Optic Nerve Damage in Experimental Mouse Ocular Hypertension

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PURPOSE. To evaluate optic nerve damage in mice after laser-induced ocular hypertension.

METHODS. Ocular hypertension was induced unilaterally in 13 NIH Black Swiss mice by laser photocoagulation of the limbus. Over the following 12 weeks, intraocular pressure (IOP) was measured at regular intervals by the microneedle method. The optic nerves of these mice and of seven normal untreated mice were then processed conventionally for electron microscopy, and cross sections of the nerve 300 μm posterior to the globe were collected. Low- and high-magnification images were collected systematically and masked before analysis. For each nerve, cross-sectional area was measured in low-magnification micrographs, and axon and glia numbers were counted in high-magnification micrographs.

RESULTS. In normal untreated mice, the average number of axons was 59,597 ± 3,112 (mean ± SD). Variation among these measurements was 5.7% ± 3.9%. After laser treatment, the duration of high IOP ranged from 2 to 12 weeks (6.2 ± 3.6 weeks, mean ± SD). The mean IOP in the treated eyes was 1.3 times greater than the mean IOP in the control eyes (P = 0.0012). The maximum IOP in the treated eyes was 1.6 times greater than that observed in the control eyes (P < 0.0001). The optic nerve cross-sectional area, mean axon density, and total number of axons in the treated eyes were significantly less than in the control eyes (28.5% ± 23.4%, 57.8% ± 37.8%, and 63.1% ± 38.1%, respectively; P < 0.005 for each). The decrease in optic nerve cross-sectional area and the positive integral of elevated IOP and duration of IOP elevation correlated significantly with total axon loss (r² = 0.79, P < 0.0001 and r² = 0.36, P = 0.040, respectively). The number of astrocytes per cross section of optic nerve was significantly greater in the treated eyes than in the control eyes (P = 0.014).

CONCLUSIONS. Laser-induced ocular hypertension in mouse eyes can induce optic nerve axon loss that correlates with the magnitude and duration of elevated IOP. (Invest Ophthalmol Vis Sci. 2003;44:4321-4330) DOI:10.1167/iovs.03-0138

Loss of optic nerve axons is characteristically associated with vision loss in glaucoma. Studies of human glaucomatous eyes after death and the eyes of monkeys and rats after induction of elevated intraocular pressure (IOP) have implicated disruption of axonal transport, excessive intraretinal (and possibly intravitreal) glutamate, increased nitric oxide, and autoimmune responses in this loss of optic nerve axons. However, the molecular mechanism underlying this loss remains unclear. The development of transgenic mouse technology raises the prospect of direct molecular investigations of axon loss associated with elevated IOP. Studies of mice that undergo spontaneous development of elevated IOP have documented associated optic nerve damage. However, the relationship between the genetic background in these mice and the axon loss mechanism is difficult to discern. In view of this, it is of particular interest to determine whether induced elevation of IOP in the normal mouse eye can cause optic nerve axon loss similar to that seen in glaucomatous optic neuropathy.

A procedure to induce elevated IOP in normal NIH Black Swiss mouse eyes is reported by Aihara et al. in the current issue. This procedure involves flattening the anterior chamber by aqueous aspiration followed by laser treatment of the limbus. IOP in these mice, normally approximately 16 mm Hg, becomes elevated to an average of 23 mm Hg and remains elevated for up to 12 weeks. Ocular side effects of this procedure were observed only occasionally, were of relatively minor significance, and were typically resolved spontaneously. The present study was undertaken to determine whether elevated IOP in the NIH Black Swiss mouse eye induces optic nerve axon loss and gliosis.

Materials and Methods

Animals

Animals used in this study were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male NIH Black Swiss mice were obtained 6 weeks after birth from Taconic Laboratory (Germantown, NY) and were 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. All mice were fed ad libitum. Two groups of mice were studied: one untreated group of 7 mice, to assess normal interindividual and intereye variability in the number of optic nerve axons and glial cells, and a second group of 13 laser-treated mice, to assess the relationship of induced ocular hypertension with optic nerve axon loss and glial proliferation.

Induction of Experimental Ocular Hypertension

The mice were anesthetized by intraperitoneal injection of a solution containing ketamine (100 mg/kg. Ketaset; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (9 mg/kg. TranquilVed; Vedco, Inc., St. Joseph, MO). Aqueous outflow in the left eye was obstructed by laser photoocoagulation at the limbus. The effect of this treatment was...
enhanced by flattening the anterior chamber before laser photocoagulation, as described by Aihara et al.12 Animal age was 8 weeks at the time of laser treatment. The fellow eye served as a control.

**IOP Measurements**

IOP was measured in both eyes after laser treatment, every week in the first 4 weeks and every 2 weeks thereafter, as described by Aihara et al.13 Mice were anesthetized with an intraperitoneal injection of a solution containing ketamine (100 mg/kg) and xylazine (9 mg/kg). A fluid-filled glass microneedle connected to a pressure transducer was inserted through the cornea into the anterior chamber to measure IOP. All the mice completed this treatment regimen, with the exception of one mouse that died of unknown causes at 6 weeks.

The mean and maximum IOPs in the contralateral control and treated eyes and the duration of IOP elevation in the treated eyes were calculated. For each animal, a graph of IOP over time was constructed for the control and treated eyes. The area under the curve of control and treated eyes was calculated in units of mm Hg-weeks. The area under the control eye curve was subtracted from the area under the treated eye curve to assess the magnitude of IOP elevation. The total area for the period during which the IOP in the treated eye was higher than in the control eye, called the positive integral IOP, provided an estimation of the total exposure of the treated eye to elevated IOP.

**Optic Nerve Axon and Glia Cell Counting**

At the conclusion of the 12-week observation period, the mice were anesthetized with intraperitoneal pentobarbital sodium (100 mg/kg, Nembutal; Abbott Laboratories, North Chicago, IL) and exsanguinated by perfusion with mammalian Ringer’s solution containing lidocaine hydrochloride (0.1 mg/mL, Xylocaine; Astra USA, Inc., Westborough, MA) and heparin sodium (500 U/mL, heparin; Elkins-Sinn, Inc., Cherry Hill, NJ). Transcardial perfusion was then continued with fixative (approximately 20 mL of 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.15 M cacodylate buffer). The optic nerves were carefully dissected and placed in this fixative overnight. The optic nerves from the animal that died at 6 weeks also were dissected and placed in fixative overnight. The optic nerves were postfixed in 1% osmium tetroxide, stained in 2% uranyl acetate, dehydrated in ethanol and acetone, and embedded in epoxy resin (Durcupan; Electron Microscopy Sciences [EMS], Fort Washington, PA). Ultrathin sections were cut perpendicular to the long axis of the optic nerves on an ultramicrotome and placed on polyvinyl formal–coated (Formvar; SPI, West Chester, PA) slotted grids. These sections were obtained at approximately 300 μm posterior to the nerve’s emanation from the globe. Sections were counterstained with 1% uranyl acetate and Sato lead, and viewed by electron microscope (model 1200 EX; JEOL, Tokyo, Japan).

The number of axons in the mouse optic nerves was assessed according the method developed by Williams et al.14 with minor modifications. For each optic nerve cross section, electron micrographs were taken at low magnification (200×) to measure the cross-sectional area. Then a series of 20 micrographs were taken at high magnification (10,000×) in a square lattice pattern in the following positions within the optic nerve: center, four micrographs; midperiphery, eight micrographs; and peripheral margin, eight micrographs (Fig. 1). No adjustments in position were made with respect to the tissues including blood vessels and glial cells. To confirm the true magnification, calibration grids were photographed at the same low (1000 mesh/in., no. 79525-01; EMS, Fort Washington, PA) and high (2160 lines/mm, no. 206; Ted Pella, Redding, CA) magnifications.

Electron micrographs were digitized using a Peltier cooled high-resolution charge-coupled device (CCD) camera (CH250; Photometrics, Inc., Tucson, AZ) and magnified at 4× in the course of digitizing. The effective magnifications were therefore 800× at low magnification and 40,000× at high magnification. The identity of the digitized images was masked before analysis, and each image was analyzed using image-processing software (NIH Image; ver.1.62; available by ftp at zippy.nimh.nih.gov/ or at http://rsb.info.nih.gov/nih-image; developed by Wayne Rasband, National Institutes of Health, Bethesda, MD). The area of the optic nerve cross section was measured three times by outlining its outer border, and the mean of these measurements was used for subsequent calculations. A counting frame (7×8 μm) was traced on the high-magnification image to facilitate measuring axon density. Then, myelinated and unmyelinated axons within the frame or that intersected the upper and left edges, were marked and counted manually by using standard unbiased counting rules15 (Fig. 2). The total area counted in the 20 micrographs analyzed for each nerve was 1120 m2. This corresponded to 2.5% to 8.4% of the total nerve cross-sectional area. Axon profiles that did not contain neurofilaments were excluded from the counts, because they may have been degenerating axons.

The typical sample area in normal mouse gave a count of 80 axons, and the typical series of 20 samples gave a total count of 1600 axons. The mean axon density was calculated, and the total number of axons per optic nerve cross section was estimated by multiplying the mean density by the area of the optic nerve cross section.

The same counting method was used to measure astrocyte density by counting the astrocyte nuclei. Astrocytes were distinguished from oligodendrocytes and microglia, based on their markedly paler cytoplasm and nuclei, the less-condensed appearance of the nuclear chromatin, and the presence of characteristic cytoplasmic glial filaments.16 The typical series of 20 sample areas in normal mouse optic nerves gave a total count of three astrocyte nuclei. The mean astrocyte nuclear density was calculated, and the total astrocyte nuclei per optic nerve cross section was also estimated by multiplying the mean density by the area of the optic nerve cross section.

To evaluate the reproducibility of the axon-counting method, three adjacent sections were analyzed from each of two different optic nerves. For each section, the position of the high-magnification micrographs was established according to measurements from the center of the optic nerve. Although corresponding images from successive sections were from similar regions, inspection confirmed that they did not
overlap. The area of optic nerve cross section, the mean axon density, and the total number of axons were compared among the three sections from each nerve.

**Statistical Analysis**

Paired t-test and linear regression were used for evaluation of study results. \( P < 0.05 \) was considered to be statistically significant.

**RESULTS**

**Reproducibility of Axon Counting**

The measurements of the area of optic nerve cross section, the mean axon density, within the three adjacent sections had coefficients of variation less than 3% (Table 1). The measurements of total number of axons within the three adjacent sections had coefficients of variation less than 1.4%, which indicated that this method effectively identified differences in cross-sectional area or mean axon density that exceeded 3% or differences in the total number of axons that exceeded 1.5%.

**Normal Mice**

The data for normal mice are shown in Table 2. The total number of axons of the optic nerve ranged from 53,079 to 65,859 (59,597 ± 3,112, mean ± SD). There were no significant intereye differences in the area of optic nerve cross section, the mean axon density, and the total number of axons. The percentage intereye variation in total number of axons ranged from 1.3% to 11.8%, and the coefficient of variation for this parameter was 6.8. The number of astrocytes per optic nerve cross section was 127 ± 58, and there were no significant intereye differences in the density and number of astrocytes. Oligodendroglia were difficult to assess, because typically there were 0 to 1 observed in a series of 20 high-magnification micrographs from an optic nerve.

**Ocular Hypertension Model Mice**

Mean IOP, maximum IOP, positive integral of IOP and time, and period of high IOP are shown for each experimental mouse in Table 3. This table also shows the optic nerve cross-sectional area, mean axon density, total number of axons, density of astrocytes, and total number of astrocytes for each mouse.

**Intraocular Pressure**

The duration of IOP elevation ranged from 2 to 12 weeks (6.2 ± 3.6 weeks, mean ± SD, Table 3). The mean IOP in the treated eyes was 1.3 times greater than the mean IOP in the control eyes (\( P = 0.0012 \), paired t-test, Fig. 3). The maximum IOP in the treated eyes was 1.6 times greater than the maximum IOP observed in the control eyes (\( P < 0.0001 \), paired t-test).

### Table 1. Reproducibility of Optic Nerve Axon Analyses

<table>
<thead>
<tr>
<th>Section No.</th>
<th>Cross-Sectional Area (( \mu m^2 ))</th>
<th>Mean Axon Density (number/( \mu m^2 ))</th>
<th>Total Number of Axons</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nerve 1</td>
<td>Nerve 2</td>
<td>Nerve 1</td>
</tr>
<tr>
<td>1</td>
<td>46,354</td>
<td>45,151</td>
<td>1.49</td>
</tr>
<tr>
<td>2</td>
<td>46,743</td>
<td>46,369</td>
<td>1.45</td>
</tr>
<tr>
<td>3</td>
<td>45,837</td>
<td>44,072</td>
<td>1.51</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>46,531 ± 455</td>
<td>45,197 ± 1,149</td>
<td>1.48 ± 0.031</td>
</tr>
<tr>
<td>Coefficient of variation</td>
<td>0.98</td>
<td>2.54</td>
<td>2.09</td>
</tr>
</tbody>
</table>

**FIGURE 2.** High-magnification (10,000×) images before (A) and after (B) analysis. A counting frame (7 × 8 \( \mu m \)) was traced on each image, and all survival myelinated and unmyelinated axons within the frame and intersecting the upper and left edges were marked and counted manually, according to standard unbiased counting rules. Each black dot indicates a counted axon.
Optic Nerve Damage

The optic nerve cross-sectional area, mean axon density, and total number of axons in the treated eyes were significantly less than in the control eyes (28.5% ± 23.4%, 57.8% ± 57.8%, and 63.1% ± 58.1%, respectively; *P < 0.005 for each, paired *t*-test; Fig. 4). Examples of low- and high-magnification images are shown in Figure 5. There was significant correlation between the area of optic nerve cross section and optic nerve damage in the treated eyes. The percentage decrease of optic nerve cross-sectional area correlated to the percentage axon loss (*r* = 0.79, *P < 0.0001, linear regression; Fig. 6). The positive integral of IOP and time correlated significantly with the percentage axon loss (*r* = 0.36, *P = 0.040, linear regression; Fig. 7). Mouse M32 was excluded from the analysis of the density and number of astrocytes because all cells, including astrocytes, were destroyed in association with the severe optic nerve atrophy present in the treated eye.

Increase in Astrocytes

The mean density of astrocytes and the number of astrocytes per cross section of optic nerve were significantly greater in the treated eyes than in the control eyes (*P = 0.0023 and *P = 0.014* respectively, paired *t*-test; Fig. 8). There was a significant correlation between the mean density of axons and astrocytes in the treated eyes (*r* = 0.38, *P < 0.053, linear regression, Fig. 9). The lower the axon density became, the higher the density of astrocytes became. Mouse M34 was excluded from the analysis of the density and number of astrocytes because all cells, including astrocytes, were destroyed in association with the severe optic nerve atrophy present in the treated eye.

DISCUSSION

These results show that induction of persistent elevation in IOP in NIH Black Swiss mice results in significant reductions in optic nerve cross-sectional area, mean axon density, and total number of axons. The reduction in total number of axons correlated with the positive integral of IOP and time, which indicates that optic nerve damage in this model depends on the magnitude and duration of elevated IOP. In addition, the lower the axon density became, the greater the density of astrocytes within the nerve became. These observations indicate that

<table>
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<th>Mouse</th>
<th>R</th>
<th>L</th>
<th>L/R</th>
</tr>
</thead>
<tbody>
<tr>
<td>M10</td>
<td>45,914</td>
<td>45,851</td>
<td>0.99</td>
</tr>
<tr>
<td>M11</td>
<td>36,654</td>
<td>43,035</td>
<td>1.17</td>
</tr>
<tr>
<td>M12</td>
<td>34,342</td>
<td>42,490</td>
<td>1.24</td>
</tr>
<tr>
<td>M13</td>
<td>27,966</td>
<td>49,923</td>
<td>1.55</td>
</tr>
<tr>
<td>M14</td>
<td>45,446</td>
<td>39,099</td>
<td>0.86</td>
</tr>
<tr>
<td>M16</td>
<td>45,715</td>
<td>41,126</td>
<td>0.81</td>
</tr>
<tr>
<td>M17</td>
<td>50,787</td>
<td>41,26</td>
<td>0.81</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>40,975 ± 8,114</td>
<td>43,455 ± 3,505</td>
<td>1.10 ± 0.25</td>
</tr>
</tbody>
</table>

R, right eye; L, left eye.

**Table 2. Normal Mouse Data**

**Table 3. Ocular Hypertension Model mouse Data**
elevation of IOP in the normal mouse eye induces axon loss in the optic nerve similar to that in human glaucomatous optic neuropathy. On average, IOP returned to baseline 6 weeks after laser treatment. Because 6 weeks in the life of a mouse that typically lives 2 years may be comparable to 5 years in that of a human who lives for 80 years, this model may be comparable to human primary open-angle glaucoma, which is typically diagnosed late in life.

In human eyes, the optic nerve head is supplied by the arterial circle of Zinn and Haller formed by the choroidal arteries. In contrast, the arterial supply of the mouse optic nerve head derives from branches of the central retinal artery, and none of the capillaries are derived from choroidal vessels. A direct effect of IOP on optic nerve circulation in the mouse may be stronger than in humans. However, the IOP model may be partially different from that in human glaucoma, Mice in the present model also had substantial axon loss. Moreover, the decrease in optic nerve cross-sectional area correlated with axon loss, as described in rat and monkey eyes with experimentally elevated IOP and in human glaucomatous eyes. In addition, optic nerve damage in the present model was related to the magnitude and duration of elevated IOP. Thus, the presently described mouse model mimics many features of chronic human glaucoma. However, the mechanism of optic nerve damage in this mouse model may be partially different from that in human glaucoma, because the mouse optic nerve head has no lamina cribrosa. The role of the lamina cribrosa in optic nerve damage in glaucoma is not clearly understood. Axonal transport is blocked in the region of the lamina cribrosa in monkey eyes with experimentally elevated IOP, which leads to axon loss in the optic nerve. The regional variation in lamina cribrosa anatomy is contrasted with susceptibility patterns of optic nerve head tissue to IOP elevation in eyes in a monkey glaucoma model.

<table>
<thead>
<tr>
<th>Total Number of Axons</th>
<th>Astrocyte Density</th>
<th>Total Number of Astrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>(number/optic nerve)</td>
<td>(number/1,000 μm²)</td>
<td>(number/optic nerve cross section)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R</th>
<th>L</th>
<th>L/R</th>
<th>R</th>
<th>L</th>
<th>L/R</th>
<th>R</th>
<th>L</th>
<th>L/R</th>
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<tbody>
<tr>
<td>56,962</td>
<td>60,733</td>
<td>1.07</td>
<td>1.8</td>
<td>2.7</td>
<td>1.50</td>
<td>82</td>
<td>123</td>
<td>1.50</td>
</tr>
<tr>
<td>62,203</td>
<td>58,388</td>
<td>0.94</td>
<td>1.8</td>
<td>2.7</td>
<td>1.00</td>
<td>65</td>
<td>77</td>
<td>1.17</td>
</tr>
<tr>
<td>65,555</td>
<td>60,229</td>
<td>0.95</td>
<td>5.4</td>
<td>2.7</td>
<td>0.50</td>
<td>184</td>
<td>114</td>
<td>0.62</td>
</tr>
<tr>
<td>55,079</td>
<td>59,565</td>
<td>1.12</td>
<td>5.6</td>
<td>2.7</td>
<td>0.75</td>
<td>100</td>
<td>114</td>
<td>1.14</td>
</tr>
<tr>
<td>60,968</td>
<td>65,859</td>
<td>1.08</td>
<td>0.9</td>
<td>2.7</td>
<td>3.00</td>
<td>41</td>
<td>134</td>
<td>3.30</td>
</tr>
<tr>
<td>59,328</td>
<td>58,564</td>
<td>0.99</td>
<td>4.5</td>
<td>5.4</td>
<td>1.20</td>
<td>204</td>
<td>210</td>
<td>1.03</td>
</tr>
<tr>
<td>57,972</td>
<td>57,156</td>
<td>0.99</td>
<td>4.5</td>
<td>2.7</td>
<td>0.60</td>
<td>227</td>
<td>110</td>
<td>0.49</td>
</tr>
</tbody>
</table>

59,152 ± 3,538 | 60,042 ± 2,830 | 1.02 ± 0.07 | 3.2 ± 1.7 | 2.9 ± 1.1 | 1.22 ± 0.86 | 129 ± 74 | 126 ± 41 | 1.32 ± 0.94 |
coma model. Although the lamina cribrosa may play an important role in glaucomatous optic nerve damage, it is interesting that axon loss of the optic nerve is induced in mouse eyes by elevated IOP despite the absence of the lamina cribrosa. This raises the possibility that the IOP-induced changes in the lamina cribrosa may not be essential for IOP-induced optic nerve damage. Further investigations may clarify whether elevated IOP in the mouse eye disrupts axonal transport in the optic nerve.

Although there was a correlation between axonal loss and the IOP integral scores, it was not strong ($r^2 = 0.36$). In particular, four mice with integral scores ranging from 38 to 48 mm Hg-weeks had axon losses ranging from 10% to 99% (Fig. 7). Of interest is that the duration of elevated IOP experienced by two of these mice that had axon losses less than 20% was 4 weeks only (M64 and M73, Table 3). In contrast, the duration of elevated IOP in the two mice with axon losses exceeding 95% was equal to or greater than 6 weeks (M31 and M109). Hence, consideration of the duration of elevated IOP, along with the integral score, may improve prediction of axon loss. It should be noted that when the integral score exceeded 48 mm Hg-weeks, axon loss always was greater than 80%. Hence, the duration of IOP elevation may be more important to consider when the integral score is less than 48 mm Hg-weeks and less important when the score exceeds 48 mm Hg-weeks. In a second example, mice M32 and M34 had different axon loss (52% and 99.8%, respectively), although IOP was similarly elevated for 6 weeks, and they had the same value for positive integral IOP. However, mouse M34 had a period of 6 weeks of normotension after the time of elevated IOP, whereas mouse M32 did not. This may be consistent with the axon loss in rat optic nerves that experienced a period of normal IOP that followed a 3-week period of significant IOP elevation. Hence, considering the duration of elevated IOP or total time since laser treatment, as well as the integral IOP score may improve prediction of axon loss. Further supporting the link of pressure elevation to axon loss is the observation of no axon loss in one treated eye in which IOP never exceeded 21 mm Hg (M113). Moreover, this eye had undergone transient hypotony, as did each of the treated eyes, suggesting that transient hypotony does not cause axon loss in this model.

Several other possible sources of variation in axon loss may exist. These include regional differences in axon density within the nerve, genetic variation among individual mice, and possible effects of environmental factors. In addition, small intereye variations were noted. These are likely to represent real differences, because they exceed by threefold the variations in repeat counts of the same optic nerve. Similar individual and intereye variations have been noted in human optic nerves. These latter sources of variation, though normal, may limit the ability to use the contralateral eye as a control for the optic nerve axon counts in the treated eye.

Astrocytes are the major glial cell type in the optic nerve in most mammalian species, and mature, quiescent astrocytes are reactivated and participate in formation of a glial scar in glaucomatous optic neuropathy. As to the proliferation of astrocytes, although there is a pool of astrocytes capable of entering the mitotic cycle, their proliferative
FIGURE 5. Low- and high-magnification images of the optic nerve cross section in the control eye (A, C, E) and the treated eye with 99.5% axon loss (B, D, F) in M33 mouse. The area of optic nerve and mean axon density in the treated eye were 50% and 1% of the control eye, respectively. Most of the remaining myelinated axons had degenerated, and prominence of glial profiles was increased (F). Magnification: (A, B) × 200; (C, D) × 4,000; (E, F) × 10,000.
capacity appears to be limited in the adult human brain. Quigley and Anderson reported that no evidence of astrocyte mitosis was observed after optic nerve transection in the monkey, and the estimated volume of astrocytes increased only slightly from normal. Furuyoshi et al. also described an increase in glial density, accompanied by a decrease in optic nerve cross-sectional area, whereas the total number of astrocytes remained nearly constant in a monkey glaucoma model. However, in the present mouse model, not only the density of astrocytes, but also the total number of astrocytes per optic nerve cross section increased, which suggests proliferation of astrocytes. This different response of astrocytes may depend on species, because there is undeniable evidence for astrocyte proliferation in response to brain injury in the mouse. Kwiecien et al. also reported glial proliferation in the optic nerve in demyelinating mutant rats. The proliferation of astrocytes in the present mouse model may be induced by a similar mechanism, because demyelination happens in association with the process of axon loss. The increase in number of astrocytes observed in the present model may indicate that they have been reactivated.

**FIGURE 6.** There was a linear correlation between the decrease of optic nerve cross-sectional area and the loss of optic nerve axons ($n = 13, r^2 = 0.79, P < 0.0001$, linear regression).

**FIGURE 7.** There was a linear correlation between the magnitude and duration of elevated intraocular pressure (IOP) and the loss of optic nerve axons ($n = 12, r^2 = 0.36, P = 0.040$, linear regression).

**FIGURE 8.** The mean density of optic nerve astrocytes (A) and the number of astrocytes per optic nerve cross section (B) in the control (■) and treated (□) eyes ($n = 12$). Compared with the control eyes, the density and number of astrocytes were significantly greater in the treated eyes (paired t-test). Bars, SE.
In conclusion, the present study confirms a positive correlation between experimental elevated IOP in the mouse eye and associated loss of optic nerve axons. Similarities between this model system and human glaucoma suggest that this model may be useful for evaluating the cellular mechanisms of glaucoma as well as the potential of new treatments for glaucoma.

Acknowledgments

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References


