Short-term effects of Q-switched ruby laser on monkey anterior chamber angle

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Three Q-switched ruby laser pulses were applied to the trabecular meshwork of 10 monkey eyes. Pulse energies ranging from 20 to 110 mJ were studied. The spot size ranged from 100 to 200 μm (in air), and the pulse duration was 28 sec. Gonioscopic examinations showed a graded response from no appreciable change at 20 mJ per pulse to marked disruption of anterior chamber angle structures at 100 mJ or more per pulse. Perfusions done within an hour of treatment showed no consistent alteration of the outflow facility. Scanning electron microscopy demonstrated the graded anterior chamber angle response. No disruption of the angle structures was seen after the 20 mJ treatment, but discrete trabecular damage occurred after treatments with 25 mJ. After pulses equal to or greater than 45 mJ the anterior chamber angle structures were markedly altered. The power density causing extensive tissue disruption was equal to or greater than 150 × 10^6 watts/cm^2. In each specimen with an identifiable trabecular lesion, tissue debris and endothelial edema were found on the adjacent inner surface of the cornea. Tearing of Descemet's membrane next to the trabecular meshwork occurred with the 100 mJ pulses. (INVEST OPHTHALMOL VIS SCI 22:310-318, 1982.)

Key words: angle recession, cornea lesions, facility of outflow, glaucoma, iridodialysis, laser, laser surgery, Q-switched laser, rhesus monkey, ruby laser, scanning electron microscopy, trabecular meshwork, trabeculectomy

Laser treatment of the trabecular meshwork has attracted interest because of the possibility that it could provide a simple, effective, noninvasive form of glaucoma therapy. Several investigators have reported successful glaucoma treatment, both with pulsed lasers1, 2 and with continuous wave lasers,3 whereas others have described varying degrees of success with laser treatment.4

The potential advantage of pulsed laser exposures for glaucoma therapy is a minimized thermal effect adjacent to the target site. The reduction of a thermal effect is an important distinction between continuous wave lasers and Q-switched lasers. With the latter, the mechanism of action is not primarily thermal.6, 8-9

An immediate lowering of intraocular pressure (IOP) after Q-switched laser pulses would most likely result from an increased outflow facility. The basis of such an increase could be the opening of outflow pathways, including penetration of Schlemm's canal. Trabecular contracture as discussed with argon lasers is thought to be less likely to occur with higher power pulsed lasers.5 At this time there is no information about the mechanisms by which Q-switched ruby lasers aid the glaucoma patient. The purpose of the
Part of the energy monitor front laser mirror head as rear mirror assembly. Figure 1. Laser system. The laser cavity is the area between front and rear mirrors. Q-switching is achieved with the Pockell's cell. Laser pulses travel from right to left. A portion of each pulse is directed into a ballistothermopile by one of the partially reflective mirrors. At the left end of the optical rail the ruby laser pulses are turned 90° toward the focusing lens. A beam splitter introduces a He-Ne aiming laser beam, which defines the target site. The target site is viewed through two mirrors with a Zeiss surgical microscope. During the exposure the mirror above the beam splitter flips toward the viewer, allowing the ruby laser pulse to pass and providing protection for the viewer.

The present study is to correlate treatment at various pulse energies from a Q-switched ruby laser with results of immediate anterior chamber perfusion and short-term morphologic changes in the anterior chamber angle.

### Materials and methods

A Q-switched ruby laser system previously described (Korad Model K-1; Korad Division, Hadron, Inc.) was used. The pulse duration was 28 nsec. On-line monitoring of pulse energy was conducted with a ballistothermopile (Model 101; Korad Div., Hadron, Inc.) and microvoltmeter (Model 102C; Korad Div., Hadron, Inc.). Pulse energy was varied by adjusting the power supply voltage to the flash lamp and by inserting partially reflective mirrors in the path of the laser pulse (Fig. 1). Treatment energies selected for this study were 20, 25, 45, 60, and 100 millijoules (mJ) per pulse. On-line dosimetry showed the energy delivered to be within ±10% of the selected values. The beam was focused to a diameter in air of 100 to 200 μm as measured with laser foot-print paper (Korad Div., Hadron).

Seven young adult rhesus monkeys were used. At least 4 months and up to 18 months before laser treatment, each monkey underwent bilateral constant-pressure anterior chamber perfusion to determine total facility by means of a technique previously reported. Ocular examination of the eyes shortly before laser exposure confirmed that the perfusion had not caused significant clinical alteration.

Each monkey was sedated with either intramuscular phencyclidine (1.0 to 1.5 mg/kg) or ketamine (3.0 to 6.0 mg/kg) and anesthetized with intravenous pentobarbital (10 to 20 mg/kg). The anterior chamber angle was viewed through an uncoated, glass, Koepp-type gonioscopy lens fabricated for use with this laser system (Jocson design; Hansen Ophthalmic Development Co., Iowa City, Iowa). Pulses were directed through the gonioscopy lens to the anterior part of the nasal trabecular meshwork. At each selected energy three pulses were delivered to each treated eye, separated by at least one-twelfth of the circumference of the eye (i.e., at least one clock-hour apart).
Fig. 2. Gonioscopic photograph immediately after exposure to a 24 mj pulse. Above the ciliary band (asterisk) a discrete oval lesion can be seen in the trabeculum (arrow). A halo around the trabecular lesion was thought to be proteinaceous material. The cornea (C) is above and the iris (I) is below. (Figure from a 35 mm color slide.)

In three of the monkeys laser treatments were bilateral, and in the other four animals only one eye was treated. In the 10 treated eyes, two were treated at each of the five preselected energy levels (see Table I). The remaining four eyes were untreated controls.

Within 1 hr after laser exposure, both eyes of each animal were cannulated with a 23-gauge needle and simultaneous constant-pressure perfusions were done. After the perfusions the intracocular pressure was maintained at 15 to 17 mm Hg, and 2.5% 0.15M phosphate buffered (pH 7.4) glutaraldehyde was introduced through a second 23-gauge cannula. After 5 min of irrigation with the fixative, an overdose of intravenous pentobarbital was administered and the eyes were enucleated immediately. The anterior segments were removed by a pars plana incision. After lens removal the anterior segments were fixed for 10 hr in the glutaraldehyde solution.

After fixation the nasal half of each anterior segment was divided into three equal-sized wedges, each containing one laser exposure site in the treated eyes. The specimens were postfixed in 1% osmium tetroxide, dehydrated in graded alcohols, critical-point dried, and coated with gold for scanning electron microscopy (SEM). Specimens were examined with either a JOEL or ETEC scanning electron microscope.

Results

The in vivo observations of the anterior chamber angle demonstrated a graded response that correlated with the laser energies used. Minimal change accompanied the 20 mj pulses; there were tiny bubbles at the trabecular meshwork, and in 2 of 6 exposures (2/6) a small dot of blood appeared at the exposure site. With the 25 mj exposures there was bubble formation within discrete trabecular lesions (Fig. 2), with reflux of blood to the anterior chamber from the canal of Schlemm. After some of the 25 mj exposures (2/6), a small local angle recession was noted. Approximately 30 sec elapsed before a mild local bleeding occurred from these recessions. Each of the 45 mj pulses resulted in bubbles in the target site, marked angle recession, local bleeding, and dispersion of pigmented tissue debris. The 60 mj pulses produced similar but more pronounced changes. The 100 mj pulses caused the greatest distortion of the target area, with bubble formation in the tissue and aqueous humor, angle recession, local iridodialysis, marked pigmented tissue dispersion, and hemorrhage. Corneal epithelial defects occurred after some (2/6) of these 100 mj exposures; these were located in the path of the laser pulse, under the gonioscopy lens.

The perfusion results are shown in Table I. The mean total facility for untreated eyes was about 0.46 μl/min/mm Hg (± 0.03 S.E.M.). No consistent immediate alteration from the pretreatment value was caused by the laser treatment.

SEM showed a graded distortion of the normal angle morphology, with more pronounced change accompanying the higher energy pulses. Four of the six sites from two eyes treated with 20 mj exposures were normal by SEM examination. The remaining two sites contained local distortions of the trabec-
Table I. Results of perfusions—total facility (μl/min/mm Hg)

<table>
<thead>
<tr>
<th>Energy (μJ)</th>
<th>Monkey No.</th>
<th>Treated eyes</th>
<th>Control eyes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>110</td>
<td>705E (R)</td>
<td>0.32</td>
<td>0.54</td>
</tr>
<tr>
<td>100</td>
<td>141 (R)</td>
<td>0.58</td>
<td>0.20</td>
</tr>
<tr>
<td>63</td>
<td>10F (R)</td>
<td>0.39</td>
<td>0.16</td>
</tr>
<tr>
<td>47</td>
<td>732J (R)</td>
<td>0.39</td>
<td>0.39</td>
</tr>
<tr>
<td>48</td>
<td>732J (L)</td>
<td>0.62</td>
<td>0.43</td>
</tr>
<tr>
<td>27</td>
<td>997E (R)</td>
<td>0.53</td>
<td>0.16</td>
</tr>
<tr>
<td>24</td>
<td>968E (L)</td>
<td>N.D.</td>
<td>0.37</td>
</tr>
<tr>
<td>21</td>
<td>3F (R)</td>
<td>0.41</td>
<td>0.54</td>
</tr>
<tr>
<td>Mean</td>
<td>0.463</td>
<td>0.365*</td>
<td></td>
</tr>
<tr>
<td>S.E.</td>
<td>0.054</td>
<td>0.046</td>
<td></td>
</tr>
</tbody>
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R = right eye; L = left eye; N.D. = no data available.
*p > 0.1.

ular tissue that could not be conclusively associated with the laser treatment. There was no evidence of bleeding. Fig. 3 and 4 show the trabecular region after exposure to 24 and 27 μJ, respectively. The major alterations were in the trabecular meshwork and adjacent corneal endothelium. At the center of the treatment site both the uveal and corneoscleral trabeculum were disrupted with free red blood cells (RBCs) and fibrin within the distorted trabeculum. There was cellular debris on the nearby corneal endothelial surface, and in a surrounding zone the endothelial cells were edematous (Fig. 5).

The SEM studies of sites treated with pulses of 100 μJ or more showed marked disruption of the angle structures with large, local iridodialyses centered on the distorted angle tissue (Fig. 6). These higher energy pulses caused a tearing of the corneal endothelium and Descemet's membrane anterior to the laser-treated site. RBCs, fibrin, tissue debris, and ruptured trabecular components were all present. Eyes treated with 45 and 60 μJ pulses showed similar but less severe changes than those in the eyes treated with the 100 μJ pulses.

Discussion

Two series of reports have presented observations of the effects of Q-switched laser pulses on the anterior chamber angle. The work of Krasnov et al.1, 2 from Moscow has emphasized the clinical effects of Q-switched ruby laser systems in glaucoma patients; the work of Van der Zypen et al.8, 9 from Switzerland has centered on the effects of a Q-switched, frequency-doubled neodymium glass laser system on the monkey anterior chamber angle. The observations reported differ to some degree from those of the present study. These will be considered under the headings of power and energy density, penetration to Schlemm's canal, effects on outflow facility, and corneal effects.

Power and energy density. In the present study, Q-switched ruby laser pulses varying in energy from 20 to 100 μJ (in air) were focused on the monkey trabecular meshwork. Both gonioscopy and SEM showed a graded response of the target tissue. Pulses equal to or greater than 45 μJ produced marked tissue damage, including hemorrhage, local angle recession, and, after 100 μJ pulses, iridodialysis. In contrast, minimal trabecular damage was found with the lowest energy pulses (20 μJ). The lesions with the most encouraging clinical appearance by gonioscopy occurred after pulses of approximately 25 μJ (Fig. 2). The effects appeared to be localized to the trabecular region, and it was possible to induce reflux of blood to the anterior
Fig. 3. Scanning electron micrographs showing the anterior chamber angle after a 24 mJ pulse. The cornea (C) is above and the iris (I) below. In this view, an arching zone of tissue debris and edematous endothelial cells are shown on the inner corneal surface. The laser target site in the trabecular meshwork is evident as a deep penetration (arrowheads). The canal of Schlemm cannot be seen. Scattered RBCs and fibrin strands are found in the damaged area of trabeculum. An iris tear is indicated (arrow) in the foreground, attributable to the primary laser effects at the trabeculum. Inset, A peripheral area of altered trabecular meshwork (asterisk) with broken and distorted trabecular beams. Bars = 100 μm.

The energy levels used by both Krasnov et al.1, 2 and Van der Zypen et al.8, 9 were within and above the upper end of the range in the present study. Krasnov has used 50 to 200 mJ per pulse (in air) delivered in a spot size of 200 to 300 μm diameter.2 The duration of the Q-switched pulse obtained from his clinical instrument is about 100 nsec (M. M. Krasnov and V. S. Akopyan, personal communication). Van der Zypen has studied effects of pulses with 110 mJ energy content (in air) and a pulse duration of 35 nsec. He estimates the spot size to be 22 μm in diameter and states that attenuation probably results in about 60 mJ being delivered to the trabecular target.9
The power density (watts/cm$^2$) of a laser pulse has been shown to be more meaningful for predicting tissue effects than is the energy in the pulse.\textsuperscript{10} Power density calculations (in air) have been done to compare the present to previous studies. From the reports of Krasnov it appears that the power density of his clinical laser system is in the range of $7 \times 10^8$ to $64 \times 10^8$ watts/cm$^2$ during glaucoma patient treatment. The power density calculation from Van der Zypen's reports were done with his $22 \mu m$ calculated (rather than measured) spot diameter. This yields a power density (in air) of about $8300 \times 10^8$ watts/cm$^2$. For the present study the power density ranged from $23 \times 10^8$ to almost $490 \times 10^8$ watts/cm$^2$. In all three laboratories the pulse durations are very short, ranging from 28 (present study) to 100 nsec (Krasnov), with power levels sufficiently high to cause nonlinear effects.

In the present study, laser pulses delivering more than $150 \times 10^8$ watts/cm$^2$ energy density (e.g., spot size in air 200 $\mu m$, energy $45$ mJ in air, duration 28 nsec) caused excessive and widespread disruption of the anterior chamber angle structure. Therefore it seems likely that the power density calculated for the work of Van der Zypen and associates is erroneously high, probably because of an underestimate of the spot diameter. Krasnov and his associates have not reported excessive tissue damage similar to that seen after the more powerful pulses in the current study. This is compatible with the power density calculations.

In all of these studies,\textsuperscript{1, 2, 8, 9} energy measurements were made for the free-in-air condition. The actual laser pulse delivery to the trabecular meshwork required use of a gonioscopic lens of special design and material. The attenuation for the lens-eye complex in the present study is not known, neither is a quantitative attenuation given in the other reports.

**Penetration to Schlemm's canal.** In glaucoma the functional problem is obstructed...
outflow from the anterior chamber. Most evidence indicates that the obstruction is located in the juxtacanalicular area, between the anterior chamber and the canal of Schlemm. McEwen calculated that a tiny opening between the anterior chamber and the canal of Schlemm would suffice to drain the flow of aqueous humor at a normal IOP. Theoretically a single pore of 12 μm diameter would be sufficient to drain 1.4 μl/min. A reason for treating the trabecular meshwork with Q-switched laser pulses is the possibility of creating openings from the anterior chamber to the canal of Schlemm. Trabecular contracture associated with argon laser treatment has not yet been linked with Q-switched pulsed lasers.

Unfortunately, it is difficult to assess by gonioscopic examination whether openings either penetrate completely to the canal of Schlemm or are functional. Even a reflux of blood from the treatment site on the trabecular meshwork does not predict the success of the therapy. In the present study, localized reflux of blood from the canal of Schlemm was seen after the 24 and 27 mJ treatments, but the total facility was normal when these eyes were perfused, and the SEM examination did not show penetrations of the trabecular tissue to the interior of the canal (Figs. 3 and 4). No satisfactory clinical method to assure the surgeon of successful laser intervention was found in the present study.
Fig. 6. Scanning electron micrographs showing the posterior iris surface (I) and iridodialysis after a 110 mJ pulse. The ciliary processes (CP) are in the foreground. The impact site for the pulse was the anterior trabecular meshwork. Through the iridodialysis a tear (asterisk) is seen in Descemet's membrane and adjacent trabecular meshwork. Inset, Tear and associated damage to the inner corneal surface and anterior trabeculum. Bars = 100 μm.

**Effects on outflow facility.** The perfusions of the treated eye were to physiologically evaluate the efficacy of Q-switched laser treatment. Ticho et al. used perfusion studies in primates to evaluate trabeculotomies performed with a continuous-wave argon laser, and no significant changes were noted. In another study, Ticho and Zauberman evaluated argon laser trabeculotomies in glaucoma patients by tonography. A biphasic response was reported. An early lower IOP with increased facility of outflow was followed by increasing IOP and decreasing outflow facility.

A Q-switched neodymium laser had been shown by Van der Zypen et al. to produce openings into the supraciliary and suprachoroidal space in primate eyes when the trabecular meshwork was treated. Histopathologic examination after an in vivo intracameral tracer substance has confirmed that the anterior chamber freely communi-
cated with the suprachoroidal space and that this communication persisted for a period of 480 days. Facility of outflow determinations, however, were not made.

The anterior chamber perfusions in the present study (Table I) show no immediate change in the facility after the laser treatment. The bleeding and the debris scattered into the anterior chamber from the laser-treated sites after pulses equal to or greater than 45 mJ were judged to be sufficient to interfere with outflow. Despite the presence of hemorrhage and debris, outflow facility was normal. However, a demonstration that trabeculotomies produced by Q-switched lasers increase facility of outflow has yet to be reported.

Corneal effects. In a previous report, the passage of high-energy laser pulses through the cornea was noted to cause corneal epithelial and endothelial lesions. The surface lesions were characterized by a circular zone of loss of epithelial cells with a small, central, conical pitting extending into the superficial stroma. Epithelial and superficial stromal lesions like this were consistently seen in the present study under the gonioscopy lens after pulse energies equal to or greater than 100 mJ. No endothelial damage was found at the site where the pulse entered the anterior chamber after it traversed the cornea.

At the target site, endothelial damage occurred secondary to interaction of the pulse with the trabecular tissue. SEM demonstrated on the endothelial surface a narrow band of tissue debris and, anterior to it, a band of edematous endothelial cells, some of which showed evidence of migration toward the wound site. These bands formed arches anterior to the target site (Figs. 3, 4, and 6). The magnitude of the alteration and the width of the bands were related to the laser pulse energy. These changes occurred after pulses with as little as 25 mJ energy content but were more pronounced after higher energy pulses. Outside and inside the arching bands the corneal endothelium appeared normal. The debris appeared to be tissue fragments.

The pattern of the endothelial edema and debris surrounding the target suggests a forceful expulsion of tissue caused by the laser pulse. Similar changes have not been previously reported; the endothelial changes observed by Worthen and Wickham were the direct result of argon laser photocoagulation and were less extensive but more damaging than the secondary effects reported in this study.

REFERENCES