Axoplasmic flow during chronic experimental glaucoma

I. Light and electron microscopic studies of the monkey optic nervehead during development of glaucomatous cupping

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The anterior optic nerve and the macular region of the retina of glaucomatous eyes of five rhesus monkeys (Macaca mulatta) have been examined by light and electron microscopy. The experimental glaucoma had been induced by argon laser treatment of the anterior chamber angle. The eyes were examined 3 to 11 weeks after the onset of sustained elevation of intraocular pressure above 20 mm Hg. Severe degenerative changes were seen in eyes with higher intraocular pressure and longer duration of glaucoma. Eyes with a lesser elevation of intraocular pressure and shorter duration of glaucoma showed changes sharply localized to the axon bundles in the scleral lamina cribrosa. Accumulation of mitochondria and dense bodies occurred anterior and posterior to collagenous septae. The location of these changes is in agreement with the localization of block of axoplasmic transport identified by autoradiographic studies. It is speculated that these cytologic changes reflect blockage of axoplasmic flow in the optic nerve of eyes with glaucoma.

Key words: glaucoma, experimental glaucoma, intraocular pressure, optic nerve damage, axon function, axoplasmic flow, axoplasmic transport, light microscopy, electron microscopy, ultrastructure, organelles

A complex movement of protein and other elements occurs bidirectionally in axons.1-3 These flows are important for axon function and integrity. Axonal insults, including those caused by compression and ischemia, result in increase, within the axon, of organelles as a result of block of axoplasmic movement. Organelles are found to accumulate in the axon both on the proximal side of the injury, nearest to the soma of the cell, and on the side of the injury distal from the soma.4-4 An example in the retina is the well-known cytoid body which occurs after microinfarction.5 After injury, the changes in the distal part of the axon progress rapidly, and eventually there is disintegration of the structure. The changes in the proximal part of the axon progress more slowly, but provided the insult is sufficiently severe and prolonged, these proximal changes also eventually progress to disintegration of the axon and the soma.6, 7 Soon after the injury the accumulation of or-
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Fig. 1. Intraocular pressure measurements in two monkeys; arrows indicate bilateral argon laser treatment of the trabecular meshwork. Dotted line represents average pressure in normal rhesus monkey eyes after phencyclidine anesthesia.

Table I. Summary of duration and amount of intraocular pressure elevations

<table>
<thead>
<tr>
<th>Monkey No.</th>
<th>Date</th>
<th>No. of consecutive days with IOP &gt;20 mm Hg before enucleation</th>
<th>No. of IOP measurements in this period</th>
<th>Intraocular pressure (mm Hg)</th>
<th>Mean</th>
<th>Range</th>
<th>Day of enucleation</th>
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<tr>
<td>456 B</td>
<td>R</td>
<td>35</td>
<td>6</td>
<td>44.7</td>
<td>28-50+*</td>
<td>50+</td>
<td>12</td>
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<tr>
<td></td>
<td>L</td>
<td>0</td>
<td>6</td>
<td>13.4</td>
<td>12-17</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>0025 P</td>
<td>R</td>
<td>25</td>
<td>6</td>
<td>50+</td>
<td>All 50+</td>
<td>50+</td>
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<td>L</td>
<td>0</td>
<td>6</td>
<td>13.6</td>
<td>12-16</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>V 953</td>
<td>R</td>
<td>78</td>
<td>12</td>
<td>36.2</td>
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<td>17</td>
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<td></td>
<td>L</td>
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<td>12</td>
<td>15.3</td>
<td>11-17</td>
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<tr>
<td>948 D</td>
<td>R</td>
<td>28</td>
<td>5</td>
<td>31.7</td>
<td>22-50+</td>
<td>50+</td>
<td>38</td>
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<tr>
<td></td>
<td>L</td>
<td>28</td>
<td>5</td>
<td>28.9</td>
<td>23-38</td>
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<td>R</td>
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<td>8</td>
<td>40.4</td>
<td>27-50</td>
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</tbody>
</table>

*50+ denotes intraocular pressure above 50 mm Hg, the upper limit of the Perkins tonometer.
† Untreated, control eyes.

ganelles and the swelling of the axon which accompanies this change are a marker of the site of the damage.

In the present study, the anterior optic nerves and the macular region of the retina of rhesus monkeys were studied by light and transmission electron microscopy during the time period 3 to 11 weeks after experimental glaucoma was induced by argon laser photocoagulation of the anterior chamber angle. Identification of abnormal organelle accumulation and axonal swelling has allowed description and localization of the axoplasmic flow blockade accompanying the early changes of glaucomatous cupping of the nervehead in these animals.

Materials and methods

Secondary glaucoma was induced in seven eyes of five rhesus monkeys by the technique of repeated argon laser photocoagulation of the anterior chamber angle. Each eye required either one or two treatments before glaucoma was induced. The monkeys used in this study weighed 3 to 5 kg and had normal eyes before the laser treatment. Three eyes, of three monkeys, were not treated and served as untreated controls.

The laser-treated monkeys were lightly anesthetized with phencyclidine (Sernylan; 1 to 2
Fig. 2. Light micrographs of normal optic nervehead; left eye, monkey 456 B, temporal side (0.5 μm plastic section, toluidine blue) A, Smooth course of axon bundles from retina to retrolaminar nerve is shown. (x160) B, Higher magnification of area in box in A. Axons pass trabecular beams without significant swelling, vacuolization, or collection of dark-staining material. (x625.)

mg/kg, intramuscular) and examined every 2 to 7 days. Intraocular pressure was measured with the Perkins applanation tonometer. The optic nerveheads were examined with the indirect ophthalmoscope, and the disc appearance was recorded photographically.

The eyes were enucleated for histopathologic examination after the intraocular pressure had been elevated above 20 mm Hg for 19 to 78 days. The eyes were enucleated immediately after administration of an intravenous overdose of pentobarbital and were fixed in 2.5% glutaraldehyde solution in pH 7.3 phosphate buffer after making an incision of the sclera and choroid over the pars plana.

The optic nervehead and the macular region of the globe were excised during the 2 hr fixation in glutaraldehyde solution; these were trimmed into smaller pieces and post-fixed in 1% osmium tetroxide solution for 2 additional hours. Tissues were then dehydrated in an ascending series of ethyl alcohol and embedded in an epoxy resin. Sections cut at 0.5 μm thick were stained with toluidine blue and examined by light microscopy. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by transmission electron microscopy.

Axon bundles in the regions of the anterior and posterior lamina cribrosa of both the nasal and temporal sides of the optic nerve were specifically examined in this study.

Results

Clinical observations. The pressure in the untreated eyes ranged from 11 to 19 mm Hg. After laser applications were completed, the pressure became elevated in the treated eyes. The amount of elevation varied from day to day; this is illustrated in Fig. 1, which shows the time course of the intraocular pressure in the two monkeys that had bilateral treatment. In general, the pressure was in a range considered clinically elevated (>20 mm Hg) for 3 to 11 weeks. A summary of the clinical course of the five monkeys is shown in Table I.

The changes in the optic nervehead of the treated eyes reflected the amount of elevation of the intraocular pressure and the duration of the elevation. For example, in one eye (monkey V 953, RE) during 175 days of repeated examinations the pressure ranged between 15 and 30 mm Hg, with isolated spikes to the 40's. The disk was normal upon clinical examination. After an additional 2 weeks of pressure between 30 and 50 mm Hg, a slight, central cone-shaped cup developed; the disc was a pink color, and the vessels were centrally located. For 78 days before enucleation, the pressure in this eye was above 20 mm Hg. At the other extreme (monkey, 0025

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Fig. 3. Electron micrograph of normal optic nerve, on the temporal side at level of anterior scleral lamina, showing axons (single arrow) containing occasional mitochondria (double arrows) and scattered dense bodies. Glial cells (G) are more dense than axons. Vitreous toward the top. (Calibration bar = 1 μm; ×15,000.)

P, RE), six examinations during 26 days indicated the pressure was above 50 mm Hg (the upper limit of the Perkins tonometer). At the end of that time, rim-to-rim, superiorly undermined, cone-shaped cupping was present; the vessels were displaced slightly to the nasal side of the center of the disk; the disk was a light pink color with a slightly paler center. The other eyes which developed glaucoma showed disk changes between these two extremes.

Morphological observations. Light microscopic examination of the normal nerveheads revealed that the bundles of axons passed from the level of the retina to the myelinated retrolaminar nerve without significant vacuolization or swelling or presence of dark staining material (Fig. 2). The electron microscopic examination of both the anterior and posterior parts of the normal axon bundles in the lamina scleralis showed a few, scattered dense bodies within the axons. In longitudinal sections the axons were of fairly regular diameter, with some interaxonal variation. The mitochondria exhibited no more than slight swelling and were scattered, occasionally being found in groups of two or three (Fig. 3).

Two of the specimens were obtained after 4 weeks, or slightly longer, of pressure elevation above 40 mm Hg (monkey 436 B and 0025 P, RE's, Table I). A third specimen had a pressure elevation above 32 mm Hg, with the pressure spiking above 40 mm Hg on occasions, for 6 weeks and also had a longer history of lower pressure elevation (monkey V 953, RE, Table I). Light and electron microscopic examination of these three specimens showed extensive pathologic change of the axons and neuroglia in the prelaminar, laminar, and retrolaminar portions of the optic nerve. The axons were swollen and there was gliosis. In the retrolaminar part of the nerve there were myelin fragmentation
and phagocytosis by histiocytes. In these specimens the ganglion cell layer of the retina appeared to be normal.

Four of the eyes had less extensive histopathologic change. These are the four eyes whose pressure is illustrated in Fig. 1. In one, the pressure ranged from 22 to 50 mm Hg during 4 weeks (monkey 948 D, RE); in the other eye of the same monkey (No. 948 D, LE) the pressure ranged from 23 to 38 mm Hg during the 4-week interval. In the third eye the pressure ranged from 27 to 50 mm Hg, with at least 11 days of pressure above 40 mm Hg, for a total of 19 days (monkey 930 D, RE). In the fourth eye the pressure ranged from 21 to 38 mm Hg for 19 days, with the majority of the measurements indicating a pressure in the low 20’s during this time (monkey 930 D, LE).

In these four eyes the retinal ganglion cell layer, the retinal nerve fiber layer, and the prelaminar optic nerve all appeared to be normal. On light microscopic examination, localized in axon bundles within the choroidal and scleral lamina cribosa were distinct swellings of axons associated with accumulation of dark-staining material. The swelling typically occurred on the proximal (anterior) side of the cribiform beams; the dark-staining material typically was found on the distal (posterior) side of the beams. When an axon bundle showed these changes, the entire cross-section of the bundle was affected (Fig. 4). Sections in a plane which crosses the site where a cribiform beam indents an axon bundle showed accumulation of dark-staining material on the distal side of the indentation (Fig. 5). To a greater or lesser extent, these changes were present in the optic nerves of all four of these eyes; the changes occurred in the entire anterior-posterior extent of the scleral lamina cribosa. The changes were more pronounced in the axial portion of the optic nerves but could be found in axon bundles near the peripheral part of the nerve and were found in both the nasal and the temporal halves of the nerve. Electron microscopic examination disclosed that the changes were similar in both the anterior and posterior scleral lamina parts of the optic nerves. The glial cells appeared to be normal. Anterior to collagenous bands of the lamina and to nearby glial structures there was a variable amount of swelling of axons, with occa-

Fig. 4. Light micrographs of right eye of monkey 948 D, temporal side (0.5 μm plastic section, toluidine blue). A, Collections of dark-staining material (arrows) in axon bundles within scleral lamina. This is more marked in axial part of nerve. (×160) B, Higher magnification of area in box in A. Dark-staining material collected on posterior side of laminar beam (single arrow). Axons anterior to beam are swollen (double arrows). (×625.)
Fig. 5. Light micrographs of right eye of monkey 930 D, temporal side (0.5 μm plastic section, toluidine blue). A, Collections of dark-staining material (arrows) in axon bundles within scleral lamina (×160) B, Higher magnification of area in box in A. Where laminar beam indents axon bundle dark-staining material accumulates (arrow). (×625.)

Fig. 6. Electron micrographs of right optic nerve of monkey 948 D, temporal side, posterior lamina sclera. Axons show reactive enlargement (single and double arrows). Mitochondria within axons are swollen; glial cells (G) are normal. (Calibration bar = 1 μm, ×15,000.) Inset: Higher magnification of area indicated by double arrows. Axonal mitochondria are swollen; nearby glial mitochondria are comparatively normal. (×37,500.) Vitreous toward the top.
Fig. 7. Electron micrographs of left optic nerve of monkey 918 D, temporal side, posterior lamina scleris. Posterior to collagenous beams of scleral lamina (LC) axons contain accumulation of swollen mitochondria (single arrow) and dense bodies (double arrow). Glial cells (G) are normal. (Calibration bar = 1 μm; ×15,000.) Inset: Higher magnification of area indicated by double arrows. Dense bodies, some of which are laminated, are accumulated inside axons; this correlates with dark-staining material seen in light micrographs. (×37,500.)

Discussion

In the present study, examination of the retina and the optic nerve of eyes with experimental glaucoma localizes the early glaucomatous changes to the level of the scleral lamina cribrosa, in the axons. Previous publications have shown that injured axons first react by exhibiting swelling on both the proximal and the distal sides of the injury site. Axoplasm and organelles accumulate. Excessive numbers of dense bodies and mitochondria, with associated mitochondrial swelling, indicate the axonal damage. The accumulation of mitochondria on both the proximal and distal sides of the injury site is due apparently to the complex, to-and-fro movement of these organelles within the axons.1–3 Reactive axonal enlargements can be identified with either the light or the electron microscope.4 As the reaction progresses after injury, electron microscopic examination
shows swelling of mitochondria, shrinkage of axoplasm, plasma membrane fragmentation, and clearing of degenerated axons from the tissue by macrophages. Three to 6 weeks after a severe injury the proximal axon segments have disappeared from the tissue. Changes observed in the present study are consistent with the glaucoma causing localized axonal injury.

Of possible importance in the present study are the observations that in eyes with milder or earlier injury the damage affects some bundles of axons more than others and that within a damaged bundle most, if not all, of the axons are affected. In this study, the optic nerves were sectioned longitudinally. In general, the earliest and the most severe injury occurred in the axially located bundles, but milder alterations from normal were identifiable across the entire extent of the optic nerve. This is in contrast to the observations in one study of the effects of acute elevation of intraocular pressure; the study showed more marked block of axoplasmic transport on the temporal side of the optic nervehead.

The present study does not resolve the controversy concerning whether the axonal injury of glaucoma or the block of axoplasmic flow from elevated intraocular pressure occurs as a result of ischemia or mechanical compression. The sharp localization of the early injury observed in the present study, with the most marked change immediately adjacent to scleral collagenous or glial laminar tissue, suggests that mechanical compression was occurring. Related to this, small blood vessels within the scleral lamina, immediately adjacent to axon bundles with injury, contain blood cells. Nonetheless, neither of these observations is sufficient to prove that mechanical constriction is the cause of the axonal injury.

Clinical experience indicates that people with ocular hypertension often withstand long periods of intraocular pressure elevation to the high 20's or low 30's without detectable clinical signs of injury. This is in contrast to the histopathologic observations of the present study. The anterior optic nerves of several young, healthy rhesus monkeys in this study showed marked damage in a surprisingly short time. Less than 4 weeks of pressure above 50 mm Hg, 5 weeks of pressure averaging in the mid-40's, and 11 weeks of pressure averaging in the mid-to-high 30's constituted a sufficient insult to cause severe damage. The change was so extensive and severe that recovery would not have been expected to occur if the intraocular pressure had been lowered to normal at that time.

Less severe morphologic alteration was found in optic nerves from eyes with a shorter-duration (3 weeks) elevation of intraocular pressure averaging 40 mm Hg or 3 to 4 weeks of intraocular pressure elevation averaging from the mid-20's to the low-30's. In these particular eyes, on the day of enucleation the pressure ranged from the mid-30's to above 50 mm Hg; the eye with the lowest average pressure (monkey 930 D, RE, Table I) had a pressure measured at 24 mm Hg 3 days before enucleation. Despite these observations of not too long or too great a pressure elevation, all four eyes in this group had histopathologic alteration of the optic nerve, localized within the scleral lamina cribrosa. In these eyes the retinal ganglion cell layer, nerve fiber layer, anterior optic nervehead, and the retrolaminar optic nerve appeared to be normal. The changes seen in these eyes are essentially identical to the morphologic changes reported by Minckler et al. after 4 to 48 hr of intraocular pressure elevation caused by a reservoir attached to an anterior chamber cannula. The localization of the changes to the scleral lamina cribrosa is consistent with the site of block of axoplasmic transport due to acute elevation of intraocular pressure in the monkey. Therefore it is speculated that the changes seen in these less severely affected eyes in the present study are due to a block of axoplasmic flow. The observations of the present study are also similar to those made by Vrabec during study of a group of glaucomatous human eyes with thick sections of the optic nerves stained with a silver method.

The present study shows that chronic ele-
vation of intraocular pressure affects some bundles of axons more than others. In monkey eyes with very prolonged, moderate elevation of intraocular pressure, damage to the optic nerve structure may be severe, but axoplasmic transport is not necessarily completely interrupted in all remaining axons (personal observations). It is therefore postulated that glaucomatous damage occurs in a progressive manner, with damage to a second bundle of axons occurring after earlier damage to a first bundle. The effect upon the second bundle may be dependent upon the structural alteration accompanying the damage to, and removal of, the first bundle. This postulate will be more acceptable if the mechanical compression mechanism of axon injury is shown to apply to the optic nerve damage of glaucoma.

REFERENCES