Regional Optic Nerve Damage in Experimental Mouse Glaucoma

Fumihiko Mabuchi,1 Makoto Aihara,1 Mason R. Mackey,2 James D. Lindsey,1 and Robert N. Weinreb1

PURPOSE. To assess the relationship between regional variation of axon loss and optic nerve head anatomy in laser-induced experimental glaucoma in the mouse.

METHODS. Experimental glaucoma was induced unilaterally in eight NIH Swiss black mice. Intraocular pressure (IOP) was measured for 12 weeks, and the mice were killed. The eyes were enucleated, and both optic nerves were dissected and processed conventionally for electron microscopy. Low- and high-magnification images of the optic nerve cross sections 300 μm posterior to the globe were collected systematically and masked before analysis. For each nerve, cross-sectional area was measured in low-magnification micrographs. Axon number and density were determined in the high-magnification micrographs. Loss of axonal density was compared between the superior and inferior and nasal and temporal areas of the optic nerve cross section. Additional cross-section micrographs were collected at 10- or 20-μm intervals throughout the optic nerve head.

RESULTS. In the treated (glaucoma) eyes, mean IOP was 44% higher than that in the control eyes. The optic nerve cross-sectional area, mean axonal density, and total axonal number were significantly less than those in the control eyes (P < 0.01 for each). Axon loss in the superior optic nerve was greater than in the inferior optic nerve in each glaucomatous eye (P = 0.012). The ratio of axonal density in the superior and inferior optic nerve (superior-to-inferior [S/I] ratio) in all treated eyes was <1.0 and significantly lower than that in the control eyes (P = 0.012). The central retinal vessels occupied approximately 20% of the central optic nerve head cross-sectional area, gradually shifted position ventrally as they progressed toward the scleral foramen (the mouse does not have a lamina cribrosa), and exited the inferior retrobulbar optic nerve adjacent to the posterior of the globe.

CONCLUSIONS. Ocular hypertension in the mouse eye sufficient to cause optic nerve damage induces preferential loss of superior optic nerve axons. Optic nerve axon loss appeared less among the axons that were near the major optic nerve blood vessels at the scleral foramen. Topographic differences in optic nerve axon loss should be considered when evaluating optic nerve damage in experimental laser-induced glaucoma in the mouse. (Invest Ophthalmol Vis Sci. 2004;45:4352–4358) DOI: 10.1167/iovs.04-0355

Optic nerve axon loss in experimental mouse models of glaucoma is related to both the magnitude and duration of intraocular pressure elevation. This relationship is similar in monkey and rat models of glaucoma. It also is similar to human glaucoma, in which axon loss occurs preferentially in the superior and inferior quadrants of the optic nerves. In the rat, however, it occurs preferentially in the superior optic nerve. It has been proposed that this reflects differences in the anatomy of human and rat eyes. Unlike human and monkey eyes, the laminar beams of the rat lamina cribrosa are primarily oriented vertically. In contrast to human, monkey, and rat eyes, however, the mouse eye has no lamina cribrosa. Thus, the potential contributions of other nerve head structural elements to preferential axon survival may be particularly evident in mouse glaucoma.

One optic nerve head feature that may influence preferential axon survival is the distribution of major blood vessels. In human eyes, the central retinal artery and vein are centrally positioned within the optic nerve head through to the retrobulbar optic nerve. In contrast, the rat central retinal artery and vein are positioned either within or near the posterior optic nerve sheath. These blood vessels are centrally positioned at the mouse optic nerve head and transition to lie adjacent to the inferior nerve sheath before exiting the scleral foramen. If the position of the major blood vessels affects preferential axon survival in glaucoma, then the asymmetric positioning of these blood vessels in the mouse may lead to asymmetric axon death during the course of experimentally induced mouse glaucoma.

In view of these vascular differences and the lack of a lamina cribrosa in the mouse eye, the present study was undertaken to determine the pattern of axonal loss in a mouse glaucoma model and to evaluate the relationship of the pattern of axonal loss to the distribution of major optic nerve head blood vessels as they pass through the scleral foramen.

MATERIALS AND METHODS

Animal Husbandry

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. The mice were anesthetized with intraperitoneal injection of ketamine (100 mg/kg, Ketaset; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (9 mg/kg, TranquilVed; Vedco, Inc., St. Joseph, MO). When a surgical plane of anesthesia was confirmed, experimental glaucoma was induced by flattening of the anterior chamber and laser photoagulation at the limbus, as described previously. The animal's age was 8 weeks at the time of laser treatment. The fellow eye was served as the control. Under the same anesthesia, IOP was measured in both eyes by the microneedle

From the 1Hamilton Glaucoma Center and 2National Center for Microscopy and Imaging Research and Department of Neurosciences, University of California San Diego, La Jolla, California.

Supported in part by the National Eye Institute EY05990 (RNW), the Margaret and Robert Boerner Glaucoma Research Fund of the Foundation for Eye Research (MA).

Submitted for publication March 30, 2004; revised June 10, 2004; accepted June 25, 2004.

Disclosure: F. Mabuchi, None; M. Aihara, None; M.R. Mackey, None; J.D. Lindsey, None; R.N. Weinreb, None

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Corresponding author: Robert N. Weinreb, Hamilton Glaucoma Center, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0946; winreb@eyecenter.ucsd.edu.

method, every week for 4 weeks and every 2 weeks for 8 weeks thereafter. The mean and maximum IOPs in the control and treated eyes, and the duration of IOP elevation in the treated eyes were calculated. All procedures were in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Optic Nerve Axon Counting

After 12 weeks observation, mice were fixed transcardially under anesthesia, and the optic nerves were carefully dissected and preserved in the fixative overnight, as described previously. A radially oriented thin strip of sclera attached to the optic nerve head was retained at the 12 o'clock position of the optic nerve to identify the orientation during subsequent preparation of the optic nerve cross sections. The optic nerves were fixed 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 (Formvar)–coated slot grids (SPI, West Chester, PA). These sections were obtained approximately 300 μm posterior to the nerve’s emanation from the globe. The sections were counterstained with 1% uranyl acetate and Sato lead and viewed with an electron microscope (model 1200 EX; JEOL USA, Peabody, MA).

The number of axons in the mouse optic nerves was assessed according the method developed by Williams et al. with minor modifications. For each optic nerve cross section, electron micrograph was taken at low magnification (200×) to measure the area of the optic nerve cross section. After identification of the superior position of the optic nerve cross section, a series of 20 micrographs...
was taken at high magnification (10,000×) in a square lattice pattern in the following positions within the optic nerve: center, four micrographs; mid-periphery, 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. The calibration grids were photographed at the same low (79525-01, 1000 mesh/in.; EMS) and high (603, 2160 lines/mm; Ted Pella, Redding, CA) magnifications, to determine true magnification.

Electron micrographs were digitized by a 1024 × 1024 charge-coupled device (CCD) camera (CH250; Photometrics, Inc., Tucson, AZ) and magnified at 4 × during 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 with 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 determined three times by outlining its outer border, as displayed by the software, and then averaged. To measure axonal density, a counting frame (7 × 8 μm) on the high-magnification image was traced, and the survival of 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. Axon profiles that did not contain neurofilaments were excluded from the counts, because they may have represented degenerating axons. The mean axonal density was determined, and the total axonal number was estimated by multiplying the mean axonal density by the area of the optic nerve cross section.

To assess whether there was a preferential axonal loss by position in the glaucomatous eyes, the percentage axonal loss in micrographs from specific regions within both optic nerves were compared. First, the mean axonal density in the superior and inferior areas of the optic nerve cross section was calculated in 10 micrographs taken in the superior and inferior half areas, as shown in Figure 1. Similar comparisons were made for axon loss in the nasal and temporal portions of the nerve. As an index of unbalanced axonal distribution, the ratio of axonal density between the superior and inferior area of each optic nerve cross section (superior to inferior [S/I] ratio) was calculated and compared between the glaucomatous and control eyes. A ratio <1.0 indicated that axonal density in the superior area of an optic nerve cross section was lower than that in the inferior area. Likewise, comparison was made between the nasal and temporal area of the treated and control optic nerve cross sections. In addition, comparison was made between the superior and inferior areas considered as one group and the nasal and temporal areas considered as a second group. In this comparison, the four micrographs from the center of the optic nerve were excluded. Hence, the mean axonal density in the superior-inferior area of the optic nerve cross section was calculated from the eight micrographs in the midperiphery and peripheral margin of the superior and inferior areas as shown in Figure 1. The mean axonal density in the nasal and temporal areas considered together was calculated in the same manner.

**Vascular Anatomy of Mouse Optic Nerve Head**

To determine the relationship between the pattern of axonal loss and the position of major blood vessels within the optic nerve head, cross-sectional images were collected at 10- or 20-μm intervals throughout optic nerve heads in two untreated eyes.

**Statistical Analysis**

Paired t-test and Wilcoxon rank sum test were used for evaluation of study results. Determination of \( P < 0.05 \) was considered statistically significant.

**RESULTS**

**IOP and Optic Nerve Damage**

The duration of IOP elevation ranged from 3 to 12 weeks (7.1 ± 4.1 weeks, mean ± SD). The mean and maximum IOP in the treated eyes were 44% and 53% times higher than that in the control eyes, respectively (\( P < 0.001 \), paired \( t \)-test; Fig. 2A). The optic nerve cross sectional area, mean axonal density, and total axonal number in the treated eyes were significantly less in treated nerves than in the control nerves by 29.9% ± 19.2%, 50.7% ± 35.2%, and 60.5% ± 33.2%, respectively (\( P < 0.01 \) for each, paired \( t \)-test, Figs. 2B–D).

**Axonal Loss Pattern**

The percentage of axon survival and axonal density in the superior area was significantly less than that in the inferior area in each glaucomatous eye (\( P = 0.012 \); Wilcoxon rank sum test, Figs. 3A, 3B). The S/I ratio in all treated eyes was <1.0 and
significantly lower than that in the control eyes (P = 0.012, Wilcoxon rank sum test; Fig. 4A). There were no statistically significant differences between the nasal and temporal, superior–inferior, and nasal–temporal areas of the optic nerve cross section (Figs. 3, 4B, 4C).

Examples of high-magnification images (3000×) from the superior and inferior regions of glaucomatous optic nerves with moderate or severe loss are shown in Figure 5. Axon density was lower in the superior area in each example.

**Vascular Anatomy of Mouse Optic Nerve Head**

Three hundred micrometers posterior to the globe, the ophthalmic artery was positioned inferior to the optic nerve (Fig. 6A). The central retinal artery branched off the ophthalmic artery, entered the eye globe inferior to the optic nerve (Fig. 6B), and penetrated the parenchyma of the optic nerve just anterior to the sclera (Figs. 6C–E). The central retinal vein was superior to the central retinal artery (Fig. 6B) and penetrated the parenchyma of the optic nerve posterior to the penetration of the central retinal artery (Figs. 6C–E). At the level of the outer retina, the central retinal artery and vein were positioned at the center of the optic disc, and these vessels occupied approximately 20% of the optic disc cross-sectional area (Fig. 6F).

**DISCUSSION**

In mouse eyes with experimental glaucoma, the axon loss in the superior optic nerve was greater than in the inferior optic nerve. The optic nerve axon loss pattern in the mouse eye exposed to elevated IOP is different from that in human and monkey eyes, in which the axons at the superior and inferior poles of the optic nerve are most susceptible to glaucomatous damage. Unlike human and monkey eyes, however, there is no lamina cribrosa in the mouse optic nerve head. Hence, pressure-associated damage of the optic nerve may reflect the effect of compressing optic axon bundles against the margin of the scleral foramen through which the optic nerve passes. Thus, differences in the position of structural elements at the level of the scleral foramen may contribute to regional differences in optic nerve axon damage.

One regionally variable element of the mouse optic nerve head is the position of the central retinal vessels. The central
retinal vessels in mouse occupied approximately 20% of the central optic nerve head cross-sectional area. The area occupied by these vessels is proportionally larger than in the human, where they occupy <5% of the cross-sectional area. Moreover, these large vessels exit the inferior optic nerve just anterior to the sclera in mouse. Because of this, the inferior axon bundles at the level of the sclera and choroid are generally closer to large blood vessels than are the axon bundles in the superior optic nerve. As blood vessels may be less rigid than the sclera, it is possible that increase of tissue pressure at the level of the scleral foramen associated with elevated IOP may have been less inferiorly than superiorly. In addition, it is possible that the efficiency of delivery of nutrients and removal of wastes or toxins is greater closer to the central retinal artery and vein than farther away. Further study is needed to determine whether this is linked with different metabolic support for the inferior axons than the superior axons.

The potentially protective influence of a nearby major blood vessel in mouse glaucoma is consistent with prior evidence supporting the beneficial effect of nearby blood vessels on axon survival. In human glaucoma, neuroretinal rim loss typically was greater in the optic nerve head sector with the longest distance to the central retinal vessel trunk than the sector closest to the trunk. Similarly, glaucomatous parapapillary atrophy was greater in the optic nerve head quadrant farthest away from the central retinal vessel trunk exit than in the quadrant that contained the central retinal vessel trunk exit. In a rat model of glaucoma, Morrison et al. reported that focal axonal degeneration was primarily located in the superior region of the optic nerve rather than in the inferior. The rat central retinal vein runs adjacently beneath the optic nerve head, although this vein does not penetrate the substance of the optic nerve. Hence, as in the mouse, close proximity of the rat central retinal artery and vein also may provide greater protection from glaucomatous damage of the axons in the inferior region of the optic nerve. Together, these observations suggest that local optic axon damage is inversely related to proximity to intact blood vessels. Additional investigations in the mouse will be needed to establish the basis of the increased axon survival nearby large blood vessels in this experimental model of glaucoma and to determine whether the pattern of axon loss is related to the distribution of retinal ganglion cell loss.

In conclusion, the present study shows that there is a characteristic pattern of optic nerve damage in mouse glaucoma that may be related to the position of the central artery and vein in the mouse optic nerve head. This indicates that topographic differences in optic nerve axon loss should be considered when evaluating optic nerve damage in experimental laser-induced glaucoma in the mouse. In addition, identifi-
cation of this pattern in the mouse will help to clarify the mechanism of glaucomatous optic nerve atrophy and the contribution of the lamina cribrosa to optic nerve damage in glaucoma.

References


