlemm’s canal are normal by light microscopic and scanning electron microscopic examination. Any effect on IOP from this particular type of pulsed laser treatment of the trabecular meshwork is probably not due to trabeculopuncture and flow of fluid through the fistula. Invest Ophthalmol Vis Sci 26:129-135, 1985
Fig. 1. Scanning electron micrograph of 26 mJ focused laser pulse on “footprint” paper. Central puncture due to laser pulse; surround due to associated effects (bar = 100 μm).

Materials and Methods

The Q-switched ruby laser used has been described (Korad Model K-1; Korad Division, Hadron, Inc.). For the present study, the laser was modified to have a ×2 beam expander in the light path. As a result the focus spot size (Fig. 1) was approximately 75–100 μm in diameter as measured on laser footprint paper (Korad Division, Hadron, Inc.). This is approximately one-half the diameter previously available. The pulse duration was 28 ns. On-line monitoring of pulse energy was done with a ballistothermopile (Model 101; Korad Division, Hadron, Inc.) and a microvoltmeter (Model 102C; Korad Division, Hadron, Inc.). Pulse energy was adjusted by varying the power supply voltage to the flashlamp and by inserting partially reflective mirrors in the light path. The laser pulse energies used for treatment in the current study were adjusted to be slightly above the threshold for gonioscopically visible lesions seen immediately after the pulse was applied to the trabecular meshwork. The trabecular injury threshold was determined for each eye treated.

Six young, adult, rhesus monkeys were used. They were housed and maintained following the ARVO Resolution on the Use of Animals in Research, and U.S. Government and N.I.H. guidelines. Pretreatment ocular examinations confirmed that the eyes were normal.

Each monkey was anesthetized with intramuscular phencyclidine (Sernylan, 1.0–1.5 mg per kg) for ocular examinations. For treatments, the same dose of phencyclidine was followed by intravenous pentobarbital (Nembutal, 10–20 mg per kg). The anterior chamber angle was viewed through an uncoated, optical crown glass, Koepppe-type monkey gonioscopy lens fabricated for use with this laser system (Jocson design; Hansen Ophthalmic Development Company, Iowa City, IA). Pulses were applied through the gonioscopy lens to the anterior part of the trabecular meshwork in the nasal half of the eye. Of the 12 eyes of the six monkeys, three were untreated controls. In each treated eye, the threshold for gonioscopically visible change of the target tissue was determined by application of single pulses to different trabecular sites. The first pulse was at an energy level of 5–6 mJ; subsequent pulses were increased in 1–2-mJ steps until the threshold was identified. The threshold was found to occur at approximately 7 mJ per pulse. Definitive treatment was done, for most eyes, using 8–12 mJ per pulse. Four eyes received three to six suprathreshold laser pulse applications. Five eyes received 10–13 suprathreshold laser pulse applications.

All animals had four clinical examinations during the 2 months after laser treatment. The examinations were done in the same dimly illuminated room at 3 days, 1 week, 1 month, and 2 months. This included external examination with a hand-held illuminator, slit-lamp examination, intraocular pressure (IOP) measurement with a Perkins applanation tonometer, gonioscopy, and indirect ophthalmoscopy. Pupil size was recorded by comparison to a circle template.

At 2 months, under pentobarbital intravenous anesthesia, both eyes of each animal were cannulated with a 23-gauge needle and simultaneous constant-pressure perfusions were done to determine facility. After the perfusions the intraocular pressure was maintained at 15–17 mmHg, and 2.5% phosphate buffered (0.15 M, pH 7.4) glutaraldehyde introduced through a second 23-gauge cannula. After 5 minutes of anterior chamber irrigation with the fixative, an overdose of intravenous pentobarbital was administered and the eyes immediately enucleated. The anterior segments were removed by a pars plana incision. The lens was removed and then the anterior segments were fixed for approximately 10 hr in the glutaraldehyde solution. After fixation the treated half of each anterior segment was divided into three or four equal-sized wedges, each containing one or more treated sites. All but one of the specimens were postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, critical point dried, and coated with gold for scanning electron microscopy (SEM). These specimens were examined with a Jeol SM35 scanning electron microscope. The remaining specimen was embedded in an
epoxy and serially sectioned at 3 μm thickness. Slides were stained with toluidine blue, and examined by light microscopy.

**Results**

Gonioscopic observation of the anterior chamber angle immediately after treatment showed localized lesions. No gross cyclodialysis occurred. Bubble formation occurred as the laser pulse was applied (Fig. 2A); the bubble cleared within minutes. There was a puncture, evidenced by reflux of blood from Schlemm’s canal to the anterior chamber, in one-half the eyes in one or more of the treatment sites. It was not possible to induce reflux bleeding to the anterior chamber from Schlemm’s canal during gonioscopy in any eyes at 1 week or at 2 months after laser treatment. Gonioscopic examinations indicated healing; there was a subtle alteration of pigmentation at the treatment sites at 2 months, but gross changes were not present (Fig. 2B). Anterior chamber inflammatory reaction was minimal and found in only five of the nine treated eyes during the examination at 3 days after laser treatment. Whether eyes were treated with a smaller (three to six) or larger (10–13) number of pulses did not significantly affect the IOP or perfusion facility (Table 1). No corneal, iris, lens, or retinal laser-induced lesions were found in the treated eyes.

Scanning electron microscopic examination of the treated anterior chamber angles shows localized distortion at treatment sites. Shallow indentations of the trabecular meshwork are present at locations where the laser pulses were focused; these measure 200–400 μm in diameter. Well-focused, small diameter laser lesions caused the trabecular meshwork to be partially punctured (Fig. 3). The trabecular beams adjacent to treated sites are bowed away from the center. At a greater distance from the center the trabecular morphology is normal, and the canal of Schlemm is open (Fig. 3). Endothelial cells smoothly cover the inner surface of all recognizable treated sites (Fig. 3; Figs. 4A, B). These cells are continuous with the corneal endothelium. Indented, smooth, endothelial-covered, healed lesions are present at one-third to one-half of the locations where laser pulses are known to have been delivered. The remaining treated sites cannot be positively identified by gross pathologic or scanning

**Table 1.** Intraocular pressure (IOP) and facility (C), from anterior chamber perfusion, after Q-switched ruby laser trabecular treatment in monkey eyes

<table>
<thead>
<tr>
<th>Number of Laser Applications</th>
<th>Number of Eyes</th>
<th>Mean IOP (range) (mmHg)</th>
<th>Mean C (range) (μl/min/mmHg)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>At 1 month</td>
<td>At 2 months</td>
</tr>
<tr>
<td>0</td>
<td>3</td>
<td>13.8 (11–17)</td>
<td>13.0 (11–14)</td>
</tr>
<tr>
<td>3–6</td>
<td>4</td>
<td>11.1 (7–19)</td>
<td>9.9 (4–20)</td>
</tr>
<tr>
<td>10–13</td>
<td>5</td>
<td>13.7 (10–20)</td>
<td>14.1 (11–20)</td>
</tr>
</tbody>
</table>
electron microscopic examination. There is no evidence of persisting perforation from the anterior chamber to the interior of the canal of Schlemm; thus no pathway was created for direct flow from the anterior chamber.

Light microscopic examination of skip serial sections of a treated site shows obliteration of the canal of Schlemm at the center of the lesion. Endothelial cells, continuous with those of the cornea, cover the anterior part of the lesion. Near the center of the treated site, the iris root is retroplaced slightly from the normal position (Fig. 5A). Sections of the tissue adjacent to the treated site show normal trabecular morphology and an open canal of Schlemm. This agrees with the observation of a sharp localization of the laser effect in the scanning electron microscopic examination.

Discussion
In the present study comparatively low energy Q-switched ruby laser pulses (8-12 mJ) are seen to cause localized trabecular lesions. In our earlier study,2 25-30 mJ per pulse were required to produce localized lesions. The explanation for the lower energy requirement in the present study is based on the smaller focal diameter obtained from an improved laser delivery system, primarily by x2 expansion of the collimated laser beam. As a consequence, a comparable energy density is achieved at the target tissue with lower energy pulses. In both studies, the energy density for the threshold of localized trabecular damage was approximately the same (between 80 and 360 J/cm²).

There was minimal ocular inflammation after application of pulsed laser energy to the monkey trabecular meshwork in the present study. There was no flare or cell reaction in the anterior chamber of four of nine treated eyes examined 3 days after laser treatment. In addition, no laser-induced change was found in untreated tissue located away from the target site.

The laser treatment caused little change of the intraocular pressure or the outflow facility in the normal monkey eyes (Table 1). Specifically, no elevation of intraocular pressure followed the pulsed laser trabecular treatment. Similarly, there is a lack of effect of argon laser trabeculopathy on intraocular pressure in normal monkey eyes.4 Pressure elevation occurs after extensive argon laser treatment of the monkey anterior chamber angle.5

Other laboratories have reported effects of pulsed lasers on the trabecular meshwork.1,6-10 Using the pulse energy, focal diameter, and pulse duration values found in the reports, an approximate energy density and power density can be calculated for trabecular meshwork treatment (Table 2). Krasnov and associates have observed that Q-switched ruby laser pulses applied to the trabecular meshwork in glaucoma patients cause sustained reduction of intraocular pressure in about 60% of those treated, but that many of the successful cases have required repeated treatment. The other 40% of the patients have required surgery.7 From the pulse energy (200 mJ) and focal diameter (200-500 μm),1,5,7 the approximate energy density applied to the eyes of their patients is estimated to be 100-400 J/cm². For a 50-ns duration, power density is about 1.0 × 10¹⁰ W/
cm². In the present study of monkeys, the values found to have an effect are very close to these. Morphologic effects of pulsed lasers on human trabecular tissues have not yet been reported.

Van der Zypen, Bebie and Fankhauser have studied the effects of a TEM₀₀ Q-switched, neodymium-glass laser on monkey anterior chamber angle tissues.⁸,⁹ Pulse energy was 60 mJ at the target tissue. Pulse duration was 35 ns. Wavelength was 1060 nm. The calculated diameter of the focus was 22 μm. When these values are used, the calculated energy density is about 15,000 J/cm² and power density about 40 × 10¹⁰ W/cm². These investigators have observed that localized lesions of the trabecular meshwork and local opening to Schlemm's canal are caused by single pulses aimed near Schwalbe's line.⁸ The punctures of the trabecular meshwork had become occluded when the eyes were examined a few weeks to 18 months after treatment. A smooth, continuous layer of endothelium and basement membrane covered the treated, scarred trabeculum.

Ticho and associates have examined the effects of frequency doubled, TEM₀₀, neodymium glass, Q-switched laser on the trabecular meshwork of baboons (Papio hamadrias).¹⁰ The energy required for localized trabecular damage was 0.5–3 mJ when pulse duration was 20 ns. The diameter of the puncture in trabecular tissue was 15 μm. The energy density is thus 280–
1600 J/cm², and the power density is 1.4–8.4 × 10¹⁰ W/cm².¹⁰ One of the three eyes studied had persistent penetrations into the canal of Schlemm at 41 days, documented by scanning electron microscopy and light microscopy. In the other two eyes the penetrations were healed.

The trabecular treatment done by Van der Zypen and associates⁸⁻⁹ was 100 times more energetic than that of Ticho and coworkers¹⁰; however, the focal diameters were nearly the same. In the latter study some of the punctures remained open. The general-ization that follows is that if the pulsed laser focus has the smallest possible diameter on the tissue, and if the pulse energy is only slightly suprathreshold, there is a higher likelihood that the puncture will stay open.

In the present study, the rhesus monkey trabecular meshwork and canal of Schlemm were obliterated in the middle of the treatment sites when examined at 2 months. The trabecular meshwork and canal of Schlemm between healed laser lesions were normal by light and scanning electron microscopy. Approxi-

Fig. 5. Light micrographs of a cross-section near the center of healed treated site seen in Figure 2B (toluidine blue stain, 3 μm section of plastic embedded tissue). A, top. Scarred trabecular tissue is collapsed and indented at treated site (arrow). Schlemm's canal cannot be identified. Iris root is retroplaced from normal position and inserts onto ciliary body (X125) (bar = 100 μm). B, bottom. Trabecular tissue remaining at treated site is collapsed and endothelial cells cover the anterior and posterior inner surface. Descemet's membrane ends abruptly (arrow); thin endothelial cells covering trabecular tissue are continuous with corneal endothelial cells (X375) (bar = 100 μm).
Table 2. Effects of Q-switched pulsed lasers on trabecular meshwork

<table>
<thead>
<tr>
<th>Author(s) and reference</th>
<th>Wavelength (nm)</th>
<th>Pulse energy (mJ)</th>
<th>Diameter focus (μm)</th>
<th>Pulse duration (ns)</th>
<th>Estimated energy density (J/cm²)</th>
<th>Estimated power density x10⁹ (W/cm²)</th>
<th>Tissue effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Krasnov MM</td>
<td>943</td>
<td>≤200</td>
<td>200-500</td>
<td>50</td>
<td>100-400</td>
<td>&lt;1</td>
<td>Not reported</td>
</tr>
<tr>
<td>Van der Zypen and Fankhauser</td>
<td>1060</td>
<td>60</td>
<td>22</td>
<td>35</td>
<td>15,000</td>
<td>43</td>
<td>Healed, sealed (1-18 months)</td>
</tr>
<tr>
<td>Ticho et al</td>
<td>530</td>
<td>0.5-3</td>
<td>15</td>
<td>20</td>
<td>280-1600</td>
<td>1.4-8.4</td>
<td>One of three open (6 weeks)</td>
</tr>
<tr>
<td>Bonney et al</td>
<td>943</td>
<td>25-28*</td>
<td>100-200</td>
<td>28</td>
<td>80-360</td>
<td>0.3-1.3</td>
<td>Open to Schlemm’s canal (Immediate)</td>
</tr>
<tr>
<td>Gaasterland et al (present report)</td>
<td>943</td>
<td>8-12*</td>
<td>75-100</td>
<td>28</td>
<td>110-270</td>
<td>0.4-1</td>
<td>Healed, sealed (2 months)</td>
</tr>
</tbody>
</table>

* Barely suprathreshold.

mately one-half the circumference of the anterior chamber angle was subjected to discrete focused laser pulses. The normal facility determined by anterior chamber perfusion indicates that the undisturbed outflow pathway tissue is sufficient for normal function.

Key words: glaucoma, pulsed laser treatment, trabecular meshwork, monkey, outflow facility, intraocular pressure

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References