Argon laser trabeculotomies in primates: evaluation by histological and perfusion studies

U. Ticho, J. C. Cadet, J. Mahler, E. Sekeles, and A. Bruchim

The continuous argon laser beam has been applied to the trabeculum at the anterior chamber angle of one eye of nine primates. The treatment was evaluated by light and scanning electron microscopy and constant rate infusion studies at different intervals following the laser treatment. The histological studies revealed coagulative necrosis, with trabeculotomies extending into Schlemm's canal in the immediate postoperative period. However, complete closure of the laser-induced trabeculotomies by healing was observed within 1 to 3 weeks following the treatment. Outflow facility data as measured by the perfusion studies revealed somewhat increased values of the treated eye during the early 2 weeks after laser treatment. On longer follow-up, no significant outflow differences were measured between the treated and untreated eyes.

Key words: argon laser, trabeculotomy, primates, histology, perfusion studies

Laser trabeculotomy is a fairly new procedure in the treatment of glaucoma. The application of both the continuous argon laser or the pulsed ruby laser beam to the anterior chamber angle were previously reported to induce a temporary hypotensive effect in cases of chronic simple glaucoma. On the other hand, other investigators induced glaucoma in rhesus monkeys by repeated and circumferential application of argon laser energy to the anterior chamber angle.

In order to better understand the effect of laser energy on the outflow channels, the effects of continuous and pulsed laser energy of various wavelengths, intensities, and duration on the outflow apparatus are being investigated. The present report will describe the histopathological and physiological changes which were induced in nine primates by trabeculotomies.

Material and methods

Animals. Nine Papio hamadrias baboons have been studied. The primates were 15 months old, weighing between 3 and 4 kg. Five primates were males, and four were females.

Anesthesia. All animals were premedicated by an intramuscular injection of atropine sulfate, 0.5 mg, and then anesthetized for the experiments by intravenous 6% phenobarbital, 0.5 ml/kg. No intubation was performed for laser surgery or the anterior chamber perfusion studies.

Laser surgery. The coherent radiation A 800 continuous argon laser unit was used as the energy source. The laser spots were of 50 μm size and 0.20 sec duration. The intensity was calibrated within the range of 1 to 2 watts until a white-yellow lesion was produced at the pigmented trabeculum. Occasionally, a gas bubble appeared anterior to the lesion immediately after the burn. Twenty-five spots were applied to the pigmented trabeculum of the right eye of each animal.

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This study has been supported by the United States-Israel Binational Foundation Grant 1084.
Submitted for publication Aug. 15, 1977.
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left untreated eye served as control. The laser beam was aimed at the lower sector of the angle through the Goldman prismatic goniols. The lesions were placed around four hours of the circumference, with about a lesion-size spacing between the two spots.

**Histopathological studies.** Enucleation was performed at various intervals following the laser application, beginning at the immediate postoperative period up to 8 months postoperatively. The anterior segment of each eye was bisected anteroposteriorly along the 6 to 12 o'clock meridian, into two equal parts which were then processed for light and scanning electron microscopy. Specimens for light microscopy were fixed in 10% buffered formalin solution and stained with hematoxylin and eosin, periodic acid-Schiff, and Masson trichrome stains. The specimens which were obtained for scanning electron microscopy were washed by phosphate buffer (pH 7.2 to 7.4) and then fixed in 1% glutaraldehyde in phosphate buffer containing 3.5% sucrose. Dehydration was performed in graded alcohol solutions and then by critical-point drying with CO2. The dried specimens were gold-coated in vacuo and observed by the Cambridge Stereoscan SW-40.

**Perfusion studies.** The constant-rate infusion method was used in the perfusion studies. The anterior chambers of both eyes were cannulated by a 23-gauge Venofix needle (H. Braun Melsungen A.G., Melsungen, Germany) by means of a needle gun as described by Sears. The needles were connected by means of polyethylene tubes to two transducers (P23Db Statham). Three-way stopcocks connected the transducer of each eye to a motor-driven syringe (Harvard Apparatus Co., Inc., Millis, Mass.) calibrated at constant infusion rates and to a two-channel recorder (Model 7782A, Hewlett-Packard Co., Palo Alto, Calif.) with a Carrier amplifier (Model 9805 B).

A diagram of the constant-rate infusion system is illustrated in Fig. 1. Infusion of phosphate buffer solution, pH 7.4, with 0.1 ml of heparin 5000 M.K./ml (Thrombelquin) was performed at different rates from 1 to 9 μl/min into both eyes. Both intraocular pressures (IOP's) were simultaneously recorded and allowed to rise up to a steady-state level at each infusion rate. When a steady-state level has been reached, the infusion was stopped, allowing the intraocular pressure to fall back to the baseline level. Then infusion at a new rate was activated up to IOP levels of 50 mm Hg.

One to three perfusion studies were performed on each of the six baboon primates during a period of 5 months following the laser surgery. The time schedule of the perfusion studies is listed in Table I.

**Results**

**Histopathological studies**

**Light microscopy.** Sections of specimens obtained at the immediate postoperative period...
Table I. Outflow facility of six primates' eyes at various periods following laser treatment

<table>
<thead>
<tr>
<th>Primate no.</th>
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<th>Untreated</th>
<th>Treated</th>
<th>Untreated</th>
<th>Days postoperative</th>
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<tr>
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<td>—</td>
<td>0.25</td>
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<td>3</td>
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<tr>
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<td>0.06</td>
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<tr>
<td>5</td>
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<tr>
<td>6</td>
<td>0.71</td>
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<td>0.09</td>
<td>16</td>
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<tr>
<td>Mean</td>
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<td>0.69</td>
<td>0.16</td>
<td>0.12</td>
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<tr>
<td>S.D.</td>
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<td>0.30</td>
<td>0.10</td>
<td>0.06</td>
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<tr>
<td>Corr. coef.</td>
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<td>-0.012</td>
<td>-0.654</td>
<td>-0.001</td>
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<30 days postoperative:

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<th>Untreated</th>
<th>Treated</th>
<th>Untreated</th>
<th>Days postoperative</th>
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<tr>
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<td>0.64</td>
<td>0.27</td>
<td>0.13</td>
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<tr>
<td>S.D.</td>
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<td>0.45</td>
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>30 days postoperative:

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<tr>
<th>Primate no.</th>
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<th>Untreated</th>
<th>Treated</th>
<th>Untreated</th>
<th>Days postoperative</th>
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<tbody>
<tr>
<td>Mean</td>
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<td>0.73</td>
<td>0.10</td>
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<tr>
<td>S.D.</td>
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<td>0.20</td>
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</table>

*C1 = Outflow facility as demonstrated by the angle formed at the flat part of the flow/IOP curve.
*C2 = Outflow facility as demonstrated by the angle formed at the steep part of the flow/IOP curve.
Correlation coefficient between outflow facility and postoperative time.

Specimens obtained 2 to 7 days following the laser treatment revealed considerable fibrocytic activity at the trabecular lesions, indicating an active healing process. Between 1 to 3 weeks following the laser treatment, the trabeculum has been replaced by connective tissue and appeared scarred (Fig. 3). Peri-

eral anterior iridocorneal adhesions were not demonstrated. The corneal endothelium showed no histopathological changes, and the iris root demonstrated superficial postnecrotic atrophy.

Scanning electron microscopy. Specimens obtained immediately following the laser treatment revealed deep, craterlike lesions of 100 to 300 μm in diameter in the innermost layers of the trabecular meshwork adjacent to the anterior chamber. The lesions were rounded with thinned, irregular edges (Fig. 4). Erythrocytes were demonstrated to pour out of the crater towards the anterior chamber (Fig. 5).

The specimens which were obtained 10 to 14 days following laser surgery revealed narrowing of the laser-induced openings to about 20 to 50 μm in diameter (Fig. 6). Those which were obtained at 3 weeks or later following the treatment showed smooth scar tissue, and no open holes could be demonstrated.

Perfusion studies. The perfusion studies performed on six primates revealed a characteristic flow/pressure curve as illustrated by the examples in Fig. 7.* At low flow rates of 1 to 3 μl/min, only a moderate increase of the

*In Table I the C1 and C2 values are given for all experiments. In Fig. 7, however, only graphs of experiments that have had all data points are displayed.
IOP has been observed up to 5 mm Hg. Thus the flow/pressure curve has been almost flat at the low flow rates. Increase in flow from 4.5 to 9 μl/min resulted in a rise of the IOP up to 50 mm Hg, demonstrated by a steep flow/pressure curve.

The outflow facility values are represented by the various angles formed by the flow/pressure curve and are recorded in Table I.* Two recordings from each eye, C₁ and C₂.

*In Table I the C₁ and C₂ values are given for all experiments. In Fig. 7, however, only graphs of experiments that have had all data points are displayed.
Fig. 4. Scanning electron micrograph of a laser-induced lesion immediately following laser treatment. Note the round craterlike lesion of 200 mm in diameter with burned edges in the inner layer of the trabecular meshwork. (×2000.)

Fig. 5. Scanning electron micrograph of a laser-induced lesion immediately following laser treatment. Note the erythrocytes flowing out of the lesion into the anterior chamber.
represent the angles formed at the flat and steep parts of the curve, respectively.

As shown in Table I, the outflow facility values in the flat curve (C₁) vary within a wide range from 0.23 to 1.7 μl/min/mm Hg, whereas in the steep part of the curve, these range between 0.06 to 0.36. When the total mean outflow facility (C₁ and C₂) of all the experiments was calculated, no significant difference could be demonstrated between the treated and untreated eye. However, analysis of the outflow values of the treated eyes related to the postoperative time period demonstrated a significant negative correlation (correlation coefficient −0.548 for C₁ and −0.654 for C₂). Thus, statistically, the outflow was reduced with time. In the untreated eye the outflow facility values did not show any correlation to this time period. In view of the time dependence, the means and standard deviation of the early (up to 30 days) and late (30 to 180 days) postoperative period have been calculated separately (Table I, bottom).

Comparison of the treated to untreated eye outflow values revealed a significant difference in C₂ (p < 0.025) and small significance in C₁ (p < 0.2) at the early postoperative period. At 30 days following laser treatment and thereafter, the outflow values of the treated eye were reduced, and no significant differences were demonstrated between both eyes. Comparison of the means of the outflow facilities of the treated eye during the early and late periods has shown reduction of outflow at the late period. However, according to Student t test, this difference in outflow had low significance for C₁ (p < 0.05) and was insignificant for C₂ (p > 0.5).

Discussion

The histopathological studies have demonstrated laser-induced trabecular lesions and trabeculotomies extending into Schlemm’s canal. This finding corresponds with other investigators’ findings in rhesus monkey eyes¹⁰⁻¹² and human cadaver eyes.¹³ Our energy levels of between 1 and 2 watts at 0.2 to 0.5 sec duration with a 50 μm spot size proved to be adequate and sufficient to produce such openings in the trabecular meshwork and disrupt the Schlemm’s canal.
Higher energy levels of the continuous argon beam at 4.2 and 6.0 watts which were utilized by other investigators are not required in order to create such an opening. Furthermore, these high energy levels were reported to induce additional damage such as iridodialysis, cycloidalysis, and ciliary body coagulation. Some of these effects may be beneficial in the treatment of glaucoma. However, in the present investigation, low levels were used in order to create a controlled puncture and to avoid the production of other factors which may affect the outflow of aqueous.

Multiple errors of perfusion studies in living animals have been thoroughly discussed by Bárany and Langham. Perfusion experiments seem to initiate much harm to a tested eye, and these certainly influence the results. It is evident that the total average of our perfusion studies did not demonstrate differences between the treated vs. untreated eyes. However, a statistical evaluation of the time dependence of the outflow facilities \( C_1 \) and \( C_2 \), using the correlation coefficient, revealed a significant difference at the early postlaser period. The inclination towards decreased outflow values with time is probably related to destruction of the trabecular meshwork and scar formation in the outflow channels.

Comparison of this model experiment to human glaucoma is lacking in some respects, since the animals had no glaucoma to begin with. Furthermore, these animals were young and possibly had high tendency for healing as compared to the older glaucoma patients. However, the experimental results were in keeping with the clinical failure in attempts to treat open-angle glaucoma with the argon laser.

The results in the present study correlate well with those of other works which utilized the continuous argon laser beam at intensities of 1 to 6 watts and demonstrated the ineffectiveness of this mode of energy, due to closure of the perforated holes within days to weeks following the surgery. At least from a theoretical analysis, shorter pulse durations utilizing higher energy levels might better succeed in improving the effectiveness of this treatment.

We wish to express our appreciation to Dr. I. Pollack and Dr. A. Patz from the Wilmer Institute of the Johns Hopkins Hospital, Baltimore, Md., for their collaboration in this study. We also wish to thank Mr. F. Pachys for the valuable electronics assistance he provided and Mrs. I. Hapun for her dedicated help.

**REFERENCES**