Translimbal Laser Photocoagulation to the Trabecular Meshwork as a Model of Glaucoma in Rats

Hana Levkovitch-Verbin, Harry A. Quigley, Keith R. G. Martin, Danielle Valenta, Lisa A. Baumrind, and Mary Ellen Pease

**Purpose.** To develop and characterize a model of pressure-induced optic neuropathy in rats.

**Methods.** Experimental glaucoma was induced unilaterally in 174 Wistar rats, using a diode laser with wavelength of 532 nm aimed at the trabecular meshwork and episcleral veins (combination treatment group) or only at the trabecular meshwork (trabecular group) through the external limbus. Intraocular pressure (IOP) was measured by a tonometer in rats under ketamine-xylazine anesthesia. Possible retinal vascular compromise was evaluated by repeated fundus examinations and by histology. The degree of retinal ganglion cell (RGC) loss was assessed by a masked, semiautomated counting of optic nerve axons. Effects of laser treatment on anterior ocular structures and retina were judged by light microscopy.

**Results.** After the laser treatment, IOP was increased in all eyes to higher than the normal mean IOP of 19.4 ± 2.1 mm Hg (270 eyes). Peak IOP was 49.0 ± 6.1 mm Hg (n = 108) in the combination group that was treated by a laser setting of 0.7 seconds and 0.4 W and 34.0 ± 5.7 mm Hg (n = 46) in the trabecular group. Mean IOP after 6 weeks was 25.5 ± 2.9 mm Hg in glaucomatous eyes in the combination group compared with 22.0 ± 1.8 mm Hg in the trabecular group. IOP in the glaucomatous eyes was typically higher than in the control eyes for at least 3 weeks. In the combination group, RGC loss was 16.1% ± 14.4% at 1 week (n = 8, P = 0.01), 59.7% ± 25.7% at 6 weeks (n = 88, P < 0.001), and 70.9% ± 23.6% at 9 weeks (n = 12, P < 0.001). The trabecular group had mean axonal loss of 19.1% ± 14.0% at 3 weeks (n = 9, P = 0.004) and 24.3% ± 20.2% at 6 weeks (n = 25, P < 0.001), increasing to 48.4% ± 32.8% at 9 weeks (n = 12, P < 0.001). Laser treatment led to closure of intertrabecular spaces and the major outflow channel. The retina and choroid were normal by ophthalmoscopy at all times after treatment. Light microscopic examination showed only loss of RGCs and their nerve fibers.

**Conclusions.** Increased IOP caused by a laser injury to the trabecular meshwork represents a useful and efficient model of experimental glaucoma in rats. (Invest Ophthalmol Vis Sci. 2002;43:402–410)

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From the Glaucoma Research Laboratory, Wilmer Ophthalmological Institute, Johns Hopkins University School of Medicine, Baltimore, Maryland.

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Corresponding author: Harry A. Quigley, Wilmer 122, Johns Hopkins Hospital, 600 North Wolfe Street, Baltimore, MD 21287; hqquigley@jhmi.edu.

Glaucoma is the second leading cause of blindness worldwide. The pathophysiology of glaucoma and its optimal treatment are still under investigation, although it is widely accepted that the level of intraocular pressure (IOP) is a consistent risk factor in its incidence, severity, and progression. Moreover, the main approach to therapy for glaucoma is IOP reduction.

Little is known about the nuclear and cytoplasmic signaling pathways that are involved in degeneration of retinal ganglion cells (RGCs) in glaucoma. Previous investigations have shown that RGCs die by apoptosis in glaucomatous eyes of humans, monkeys, and rats. The stages leading to apoptosis and potential inhibitors of this process are of broad interest among neuroscientists. The elucidation of apoptotic pathways in glaucoma may suggest neuroprotective therapies other than IOP reduction. The study of mechanisms and the molecular basis of glaucoma will be enhanced by reproducible, efficient animal models.

Because the level of IOP is a major risk factor in human glaucoma, one experimental approach has been to increase IOP to a level that preferentially kills RGCs without causing ancillary injury to the retina and ocular structures. Both induced and spontaneous increases in IOP simulate important aspects of RGC loss in nonhuman primates, rats, rabbits, mice, and dogs. Cioffi and Sullivan have taken an alternative approach, inducing vasoconstriction in posterior orbital blood vessels by prolonged pharmacologic exposure. Experimental IOP elevation in monkeys using laser application was suggested by Gaasterland and Kupfer and later refined. Although the monkey model of laser-induced IOP increase is robust and has been used by many laboratories, it has become too expensive for investigations that require large numbers of animals to test mechanisms of RGC death and its prevention.

The only reported methods for measuring IOP in the mouse involve invasive cannulation and methods for increasing IOP in the rat have therefore been investigated. Johnson et al. and Morrison et al. increased rat IOP by hyperosmotic saline microinjection into limbal veins, calibrating the tonometer (Tonopen XL; Mentor, Norwell, MA) to measure IOP. Shareef et al. and others treated large veins draining the anterior rat eye and used the pneumotonometer to estimate IOP. WoldeMussie and Feldman and Wijono and Ruiz treated the limbal vessels of rat eyes with a laser to decrease outflow, whereas Ueda et al. enhanced the laser uptake by ink injections into the anterior chamber before treatment.

We have attempted to produce experimental IOP elevation in more than 100 rats with each of the four approaches just described. Some of them produced elevated IOP and optic nerve damage, but the consistency of IOP elevation and the ease of production of these models were not ideal in our experience. As a result, we modified the delivery of laser energy to the anterior structures of the rat eye to develop a reliable model of glaucoma in rats. As described in the current report, our model has its own advantages and weaknesses. We present herein extensive observations on how variation in laser delivery affects the height and duration of IOP increase. In
addition, we demonstrate the RGC loss pattern over time and the effect of laser treatment on the outflow channels of the rat by histologic examination.

**MATERIALS AND METHODS**

**Animals**

Wistar rats weighing 375 to 400 g were treated with procedures approved and monitored by the Animal Care Committee of the Johns Hopkins School of Medicine and according to the procedures outlined in the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Animals were housed under a 14-hour light-10-hour dark cycle with standard chow and water provided ad libitum.

**Chronic Experimental Glaucoma Model**

To produce increased IOP we used a diode laser (Coherent Radiation, Clement-Ferrand, France) at 532 nm. Animals were anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (5 mg/kg) and topical proparacaine 1% eye drops. The laser treatment was administered unilaterally and was repeated after 1 week if the IOP difference between the treated eye and the fellow eye was less than 6 mm Hg. There were four treatment groups. The laser protocols used for each group are summarized in Table 1. Groups 1 and 2 were treated by a combination treatment of laser burns directed both at the trabecular meshwork (TM) and the episcleral veins that drain the perilimbal plexus of vessels (combination therapy; Fig. 1A). These methods differed markedly from previous rat models, in that much of the energy was directed at the TM through the cornea. Having previously observed that translimbal trabecular laser treatment causes significant trabecular damage, our purpose was to maximize angle damage, whereas minimizing injury to ciliary body and blood vessels. Groups 1 and 2 were treated using different laser settings to help establish optimal parameters. In group 1, the laser was set to deliver spots of 0.4-W power and 0.7-second duration whereas in group 2 the settings were 0.6 W and 0.5 seconds. Group 3 was treated by a combination treatment of laser burns focused directly at the limbal plexus of the veins and not diagonally at the trabecular meshwork.

**Table 1. Treatment Groups 1–4**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment Type</th>
<th>Deliveries to TM (n)</th>
<th>Deliveries to Episcleral Veins (n)</th>
<th>Laser Setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Combination</td>
<td>60–80</td>
<td>15–20</td>
<td>0.7 sec/0.4 W</td>
</tr>
<tr>
<td>2</td>
<td>Combination</td>
<td>60–80</td>
<td>15–20</td>
<td>0.5 sec/0.6 W</td>
</tr>
<tr>
<td>3*</td>
<td>Combination</td>
<td>60–80</td>
<td>15–20</td>
<td>0.2 sec/1 W</td>
</tr>
<tr>
<td>4</td>
<td>Trabecular</td>
<td>60–80</td>
<td>0</td>
<td>0.7 sec/0.4 W</td>
</tr>
</tbody>
</table>

*In group 3 the 60 to 80 burns were focused directly on the limbal plexus of the veins and not diagonally at the trabecular meshwork.

The laser beam was delivered with a slit lamp system without additional lenses. The rat eye was rotated manually to allow the laser beam to be directed in a sharp angle to the TM (Fig. 2). Treatment took

**Figure 1.** There were four laser treatment groups. Groups 1 and 2 were treated by a combination treatment of laser deliveries directed both at the TM and the episcleral veins. (A) Each circle represents an area of laser delivery. In contrast, group 3 was treated by laser deliveries focused directly at the limbal plexus and at the episcleral veins, but not at the TM (B). Group 4 was treated only by deliveries directed at the TM (C).
approximately 5 minutes per eye, performed by an experienced researcher; therefore, up to 60 rats could be comfortably treated in 1 day.

Acute Elevation of IOP
To compare brief and chronic IOP elevation, we produced acute unilateral IOP elevations for 6 to 8 hours in rats anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg; Sigma Chemical Co., St. Louis, MO). Baseline IOP measurements had been performed with the tonometer (Tonopen; Mentor). To monitor blood pressure (BP) during acute experiments, a cannula of PE-50 polyethylene tubing was placed in the femoral artery and connected to a pressure transducer. A blunt 30-gauge needle connected to a variable-height reservoir was placed in the anterior chamber to control IOP. The perfusion pressure (PP) of the eye was defined as the mean BP minus IOP. BP was monitored every 15 minutes, and IOP was adjusted by altering reservoir height to maintain a constant PP. Respiration rate and response to stimuli were monitored to aid in the appropriate administration of supplemental pentobarbital sodium. PP was maintained at 15 to 20 mm Hg, and fundus examination with indirect ophthalmoscopy was repeated every 15 minutes to assure that blood flow to the retina was not compromised. Animals were killed 6 weeks after the acute increase in IOP.

IOP Measurement in a Chronic Glaucoma Model
IOP was measured with the tonometer in both eyes before and immediately after laser treatment, every 3 to 4 days in the first 2 weeks and weekly thereafter. Rats were anesthetized with ketamine (50 mg/kg), xylazine (5 mg/kg), and topical 1% proparacaine hydrochloride. Ten measurements were obtained in each eye and the mean was calculated, as suggested by Moore et al.24,31 To standardize the measurements, we always measured both eyes in the same session and waited 5 to 8 minutes after the intraperitoneal injection so that the animal was deeply anesthetized.

For each animal, a graph of IOP over time was constructed for the treated and fellow eye. The area under the curve of treated and control eyes was calculated in units of mm Hg-days. The area under the control eye curve was subtracted from the curve of the area under the treated eye. The total area for periods when the IOP in the treated eye was higher than in the control eye was called the positive integral. The positive integral provided an estimation of the total exposure of the treated eye to increased IOP. The peak IOP increase and the mean IOP in treated eyes were also calculated.

Clinical Retina Examination
The retinal and choroidal blood vessels were observed by indirect ophthalmoscopy immediately after laser treatment and at 1 day, 3 days, 1 week, and 2 weeks afterward. Particular attention was paid to patency of vessels and to signs of retinal edema and hemorrhage.

Optic Nerve Axon Counting
Rats were killed by exsanguination under deep ketamine-xylazine anesthesia. They were perfused through the heart briefly with 4% paraformaldehyde followed by 5% glutaraldehyde in 0.1 phosphate buffer (pH 7.2), and the eyes and attached orbital optic nerves were removed. A cross section of the optic nerve from both experimental and control eyes was removed 1.5 mm posterior to the globe and postfixed in 1% osmium tetroxide in phosphate buffer. Nerves were processed into epoxy resin, sectioned at 1 μm, and stained with 1% toluidine blue.

The area of the optic nerve cross-section was measured by outlining its outer border at ×10 magnification on an image analysis system (Sensis digital camera and Metamorph software; Universal Imaging Corp., West Chester, PA). Three area measurements were taken and the mean was used. To measure the density and fiber diameter distributions, we captured images with a ×100 phase-contrast objective from 10 randomly spaced nerve regions. These were edited to eliminate non-neural objects, and the size of each axon internal to its myelin sheath (minimum diameter) and the density of axons per square millimeter were calculated for each image and for the entire nerve. The mean density was multiplied by nerve area to yield fiber number for each nerve. The total axon number in the glaucomatous eye was compared with the control fellow eye to yield a percentage loss value. The number of axons counted among the 10 images of each nerve was approximately 20% of the total optic nerve area. The counting process was performed by observers masked to the protocol used in each nerve.

Anterior and Posterior Segment Evaluation
Cross-sections 1 mm in thickness from the meshwork area and from the posterior segment of both glaucomatous and control eyes were removed and postfixed in 1% osmium tetroxide in phosphate buffer. These were processed into epoxy resin, sectioned at 1 μm, and stained with 1% toluidine blue.

Statistical Analysis
Paired and unpaired t-tests and linear regression were used for evaluation of study results.

RESULTS
In this study, we treated 174 rats with trabecular photocoagulation, using the diode laser, and 5 rats had acute IOP elevation.

Intraocular Pressure
The mean baseline IOP in 135 animals (270 eyes) before treatment and under general anesthesia was 19.4 ± 2.1 mm Hg. All treated eyes in the study groups 1 through 4 had elevated IOP during the first 48 hours after the laser treatment (Table 2). The mean IOP in the glaucomatous eyes was significantly higher than in the control eyes in groups 1, 2, and 4, but not in group 3. Group 1 eyes had the highest mean IOP difference between glaucomatous and control eyes, as well as the highest mean peak IOP. In groups 1, 2, and 4, IOP was consistently elevated for 21 days (Fig. 3), whereas group 3 eyes showed a briefer IOP spike. The eyes in group 3 were treated with burns directed at the limbal vessels with no direct treatment to the anterior chamber angle.

The percentage of eyes that were treated twice with the laser differed slightly among the groups. In group 1, 75% were treated twice (81/108 eyes), in group 2, 42% received two treatments (5/12 eyes), in group 3 all eight rats had two treatments, and in group 4, 76% (28/37) had two laser sessions.

Optic Nerve Damage
According to our counting system, normal Wistar rats eyes have a mean axon count of 87,318 ± 4,955. The 95% confi-
The evidence limit for normal axonal number is thus 11% of the mean count. We can say that a difference greater than 11% is indicative of loss, with a probability of 97.5%. The percentage loss of optic nerve axons in the glaucomatous eyes increased significantly over time in groups 1 and 4 (Fig. 4; Table 2). Groups 2 and 3 were studied only at 9 weeks after treatment, and the time course of damage therefore could not be presented. Eyes treated with a combination treatment and laser setting of 0.7 seconds and 0.4 W (group 1) had more damage than eyes with similar laser settings limited only to the TM (group 4). In group 1, the mean axonal damage at 1 week was 16.1% (H11006 14.4% (n = 8, P = 0.01, paired t-test), with six of eight eyes showing axonal loss (using the definition above). Mean axonal loss increased to 59.7% (H11006 25.7% (n = 8, P < 0.001, paired t-test) at 6 weeks and to 70.9% (H11006 23.6% (n = 12, P < 0.001, paired t-test) at 9 weeks. All treated eyes had axonal damage by 6 and 9 weeks after treatment.

The mean axonal loss with trabecular treatment (group 4) was milder than that in group 1: 19.1% (H11006 14.0% at 3 weeks (n = 9, P = 0.004, paired t-test), 24.3% (H11006 20.2% at 6 weeks (n = 25, P < 0.001, paired t-test), and 48.4% (H11006 32.8% at 9 weeks (n = 12, P < 0.001, paired t-test). Eight of 9 eyes in the 3-week group, 23 of 25 at the 6-week group, and 11 of 12 eyes in the 9-week group had axonal loss.

There was a statistically significant correlation between the amount of optic nerve damage and three assessments of IOP exposure. The strongest correlation was with the positive

### Table 2. Mean IOP, Peak IOP, and Optic Nerve Damage in the Treatment Groups

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>Eyes (n)</th>
<th>Mean IOP after 9 Weeks (mmHg ± SD)</th>
<th>Peak IOP (mmHg ± SD)</th>
<th>Axonal Loss (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glaucoma</td>
<td>Control</td>
<td>Glaucoma</td>
</tr>
<tr>
<td>Group 1: combination, 0.7 sec/0.4 W</td>
<td>108</td>
<td>25.5 ± 2.9</td>
<td>19.8 ± 1.6</td>
<td>49.0 ± 6.1</td>
</tr>
<tr>
<td>Group 2: combination, 0.5 sec/0.6 W</td>
<td>12</td>
<td>22.7 ± 3.4</td>
<td>19.5 ± 1.3</td>
<td>42.9 ± 4.0</td>
</tr>
<tr>
<td>Group 3: combination, 0.2 sec/1 W</td>
<td>8</td>
<td>19.0 ± 4.2</td>
<td>19.3 ± 3.1</td>
<td>30.1 ± 6.9</td>
</tr>
<tr>
<td>Group 4: trabecular meshwork, 0.7 sec/0.4 W</td>
<td>37</td>
<td>22.0 ± 1.8</td>
<td>19.2 ± 0.9</td>
<td>34.0 ± 5.7</td>
</tr>
<tr>
<td>Acute model (8 h)</td>
<td>5</td>
<td>65 ± 5.6</td>
<td>NR</td>
<td>70 ± 2.3</td>
</tr>
</tbody>
</table>

All data are the mean ± SD. NA, not available; NR, not recorded.
None of these eyes showed signs of impairment of the optic nerve. The mean optic nerve damage was 70.9% (group 1, n = 108) compared in glaucomatous and control eyes. One week after laser treatment, when a mean of 16.1% of axons had died, there was no clear loss of height in either layer in the glaucomatous eyes. The only visible alterations were loss of the nerve fiber layer and ganglion cell layer thickness. No change in choroidal structure was detected in any of the laser-treated eyes. The posterior segments of 36 eyes of 18 rats were examined by indirect ophthalmoscopy immediately after laser treatment and light microscopy at 9 weeks after treatment. There was no similarity to that of the human, with trabecular beams that are integral, our estimate of cumulative IOP elevation ($P < 0.0001$, $r^2 = 0.40$, linear regression; Fig. 5A). Damage was also related to peak IOP after treatment ($P < 0.0001$, $r^2 = 0.28$, linear regression; Fig. 5B). The correlation between the amount of damage and the maximal IOP difference between glaucomatous and control eyes was also statistically significant ($P = 0.003$, $r^2 = 0.16$, linear regression; Fig. 5C).

Acute IOP Elevation

We induced acute IOP elevation in five eyes of five rats for 6 to 8 hours at IOP of 15 to 20 mm Hg (mean IOP, 65.0 ± 6.0). Six weeks later, the mean axonal loss among these eyes was −1.4% ± 10.8% (n = 5, $P = 0.9$, pair $t$-test), indicating no optic nerve damage. None of these eyes showed signs of impairment of blood flow in the retina or choroid.

Axonal Size Distribution

To compare the loss of axons by diameter group, the proportionate loss in each size group from 0.1 to 1.0 μm was compared in glaucomatous and control eyes. One week after laser treatment, when a mean of 16.1% of axons had died, there was a significant trend toward greater loss among large-diameter fibers (Fig. 6A, $P < 0.0001$, $r^2 = 0.65$, n = 8, linear regression). By 9 weeks after treatment, when an average of 70.9% of axons were gone, there was no selective loss (Fig. 6B, $P = 0.59$, $r^2 = 0.0050$, n = 12, linear regression).

Retinal Examination during Chronic Glaucoma Experiments in Rats

Indirect ophthalmoscopy immediately after laser treatment and during the first 2 weeks thereafter revealed no clinical abnormality in blood flow, and no retinal edema was observed ($n = 88$ rats).

Histologic Examination of the Retina

The posterior segments of 36 eyes of 18 rats were examined by light microscopy at 9 weeks after treatment. There was no difference between control and eyes with elevated IOP in the outer retinal layers (Fig. 7). The heights of the outer and inner nuclear layers were measured in several specimens, but there was no clear loss of height in either layer in the glaucomatous eyes. The only visible alterations were loss of the nerve fiber layer and ganglion cell layer thickness. No change in choroidal structure was detected in any of the laser-treated eyes. The specific features of the choroid that were examined were areas devoid of blood vessels or with occluded blood vessels and areas that were dramatically thinner than normal. The mean axonal loss in the eyes of these rats with chronic IOP elevation was 69.0% ± 23.0%.

Anterior Segment Histology

Specific attention was given to examination of the outflow channels, the ciliary body, the iris, and the episcleral area of 20 eyes of 10 laser-treated rats. Six rats were treated by a combination treatment (group 1) and four rats were treated by trabecular treatment (group 4), and all were killed 6 weeks after laser treatment. The normal rat outflow area has some similarity to that of the human, with trabecular beams that are
covered by endothelial cells leading to a large channel that is the equivalent of Schlemm’s canal. Outflow vessels are occasionally seen connecting from this channel to the episcleral vessels. The iris inserts posterior to this trabecular area, and the ciliary body with its two layers of epithelium is located on the peripheral iris, somewhat more anteriorly than in the human eye.

In most of the laser-treated animals, there were clear abnormalities in the outflow channels. These included loss of intertrabecular spaces, compaction of trabecular beams, and partial or complete obliteration of the Schlemm’s canal–like channel. In some areas, the iris was scarred to the angle over the meshwork (peripheral anterior synechia). In two treated eyes, there were red blood cells in the anterior chamber remaining from hyphema. In the six rats in group 1, the ciliary body was partially atrophied, whereas in the four rats in group 4 the gross appearance of the ciliary body was normal (Fig. 8).

Complications

After laser treatment, some ocular complications were noted. In group 1, 8% of the treated eyes showed development of hyphema that resolved clinically in less than 1 week. Corneal opacities severe enough to prevent a clear view of the posterior segment were noted in only 7%. In group 2, 40% had hyphema, which resolved in most within 48 hours. Severe corneal opacities were noted in 8%. In group 3, 30% had hyphema and 20% had severe corneal changes. Thirteen percent of the eyes in group 4 had hyphema, and none had severe corneal changes. Eyes treated in the acute model did not have any complications.

**Discussion**

The ideal glaucoma model in rats should be simple to produce, inexpensive, reproducible, and as similar to human glaucoma in its effects as possible. We suggest that the translimbal photocoagulation model in rats meets many of these criteria. The model produced elevated IOP in nearly every treated eye. Six weeks after treatment, 50% to 60% of RGCs had died, and some RGC loss was present in most eyes. The significant damage was confined to the RGC layer, nerve fiber layer, and optic nerve axons. The outflow channels in the anterior chamber were clearly abnormal. No clinical fundus abnormality was detected by indirect ophthalmoscopy, despite IOP as high as 50 mm Hg. The mean blood pressure of the rat is typically higher than 70 mm Hg (measured under anesthesia by arterial cannulation in our acute experiments). In the awake state, blood pressure may be higher. Thus, the IOP generated by this model is unlikely to be high enough to occlude major retinal or choroidal vessels. Consistent with this conclusion, we found no loss of the midretinal architecture in the area supplied by retinal arteries and no sign of choroidal infarction. Although the rat RGC and optic nerve head structure differ substantially from the human, it appears that elevated IOP causes a selective loss of RGCs, due at least in part to axonal injury at the area of the optic nerve just at and behind the eye.

Groups 1, 2, and 4 showed significant optic nerve damage at 6 weeks. Group 3, in which treatment was directed toward the vessels and not at the TM, showed insignificant damage. In addition, animals treated by acute elevation of IOP did not show any axonal damage. The laser treatment of group 1 lead...
to the most significant optic nerve damage associated with the highest mean and peak IOP. Damage to optic nerve axons therefore was clearly related to IOP elevation, and more than a brief, high increase in IOP was necessary to produce damage. This type of treatment (group 1) may be used for investigating cellular processes and mechanisms of RGC death in glaucoma, because it produced substantial damage in a relatively short period. If the neuroprotective effect of a new drug were to be investigated, it may be beneficial to use the model with the laser setting used in group 4, because the damage in these animals was substantial but occurred more slowly. This would facilitate the detection of the effect of a proposed therapy better than in eyes with rapid, severe optic nerve injury.

The translimbal photocoagulation technique is simple to perform, and a large number of animals can be treated in 1 day. For those who are familiar with slit lamp and laser use, the method could be learned in 1 hour with direct supervision. If an investigator were not familiar with ophthalmic instrumentation, the learning time would be longer. However, it requires access to a diode or similar continuous wave laser. In our opinion, this method is simpler to perform than the saline injection or the vein cautery methods, based on our personal experience with performing each of these other methods in more than 150 rats each. The saline injection model described by Morrison et al. requires extensive construction of fine glass needles and tubing, as well as excellent microsurgical skills. The vein cautery model also requires a sterile approach, surgical instruments, magnification through loupes or operating microscope, and considerable experience to recognize the surgical landmarks and variations in vein anatomy in the rat.

The expense of any rodent model is favorable compared with the use of monkeys, yet even the rat models have substantial equipment, facilities, and personnel costs. Our approach requires a laser costing thousands of dollars. The saline and vein cautery methods require animal surgical facilities and surgical instruments. Our method is performed by one investigator, whereas the other methods are possible to perform alone, but are more conveniently accomplished with an assistant, thus increasing the cost of personnel.

**Figure 7.** Histologic examination by light microscopy of the retina in a glaucomatous rat eye (group 1) with more than 60% RGC loss at 9 weeks after treatment (B, D) and a normal rat eye (A, C). The RGC and nerve fiber layers were affected in the glaucomatous eye with obvious RGC loss (D). 1% Toluidine blue; magnification, (A, B) ×20; (C, D) ×40.
to have continued IOP elevation over longer periods, 3 weeks of elevated IOP produces substantial RGC loss, sufficient for most investigations. If longer IOP elevation is desired, another laser treatment can be performed, but this increases the occurrence of corneal decompensation. We were concerned that all the injury to RGCs may have been due to very brief, high IOP in the first day after laser treatment. To investigate this possibility, we performed 6- to 8-hour IOP elevations. Because these did not damage the RGCs, it is clear that chronic IOP elevation is needed to cause damage. It can be argued that 3 weeks of elevated IOP is not really a chronic model. Considering that the life span of a rat is 2 years, 3 weeks of its life may be comparable to 2 years in the life of a human who lives to be 80 years old.

We measured IOP in rats under ketamine-xylazine anesthesia with a tonometer (Tonopen XL; Mentor) immediately after laser treatment, every 3 days for 2 weeks, and weekly thereafter. It would be ideal to have as many measurements as possible. Jia et al.44 trained Brown Norway rats to allow daily tonometry without anesthesia. Unfortunately, we have found that this is not possible with Wistar rats. General anesthesia lowers IOP in the rat, but all experiments are performed by comparing the IOP of the treated to the fellow, normal eye. Thus, anesthesia affects the absolute, but not the relative, IOP difference. IOP in rats varies in a circadian fashion, higher in the evening and at dark than in the morning and in light.35,36 We kept the rats in a normal lighting rhythm to allow their normal IOP diurnal pattern, reasoning that unknown effects may be caused by severe alteration of the animals' environment. Although Jia et al.45 found no damage to photoreceptors in rats from constant light exposure, our albino rats may be more susceptible to light toxicity.

Over time, corneal abnormalities developed in the treated eyes, including dry eyes, opacities, and even atrophic ulcers. These complications were also noted by us (unpublished observations, 2000) in other glaucoma models in rats.

Of particular interest, we found that RGC loss continued after the period of major IOP elevation. Possibly, neuronal death from primary injury lags behind the initial event of IOP increase. Alternatively, the continued loss of RGCs weeks after restoration of normal IOP may be due to secondary degeneration. This process can be defined as death of RGCs that survive the primary insult but are injured by toxic effects of the primary degenerating neurons. Secondary degeneration has been shown to occur in RGCs after partial optic nerve transection.57,58

We have shown that the amount of optic nerve damage is correlated with the cumulative IOP exposure, as well as with peak IOP and maximal IOP difference between the glaucoma and control eye. Johnson and al.59 have also found that optic nerve damage is linearly correlated with IOP.

Previous studies have shown a preferential loss of large optic nerve axons in human glaucomatous eyes and in monkey eyes with modest optic nerve damage.40–42 In the present investigation, there was selectively greater loss of larger RGC axons at 1 week after treatment, when damage was mild, but the selectivity was not still present when three fourths of the nerve was gone. This is logical, because larger axons make up a small proportion of the rat, monkey, and human optic nerve. Clearly, after loss of the majority of axons, it would be surprising to find any selectivity, because all RGC sizes would have to be affected. We were somewhat surprised to find any selectivity in this rat model, because the rat appears to have less distinct segregation of RGCs by size, and because the damage was produced quite rapidly, minimizing the chance of detecting subtle differences in RGCs loss. In every investigation of human eyes with glaucoma that has studied this question, selective loss of larger RGCs was found at the stage of mild damage. It has been suggested that this finding may be due to

A method that does not reliably produce increased IOP and optic nerve damage has the obvious cost of wasted animals and the time and material spent on animals in which no IOP elevation occurs. Our experience with the saline and vein cauterity models indicated that approximately half the animals had IOP increases insufficient to produce RGC loss. This led to a waste of half the time and expense for IOP measurement and histologic counting of RGCs or axons. In contrast, one of the main advantages of the translimbal photocoagulation model is that it is effective at producing IOP elevation and damage in most animals.

In the laser model, mean IOP returned to baseline in many animals by 3 weeks after treatment. Although it would be ideal

**Figure 8.** Histologic examination by light microscopy of the anterior chamber angle of a normal rat eye (A) and a group 4 rat eye 6 weeks after laser treatment (B). In the normal eye, the TM had large spaces, and the outflow channel was wide. Arrowhead: posterior aspect of large canal for aqueous humor. Arrow: anterior chamber side of the trabecular system, containing two to four cords of tissue between the anterior chamber and the outflow canal. In the experimental glaucoma eye (B), there was loss of intertrabecular spaces and narrowing of the outflow channel. Arrowhead: the posterior limit of a much-reduced canal just below it in this view; arrow: position at which the normal trabecular cords with their intertrabecular spaces are found in the normal eye. Instead, the laser-treated eye had solid fibrous tissues and the iris was adherent along the entire length of the meshwork in this area (from the arrow to the arrowhead). 1% Toluidine blue; magnification, ×40.
shrinkage of axons rather than selectivity; however, there was no shift of the distribution of axonal diameter to smaller sizes. In summary, we have shown that translimbal laser photocoagulation to the TM reliably causes sufficient IOP elevation to produce significant optic nerve damage. Thus, this glaucoma model can be used to investigate cellular events that lead to RGC death and to evaluate the effect of new treatment strategies.

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References