Posterior Scleral Thickness in Perfusion-Fixed Normal and Early-Glaucoma Monkey Eyes

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PURPOSE: To characterize posterior scleral thickness in the normal monkey eye and to assess the effects of acute (15- to 80-minute) and short-term chronic (3- to 7-week) intraocular pressure (IOP) elevations.

METHODS: Both eyes of four normal monkeys (both eyes normal) and four monkeys with early glaucoma (one eye normal and one eye with induced chronic elevation of IOP) were cannulated. In each monkey, IOP was set to 10 mm Hg in the normal eye and 30 or 45 mm Hg in the contralateral eye (normal or early glaucoma) for 15 to 80 minutes. All eight monkeys were perfusion fixed, yielding eight low-IOP–normal eyes, four high-IOP–normal eyes, and four high-IOP–early glaucoma eyes. Posterior scleral thickness was measured histomorphometrically at 15 measurement points within each eye, and the data were grouped by region: foveal, midposterior, posterior–equatorial, and equatorial.

RESULTS: Overall, posterior scleral thickness was significantly different in the various regions and among the treatment groups (P < 0.0001). In the low-IOP–normal eyes, the posterior sclera was thickest in the foveal region (307 μm) and thinnest in the midposterior (199 μm), posterior–equatorial (133 μm), and equatorial (179 μm) regions. In the high-IOP–normal and high-IOP–early glaucoma eyes, the posterior sclera was thinnest overall and within specific regions, compared with the low IOP–normal eyes.

CONCLUSIONS: The posterior sclera in the perfusion-fixed normal monkey eye thins progressively from the fovea to the equator and is thinnest just posterior to the equator. Acute and short-term chronic IOP elevations cause regional thinning within the posterior sclera of some monkey eyes, which significantly increases stresses in the scleral wall. (Invest Ophthalmol Vis Sci. 2001;42:3202–3208)

Scleral thickness is a critical component in determining the stresses (force/cross-sectional area) and strains (deformation under load) in the scleral shell due to intraocular pressure (IOP). For a given level of IOP, stress and strain in the scleral wall increase proportionally with a decrease in scleral thickness, according to the standard formula governing pressure vessel theory.

In glaucoma, thinning of the sclera may occur in response to chronic elevation of IOP. In this circumstance, scleral wall stress that is already increased due to elevated IOP is further increased in those regions of glaucomatous scleral thinning. This suggests that not only the initial thickness but also the behavior (response to chronic IOP elevation) of the peripapillary sclera may contribute to the susceptibility of an individual optic nerve head (ONH) to a given level of IOP.

As part of an ongoing attempt to study the ONH as a biomechanical structure, we are building finite element models of the load-bearing connective tissues of the perfusion-fixed monkey lamina cribrosa, scleral canal wall, and peripapillary sclera. Finite element modeling is a computational technique that is used to estimate the stresses and strains within a complex, load-bearing structure. We have previously used this technique to describe IOP-related stress in models of an idealized human posterior scleral shell and ONH. We are currently constructing three-dimensionally accurate models to characterize the manner in which the load-bearing connective tissues of the lamina cribrosa and scleral canal wall are influenced and then damaged by a given level of IOP.

To construct a finite element model of the scleral canal wall and lamina cribrosa requires a model of the larger posterior scleral shell, to establish boundary conditions for the connective tissues of the peripapillary sclera. The response of a model of the ONH is not only determined by the level of applied IOP, but is also greatly influenced by the stresses and strains transmitted to the edge (boundary) of the ONH and peripapillary sclera by the adjacent posterior sclera. The purpose of this investigation was to characterize the thickness of the posterior sclera within perfusion-fixed normal monkey eyes and then to assess whether acute and/or short-term chronic elevations of IOP influence that thickness.

MATERIALS AND METHODS

Animals

All animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Scleral specimens from eight male rhesus monkeys (estimated age, 5–11 years) killed as part of another study were obtained (Table 1). Four of these monkeys, with two normal eyes, were perfusion fixed using buffered, hypertonic aldehyde solutions, with one eye set to low IOP (10 mm Hg) and one eye set to high IOP (either 30 or 45 mm Hg) for 15 to 80 minutes before fixation. In the other four monkeys, chronic elevated IOP had been induced in one eye 1 to 2 months earlier. Each of these monkeys was perfusion fixed with the normal eye set to low IOP (10 mm Hg) and the experimental glaucomatous eye set to high IOP (either 30 or 45 mm Hg) 15 to 80 minutes before perfusion (Table 1). Spontaneous IOPs were measured using an application tonometer (Tonopen XL; Bio-Rad, Glendale, CA), and axial length measurements were made with an A-scan ultrasonometer (A-1500 A-Scan; Sonomed, Lake Success, NY).

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### Early Experimental Glaucoma

In both eyes of four monkeys, ONH surface compliance testing was performed on three separate occasions to characterize normal ONH surface position and compliance. Early experimental glaucoma was then induced in one eye of each animal by lasering the trabecular meshwork every 2 weeks until a statistically significant elevation in IOP was observed (Table 1). Each lasered eye was then compliance tested every 2 weeks to detect the onset of early glaucomatous damage to the ONH tissues. Early glaucomatous damage was defined as the onset of fixed posterior deformation of the ONH surface and/or the onset of ONH surface hypercompliance within the data from two consecutive compliance tests. All four monkeys were perfusion fixed within 2 weeks of the onset of early glaucomatous damage.

### Scleral Specimen Preparation

After perfusion fixation, the scleral tissues of the various monkeys were stored for different lengths of time in 5% glutaraldehyde solution before processing. However, both scleral shells of a given monkey were always processed for scleral specimen generation on the same day, as follows.

A 10-0 polypropylene suture was placed through the sclera to mark the position of the fovea, and the ONH and peripapillary sclera were then removed with a 6-mm trephine. The clean scleral shell was placed on a fixed polyethylene ball corresponding to the diameter of the shell, with the fovea at the apex. Vinyl stencils were then pinned at the fovea and the equator along the superior, inferior, nasal, and temporal axes. The stencils established the positions of the eight 2-mm-wide scleral strips, which were cut with a pointed end and marked with a 1040 polypropylene suture passed 3 mm from the tip. The eight scleral strips for each eye were then dehydrated, infiltrated, and embedded in historesin (Technovit 7100; Kulzer, Wehrheim, Germany) perpendicular to the face of the embedding block to allow for serial sagittal sectioning.

### Scleral Specimen Sampling

A preliminary study determined that a single group of eight sagittal sections was adequate to characterize scleral thickness throughout the entire 2-mm width of a scleral specimen (data not shown). For each scleral specimen, a microtome (model RM2165; Leica, Bensheim, Germany) was used to cut 200-μm-thick sections to embed the tissues. Eight serial, sagittal, 3-μm-thick sections were then cut, mounted on glass slides, and stained with Van Gieson stain.

### Section Image Acquisition

A digital color image of each section was acquired under a light microscope (Optiphot-2; Nikon, Tokyo, Japan) fitted with a charge-coupled device (CCD) color camera (HV-C20; Hitachi, Tokyo, Japan), along with a companion image of a slide-mounted micrometer scale to allow calibration of the exact pixel size. All images were generated at a resolution of approximately 5 μm/pixel (Fig. 2).

### Scleral Thickness Measurements within Each Image

A total of 1024 section images (16 eyes with 8 scleral strips per eye and 8 sections per strip) were processed as follows. First, the exact pixel size was calculated using the companion slide-mounted micrometer image. For each cardinal strip image, three pairs of opposing marks (foveal, midposterior, and posterior-equatorial) were placed on the anterior and posterior scleral surfaces (Figs. 1A, 2). For the equatorial section images, one pair of opposing marks was made on the anterior and posterior surfaces at the midpoint of the scleral section. Thus,
Scleral thickness was measured at 15 measurement points in each eye (Fig. 1A).

Each image was marked by an operator who was masked to treatment group. After marking, the coordinates of each pair of points were output to a custom routine (Mathematica software; Wolfram Research, Champaign, IL) that calculated the Cartesian distance between the points.

Assignment to Treatment Groups

Data from each eye were assigned to one of three treatment groups: low IOP–normal eyes (n = 8), high IOP–normal eyes (n = 4), and high IOP–early glaucoma eyes (n = 4), where low IOP and high IOP signify acute IOP settings, and early glaucoma indicates chronic IOP elevation.

Analysis of Variance Testing to Assess the Effects of Treatment, Region, and Monkey

A nested analysis of variance (ANOVA) was used to assess the effects of treatment and region on the dependent variable, scleral thickness. Within this ANOVA, overall effects of acute elevation of IOP were first assessed by comparing the pooled data from the low IOP–normal and high IOP–normal treatment groups. The effects of acute IOP elevation were secondarily assessed in individual monkeys by comparing the low IOP–normal eye with the high IOP–normal eye in monkeys 1, 2, 3, and 4.

The overall effect of chronic IOP elevation (early glaucoma) was assessed by comparing the pooled data from the high IOP–normal and high IOP–early glaucoma treatment groups.

Finally, the combined effects of both acute and chronic IOP elevation were first assessed by comparing the pooled data from the low IOP–normal and high IOP–early glaucoma treatment groups, and secondarily assessed by comparing the low IOP–normal eye with the high IOP–early glaucoma eye in monkeys 5, 6, 7, and 8.

RESULTS

Reproducibility of Scleral Thickness Measurements

The results of three separate measurements of scleral thickness made on different days by a single observer at four measurement points on scleral strips from each of the two eyes of two monkeys are reported in Table 2. By ANOVA, the effect of measurement day was not significant overall or within the data from monkey 8, but was significant in the data from monkey 1 (P < 0.001). Even in the face of the variability due to measurement day, the effects of monkey, eye (treatment), measurement point, and region remained significant (P < 0.0001). Additionally, the measurement day differences in monkey 1, although statistically significant, were small relative to the magnitude of the significant differences observed between eyes, measurement points, and regions (Figs. 3, 4, and 5; Table 3).

Mean Posterior Scleral Thickness by Measurement Point

The mean posterior scleral thickness at each measurement point is displayed for all 16 eyes (overall) and for each treatment group in Figure 3. Because the effect of treatment was significant by ANOVA (P < 0.0001), the data for each treatment group are emphasized throughout the remainder of this report.

Mean Posterior Scleral Thickness by Region

The mean posterior scleral thicknesses for the four regions (foveal, midposterior, posterior-equatorial, and equatorial) were compared by ANOVA (P < 0.0001) and are displayed in Figure 4. The foveal marks (1 and 2) were placed 1 mm toward the fovea from the orientation suture, the posterior-equatorial marks (3 and 4) were placed at the equatorial end, and the midposterior marks (5 and 6) were placed equidistant between the foveal and posterior-equatorial marks.
Overall, mean scleral thickness was significantly less in the high IOP–normal eyes (186 μm), compared with the low IOP–normal eyes (204 μm; Table 3). By region, scleral thickness was indistinguishable in three of the four regions; however, in the foveal region, the sclera was significantly thinner in the high IOP–normal eyes (265 μm), compared with the low IOP–normal eyes (307 μm; P < 0.0001, ANOVA). Within the four normal monkeys (monkeys 1, 2, 3, and 4) considered individually, the mean posterior scleral thickness was significantly greater in the normal eye fixed at low IOP than in the contralateral normal eye fixed at high IOP in monkeys 1 and 2, but not in monkeys 3 and 4 (data not shown). Finally, scleral thickness was significantly less at 6 of the 15 measurement points in the high IOP–normal treatment group, compared with the low IOP–normal group (Fig. 4A). Statistically significant differences in scleral thickness between the low IOP–normal eyes and the contralateral high IOP–normal eyes of monkeys 1 to 4 individually are shown for each measurement point in Figure 5A. Although the pooled data in Figure 4A show a trend toward posterior scleral thinning in the high IOP–normal treatment group, the individual monkey data, as shown in Figure 5A, are equivocal.

**Effect of Short-Term Chronic IOP Elevation (Early Glaucoma) on Posterior Scleral Thickness**

Overall and within three of the four regions, mean posterior scleral thicknesses in the high IOP–normal eyes and high IOP–early glaucoma eyes were indistinguishable (Table 3). In the midposterior region, however, mean scleral thickness was significantly less in the high IOP–early glaucoma eyes (168 μm) than in the high IOP–normal eyes (189 μm; Table 3). Mean scleral thickness was significantly less at 4 of the 15 measurement points and significantly greater at 2 of the 15 measurement points in the high IOP–early glaucoma eyes than in the high IOP–normal eyes (Fig. 4B).

**Combined Effect of Acute and Short-Term Chronic IOP Elevation on Posterior Scleral Thickness**

Overall, the posterior sclera was significantly thinner in the high IOP–early glaucoma eyes (182 μm) compared with the low IOP–normal eyes (204 μm; Table 3). By region, scleral thickness was indistinguishable between the two groups in the equatorial region, but was significantly less in the foveal, mid-posterior, and posterior-equatorial regions in the high IOP–glaucoma eyes (Table 3). Within the four monkeys with early glaucoma (monkeys 5, 6, 7, and 8) considered individually, the posterior sclera was significantly thinner in the high IOP–early glaucoma eye in three of the four monkeys (data not shown).

Finally, scleral thickness was significantly less at 9 of the 15 measurement points and significantly greater at 1 of the 15 measurement points in the high IOP–early glaucoma eyes than in the high IOP–normal eyes (Fig. 4B).
measurement points in the high IOP–early glaucoma eyes, compared with the low IOP–normal eyes (Fig. 4C). Statistically significant scleral thickness differences between the high IOP–early glaucoma eyes and low IOP–normal eyes of monkeys 5, 6, 7, and 8 are shown in Figure 5B. Within these data, the overwhelming trend is toward thinning of the sclera in the high IOP–early glaucoma eyes compared with their contralateral low IOP–normal control eyes.

**DISCUSSION**

The purpose of this study was to characterize the thickness of the posterior sclera in normal (perfusion-fixed) monkey eyes and secondarily to assess whether acute and short-term chronic elevations of IOP induce detectable posterior scleral thinning. The principal findings of this report are as follows. First, in the low IOP–normal eyes, the posterior sclera was thickest in the foveal region (307 μm) and thinner in the midposterior (199 μm), posterior-equatorial (133 μm), and equatorial (179 μm) regions. Second, compared with the low IOP–normal eyes, the posterior sclera in both the high IOP–normal eyes and the high IOP–early glaucoma eyes was thinner, both overall and in specific regions. Third, although scleral thinning appeared to be greater in the high IOP–early glaucoma eyes than in the high IOP–normal eyes, the differences were not clear enough to separate the effects of acute IOP elevations and short-term chronic IOP elevations (early glaucoma) on posterior scleral thickness in the monkey eye.

The central feature of our data is the profound thinning of the sclera, when moving anteriorly from the fovea toward the
equator, with the posterior sclera being thinnest just posterior to the equator. This trend is present within the data from all 16 eyes combined (overall), the data for each of the three treatment groups, and the data for each of the 16 eyes considered individually. A similar pattern has been reported for the posterior sclera in human eyes. Olson et al. reported scleral thickness in humans of 900 to 1000 μm in the foveal region and 390 μm near the equator. Fine and Yanoff reported human scleral thicknesses of 1000 and 400 to 500 μm in the foveal and equatorial regions, respectively. Although our data indicate that perfusion-fixed monkey sclera is thinner in all regions than human sclera, the relative decrease (approximately 57%) in scleral thickness from the foveal region to the posterior equatorial region is comparable to that reported in human eyes (approximately 60% in the study by Olson et al. and 50% to 60% in the study by Fine and Yanoff).

Comparison of the high IOP–normal data (n = 4) with the low IOP–normal data (n = 8) suggests that thinning of the posterior sclera can be detected after acute (15–80 minutes) elevation of IOP in some monkey eyes. This effect can be seen in the overall and regional data (Table 3) and at some of the individual measurement points, principally within the foveal region (Fig. 4). However, in the four normal monkeys considered individually, scleral thinning was not consistently present in the high-IOP eye (Fig. 5A). These findings suggest that acute IOP elevations induce posterior scleral thinning in some, but not all, normal monkey eyes.

By contrast, scleral thinning resulting from the combined effects of acute and short-term chronic IOP elevation was clearly present within both the pooled and individual monkey data of the four early glaucoma monkeys. Nemeth used B-scan ultrasonography to detect thinning of the posterior coat volume (total tissue volume of the retina-choroid-sclera) in human glaucomatous eyes. However, that study is limited by the relatively small number of patients (n = 15), a questionable statistical strategy, and the inability of B-scan ultrasonography to isolate scleral thickness within its measurement of posterior coat thickness.

The histologic data in our study suggest that short-term chronic (mean of 4.3 weeks) IOP elevations of moderate magnitude (mean maximum measured IOP, 27.3 mm Hg) cause detectable thinning of the posterior sclera in a majority of monkey eyes. Two distinct phenomena may underlie these findings. In the first scenario, the material properties of early glaucomatous sclera in the monkey are unaltered, and scleral thinning is due either to the Poisson effect (thinning of the wall due to expansion of the shell) or radial compression of the sclera (which squeezes out fluid, causing a loss in scleral tissue volume), or a combination of the two. In the second scenario, the extracellular matrix of the posterior sclera is damaged or remodeled in early-glaucoma monkey eyes. In this case, the scleral thinning we detected in early-glaucoma eyes was due to an abnormal response to the acute IOP elevation that preceded fixation. Quigley et al. reported a decrease in collagen fibril density within the lamina cribrosa and peripapillary sclera in human eyes with glaucomatous damage. Although the same study did not detect this effect in monkeys, it is possible that extracellular matrix alterations within the posterior sclera occur early in response to chronic elevation of IOP. In this connection, initial results from ongoing studies in our laboratory suggest that the viscoelastic material properties of the peripapillary sclera are fundamentally altered in early-glaucoma eyes.

Our present study is limited by several considerations. First, we used perfusion fixation to best ensure that the connective tissues of the scleral shell and ONH were captured in their in vivo state at controlled levels of IOP. Pandajonas et al. reported 12.5% linear shrinkage in the optic disc after fixation. In a recent study of human sclera, thickness did not change significantly in response to fixation. Regardless of the degree of shrinkage or swelling, assuming that it occurs evenly over all specimens, our scleral thickness measurements should still legitimately model the relative variation in scleral thickness by location and treatment that would be present in nonfixed monkey eyes.

Second, our perfusion conditions did not allow us to have a group of monkeys in which both normal eyes were perfusion fixed at an IOP of 10 mm Hg. Such a group of monkeys would have allowed us to characterize the magnitude of scleral thickness differences between the two eyes of a normal monkey—overall, regionally, and at each measurement point—because of physiologic differences alone. Also, we did not have monkeys with low IOP–normal and low IOP–early glaucoma eyes; this comparison would have enabled us to isolate the effect of short-term, chronic IOP elevation from that of acute IOP elevation immediately preceding fixation. In addition, we did not have monkeys with low IOP– and high IOP–early glaucoma eyes, which would have allowed evaluation of the effect of acute IOP elevation in early-glaucoma eyes.

Finally, perfusion of the scleral vessels (and therefore fixation) may have been inhibited in the high-IOP eyes. To ensure good fixation at high pressure, animals were left undisturbed (with high IOP maintained) for 1 hour before dissection. However, after enucleation, the vortex and retinal veins were partially filled with blood in a subset of the high-IOP eyes. In addition, when serial sagittal sections of the ONH of these same monkey eyes were viewed as part of another study, the choroid also was intermittently filled with blood. Taken together, these findings suggest that in some high-IOP eyes, scleral thickness may not have been captured at the time of initial perfusion (at high IOP), but instead may include some

### Table 3. Scleral Thickness Overall and by Region and Treatment and Calculated Increase in Scleral Wall Stress Due to Scleral Thinning

<table>
<thead>
<tr>
<th>Region</th>
<th>Low IOP–Normal (n = 8 eyes)*</th>
<th>High IOP–Normal (n = 4 eyes)*</th>
<th>Increase in Tangential Scleral Stress (%)</th>
<th>High IOP–Early Glaucoma (n = 4 eyes)*</th>
<th>Increase in Tangential Scleral Stress (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foveal</td>
<td>307 ± 9</td>
<td>265 ± 12†</td>
<td>14</td>
<td>275 ± 12†</td>
<td>11</td>
</tr>
<tr>
<td>Midposterior</td>
<td>199 ± 7</td>
<td>189 ± 10</td>
<td>NS</td>
<td>168 ± 10†</td>
<td>16</td>
</tr>
<tr>
<td>Posterior-equatorial</td>
<td>133 ± 7</td>
<td>122 ± 10</td>
<td>NS</td>
<td>116 ± 10†</td>
<td>13</td>
</tr>
<tr>
<td>Equatorial</td>
<td>179 ± 7</td>
<td>168 ± 12</td>
<td>NS</td>
<td>169 ± 10</td>
<td>NS</td>
</tr>
<tr>
<td>Overall</td>
<td>204 ± 5</td>
<td>186 ± 4†</td>
<td>9</td>
<td>182 ± 4†</td>
<td>11</td>
</tr>
</tbody>
</table>

Data are mean micrometers ± 95% confidence interval. NS, nonsignificant change in thickness compared with the low IOP–normal group.

* Regions are different within each treatment group (P < 0.0001, ANOVA) except for the mid-posterior versus equatorial comparison in the high IOP–early glaucoma group.

† Different from corresponding low IOP–normal value (P < 0.01, ANOVA).

‡ Different from corresponding high IOP–normal value (P < 0.01, ANOVA).
degree of artifactual scleral swelling that occurred between perfusion, enucleation, and placement of the posterior globes into fixative. If such swelling is present, it suggests that our study may underestimate the effect of acute and short-term chronic IOP elevation on posterior scleral thickness in the normal and early-glaucoma monkey eye.

IOP-induced alterations in scleral thickness are important because stress and strain in the scleral wall increase proportionally with a decrease in scleral thickness. Thus, if the sclera thins in response to chronic IOP elevations, the increases in scleral wall stresses due to the elevated IOP are further exacerbated by the increases in stress due to scleral thinning.

Spherical thin-walled pressure vessel theory states that circumferential scleral wall stress equals IOP multiplied by the internal radius of the scleral shell, divided by two times the wall thickness. As a result, a decrease in scleral thickness of a certain percentage induces approximately the same percentage increase in scleral wall stress. Although the scleral shell is not exactly spherical and the material properties of scleral tissue are viscoelastic and anisotropic, pressure vessel theory can be used to approximate the increases in scleral wall stress induced by scleral thinning. Thus, on the basis of our observed differences in scleral thickness, estimated scleral wall stress was increased approximately 11% in the foveal region and 9% overall in the high IOP–normal group; and 11%, 16%, and 13% in the foveal, midposterior, and posterior equatorial regions and 11% overall in the high IOP–early glaucoma group (Table 3). These data are for scleral regions away from the ONH; similar data for the peripapillary sclera will be the subject of a future report.15

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