Primary Open-Angle Glaucoma Is Not Associated With Photoreceptor Loss

Kurtis R. Kendell, Harry A. Quigley, Lisa A. Kerrigan, Mary E. Pease, and Erica N. Quigley

Purpose. To determine if photoreceptors die in primary open-angle glaucoma.

Methods. Retinas were examined in a masked fashion from nine standard locations of 14 eyes with documented open-angle glaucoma and from nine age-matched control eyes. The number and density of photoreceptors, as well as the area and height of the outer nuclear layer, were calculated with an automated image analysis system. The number of photoreceptors per 0.1 mm of retina was determined.

Results. No significant difference was seen between control and glaucomatous eyes in comparisons of photoreceptor density, outer nuclear layer height, or photoreceptors per 0.1 mm of retinal length in nine retinal zones. There was no detectable association between photoreceptor number and severity of glaucoma (defined as mild, moderate, or severe), visual field, and optic nerve fiber loss. In eyes in which damage predominated in the upper or lower visual field, no corresponding difference in photoreceptor number in upper compared to lower retinal zones was observed.


The predominant damage to the retina in open-angle glaucoma is loss of retinal ganglion cells and their axons.1-3 The physical features of optic disc excavation and atrophy of the retinal nerve fiber layer support the concept that ganglion cell loss occurs.45 The pattern-evoked electroretinogram, a response that is dependent on ganglion cell presence,6 declines with damage from glaucoma, both in human eyes7 and in experimental monkeys.89 There have been no demonstrations of either primary or transsynaptic degeneration of mid-retinal or outer retinal cells in open-angle glaucoma. Indeed, the flash-evoked electroretinogram is normal even in advanced glaucoma,10 indicating no major effect on the rods, cones, and mid-retinal cells that generate this response. However, to our knowledge, no previous investigation has attempted to quantify the number of rods and cones in primary glaucomatous eyes. Recently, Panda and Jonas11 reported a decrease in photoreceptor number in eyes with advanced secondary glaucoma caused by traumatic injury.

Because potential photoreceptor loss in glaucoma may have clinical or pathophysiological significance, we designed the present investigation to compare the estimated number of photoreceptors in human eyes with a history of primary open-angle glaucoma to age-matched, normal eyes.

METHODS

The eyes for this study were obtained by postmortem donations to eye banks with informed consent of next of kin and in accordance with the tenets of the Declaration of Helsinki. We collected ophthalmic histories pertaining to glaucoma in all 14 eyes of 13 donors (Table 1). Either visual field studies (12 eyes) or optic nerve histology (11 eyes), or both, was available to provide staging and corroboration of damage from
glaucoma. Nine control eyes matched for age were selected from nine persons without a known history of eye disease (age: 72 ± 15 years for controls, 76 ± 18 years for those with glaucoma, mean ± 1 SD, P > 0.05, two-tailed Rest). As detailed below, control eyes also had normal retina and optic nerve head appearance on examination with the dissecting microscope, normal retinal and optic nerve examination by light microscopy, and an optic nerve area of neural tissue within the range we previously reported for identically prepared tissues in normal eyes. It is, therefore, unlikely that control eyes had glaucoma. All persons whose eyes were studied were white. There was a slightly higher proportion of male donors in the control group. The mean time to fixation of the glaucomatous eyes (8.5 ± 8.9 hours) was not significantly different from that of control eyes (9.2 ± 8.0 hours, two-tailed Rest, P = 0.85). The glaucomatous eyes were most often fixed in phosphate-buffered aldehydes, whereas the control eyes were typically fixed in aqueous aldehyde solutions (see last paragraph, Methods). All eyes were examined under the dissecting microscope, and the location of the fiber loss was recorded to correlate its location with the location of visual field defects and potential visual field loss.
photoceptor loss. Each control eye had a neural area within 25% of the normal mean value.

The visual field tests were staged as follows: no defect = normal, small defect or nasal loss = mild, major loss in one hemifield = moderate, and major loss in upper and lower fields = severe. Evaluation of the field test and of neural loss was performed independently and in a masked fashion. The data were then compared to give a final degree of injury estimate; where the field and nerve estimates differed, the nerve estimate was used (for example, field = moderate, nerve = mild, final grade = mild).

Nine samples of the retina were taken from each of the eyes at the locations shown in Figure 1 using a 3-mm trephine. The samples were kept flat using a nylon screen in a filter during dehydration in graded ethanol. They were embedded in JB-4 resin (Polysciences, Warrington, PA), and sections were stained with 0.5% hematoxylin for 30 minutes.

The number of photoreceptor nuclei per unit area (density) and the height of the outer nuclear layer (ONL) were measured, in a masked fashion, using the VIDAS microscopic counting and measurement system (Roche Image Analysis Systems—Elon College, NC, and Carl Zeiss, New York, NY). This system acquires a digital video image of the tissue, with nuclei seen as dark areas whose size and number can be estimated with appropriate software. The counts were made in an area circumscribed by the ONL boundaries in randomly chosen continuous retinal strips (Fig. 2). We multiplied the density of photoreceptor nuclei within the ONL by the ONL height by 0.1 mm to estimate the photoreceptor number in a defined length of retina in each region. The 0.1-mm length seemed an appropriate length to account for local variation. We examined all three variables, density, ONL height, and nuclei per 0.1 mm, because photoreceptor loss could be manifest in at least two different ways. First, the ONL might remain the same height with a decrease in its cell density as cells die. Or, the ONL might decrease in height as cells die, preserving the same cell density. Unless both possibilities were evaluated, a true difference between glaucoma and normal might be missed. Each of the three measurements was averaged from five different zones of each of the nine retinal locations. We did not attempt to differentiate between rod and cone nuclei (see Discussion).

Four persons performed the counting process. Each evaluated 15 retinal areas on three separate occasions to estimate intraobserver and interobserver variation. The coefficient of variation was approximately 5% for interobserver variation in nuclei per 0.1 mm and ONL height counts.

The total number of photoreceptors counted was 67,697. The mean number of photoreceptor nuclei counted per eye was 2904 (SD = 776; n = 23); range, 1607 to 3606). These data were gathered from 45 locations (five from each of nine retinal samples), each location comprising an area of approximately 5000 square micrometers, for a total area of about 0.225 mm² per retina.

We measured both eyes of one person with glaucoma. Because intradonor variation may be less than that seen among donors, all summary calculations for the groups with glaucoma were made with one of these eyes excluded and with both eyes included. No sig-
TABLE 2. Mean Density, ONL Height, and Nuclei/0.1 mm for Control and Glaucoma Eyes

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>All Glaucoma</th>
<th>Glau: normal</th>
<th>Glau: mild</th>
<th>Glau: moder</th>
<th>Glau: severe</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N)</td>
<td>(9)</td>
<td>(14)</td>
<td>(4)</td>
<td>(4)</td>
<td>(3)</td>
<td>(3)</td>
</tr>
<tr>
<td>Density</td>
<td>187 (23)</td>
<td>219 (26)</td>
<td>225 (8)</td>
<td>321 (18)</td>
<td>237 (9)</td>
<td>178 (13)</td>
</tr>
<tr>
<td>ONL Height</td>
<td>40.3 (5.8)</td>
<td>37.1 (4.5)</td>
<td>38.8 (2.3)</td>
<td>36.1 (4.3)</td>
<td>36.0 (1.9)</td>
<td>37.1 (7.2)</td>
</tr>
<tr>
<td>Nuclei/0.1 mm</td>
<td>75.3 (13.1)</td>
<td>80.8 (12.9)</td>
<td>86.1 (4.9)</td>
<td>82.4 (12.1)</td>
<td>85.1 (4.0)</td>
<td>67.4 (17.1)</td>
</tr>
</tbody>
</table>

ONL = outer nuclear layer; Glau = glaucoma; moder = moderate. Difference in density between control and all glaucoma significant (P = 0.007); other differences between these two groups not significant (P > 0.05). Differences between subgroups of glaucoma and normal not tested because of small samples. Units for mean (standard deviation) in density = nuclei per 100 square micrometers; in ONL height = micrometers; in nuclei/0.1 mm = estimated cell number in 100 micrometer length of retina.

significant difference was seen in any of the calculations; both eyes were included in the analyses presented.

Because the type of fixation might affect the counting of photoreceptor nuclei, we have evaluated six retinal samples from each of five further control eyes whose data are not included here. These were obtained without prior fixation; half the retina of each eye was fixed in aqueous formalin and half in phosphate-buffered 4% glutaraldehyde/2% paraformaldehyde. Mean photoreceptor nuclei per 0.1 mm was not statistically different between the fixation protocols.

RESULTS

The density of photoreceptors was actually higher in glaucomatous than in control eyes (t-test, P = 0.007; Table 2). There were no significant differences between glaucomatous and control groups in the ONL height or photoreceptor nuclei per 0.1 mm (P > 0.05; Table 2). The ONL height of the glaucomatous eyes was somewhat less than the control ONL height. However, because of the higher density of photoreceptors in glaucomatous eyes, when the number of photoreceptors in a linear segment of retina was calculated, the mean number of nuclei per 0.1 mm in the glaucomatous eyes (81 ± 15 nuclei) was numerically greater than the control mean (75 ± 13 nuclei), though not significantly so (P > 0.05). These data have an 80% power to determine a mean difference between groups of 16 nuclei per 0.1 mm and a 95% power to detect a mean difference of 21 nuclei per 0.1 mm.

With stratification of the glaucomatous eyes by severity of damage, all groups except the most severe had values of density and nuclei per 0.1 mm that exceeded the normal mean (Table 2). The single exception was the group with severe glaucoma, in which the values were lower. The value for nuclei per 0.1 mm in the group with severe glaucoma can be attributed solely to one outlier specimen, because the other two members in this group had values at or above the normal range.

One glaucomatous eye had selective superior visual field loss, whereas two others had selective inferior visual field loss. Comparisons of photoreceptor density and ONL height in superior versus inferior zones show no tendency for photoreceptor numbers to match the hemiretinal ganglion cell loss (Table 3).

The retinal zone, including the fovea (#9), had the lowest density and the lowest number of photoreceptor nuclei per 0.1 mm in control and glaucomatous eyes (Table 4). None of the individual zones had a significantly different number of photoreceptor nuclei between glaucomatous and control eyes (Table 4).

DISCUSSION

We found no evidence that there are fewer rods and cones in the retinas of persons with primary open-angle glaucoma than in control eyes in persons of similar ages. The mean estimate for photoreceptor number was actually higher in the group with glaucoma, though the difference was not statistically significant. There was no single region among the nine zones that were studied in which the group with glaucoma substantially differed from normal. There was no consistent trend toward fewer photoreceptors with increasing damage from glaucoma. The values of one retinal specimen with glaucoma differed dramatically from those of the others. The severe loss in mid-retinal cells and in overall retinal thickness in this specimen suggested the possibility that it had suffered a vascular occlusion, though we have no evidence of this in its history or in its gross or microscopic appearance. There was no trend toward fewer photoreceptors in the more damaged half of three specimens with altitudinal glaucoma injury. In summary, we find no reason
to conclude that photoreceptor atrophy is characteristic of primary glaucoma. It is possible that detrimental effects on retinal neurons might occur that would not be expressed as loss of cells, but such functional changes would not have been detected by this study.

It has been speculated that loss of photoreceptors might provide evidence for impaired chorioidal vascular function in glaucoma. Alternatively, loss of photoreceptors might occur in glaucoma as a transsynaptic degeneration after loss of ganglion cells and the intervening bipolars. Our study does not support either of these as having a role in primary open-angle glaucoma.

A previous report that found a loss of rods and cones in eyes with secondary glaucoma had several differences from the present investigation. Perhaps most important, the glaucomatous eyes in that study were blind, painful eyes enucleated because of secondary glaucoma after severe injury. Those eyes may have suffered undetected vascular occlusion, despite any direct evidence for this at enucleation and histologic examination. Furthermore, concussive trauma routinely leads to loss of photoreceptors, as demonstrated in experimental models. In addition, several of the eyes in that report had undergone glaucoma surgery that might have been associated with transient chorioidal and/or serous retinal detachment, either of which also could lead to loss of rods and cones. There were no estimates of the optic nerve fiber loss in the report of Panda and Jonas, though we might assume that there were few ganglion cells remaining in eyes that were considered appropriate for enucleation. Thus, the difference in our results may be an accurate reflection of differences between the consequences of secondary glaucoma and their absence in eyes with primary glaucoma, such as the ones we studied.

We included only eyes with documented histories of primary glaucoma. We think that in every case the mechanism was open angle, though one eye had had a laser iridectomy. We used eyes with either an available visual field test or a detailed estimation of optic nerve fiber number by histologic examination. A large number of photoreceptor nuclei were counted with an image analysis system from a variety of locations in the posterior retina in each specimen. Our method has acceptable reproducibility both within and among observers. The use of a plastic embedding medium instead of paraffin improved the resolution of individual photoreceptor nuclei.

Our study might have failed to detect differences between glaucoma and normal eyes because of its modest sample size. However, a power calculation indicates that we had a 95% chance to detect a 29% loss in photoreceptor density if it had occurred. This is a difference of the magnitude reported by Panda and Jonas. Although there may be autolytic changes in eye bank tissue that could obscure some findings, we matched the control tissues for all relevant parameters, including time from death to fixation. A slight difference between some members of the control group and the group with glaucoma in the solutions in which the aldehyde fixatives were used is unlikely to be of importance. Although retinal area is related to photoreceptor number, glaucomatous and normal eyes in this study did not differ in axial length measurements, suggesting that we controlled adequately for eye size.

The finding that there are fewer photoreceptors in our specimens in the zone centered on the fovea

<table>
<thead>
<tr>
<th>Zone</th>
<th>All Glaucoma</th>
<th>Control</th>
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<tbody>
<tr>
<td>1</td>
<td>90.8 (16.6)</td>
<td>98.5 (14.4)</td>
</tr>
<tr>
<td>2</td>
<td>85.0 (14.3)</td>
<td>88.7 (6.8)</td>
</tr>
<tr>
<td>3</td>
<td>97.4 (16.8)</td>
<td>93.3 (10.7)</td>
</tr>
<tr>
<td>4</td>
<td>81.4 (20.9)</td>
<td>94.0 (7.7)</td>
</tr>
<tr>
<td>5</td>
<td>80.8 (20.5)</td>
<td>78.4 (15.5)</td>
</tr>
<tr>
<td>6</td>
<td>79.0 (19.3)</td>
<td>91.1 (21.6)</td>
</tr>
<tr>
<td>7</td>
<td>77.6 (15.9)</td>
<td>80.5 (16.1)</td>
</tr>
<tr>
<td>8</td>
<td>74.0 (15.2)</td>
<td>84.7 (11.7)</td>
</tr>
<tr>
<td>9</td>
<td>62.8 (19.7)</td>
<td>71.1 (13.9)</td>
</tr>
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</table>

Units are mean (±standard deviation) nuclei per 100 μm linear length of retina. In the group with glaucoma, (n = 14), zone 9 is significantly lower (P < 0.05) than all zones except zone 8. In control group (n = 9), zone 9 is significantly lower than all zones except zones 5 and 7. None of the individual zone data are significantly different between the group with glaucoma and the control group (all P > 0.05).
was initially puzzling to us. We then compared the available information on regional distribution of rods plus cones and realized that our data are consistent with the estimates of Osterberg and Curcio. Within the central 30° of the retina, the density of rods is relatively constant except in the centralmost 1° (the fovea), where there are only cones. The cone number is small relative to rods outside the fovea. Hence, the rod density largely determines the photoreceptor density in our data. Only in the foveal zone does the cone density reach a high level, but it still has a lower value than the density of rods (plus cones) elsewhere in the posterior retina.

Because our study and the previous report of histologic counts of photoreceptors concentrated largely on areas of the retina in which rods predominate, it is possible that a subtle loss of cones—for example, a selective decrease in the number of short-wavelength sensitive (blue) cones—might not have been detected. To approach this question, we have studied the number of cones in monkey eyes with chronic experimental glaucoma (data not shown) in the area 900 μm to 1200 μm from the foveal center. The number of cones in glaucomatous eyes was within 4% of that in the normal fellow eyes of these monkeys. It is unclear whether further investigation of photoreceptor differences between glaucomatous and normal eyes will be productive. We hope that continued study of the anatomic changes characteristic of glaucoma will stimulate correlative research into the improved functional testing of this disorder.

Key Words
glaucoma, pathology, photoreceptor, pathogenesis, image analysis

References