Ultrastructural Changes in the Aqueous Outflow Apparatus of Beagles with Inherited Glaucoma

Don A. Samuelson, Glenwood G. Gum, and Kirk N. Gelatt

Spontaneous glaucoma in the beagle was exhibited after 6 months of age by elevated intraocular pressures and open iridocorneal angles followed by secondary changes. In order to appreciate alterations of the aqueous outflow apparatus in dogs with this autosomal recessive disorder, the eyes of beagles with inherited glaucoma at ages 1 day through 34 months were examined by light, scanning and transmission electron microscopy. Developmentally, no notable differences were observed between normal and preglaucomatous outflow channels through 7 months of age. In 12-month-old glaucomatous chamber angles clustered basement membrane-like material was found scattered throughout the outer corneoscleral trabecular meshwork. In this region elastin-like fibers appeared to be more numerous and arranged less regularly than age-matched normal eyes. Occasional trabecular cells within the corneoscleral trabecular meshwork possessed small clusters of serrated, opaque rods within their cytoplasm. In the older glaucomatous dogs these changes were more generalized and extensive throughout the entire corneoscleral trabecular meshwork. In some individual eyes the anterior chamber angles were observed to be narrow both clinically and histologically. These outflow apparatuses were additionally characterized by compressed, less organized trabeculae with a concomitant build-up of extracellular materials. No correlation was found between the shallowness of the iridocorneal angle and increase in intraocular pressure. Primary glaucoma in the beagle during its earlier phases compared more positively to open-angle glaucoma in man than any of the other spontaneous types in animals. Invest Ophthalmol Vis Sci 30:550-561, 1989

Glaucoma represents a sustained elevation in intraocular pressure (IOP) that results in damage to the optic nerve and concomitant loss in vision. Among nonprimate species glaucoma has been most frequently observed in the dog and is less common in the cat. In the dog, glaucoma is often primary, being either familial or inherited. Glaucoma in the beagle is inherited as an autosomal recessive trait and begins as a chronic bilateral elevation in IOP between 9 to 12 months of age. The early stages of this disease progress for the next 6 to 9 months. Intraocular pressures rise from 20-22 mm Hg (normal in the canine eye) to 30-35 mm Hg with reduced tonographic (C) values. The iridocorneal angle remains open, and the optic nerve head appears normal. As the disease progresses without treatment to moderate and advanced stages, corneal edema, episcleral congestion, buphthalmos and lens luxation, closure of the iridocorneal angle and retinal degeneration can occur.

All studies have strongly supported the hypothesis that spontaneous glaucoma in the beagle should be classified as a primary open angle glaucoma. The present investigation examines this hypothesis by ultrastructural observations of aqueous humor outflow pathways in the beagle with inherited glaucoma at different stages of this disorder and compares these findings with aqueous humor outflow pathways in normal, laboratory-quality beagles.

Materials and Methods

Clinical examinations including gonioscopy, tonometry, and fundoscopy were performed on all dogs 3 months of age and older. All affected dogs that were used in this study were offspring of glaucomatous parents, who both were affected with the recessive trait. Eyes of 14 affected beagles at ages 1 day, 1 week, 3, 7, 12, 22, 24, 25, 26, 28 and 34 months and three normal beagles at 6, 12 and 18 months of age were immediately fixed in 2.0%
Table 1. Summary data of clinical observations of different-aged beagles with inherited glaucoma and normal beagles

<table>
<thead>
<tr>
<th>Animal number</th>
<th>Age (months)</th>
<th>Condition</th>
<th>Eye</th>
<th>IOP (mm Hg)</th>
<th>Gonioscopy</th>
<th>Fundus</th>
</tr>
</thead>
<tbody>
<tr>
<td>106</td>
<td>3</td>
<td>Affected</td>
<td>OS</td>
<td>24</td>
<td>Open angle</td>
<td>Normal</td>
</tr>
<tr>
<td>127</td>
<td>7</td>
<td>Affected</td>
<td>OD</td>
<td>20</td>
<td>Open angle</td>
<td>Normal</td>
</tr>
<tr>
<td>123</td>
<td>12</td>
<td>Affected</td>
<td>OS</td>
<td>22</td>
<td>Open angle</td>
<td>Normal</td>
</tr>
<tr>
<td>5021</td>
<td>22</td>
<td>Affected</td>
<td>OS</td>
<td>25</td>
<td>Narrow angle</td>
<td>Optic disc cupping with pigmentation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OD</td>
<td>23</td>
<td>Open angle</td>
<td>Mild cupping and pigmentation of optic disc</td>
</tr>
<tr>
<td>5022</td>
<td>24</td>
<td>Affected</td>
<td>OS</td>
<td>35</td>
<td>Open angle</td>
<td>Slight cupping of optic disc</td>
</tr>
<tr>
<td>5017</td>
<td>25</td>
<td>Affected</td>
<td>OS</td>
<td>39</td>
<td>Narrow angle</td>
<td>Slight cupping and optic disc atrophy</td>
</tr>
<tr>
<td>72</td>
<td>26</td>
<td>Affected</td>
<td>OD</td>
<td>30</td>
<td>Narrow angle</td>
<td>Moderate disc cupping</td>
</tr>
<tr>
<td>63</td>
<td>26</td>
<td>Affected</td>
<td>OS</td>
<td>33</td>
<td>Narrow angle</td>
<td>Moderate disc cupping</td>
</tr>
<tr>
<td>80</td>
<td>28</td>
<td>Affected</td>
<td>OS</td>
<td>32</td>
<td>Open angle</td>
<td>Moderate cupping and optic disc atrophy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OD</td>
<td>36</td>
<td>Open angle</td>
<td>Advanced cupping and optic disc atrophy</td>
</tr>
<tr>
<td>60</td>
<td>34</td>
<td>Affected</td>
<td>OS</td>
<td>34</td>
<td>Open angle</td>
<td>Slight cupping and optic disc atrophy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OD</td>
<td>29</td>
<td>Open to Narrow angle</td>
<td>Slight cupping and optic disc atrophy</td>
</tr>
<tr>
<td>5013</td>
<td>34</td>
<td>Affected</td>
<td>OS</td>
<td>28</td>
<td>Open angle</td>
<td>Moderate cupping and optic disc atrophy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OD</td>
<td>27</td>
<td>Open angle</td>
<td>Slight cupping of optic disc</td>
</tr>
<tr>
<td>9044</td>
<td>16</td>
<td>Normal</td>
<td>OS</td>
<td>19</td>
<td>Open angle</td>
<td>Normal</td>
</tr>
<tr>
<td>9032</td>
<td>18</td>
<td>Normal</td>
<td>OD</td>
<td>20</td>
<td>Open angle</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Glutaraldehyde buffered (0.1 M sodium cacodylate, pH 7.2) solution after euthanasia and enucleation.\textsuperscript{11} Constant pressure-perfusion at intraocular pressures, that were determined for each glaucomatous beagle (depending on the preceding 3 days of mean tonometric results) with normal saline solution followed by the glutaraldehyde solution through cannulation of the anterior chamber, was performed on two affected beagles at 24 (no. 5022) and 26 (no. 63) months of age. The eyes were enucleated, and 30 min later the cornea and posterior half of all globes were removed, leaving a ring of anterior uvea which was sectioned longitudinally into pie-shaped pieces along dorsal, ventral, nasal and temporal planes and placed in fresh fixative for two hours at room temperature. Preparation for light microscopy and transmission and scanning electron microscopy followed the methodology previously reported.\textsuperscript{11} This study was performed in accordance with the ARVO Resolution on the Use of Animals in Research.

Results

Clinical findings of the glaucomatous beagles are listed in Table 1. At 1 day postpartum, the iridocorneal angle was not markedly differentiated microscopically, having been mostly cellular. The uveal trabecular meshwork consisted of a loose, spongy mes-
enchyme that was bordered externally by a more compact corneoscleral meshwork (Fig. 1A). The outermost region of the corneoscleral trabecular meshwork was lined by endothelial cells of the angular aqueous plexus. Anteriorly, along the iridocorneal junction, an irregular fibrillar sheet covered the iridocorneal angle, having loosely fitted over the peripheral extension of the corneal endothelium and adjacent meshwork. By 1 week of age, trabecular beams appeared only in the uveal meshwork (Fig. 1B). Occasional macrophage-like cells were found in the developing intertrabecular spaces (Fig. 1C). The anterior fibrillar sheet was weakly organized, having consisted of irregular strands extending from the iris to the anterior meshwork.

In 3-month-old eyes, the iridocorneal angles were fully differentiated (Fig. 1D). The uveal meshwork was comprised of widely spaced trabeculae that consisted of variously sized bundles of collagen and elastic-like material and lined by trabecular cells. The corneoscleral meshwork continued to be more compact than the uveal meshwork, having been comprised of flattened, radially aligned collagen beams enveloped by attenuate trabecular cells (Fig. 2A). At 7 months of age, the iridocorneal angles widened mostly within the uveal meshwork as the intertrabecular spaces expanded (Fig. 2B). Cellular intertrabecular bridges attached adjacent trabeculae throughout the uveal and corneoscleral meshworks. The angular aqueous plexus was consistently observed in each section of each specimen through 7 months of age. Intertrabecular spaces and the region immediately adjacent to the inner endothelial wall of the angular aqueous plexus were free of any extracellular...
material in the eyes of the 3- and 7-month-old pre-glaucanoma dogs.

In 12-month-old early glaucomatous eyes (no. 123), the iridocorneal angle appeared to be largely similar anatomically to the 7-month-old eyes. Distinct morphologic barriers between the anterior chamber and iridocorneal angle were not detected by light microscopy or scanning electron microscopy.

Broad primary pectinate ligaments attached the anterior base of the iris to the periphery of the cornea. The uveal meshwork remained unaltered having been comprised of large trabeculae that branched more extensively next to the ciliary musculature. The trabeculae of inner corneoscleral meshwork were separated by distinct intertrabecular spaces (Fig. 3A). The outer corneoscleral meshwork was less uniformly orga
Fig. 3. (A) In the anterior outer corneoscleral meshwork (OCM) of 12-month-old affected eye (no. 123), numerous, irregularly arranged, darkly stained bodies (arrows) are seen. ICM—inner corneoscleral meshwork, AAP—angular aqueous plexus, X400. (B) Anterior outer corneoscleral meshwork (OCM) of 12-month-old normal eye. Arrows point to linearly aligned darkly stained bodies. AAP—angular aqueous plexus, X400.

tin-like fibers appeared to be more numerous and arranged less regularly than age-matched normal eyes (Fig. 4B, C). These elastin-like bodies appeared to correlate positively with the dark-staining bodies described in Figure 3A and B. Endothelial cells of the angular aqueous plexus were bordered occasionally by fibrils which measured 10–12 nm in diameter and were often associated with adjacent elastin-like fibers (Fig. 4D). Clustered basement membrane-like material was found to be scattered in isolated areas within the outer corneoscleral trabecular meshwork (Fig. 5A, B). The uveal trabecular meshwork changed very little substructurally. Although separated further by widened intertrabecular spaces, trabecular cells remained largely oriented in longitudinal sheets.

In dogs with moderately advanced glaucoma (22 through 34 months of age) two alterations of the iridocorneal angles were observed both clinically and histologically. In some instances (nos. 5021-OS, 60-OD, 63-OS, 5017 in Table 1) the iridocorneal angles were considerably narrow with compact uveal and corneoscleral meshworks (Fig. 6). Intertrabecular spaces were markedly reduced in size in the uveal meshwork and to a lesser extent in the corneoscleral meshwork (Fig. 6, inset). Nevertheless, channels for aqueous humor outflow appeared to be open in both meshworks. In most of the affected animals, ranging from 24–34 months of age (nos. 72, 80, 5013, 5022 and 5024 in Table 1) the iridocorneal angles of both eyes remained deep and well-formed (Fig. 7A). In one of these eyes (no. 5022-OS) several small epithelial sheets extended from the iris to the peripheral cornea (Fig. 7B, C). These sheets consisted of pigment cells that were contiguous with anterior epithelium of the iris. Similar cellular sheets were viewed with greater frequency in both eyes of the affected 28-month-old beagle (no. 80) and were similarly characterized by the presence of flattened pigment cells. The corneoscleral meshwork in the iridocorneal angles that remained open possessed prominent intertrabecular spaces (Fig. 8). Distinct morphologic barriers were neither found in open nor narrow iridocorneal angles.

In the narrow iridocorneal angles compaction of the uveal and corneoscleral meshworks was very apparent both light microscopically and ultrastructurally (Figs. 6, 9A). Although trabecular cells frequently contacted each other in the uveal and inner corneoscleral meshwork, distinct intertrabecular spaces remained visible. By comparison, intertrabecular spaces within the outer corneoscleral meshwork were more difficult to distinguish. Within trabeculae of the outer corneoscleral meshwork a thickened band of granular material, interspersed with small irregular
Fig. 4. (A) Presence of aggregates of opaque serrated rods (arrows) within a trabecular cell of the outer corneoscleral meshwork in a 12-month-old affected beagle (no. 123). TEM, ×38,000. (B) Numerous elastin-like fibers (arrows) in the corneoscleral meshwork of 12-month-old affected eye (no. 123). TEM, ×4000. (C) Fewer, more regularly arranged elastin-like fibers (arrows) in the corneoscleral meshwork of 12-month-old normal eye. TEM, ×4000. (D) Fibrils occasionally lined the inner face of the endothelium (EN) of the angular aqueous plexus (AAP) and were associated with adjacent elastin-like fibers (EL). TEM, ×25,000.

collagen fibrils, separated the collagenous cores from the trabecular cells (Fig. 9A, inset). A uniform layer of fibrils, approximately 10–12 nm in diameter, and amorphous material coated much of the inner endothelial walls along the angular aqueous plexus (Fig. 9B). Elastin-like fibers frequently pressed against the endothelium of the angular aqueous plexus.

In the glaucomatous eyes that possessed the open iridocorneal angle, delicate fibrils and basement membrane-like material were frequently detected within the trabeculae of the outer corneoscleral meshwork (Fig. 10A). Occasional larger quantities of the fibrils and amorphous material were found immediately adjacent to the inner endothelial wall of the angular aqueous plexus (Fig. 10B). These fibrils were the same size as that observed in 12-month-old affected eyes and at various places appeared to have entered the endothelial vacuoles.

In both narrow and open advanced glaucomatous eyes, the collagen fibers that comprised the cores of the trabecular beams of the outer corneoscleral meshwork were consistently less even in diameter, having ranged from 30 to 140 nm versus 30 to 80 nm in the age-matched animals. Trabecular cells along the outer uveal and inner corneoscleral meshworks in the older affected dogs (nos. 60, 63, 72, 80 and 5013) possessed numerous aggregates of the opaque serrated rods (Fig. 11).
Fig. 5. (A) Clusters of basement membrane-like material (arrow) are scattered throughout the posterior outer corneoscleral meshwork in a 12-month-old affected beagle (no. 123). TEM, \( \times 4000 \). (B) Close-up of the material (asterisk) pointed to in Figure 6A. TEM, \( \times 22,000 \).

Discussion

The ontogeny of the iridocorneal angles of beagles with inherited glaucoma through 6 months of age is identical to that of normal laboratory quality beagles.\(^{12}\) Development of the aqueous humor outflow channels appeared to be complete by the third month as the pectinate ligaments, corneoscleral and uveal trabecular meshworks, and angular aqueous plexus were well-defined by this age. Congenital anomalies or abnormal postnatal structures that could have contributed to impedance of aqueous humor drainage were not detected. These findings were consistent with previous gross and light microscopic observations of young affected beagles.\(^{7}\)

Possible abnormal morphology was first detected in the 12-month-old glaucomatous dog (no. 123) being restricted to local areas within the corneoscleral trabecular meshwork. Changes involved mostly the extracellular components and, to a lesser degree, the trabecular cells. Light microscopically, the outer corneoscleral trabeculae appeared to be aligned less radially than that observed in age-matched normal eyes. Perhaps significantly, the elastin-like bodies associated with these trabeculae were more prominent in this region in the affected dog. The most ultrastructurally distinct extracellular alteration at this age was the presence of scattered accumulations of small fibrils within the outer corneoscleral trabecular meshwork and next to the inner endothelial wall of the angular aqueous plexus. The presence of small clusters of serrated, opaque rods among occasional trabecular cells was the most notable cellular change that was observed in the affected animals. These structures, which were more numerous in older affected individuals, were not observed in age-matched normal dogs in current and previous studies.\(^{11-14}\) The association of cellular serrated rods and extracellular changes remains to be ascertained.

In the older and more advanced glaucomatous dogs cellular and extracellular changes were more generalized and extensive throughout the corneoscleral trabecular meshwork. Individuals with narrower anterior chamber angles were additionally characterized by compressed, less organized trabeculae and more uniform build-up of amorphous material and small fibrils within trabeculae of the outer corneoscleral meshwork and next to individual trabecular veins of the angular aqueous plexus. The extracellular materials found in the affected dogs were identical to that previously described in normal beagles and other canine breeds.\(^{12,14}\) The amounts of these materials, however, appeared to be markedly greater than that observed in age-matched normals. The presence of epithelial sheets, which were found in two individuals, most likely arose secondarily and did not appear to alter aqueous humor outflow appreciably. Interestingly, the present study did not find a strong correlation between shallowness of the iridocorneal angle (and anterior chamber) and increase in IOP. Intracocular pressures of eyes with deep, open iridocorneal angles were similar to or in some instances even higher than the IOPs of eyes with narrowed iridocorneal angles. These findings supported previous observations of the glaucomatous beagles based on gross and histologic examination.\(^{7}\)
Inherited glaucoma in the beagle, which occurs well after birth, is documented at the ultrastructural level in the present study to be of the primary open-angle type. Narrowing of the iridocorneal angles, which can lead to acute closure, occurred for the most part 12 to 24 months after the elevation of intraocular pressure. Primary glaucoma in the beagle, particularly in its earlier phases, compares more positively to open-angle glaucoma in humans than any of the other spontaneous types in animals. 

The morphologic changes of human iridocorneal angles with advanced primary open-angle glaucoma have been well-documented. The most notable and consonant change consisted of a deposition of a considerable amount of extracellular material in the juxtacanalicular region adjacent to the endothelium of Schlemm’s canal. Early ultrastructural studies described the extracellular material to be amorphous and nonfibrillar. On the basis of having examined iridocorneal angles with POAG sagittally, one study proposed the material was composed of three types of plaques. However, further investigations using tangential sections, in part, determined the presence of two components: a sheath and core of the elastic-like fibers; and basement membrane-like material. These components were found in normal and glaucomatous eyes, alike, but occupied a significantly larger area of the juxtacanaliculic tissue in glaucomatous eyes. Further histochemical analysis of the tangential sections of normal and glaucomatous specimens revealed: that the sheaths surrounding individual cores of elastic-like fibers were sensitive to both testicular hyaluronidase and chondroitinase AC; and that collagen and collagenase-insensitive fibrils were present within the sheaths. By comparison, the homogenous, basement membrane-like material that lined the endothelium of Schlemm’s canal was chondroitinase ABC-digestible. After removing the homogenous material, fine fibrils were revealed in the glaucomatous eyes only. The beagle iridocorneal angle with POAG shares three specific microscopic morphologic similarities with the human counterpart: the maintenance of an open aqueous humor pathway in the corneoscleral trabecular meshwork during the genesis of glaucoma, ie, usually definable and often confluent intertrabecular spaces; a progressive, generally uneven accumulation of extracellular material within the outer corneoscleral trabecular meshwork and next to the endothelial lining of the angular aqueous plexus/canal of Schlemm, ie, juxtacanaliculic tissue; and an absence of any new or different observable extracellular material found in glaucomatous iridocorneal angles that might be responsible, in part, for an in-
crease to resistance of aqueous outflow. On the other hand, macroscopic morphologic differences exist between POAG in the human and canine iridocorneal angles. The uveal meshwork, which is prominent in the dog and nearly absent in humans, may contribute to or influence the canine disorder in some capacity.
Fig. 8. Outer corneoscleral meshwork (CM) in the open iridocorneal angle of an eye with moderately advanced glaucoma possesses wide inter trabecular spaces. S—sclera, X250.

Fig. 9. (A) Closely packed and irregularly positioned trabecular (T) and trabecular cells of the outer corneoscleral meshwork of a narrowed iridocorneal angle of a beagle (no. 63) with moderately advanced glaucoma. TEM, X6100. Inset: Thickened band of granular material (GM) interspersed with small fibers lined the core of the trabecular beams which consisted of collagen fibers (arrows) of irregular diameters. TC—trabecular cell, TEM, X12,000. (B) Numerous small fibrils and amorphous material (AM) coated much of the inner endothelium (EN) of the angular aqueous plexus (AAP). TC—trabecular cell, TEM, X44,000.
The sheaths that encapsulate the cores of elastin-like fibers in the juxtacanalicular tissue in normal human eyes and become more prominent in glaucomatous individuals have not been observed in normal or affected dogs. However, the apparent increase of elastin-like fibers and adjacent fibrils among the outer corneoscleral trabeculae and next to the trabecular veins of the angular aqueous plexus in the beagles with moderately advanced glaucoma may be analogous to the build-up of elastin-like fibers and associated sheaths in human POAG. The influence of the material along the endothelial lining of the angular aqueous plexus in the dog on aqueous humor outflow may differ than that in humans as the outflow vessels are anatomically different in these species.12,28,29

The presence of the accumulation of extracellular materials within human juxtacanalicular tissue and its relationship to POAG has been debated for some time.24-27,30-32 Most recently, an exquisite morphometric study on the concentration of electron-dense materials within the juxtacanalicular tissue of normal individuals and those with POAG revealed that these materials increased with age in normal eyes and was significantly greater (23%) in older glaucomatous tissues only.32 The study proposed that the increase in extracellular dense materials in POAG eyes was not sufficient to account for a substantial reduction in aqueous humor outflow and that other factors, including alterations in cellularity, glycosaminoglycans and glycoproteins, may be stronger influences on outflow resistance. These same factors as well as the progressive build-up of extracellular material within outer portion of the iridocorneal angle are being examined in the affected beagle.33-35 In spite of macroscopic anatomical differences between the human and canine aqueous humor outflow apparatuses, the similar pattern of microscopic and ultrastructural changes associated with aqueous humor outflow pathways make the glaucomatous beagle the most useful animal model identified to date for conducting additional studies on early pathogenetic events of this biochemical disorder.

Key words: glaucoma, canine, ultrastructure, iridocorneal angle, inherited
Acknowledgments

We would like to express our deepest gratitude to Ms. Patricia Lewis and Ms. Fern Flake for their technical assistance in this study and to Drs. J. Alvarado, K. Barrie and M. Sherwood for their review and suggestions of this manuscript.

References