Experimental Mouse Ocular Hypertension: Establishment of the Model

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PURPOSE. To establish an experimental model of ocular hypertension in the mouse.

METHODS. Twenty-two black Swiss mice were used. After anesthesia and pupil dilation, the anterior chamber was flattened by the aspiration of aqueous humor. Laser photocoagulation (532-nm wavelength, 200-mW power, 0.05-second duration, 200-μm spot size) then was performed at the limbus. Intraocular pressure (IOP) was measured weekly for 4 weeks and biweekly for 12 weeks, by a microneedle method. Slit lamp biomicroscopy was performed throughout the period and the structural changes were assessed histologically. A treatment response was considered to be a success if either the mean of IOP measurements collected during the first 4 weeks was increased by 30% or more, or the mean of all measurements collected during the 12 week study period was increased by 30% or more.

RESULTS. Laser-treated eyes showed significantly higher IOP than control eyes from 1 to 6 weeks (P < 0.001). The average IOP in treated eyes during the first 4 and 12 weeks was significantly higher than the control IOP (P < 0.001). These IOP increases were 7.1 and 3.8 mm Hg, respectively. During the first 4 weeks, sustained elevation of IOP was obtained in 64% (14/22) of the treated eyes. During the entire 12-week study, increased IOP was successfully maintained in 37% (7/19) of the treated eyes. After 6 weeks, elevated IOP often returned to normal or several mm Hg below normal. Histologic analysis at the end of the 12-week study showed no inflammatory cells in the anterior segment and confirmed that the angle was closed by the laser photocoagulation treatment.

CONCLUSIONS. This method produces persistent IOP elevation in mouse eyes and may be a promising experimental model for the investigation of the molecular mechanisms of glaucomatous optic neuropathy. (Invest Ophthalmol Vis Sci. 2003;44:4314–4320) DOI:10.1167/iovs.03-0137

Persistent elevation of intraocular pressure (IOP) is the leading risk factor for optic disc damage in glaucoma. However, the molecular mechanisms of these variations or of pressure-induced optic nerve damage in general remain poorly understood.

The ability to alter levels of specific proteins in vivo by transgenic technology offers considerable promise in clarifying the molecular mechanisms of glaucomatous optic neuropathy. This approach has been strengthened recently by observations that the mouse eye has a well-defined trabecular meshwork, conventional and uveoscleral outflow of aqueous humor, differentiated Schlemm’s canal and ciliary muscle, and a vascularized retina. In addition, many physiological parameters and responses are similar in mouse and human eyes. For example, a recent survey of more than 30 mouse strains found that average daytime intraocular pressure (IOP) ranges between 10 and 20 mm Hg. We have further developed the method used in this study for the measurement of IOP in the mouse and reported the drug response in mouse eyes to the IOP-lowering agent, latanoprost. These reports suggest IOP regulation is similar in mouse and human eyes. Together, the findings suggest that an experimental treatment that elevates mouse IOP may be useful for clarifying the mechanism of pressure-induced loss of optic nerve axons in glaucoma.

A well-studied method for elevating IOP in monkey eyes is the application of laser burns to outflow tissues. However, monkeys are a limited resource and thus not suitable for experiments requiring large numbers of subjects. Moreover, transgenic technology is not practical in the monkey. Elevation of IOP in rat eyes has been achieved by injection of hypertonic saline into episcleral veins, catarization of episcleral veins, or laser treatment. However, few transgenic rat models are presently available. In contrast, many transgenic mouse models are available. Thus, the development of a treatment to obtain sustained elevation of IOP in the mouse could be advantageous for transgenic studies of the molecular mechanisms of glaucomatous optic nerve damage. There is no prior report describing a method to achieve persistent elevation of mouse IOP. The present article describes the use of laser treatments to obtain sustained IOP elevation in mouse eyes.

MATERIALS AND METHODS

Animal Husbandry

All animals were performed in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Twenty-two Black Swiss mice were obtained from Taconic Laboratories (Germantown, NY). Mice were bred and housed in clear cages covered loosely with air filters and containing white pine shavings for bedding. The environment was kept at 21°C with a 12-hour light–dark cycle (6 AM to 6 PM). All mice were fed ad libitum. Animals ranged in from 8 to 12 weeks.

Anesthesia

The mice were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg; Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (9 mg/kg; TranquiVed; Vedco Inc., St. Joseph, MO), prepared at room temperature. They were gently restrained in a clear plastic film, truncated cone (Decapicone; Braintree Scientific, Inc., Braintree, MA) to avoid stress, and anesthesia was administered with a 30-gauge needle. Each mouse was monitored carefully to assess the state of anesthesia. If the mouse displayed no response to pinching of...
the back skin, it was placed on the platform for IOP measurement and the surgical procedure.

**Procedure for the Obstruction of Aqueous Outflow**

Aqueous outflow was obstructed by laser photoocoagulation of the corneal limbus. For enhancement of the effect of this treatment, mydriasis was induced, and the anterior chamber was flattened before laser photoocoagulation (Fig. 1). The specific procedures are described in the following sections.

**Mydriasis.** One eye in each mouse was dilated by topical treatment with 4 μL of a mixture of mydriatic agents (atropine, tropicamide, phenylephrine, and cyclopentolate; 0.25% each) applied 30 minutes before the procedure was initiated.

**Flattening of the Anterior Chamber.** After anesthesia, the mouse was placed on a platform under a stereo microscope. A drop of phosphate-buffered saline (PBS) was placed on each cornea to avoid desiccation. The microneedle was made of borosilicate glass tubing (outside diameter 1.0 mm, inside diameter 0.58 mm; Kwik-Fil; World Precision Instruments Inc., Sarasota, FL), pulled with a pipette puller (P87; Sutter Instruments, Novato, CA), and the tip was beveled to 30° with a microgrinder (Micropipette Beveler; WPI). The outer diameter of the tip was 75 to 100 μm. The microneedle was connected to a 1-mL syringe that was mounted on a micromanipulator (WPI). The microneedle was then inserted into the anterior chamber with the bevel upward, and the aqueous fluid was aspirated. After verification that the anterior chamber was flattened, the needle was withdrawn.

**Laser Photoocoagulation of Limbus.** Immediately after the anterior chamber was flattened, the anesthetized mouse was placed on the platform of a biomicroscope with a diode laser system (532 nm, 100 mW, 0.05 seconds, and 200 μm, respectively). The laser power, duration, and spot size were 100 mW, 0.05 seconds, and 200 μm, respectively. Mydriasis, such as atropine, tropicamide, phenylephrine, and cyclopentolate (0.25% each) was applied 30 minutes before laser treatment.

**Laser treatment**

200 μm spot, 100 mW, 0.05 sec

60 shots on the limbus

**IOP Measurement**

After laser treatment, IOP of the treated eye (IOPtx) and nontreated control eye (IOPc) was measured by our previously described microneedle method every week for the first 4 weeks and biweekly thereafter until 12 weeks had elapsed. The cornea, anterior chamber, and optic disc were then examined with a slit lamp biomicroscope.

**Evaluation of IOP Increase**

The change in IOP (IOPtx − IOPc) was calculated, and the percentage increase was determined according to the following formula: ΔIOP(%) = 100 × (IOPtx − IOPc)/IOPc. The difference between IOPtx and IOPc was statistically analyzed by paired t-test. A treatment response was considered to be a success if either the mean of IOP measurements collected during the first 4 weeks was increased by 30% or more or the mean of all measurements collected during the 12-week study period was increased by 30% or more.

**Histologic Analysis**

For evaluation of the effect of laser treatment and the inflammatory response after the treatment, the anterior segments of the treated and nontreated eye were fixed with 2% paraformaldehyde, and frozen sections of the eye were prepared for microscopic analysis. The sections were stained by toluidine blue to aid visualization of optic structures.

**Recovery of Anterior Chamber and IOP.** The flattened anterior chamber and the laser photoocoagulation may induce brief temporal hypotension, which could affect the sensitivity to axonal damage from ocular hypertensive damage. To assess the recovery of anterior chamber depth after flattening and laser photoocoagulation, images of the anterior chamber were taken before, during, 30 minutes after, and 60 minutes after the procedure. IOP was measured 5 hours after the procedure to assess the recovery of IOP. Moreover, IOP in the next 5 days after the procedure was measured in one group that underwent the procedure for flattening the anterior chamber only (n = 6) and in another group that underwent flattening of the anterior chamber and laser photoocoagulation (n = 6).

**FIGURE 1.** Diagram of the procedure for the elevation of IOP. First, mydriatic agents and atropine were applied 30 minutes before laser treatment (left). Second, aqueous fluid was aspirated by a microneedle to flatten the anterior chamber and close the angle (middle). Third, laser beams with a 532-nm wavelength were applied to the limbus around the cornea (right). The laser power, duration, and spot size were 100 mW, 0.05 seconds, and 200 μm, respectively.

**FIGURE 2.** Changes in anterior chamber depth before and after flattening of the anterior chamber. (A) Before the flattening of the anterior chamber. (B) Flattening of the anterior chamber by aspiration of the aqueous fluid with a microneedle caused the iris to move forward. (C) By 30 minutes after the procedure, the anterior chamber had deepened. (D) By 60 minutes after the procedure, the anterior chamber had recovered its depth. Magnification, ×14.
RESULTS

Effect of Laser Treatment Alone

Pilot studies were conducted in 48 mice to determine whether laser photocoagulation to obstruct Schlemm’s canal could induce elevated IOP. The spot size, laser power, duration, and spots were 50 to 200 μm, 100 to 500 mW, 0.05 to 0.2 second, and 30 to 80 spots, respectively. No persistent elevation of IOP over 2 weeks was induced in any of the treated eyes, and hyphema or phthisis was observed in 26 (54.2%) of the eyes. In the remaining mice, the average IOP of NIH Black Swiss mice was 16.3 mm Hg (mean ± SD) to 16.9 ± 3.9 mm Hg during the entire 12-week study period. Maximum IOP throughout the period was 39.6 mm Hg.

Fifteen (68%) of the 22 treated eyes had more than a 30% IOP increase at 1 week (mean IOP increase: 91% ± 39%). After 4 and 12 weeks, 9 (41%) of 22 and 5 (26%) of 19 eyes maintained more than a 30% IOP increase and the mean increase was 62% ± 17% and 47% ± 20%, respectively (Fig. 3B).

To account for the fluctuation of IOP in each eye, the average IOPs during the first 4 weeks and during the entire 12 weeks were determined (Table 2). Average IOPtx was significantly higher than the average IOPc during both periods ($P < 0.001$).

During the first 4 weeks, 14 (64%) of 22 eyes had an IOP increase of more than 30%. During the entire 12-week study, 7 (37%) of 19 eyes maintained an IOP increase of greater than 30%. In the eyes with successfully maintained high IOP, a dilated pupil and peripheral anterior synechia around anterior chamber angle were observed by biomicroscopy (Fig. 4).

Preliminary Studies with Anterior Chamber Flattening

In considering flattening of the anterior chamber by aqueous aspiration before laser treatment, it was important to determine how quickly the anterior chamber depth would recover and how quickly IOP would begin to increase toward normal. Aspiration and recovery of the anterior chamber were followed by biomicroscopy, as shown in Figure 2. The anterior chamber was flattened by the aspiration of aqueous fluid with a microneedle (Fig. 2B). It partially recovered its depth by 30 minutes after aqueous aspiration (Fig. 2C) and, by 60 minutes (Fig. 2D), showed a depth similar to the original depth (Fig. 2A).

To assess IOP recovery, measurements were obtained at 5, 24, 48, and 72 hours after flattening. Normal IOP of NIH Black Swiss mice was 16.3 ± 3.0 mm Hg (mean ± SD, $n = 6$). IOP measured 5 hours after flattening of the anterior chamber was 8.9 ± 1.1 mm Hg (mean ± SD, $n = 4$). As shown in Table 1, IOP after flattening the anterior chamber returned to normal in the next day (16.0 ± 2.8 mm Hg) and was maintained over the next 2 days. Eyes also were treated by flattening followed by laser treatment as described earlier. As shown in Table 1, IOP in these eyes either was the same as before treatment, or moderately increased, but to less than 36 mm Hg.

Long-Term IOP Changes Induced by Laser Treatment

One week after laser treatment of 22 eyes, IOP was markedly elevated (Fig. 3). The average IOP of the treated eyes (IOPtx) remained significantly higher than that of IOPc until 6 weeks after laser treatment ($P < 0.001$, Fig. 3A). Three mice died of unknown causes between 5 and 12 weeks after treatment. In the remaining mice, the average IOPc was stable and ranged from 15.9 ± 3.0 (mean ± SD) to 16.9 ± 3.9 mm Hg during the entire 12-week study period. Maximum IOP throughout the period was 39.6 mm Hg.

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To account for the fluctuation of IOP in each eye, the average IOPs during the first 4 weeks and during the entire 12 weeks were determined (Table 2). Average IOPtx was significantly higher than the average IOPc during both periods ($P < 0.001$).

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![Image](image-url)
Complications of the Laser Treatment

All observed complications of laser-treated eyes are summarized in Table 3. The 22 laser-treated eyes were separated into two groups by the extent of IOP elevation. Group A included 14 eyes with more than a 30% increase of IOP for 4 weeks and group B included 8 eyes that had less than a 30% increase. Throughout the total study period, untreated eyes did not have any complication. In the laser-treated eyes, corneal edema, corneal opacity, and cataract were observed during the study period, but there was no observable change of the retinal vessels or evidence of ocular infection. Immediately after the laser treatment, hyphema was observed in one eye of each group. This was no longer apparent by 1 week after treatment. One week after treatment, corneal edema was seen in 4 of 14 eyes in group A. This edema had resolved in each animal by the second week after treatment. Corneal opacity was present in 2 of 14 of the group A eyes at the second week and was completely resolved by the sixth week of the study. opacity also was seen in two of eight eyes in group B, but did not resolve by the end of the study. Cataract formation was observed only in one group A eye and in two group B eyes.

Histologic Analysis of the Anterior Segment

Histologic analysis of paraffin-embedded eye sections showed that the angle of laser-treated eyes was completely closed (Fig. 5). In addition, Schlemm’s canal was not observable in the laser-treated eyes. Inflammatory cells were not observed around the limbus.

DISCUSSION

Flattening of the anterior chamber followed by laser photocoagulation successfully induced persistent elevation of IOP for at least 6 weeks in mouse eyes. More than a 30% increase of mean IOP elevation during 4 and 12 weeks was detected in 64% and 37% of treated eyes, respectively, without any severe complications. Several methods have been reported to increase IOP in other animals, and we tried to apply them to the mouse eye. Currently methods used are laser photocoagulation to the trabecular meshwork in monkey and rat, catarization to episcleral veins in rat, and hypertonic saline injection in rat. However these methods are challenging to apply to the smaller mouse eyes. Moreover, we found that the outflow vessels of

Table 2. Average IOP in Laser-Treated Eyes (IOPtx) and Contralateral Control Eyes (IOPc) during the First 4 Weeks and during the Entire 12-Week Study Period

<table>
<thead>
<tr>
<th>Mouse</th>
<th>IOPtx (mmHg)</th>
<th>IOPc (mmHg)</th>
<th>ΔIOP (mmHg)</th>
<th>ΔIOP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.7 ± 4.1</td>
<td>15.0 ± 1.8</td>
<td>8.7 ± 2.6</td>
<td>57.8 ± 14.4</td>
</tr>
<tr>
<td>2</td>
<td>29.0 ± 5.1</td>
<td>15.0 ± 1.0</td>
<td>14.0 ± 4.7</td>
<td>93.1 ± 31.4</td>
</tr>
<tr>
<td>3</td>
<td>24.8 ± 7.4</td>
<td>15.4 ± 0.5</td>
<td>9.4 ± 7.2</td>
<td>60.6 ± 46.2</td>
</tr>
<tr>
<td>4</td>
<td>268 ± 4.6</td>
<td>17.5 ± 1.4</td>
<td>9.3 ± 5.7</td>
<td>55.0 ± 56.7</td>
</tr>
<tr>
<td>5</td>
<td>18.6 ± 3.5</td>
<td>12.1 ± 0.6</td>
<td>6.5 ± 3.7</td>
<td>54.6 ± 32.0</td>
</tr>
<tr>
<td>6</td>
<td>261 ± 8.6</td>
<td>15.9 ± 1.0</td>
<td>10.2 ± 8.9</td>
<td>64.8 ± 59.2</td>
</tr>
<tr>
<td>7</td>
<td>19.8 ± 2.7</td>
<td>16.3 ± 2.7</td>
<td>3.5 ± 4.0</td>
<td>21.4 ± 27.8</td>
</tr>
<tr>
<td>8</td>
<td>18.7 ± 7.6</td>
<td>11.8 ± 2.0</td>
<td>7.0 ± 5.9</td>
<td>55.9 ± 41.7</td>
</tr>
<tr>
<td>9</td>
<td>225 ± 8.1</td>
<td>13.0 ± 3.5</td>
<td>9.5 ± 5.5</td>
<td>71.5 ± 28.4</td>
</tr>
<tr>
<td>10</td>
<td>18.7 ± 2.3</td>
<td>16.2 ± 1.9</td>
<td>2.5 ± 1.4</td>
<td>15.8 ± 8.3</td>
</tr>
<tr>
<td>11</td>
<td>23.9 ± 4.0</td>
<td>15.0 ± 1.3</td>
<td>9.0 ± 4.5</td>
<td>61.3 ± 34.6</td>
</tr>
<tr>
<td>12</td>
<td>20.1 ± 2.3</td>
<td>15.5 ± 0.6</td>
<td>4.7 ± 2.0</td>
<td>30.1 ± 12.3</td>
</tr>
<tr>
<td>13</td>
<td>19.0 ± 2.3</td>
<td>15.5 ± 0.7</td>
<td>3.5 ± 2.2</td>
<td>22.8 ± 14.2</td>
</tr>
<tr>
<td>14</td>
<td>18.4 ± 3.4</td>
<td>15.8 ± 0.8</td>
<td>2.7 ± 4.0</td>
<td>17.6 ± 26.9</td>
</tr>
<tr>
<td>15</td>
<td>18.6 ± 6.8</td>
<td>15.6 ± 0.5</td>
<td>3.0 ± 6.6</td>
<td>18.7 ± 41.8</td>
</tr>
<tr>
<td>16</td>
<td>17.1 ± 3.5</td>
<td>17.6 ± 0.7</td>
<td>0.5 ± 3.6</td>
<td>2.4 ± 21.0</td>
</tr>
<tr>
<td>17</td>
<td>32.0 ± 3.8</td>
<td>19.9 ± 0.8</td>
<td>12.2 ± 4.3</td>
<td>61.8 ± 23.1</td>
</tr>
<tr>
<td>18</td>
<td>28.6 ± 5.7</td>
<td>19.3 ± 1.0</td>
<td>9.3 ± 6.5</td>
<td>49.4 ± 35.8</td>
</tr>
<tr>
<td>19</td>
<td>18.8 ± 2.5</td>
<td>19.8 ± 0.9</td>
<td>-1.1 ± 1.8</td>
<td>-5.6 ± 9.2</td>
</tr>
<tr>
<td>20</td>
<td>24.6 ± 9.2</td>
<td>19.2 ± 1.7</td>
<td>5.4 ± 8.6</td>
<td>27.3 ± 42.4</td>
</tr>
<tr>
<td>21</td>
<td>29.9 ± 6.9</td>
<td>18.5 ± 2.5</td>
<td>11.4 ± 6.7</td>
<td>65.2 ± 42.3</td>
</tr>
<tr>
<td>22</td>
<td>35.3 ± 4.7</td>
<td>18.2 ± 1.6</td>
<td>17.1 ± 3.8</td>
<td>93.9 ± 19.5</td>
</tr>
<tr>
<td>Total</td>
<td>23.4 ± 5.1</td>
<td>16.3 ± 2.3*</td>
<td>7.1 ± 4.6</td>
<td>45.1 ± 27.6</td>
</tr>
</tbody>
</table>

Three mice died after 5 weeks. All data are shown as the mean ± SD. Shaded numbers in the ΔIOP (mm Hg) columns indicate a greater than 5-mm Hg increase. Shaded numbers in the ΔIOP (%) columns indicate a greater than 30% increase.

*Statistically significant difference between IOPtx and IOPc by the paired t-test (P < 0.001).

FIGURE 4. Anterior segment in a treated eye 4 weeks after treatment observed by biomicroscopy. The pupil was dilated (arrow) and the peripheral anterior synchiae were present (arrowheads). Magnification, ×20.
the mouse eye form a fine plexus, so that large episcleral veins suitable for this surgical treatment were not readily observable. In addition, we found that the mouse sclera was too thin to receive cautery treatments without also inducing undesired complications. Intracameral injection of collagen gel and beads to raise the outflow resistance and the subconjunctival injection of growth factors to promote the production of extracellular matrix in outflow tissues also failed to elevate IOP, as shown in Table 4. Direct laser photocoagulation of the angle also induced severe bleeding in all cases, which persisted for more than 1 week. Moreover, half of treated eyes showed phthisis. Laser photocoagulation without flattening of the anterior chamber was applied to Schlemm’s canal and venous plexus. As a result, some of eyes showed increased IOP. However, the intensity, persistence, and reproducibility of IOP elevation were insufficient to use these treated eyes as high-IOP models. Finally, the procedure described herein, with induced angle closure achieved by flattening of the anterior chamber and subsequent laser photocoagulation to the corneal limbus, achieved consistent elevation of IOP in the mouse.

The persistent increase of IOP with this procedure appears to be based on the obstruction of the outflow pathway by angle closure (Figs. 4, 5). The intentional closing of the angle by flattening of the anterior chamber may be useful in enhancing the effect of photocoagulation to obstruct the outflow through the angle by direct attachment of the iris root to the peripheral cornea. Moreover, the closed angle space may be useful in localizing the bleeding space, even if the bleeding occurred. In the initial reaction of eyes to this treatment, severe inflammation due to breakdown of the blood–aqueous barrier and a substantial increase of IOP were not observed. This may reflect both the anti-inflammatory effect of atropine and the aqueous leakage through the small hole in the cornea that was created when it was perforated to flatten the anterior chamber. This indicates that the present model appeared to avoid the complications and abrupt increase of IOP that typically occur during the initial few hours in human acute angle-closure glaucoma. Moreover, IOP in the laser-treated group was less than 36 mm Hg during the first 3 days (Table 1) and less than 40 mm Hg throughout the observation period (Fig. 5B). Thus, this model differs from a model of acute ischemia. Although the brief hypotony induced by this procedure may affect the optic nerve damage, the anterior chamber was deep 1 hour later (Fig. 2), and IOP was 9 mm Hg by 5 hours later. IOP was normal by the day after the procedure in the group that underwent flattening of the anterior chamber only. Also, in the group that underwent flattening of the anterior chamber and laser treatment, IOP was greater than 20 mm Hg by the next day (Table 1). These data indicate that the hypotony associated with anterior chamber flattening lasted for only a short time and thus was unlikely to contribute to the overall damage.

There are several advantages and limitations associated with this procedure. One advantage is the relatively high success rate of IOP elevation after a single procedure. Sufficient elevation of IOP using laser application alone in monkey or hypertonic saline injection in rat requires multiple procedures.9,10,14,16–21 Repeated procedures may increase the risk of complications and affect the outcome.

Another advantage is the transparency of the anterior segment throughout the observation period, which allows biomicroscopic evaluation of the anterior chamber throughout the study and may facilitate clinical evaluation of the damage to the retina and optic disc. With this method, a partial opacity of the cornea in some eyes disappeared relatively quickly and cataract formation was observed in only one eye of the successfully treated eyes (group A, Table 3). Application of mydriatic agents and atropine is useful, not only to reduce the complications such as hyphema and inflammation, but to maintain the mydriatic state, which provides for observation of the fundus throughout the duration of the study (Fig. 4).

A limitation of our method is that laser treatment and flattening of the anterior chamber may induce inflammation. At 1 week after the procedure, 6 of 22 animals could not be examined with slitlamp biomicroscopy due to an edematous or opaque cornea. After 2 weeks, however, 18 (82%) or more of the animals did not have any anterior chamber inflammation. In comparison, extrabulbar procedures performed in rats, such as cautercization of episcleral vessels and injection of hypersonic saline, might be less inflammatory, but obstruct the blood flow out of the eye which can affect the magnitude of the retinal damage.

Another limitation is the variability of IOP magnitude and duration. We observed that the time course of IOP was different in each mouse eye, as shown in Figure 5, even though each

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**Table 3. Complications Associated with IOP Elevation**

<table>
<thead>
<tr>
<th>Group</th>
<th>Complications</th>
<th>Time after Treatment (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>A: ΔIOP &gt; 30% in 4 weeks</td>
<td>Corneal edema</td>
<td>4/14</td>
</tr>
<tr>
<td></td>
<td>Corneal opacity</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cataract</td>
<td>0</td>
</tr>
<tr>
<td>B: ΔIOP &lt; 30% in 4 weeks</td>
<td>Corneal edema</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Corneal opacity</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Cataract</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are eyes with the complication over the total eyes. 0, no complications.
eye underwent the same procedure. Moreover, by 6 weeks after treatment, IOP often returned to normal or several mm Hg below normal. Thus, the mean IOP during 4 and 12 weeks may not be related to the magnitude of elevated IOP during the latter weeks of the study. The integral of IOP elevation and duration provides a rudimentary basis for comparing the cumulative effect of IOP elevation. However, it does not differentiate between eyes with high IOP for a short period or moderate IOP elevation for a longer period. The reason for the IOP reduction at 6 weeks after treatment is unknown. It may be related to compensatory changes in aqueous humor inflow or outflow pathways. Although the lower IOP was typically within 5 mm of normal IOP, it is possible that even this minor reduction could reduce the rate of optic nerve damage.

Because there is no prior report of an experimental mouse model of sustained high IOP, the precise level and duration of elevated IOP that can induce a specific amount of optic nerve damage is unknown. Based on reports in other animals, a successful IOP elevation in our mouse model was defined as a 30% increase compared with control IOP. In a rat glaucoma model of eyes with the episcleral vessels cauterized, 10% to 20% of axons were damaged within 4 weeks, with a 50% increase of IOP.22 Also in the rat laser model, a 50% increase in IOP induced 19% of axon loss in 3 weeks. Loss of lateral geniculate nuclear cells, loss of neurotrophic factors, changes of extracellular matrix in the optic nerve head, and abnormality of the electoretinogram also were shown after 1 month in rat models of elevated IOP.23 In monkeys, IOP elevation induced optic disc changes that were detected 4 weeks after treatment. In our results, at least 60% of treated mouse eyes sustained more than a 30% increase of high pressure for at least 4 weeks. Thus, persistent elevation by our method may be useful for investigation of optic nerve damage in mice. However, it should be noted that these responses for IOP elevation could vary by both strain and individual.

To determine whether this technique induces optic nerve damage similar to glaucomatous optic neuropathy, the relationship between IOP elevation and loss of optic nerve fibers must be evaluated. As a first step in this process, we have studied the nerves of mouse eyes 12 weeks after IOP was elevated by the present technique. As reported in the current issue, we found that optic nerve damage after the present laser treatment procedure was directly related to the magnitude and duration of IOP elevation. Additional studies evaluating the time course of axon loss will further clarify the utility of this model for studying the effect of elevated IOP on the survival of optic nerve fibers.

In conclusion, the present study documents persistent elevation of mouse IOP after anterior chamber flattening and laser treatment. This model may be useful to investigate the relationship between IOP elevation and optic nerve damage.

Acknowledgments

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References