Decreased Photoreceptor Count in Human Eyes With Secondary Angle-Closure Glaucoma

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Glaucoma has been known to be associated with a loss of retinal ganglion cells and their axons throughout the fundus and a decreased count of photoreceptors and retinal pigment epithelial (RPE) cells in the parapapillary region. This study investigated whether glaucomatous changes of the deep retinal layer occur outside the parapapillary region. The nuclei of the retinal photoreceptors and RPE cells were counted in histologic slides of 23 eyes with painful secondary angle-closure glaucoma resulting from perforating corneal injuries. Fourteen eyes with malignant choroidal melanoma not involving the ciliary body or trabecular meshwork served as the control group. No surgical procedure, including laser treatment, had been performed posterior to the ora serrata. There were no hints of retinal vessel occlusion and localized traumatic retinopathy, historically, ophthalmoscopically, or histologically. Photoreceptor count was significantly lower ($P < 0.05$) in the glaucoma eyes than in the control group. Count of RPE cells did not differ between the two groups. This may indicate that glaucoma can be associated with a loss of photoreceptors. This could be important for psychophysical testing and may point to a more widespread involvement of ocular tissues in glaucoma than believed. Invest Ophthalmol Vis Sci 33:2532-2536, 1992

Glaucoma, as a disease process, has been shown to affect the inner retinal layer. This is associated with a loss of retinal ganglion cells and their axons, a decreased visibility of retinal nerve fiber bundles, and a diminution of neuroretinal rim area in the optic nerve head. Glaucoma has been connected with various psychophysical deficits, including glaucomatosus visual field defects and, in some patients, acquired cyanopsychromatopsia. Besides these alterations in the superficial retinal layers, changes have been described in the deeper layers of the retina close to the optic nerve head. Ophthalmoscopically, the abnormalities appear as irregular hypopigmentation and hyperpigmentation or as increased visibility of denuded Bruch's membrane, large choroidal vessels, and the sclera. They are correlated with an enlarged blind spot. Histomorphometry has shown that this parapapillary chorioretinal atrophy in patients with glaucoma is associated with irregular pigmentation, diminution, and complete loss of RPE cells as well as a decreased count, or absence, of retinal photoreceptors.

Only a few investigations have been directed at morphological variations in the retinal tissue deeper than the ganglion cell layer outside the parapapillary region. The purpose of the present study was to evaluate glaucomatous changes in those tissue layers. This may be important for interpreting results of psychophysical tests and may give hints for a more widespread involvement of ocular tissues by the glaucomatous process. Although traumatic secondary angle-closure glaucoma represents only the high-pressure end of the spectrum of chronic glaucomas, we chose these eyes as study objects. They can be obtained perfectly fixed and in sufficient numbers, whereas eyes of patients with primary open-angle glaucoma are rarely enucleated post mortem and often show autolytic alterations, especially at the level of the retinal pigment epithelium and the retinal photoreceptors. This renders them unsuitable for such an examination.

Material and Methods

Twenty three eyes (8 right eyes, 15 left eyes) with secondary angle-closure glaucoma resulting from perforating corneal injuries were included in the study. Mean age of the 14 men and 9 women was $65.6 \pm 17.5$ yr (mean $\pm$ standard error of the mean; range $30-87$ yr). Preoperatively, intraocular pressure usually had been persistently higher than $35$ mmHg for several years. It resulted in painful bullous keratopathy. Vi-
sion was reduced to defective light perception or amaurosis. Antiglaucomatous operations, including cyclocryocoagulation, were performed in nearly all patients but had not sufficiently reduced the intraocular pressure.

No history of central retinal vessel occlusion nor surgical procedures performed posterior to the ora serrata, including laser treatment, could be elicited. Similarly, no sign of such a disease or therapy could be detected ophthalmoscopically or histologically. Because the corneal perforation prevented a detailed fundus examination at the time of laceration, traumatic retinopathy could not be excluded with certainty. Gross examination of the fixed globes and microscopic evaluation, however, did not reveal signs or sequences of traumatic involvement of the retina, such as Berlin’s edema, formation of retinal tears, retinal detachment, chorioretinal atrophic areas, migration of RPE cells into the retinal tissue, disruption of the normal anatomic relationship between the retinal pigment epithelium and the photoreceptors, direct or indirect uveal and retinal ruptures, and central subretinal neovascularization.24 The cross sections of the optic nerves showed a total loss of nerve fibers with subsequent disorganization of the normal oligodendrocyte-astrocyte-nerve fiber architecture. There was no avulsion or division of the optic nerve nor were there signs of direct or indirect trauma. Eyes with obvious generalized or localized retinal atrophy were excluded to avoid any bias resulting from inclusion of eyes with unknown or undetected central retinal vessel occlusion or traumatic retinopathy in addition to glaucoma. Furthermore, the macular region, the location with the highest frequency of commotio retinae, was spared from the examination.

Fourteen eyes (eight right eyes, six left eyes) of five men and nine women with a mean age of 61.3 ± 14.8 yr (range 35–83 yr) had been removed because of a malignant melanoma of the choroid. They served as the control group. Some of the patients had received daily radiation of 4 gray for 5 d preoperatively. Histologically, the tumor broke through Bruch’s membrane and invaded the retina in some eyes. In all eyes of the control group, no evidence of tumor involvement of the ciliary body or trabecular meshwork was detected. Intraocular pressure was less than 22 mmHg.

The glaucoma and control group did not significantly differ in sex (P = 0.08), age (P = 0.38), sagittal diameter (24.5 ± 2.00 mm versus 24.5 ± 0.51 mm; P = 0.85), horizontal diameter (24.0 ± 1.35 mm versus 23.5 ± 0.78 mm; P = 0.46), or vertical diameter (23.7 ± 1.24 mm versus 23.5 ± 1.05 mm; P = 0.72) of the eye.

Immediately after enucleation, the globes were fixed in a routine manner for light microscopy. An anterior-posterior segment going through the pupil, the optic nerve, and any other abnormality, ie, the perforating injury or the tumor, was cut out of the fixed globes. These segments were dehydrated in alcohol, imbedded in paraffin, and sectioned for light microscopy.

Using a high-power objective (100×), with the numerical aperture of the condenser optimally adjusted, the thickness of the section was evaluated by bringing the bottom and top of the section sequentially into focus. The section thickness was read off the micrometer scale with which the fine focusing knob of the microscope was provided. Mean slide thickness did not significantly differ between the glaucoma and control group (8.9 ± 1.2 μm versus 9.0 ± 0.8 μm).

The nuclei of the retinal photoreceptors and RPE cells were counted and thickness of the retinal outer nuclear layer was measured in regions “A” to “H” (positions G and H only being present in eyes with greater axial length), regardless of whether the nucleolus of the cell was included in the section or not. The regions “A” to “H” were of equal width, each measuring 0.15 mm. They were located on concentric circles, equidistant (2.7 mm) from each other, starting at a distance of 0.9 mm from the optic disc border (Fig. 1). The parapapillary region containing the parapapillary chorioretinal atrophy was excluded. To determine the thickness of the retinal outer nuclear layer, a planimeter’s cursor marked by a red light (Morphomat 30; Kontron, Eching/Munich, FRG) was mirrored into the visual field of the microscope using an optical device supplied by Zeiss (Oberkochen, FRG).

The length of the retina taken from the ora serrata to the posterior fundus and forward to the ora serrata on the other side was not significantly different between the two groups (45.7 ± 2.3 mm versus 47.4 ± 4.5 mm). Orientation of the sections regarding the macula also did not vary significantly. The macular region defined as the area situated between 15° superior and 15° inferior to the temporal horizontal fundus raphe was excluded from the examination. This was done to avoid the effects of an undetected retinal commotio at the macula and the high regional variability in cell density at the fovea. The morphological variability requires that the orientation of the histologic section through the central fundus region be precisely known. Because the globes had been opened and sectioned in a routine way without direct on-sight onto the fovea, determining the meridian of the slide with an accuracy of a few degrees was not possible.

One section per eye passing closest through the optic disc center was taken for histomorphometrical
Results

In the control group, counts of photoreceptor cells and RPE cells and thickness of the retinal outer nuclear layer were statistically independent of sex, right or left eye, axial length, horizontal and vertical diameter of the globe, and age (P > 0.10). There was a tendency toward an age-related decrease in the photoreceptor count in the peripheral fundus regions. This correlation did not reach the significance level of 5%.

In the glaucoma group, count of photoreceptors was significantly lower than in the control group (Table 1). The differences were significant if they were taken as a sum and taken separately for the regions “A” to “D” (Table 1). There was a tendency toward larger differences in regions closer to the posterior part of the fundus than in regions closer to the ora serrata. Parallel to the photoreceptor cell count, thickness of the retinal outer nuclear layer was smaller in the glaucomatous eyes than in the control group. This difference was not significant at a 5% level.

The number of RPE cells did not differ significantly between the groups, neither as a sum nor as measurements at single regions.

Discussion

Traumatic secondary angle-closure glaucoma is a special form of glaucoma. Compared to primary open-angle glaucoma, it is characterized by higher intraocular pressure readings, a shorter passage of time

Table 1. Count of retinal photoreceptors (means and standard deviations) at different regions “A” to “H,” located on concentric circles, equidistant from each other, starting at a distance of 0.9 mm from the optic disc border

<table>
<thead>
<tr>
<th>Region</th>
<th>Glaucoma</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>145 ± 64</td>
<td>192 ± 48</td>
<td>0.02</td>
</tr>
<tr>
<td>B</td>
<td>171 ± 60</td>
<td>221 ± 50</td>
<td>0.02</td>
</tr>
<tr>
<td>C</td>
<td>160 ± 52</td>
<td>211 ± 29</td>
<td>0.001</td>
</tr>
<tr>
<td>D</td>
<td>142 ± 48</td>
<td>183 ± 42</td>
<td>0.01</td>
</tr>
<tr>
<td>E</td>
<td>134 ± 45</td>
<td>154 ± 43</td>
<td>ns</td>
</tr>
<tr>
<td>F</td>
<td>105 ± 43</td>
<td>111 ± 41</td>
<td>ns</td>
</tr>
<tr>
<td>G</td>
<td>77 ± 22</td>
<td>89 ± 50</td>
<td>ns</td>
</tr>
<tr>
<td>H</td>
<td>62 ± 23</td>
<td>80 ± 22</td>
<td>ns</td>
</tr>
<tr>
<td>Sum</td>
<td>316 ± 103</td>
<td>414 ± 91</td>
<td>0.02</td>
</tr>
</tbody>
</table>

ns, not significant.
until occurrence of complete optic nerve atrophy, often unilaterality, and extraocular reasons of formation of anterior peripheral synechias with secondary elevation of intraocular pressure. It can be considered to be one extreme of the disease process glaucoma ranging from eyes with marked elevated intraocular pressure to eyes with so called normal-pressure glaucoma. Although they represent only a small fraction of all glaucoma eyes, barotraumatically induced changes observed in eyes with secondary angle-closure glaucoma also may occur in eyes with primary open-angle glaucoma. Thus, evaluation of secondary angle-closure glaucoma may offer hints regarding primary open-angle glaucoma.

In the present study, eyes with secondary angle-closure glaucoma had a smaller photoreceptor cell count than eyes of a nonglaucomatous control group. Despite the statistical significance of that finding, certain factors limit the present study. Although all eyes with any hint or history of retinal vessel occlusion were eliminated from our investigation (ie, eyes with obvious retinal atrophy or disorganization) it cannot be assured that some eyes did not have such a history. Also, the trauma leading to the corneal laceration and secondary anterior chamber angle closure also might have affected the retina. However, neither gross examination nor microscopic evaluation revealed hints of traumatic involvement of the retina. Eyes with significant localized or generalized retinal atrophy had been excluded. The macular region where commotio retinæ most often occurs was spared from examination. The preoperative radiation of the melanoma eyes or the tumor by itself could have resulted in cell loss in the retina. However, this only emphasizes the significance of the cell loss in the study group.

In the literature, only a few studies have been directed at such glaucomatous changes in the deep retinal layers. Kalvin, Hamasaki, and Gass injected alpha-chymotrypsin into the posterior chamber of owl monkeys in miosis to elevate the intraocular pressure to 24–72 mmHg. They reported on severe retinal damage with the absence of retinal photoreceptors or significant alteration of this layer and grossly abnormal outer and inner nuclear layers of the retina. An electroretinogram could not be evoked from those eyes with moderate and severe glaucoma. Injection of the enzyme into the suprapapillary vitreous resulted in damage to the retina without elevation of intraocular pressure and without cupping of the optic disc. This led the authors to argue that the marked damage to the photoreceptors and the outer retinal layers was caused, at least in part, by the enzyme itself, perhaps in combination with the elevated intraocular pressure.

De Carvalho increased the intraocular pressure in rabbits by injecting cotton fragments into the anterior chamber. He observed a marked edema of the retinal ganglion cell layer in the first days after the procedure, followed by a vacuolization and disintegration of the same layer after the first two weeks. Two weeks later, the whole retina showed marked diffuse degeneration and atrophy. These changes were observed only in those animals in which the injection of the cotton fragments had caused a pressure rise. They were not present in animals with cotton fragments in the anterior chamber without elevation of the intraocular pressure. The author concluded that the degeneration of the whole retina was the result of the pressure rise. It remains unclear, however, whether the pressure rise or the marked inflammatory response provoked by the cotton fragments that led to endophthalmitis in 11 out of 42 animals was responsible for the retinal changes. Maumenee exposed two relatively freshly obtained retinæa to alpha-chymotrypsin in vitro and detected no significant changes. This may indicate that the alterations of the retinal outer layer observed in vivo in the eyes after injection of alpha-chymotrypsin and elevation of intraocular pressure may be caused by the pressure rise and not by the enzyme directly. However, retinal reaction in vitro as examined by Maumenee may have differed from retinal changes that occurred in vivo as observed by Kalvin and coworkers.

A rubber band around the equator of the eye increased the intraocular pressure to values of between 35 and 50 mmHg in a study conducted by Flocks, Tsukahara, and Miller. They reported on marked changes in all the layers of the retina. As with the results of Maumenee, Kalvin and associates, this also suggests that the pressure rise might have affected the retinal photoreceptors.

Besides these few studies, glaucomatous changes of the deeper retina outside the parapapillary region have not been mentioned in the general ophthalmic literature. As already suggested by Naumann and Ueno, the observed decreased photoreceptor count in glaucomatous eyes may indicate that glaucomatous tissue damage may not be confined solely to the inner retinal layers throughout the fundus and pigment epithelial cells and photoreceptors in the parapapillary region. The decreased count may point at a more widespread glaucomatous involvement of ocular cells, including changes of the deep retinal layers outside the area around the optic disc.

Psychophysically, glaucomatous sensory deficits—ie, acquired blue color deficiency—may be assumed also to be based upon the loss of photoreceptors and not only upon a decrease of the ganglion cell population. Pathogenetically, an altered perfusion of the
choriocapillaries could be held responsible for photoreceptor diminution in secondary angle-closure glaucoma. Moderate increments in intraocular pressure cause concomitant reductions in choroidal blood flow in cats and monkeys.\(^{30-32}\) This lack of autoregulation of the choroidal circulation contrasts the autoregulation of the retinal blood flow that has been reported to be independent of the intraocular pressure up to 60 mmHg.\(^{30,31}\)

**Key words:** glaucoma, glaucomatous optic nerve damage, retinal photoreceptors, retinal pigment epithelium, cyan-dyschromatopsia

**Acknowledgment**

We thank Prof. Dr. med. G. O. H. Naumann for the use of his laboratory.

**References**