Biomechanical Effects of Intraocular Pressure Elevation on Optic Nerve/Lamina Cribrosa before and after Peripapillary Scleral Collagen Cross-Linking

Ivey L. Thornton,1,2 William J. Dupps,1,3 Abhijit Sinha Roy,1 and Ronald R. Krueger1

Purpose. To evaluate the biomechanical effect of intraocular pressure (IOP) elevation on the optic nerve/lamina cribrosa complex (ON/LC) and peripapillary sclera (PS) of porcine eyes before and after localized collagen cross-linking.

Methods. Eighteen porcine globes were divided evenly into three groups. The optic nerves were transected to expose the ON/LC, and each globe was infused through an in-line pressure transducer for direct IOP control. Surface wave velocity, a nondestructive measure of tissue stiffness, was measured across the ON/LC and PS before and after collagen cross-linking at IOPs of 10 and 30 mm Hg (groups 1 and 2) and at each globe’s preinflation IOP and 80 mm Hg (group 3). In group 3, papillary strain was measured by analyzing the displacement of fiducial marks immediately adjacent to the ON/LC by using digital photography. Cross-linking in group 1 was achieved with riboflavin-ultraviolet A (UVA) delivery to the entire ON/LC and PS and, in groups 2 and 3, with an annular sponge soaked in glutaraldehyde (GTA) and applied only to the ON/LC and PS and, in groups 2 and 3, with an annular sponge soaked in glutaraldehyde (GTA) and applied only to the PS.

Results. Native PS was significantly stiffer than the ON/LC across all experiments. Before cross-linking, IOP elevation caused significant stiffening of both the ON/LC and PS. After cross-linking with either technique, IOP elevation stiffened the PS but not the ON/LC region. In group 3, papillary strain during IOP elevation was significantly reduced after PS cross-linking.

Conclusions. Stiffening of the peripapillary scleral ring reduces the biomechanical sensitivity of the ON/LC complex to IOP elevation and may represent a novel mechanism for neuroprotection in glaucoma. (Invest Ophthalmol Vis Sci. 2009;50:1227–1233) DOI:10.1167/iovs.08-1960

Glaucoma is among the leading causes of blindness in the United States and worldwide. It is estimated that more than 2.5 million people in the United States have glaucoma and that more than 130,000 people with the disease are legally blind.1 By 2010, the estimated worldwide prevalence of glaucoma will be 60 million, with 4.5 million being legally blind.2 Glaucoma is commonly associated with the presence of high intraocular pressure (IOP), optic nerve damage, and patterned visual field loss, but many other risk factors for glaucomatous optic neuropathy have been identified.1 In a recent review of the Ocular Hypertension Treatment Study, the most significant risk factors for the development of glaucoma included age, IOP, cup/disc ratio, and thin central corneal thickness, where the latter remained an important predictor in both univariate and multivariate analyses.3 This latter finding has generated increased interest in the biomechanical properties of the ocular coat and its role in the pathophysiology of glaucoma.

In studies such as the Ocular Hypertension Treatment Study and the European Glaucoma Prevention Study, evidence suggests that a history of diabetes mellitus is inversely associated with the risk of glaucoma 1,3 and may therefore be protective. Hyperglycemia is associated with a naturally occurring form of collagen cross-linking, resulting from nonenzymatic glycation of proteins, that leads to tissue stiffening.4 Computational simulations have suggested that mechanical strain in prelaminar optic nerve tissue, a potential mechanism of injury in glaucomatous optic neuropathy, is greatest as a function of IOP when peripapillary scleral stiffness is lowest.5 We hypothesize that natural cross-linking of collagen in the lamina cribrosa or peripapillary sclera may contribute to the protective effects of diabetes mellitus against glaucoma. In the anterior segment, similar effects in corneal collagen may account for the protective effect of diabetes in keratoconus,6,7 a disease characterized by abnormal corneal elasticity8 and progressive instability of corneal shape. Collagen cross-linking has been introduced as a technique for stiffening the cornea,9 and clinically, topical riboflavin coupled with ultraviolet-A (UVA) light exposure, has been used effectively as a stabilizing treatment for keratoconus.10

We propose that collagen cross-linking of the lamina cribrosa and/or peripapillary sclera might be a plausible method for modulating biomechanical stress and strain-based injury mechanisms in the laminar region, thereby preventing the onset or slowing the progression of glaucomatous optic neuropathy. Recent experiments have demonstrated that direct application of cross-linking agents such as glyceraldehyde and methyglyoxal to the lamina cribrosa and peripapillary sclera can increase the tensile strength of these structures in explants subjected to extensometric analysis.11 In this study, we cross-linked the in situ optic nerve/lamina cribrosa (ON/LC) complex and/or the peripapillary sclera (PS) of porcine globes using riboflavin/UVA and glutaraldehyde (GTA), a dialdehyde that is a potent positive control for cross-linking effects.9 Local stiffness and deformation in the ON/LC and PS regions were measured before cross-linking at low and high IOPs to (1) measure the regional strain and stiffness changes generated.
during an IOP increase, and then again after cross-linking to (2) assess the degree of cross-linking achieved under constant IOP, and (3) to study the potential protective effect of ON/LC and/or PS cross-linking against ON/LC stiffening and strain during an IOP increase.

METHODS

PS Cross-Linking Methodology

Surface wave velocity (SWV) is a measure of tissue stiffness that has been used in dermatologic applications to measure age-related changes, the softening effects of tissue hydration and skin creams, and the sclerosing effects of radiation on breast skin. When acoustic waves in the 0.5- to 30-kHz frequency range are used, propagation speed in the skin is related to the density and stiffness of the tissue. A prototype handheld ocular surface elastometer (Sonic Eye; Priavision, Inc., Menlo Park, CA) with an operating frequency within this range (4 kHz) and a fixed 4.5 mm wave propagation distance has been described in detail elsewhere. The sonic eye was used in this study as a nondestructive means of measuring local in situ tissue stiffness across and around the optic nerve head.

Eighteen porcine eyes were obtained from a local abattoir (Helfingers Meats, Inc., Jeromesville, OH) and were used within 24 hours postmortem. The optic nerves were dissected under microscopic visualization to expose the optic nerve and potentially the lamina cribrosa. As shown in Figure 1, vitreous infusion of normal saline through a 23-gauge needle (BD Biosciences, Franklin Lakes, NJ) and in-line pressure transducer (Biotrans 2; Biosensors International, Inc., Singapore) provided direct IOP control and measurement capabilities (Infinity SC9000XL digital pressure monitor; Drager Medical, Lubeck, Germany). Three SWV measurement positions were defined as illustrated in Figure 2 to provide comparable papillary (ON/LC) and circumferentially oriented peripapillary stiffness measurements across experiments. During the papillary measurements, the SWV probe was within the outer margin of the ON/LC, defined as area 1, avoiding direct contact with the adjacent PS. Replicate SWV measurements were obtained at a rate of 1 per second with a triggering foot pedal to produce at least five measurements that were averaged automatically for each region of interest, and a total of three averaged measurements were taken and subsequently averaged again for each area. A PC software interface converted the time-of-flight data to velocity in meters per second (m/s), and the velocity data were exported (Excel ver. 11, SP2; Microsoft Corp., Redmond, WA, and Minitab ver. 14.20; Minitab Inc, State College, PA) for statistical analysis.

Three experimental groups of six eyes each were defined. In group 1, SWV across the ON/LC complex and PS regions of interest were measured at 10 and 30 mm Hg to represent a physiological range of pressures. The PS measurements were obtained along two quadrants immediately adjacent to the ON/LC (Fig. 2). Next, both the ON/LC and PS regions were treated with topical riboflavin 1% every 5 minutes during 30 minutes of continuous UVA irradiation, using a light source at a wavelength of 365 nm and energy of 3 mW/cm² (Priavision, Inc.). SWV was then remeasured at each region of interest at 10 and 30 mm Hg.

In group 2 (n = 6), a protocol similar to that described in group 1 was repeated at 10 and 30 mm Hg. However, instead of applying riboflavin/UVA irradiation, we soaked an annular sponge (inner diameter 3.5 mm, outer diameter 8 mm) in 4% GTA diluted from 8% electron microscopy grade stock (Polysciences, Warrington, PA) and positioned it over the PS for 30 minutes with deliberate avoidance of the ON/LC. Measurements were then repeated at both IOP levels.

In group 3 (n = 6), the same procedure described for group 2 was implemented with the following exceptions: First, measurements were acquired at each eye’s preinflation pressure (between 2 and 4 mm Hg) and at 80 mm Hg, to ensure that measurable changes in SWV and ON/LC geometry would be generated in at least one experimental group. Second, to facilitate calibration, four fiducial ink marks were

![Figure 1. In-line pressure transducer infused with normal saline for direct IOP control.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932958/)

![Figure 2. SWV measurements were taken between two Points using the sonic eye elastometer atop the ON/LC complex (area 1) and the PS (areas 2 and 3). Note the fiducial marks placed immediately adjacent to the ON/LC complex in the group 3 eyes.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932958/)
Table 1. Stiffness (m/s) of the Optic Nerve/Lamina Cribrosa Complex and Peripapillary Sclera before and after Cross-Linking at Low and High IOPs

<table>
<thead>
<tr>
<th></th>
<th>Optic Nerve/Lamina Cribrosa Complex</th>
<th>Peripapillary Sclera</th>
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<tbody>
<tr>
<td></td>
<td>Pre–cross-linking</td>
<td>Post–cross-linking</td>
</tr>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low IOP</td>
<td>28.1 ± 1.8</td>
<td>28.0 ± 1.6</td>
</tr>
<tr>
<td>High IOP</td>
<td>25.7 ± 4.0</td>
<td>27.1 ± 1.8</td>
</tr>
<tr>
<td>P</td>
<td>0.23</td>
<td>0.39</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low IOP</td>
<td>29.9 ± 1.8</td>
<td>29.1 ± 2.5</td>
</tr>
<tr>
<td>High IOP</td>
<td>27.3 ± 2.4</td>
<td>27.4 ± 2.4</td>
</tr>
<tr>
<td>P</td>
<td>0.07</td>
<td>0.038</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low IOP</td>
<td>25.7 ± 1.6</td>
<td>29.5 ± 2.9</td>
</tr>
<tr>
<td>High IOP</td>
<td>29.4 ± 2.1</td>
<td>29.3 ± 1.9</td>
</tr>
<tr>
<td>P</td>
<td>0.003</td>
<td>0.87</td>
</tr>
</tbody>
</table>

Data are the mean meters per second ± SD. All, combined data of groups 1, 2, and 3; bold, P < 0.05.

Comparison of Sonic Wave Velocity Measurements and Uniaxial Extensometry

To investigate the validity of surface wave elastometry as a nondestructive technique for measuring scleral tissue stiffness, we performed surface wave elastometry and tensile mechanical testing of the same scleral strips in an additional set of experiments. Fresh porcine eye globes were acquired and stored at 4°C. Nine 4 mm scleral strips in an additional set of experiments. Fresh porcine eye globes posterior to the globe equator were excised from nine porcine globes. The specimens were then preconditioned to a solution in 0.9% saline.

The specimens were then pre-conditioned to a load peak of 250g over three cycles to obtain a recoverable reference state at the beginning of the actual tensile test. Each specimen was then stretched up to a maximum load of 250g over a time of 3 seconds. This measurement was taken three times and force/displacement data were recorded at a sampling rate of 40 Hz. The average of the three force/displacement curves was then used to obtain the engineering stress-versus-strain data for each specimen. The stress was calculated as the ratio of force and cross-sectional area at the start of the test (after 1 g tensile loading of the specimen). The strain was calculated as the ratio of instantaneous distance between the clamps and distance at the start of the test. The stress-versus-strain data were then curve-fitted to the equation: σ = A(eE − 1), where σ is the stress, e is the strain, and A and B are the material constants. The constants A and B were determined by using a least-squares minimization routine (MatLab; The MathWorks, Inc., Natick, MA). The Young’s modulus at strain e was calculated as E = dσ/de = A × B × eE, where E, the modulus, is the slope of the stress-versus-strain curve.

RESULTS

PS Cross-Linking Results

Table 1 summarizes SWV measurements in each group at low and high IOP before and after collagen cross-linking. Across all groups, native PS stiffness exceeded that of the ON/LC complex by at least a factor of 2 at low IOP (59.2 ± 4.6 m/s for PS vs. 27.9 ± 2.4 m/s for ON/LC; P < 0.001) and more at high IOP (125.7 ± 21.5 m/s for PS vs. 27.5 ± 3.2 m/s for ON/LC; P < 0.001). In group 1, riboflavin/UVA exposure of the entire peripapillary and peripapillary region had no measurable effect on the stiffness of the ON/LC (P = 0.57), but significantly increased the PS stiffness at 10 mm Hg (58.9 ± 2.3 to 121.1 ± 17 m/s; P = 0.004). No significant cross-linking-induced changes in stiffness were observed at an IOP of 30 mm Hg in the ON/LC (P = 0.33) or PS (P = 0.35). Only the PS stiffened significantly with an increase in IOP both before (P < 0.001) and after (P = 0.002) cross-linking.

In group 2, only an annulus of PS was exposed to GTA, with deliberate avoidance of the ON/LC. Focused PS cross-linking had no significant impact on ON/LC stiffness at measurement pressures of 10 mm Hg (P = 0.56) and 30 mm Hg (P = 0.9). GTA-mediated increases in PS stiffness, however, were significant at 10 mm Hg (from 64.0 ± 1.8 to 148.4 ± 43.0 m/s, P = 0.005) and marginally significant at 30 mm Hg (141.9 ± 20.5 to 197.0 ± 35.6 m/s, P = 0.05). In addition, as IOP was increased, ON/LC stiffness actually decreased slightly when the PS was cross-linked (P = 0.038), an effect that was not present before cross-linking (P = 0.07). As in group 1, IOP increases caused
significant PS stiffening both before ($P < 0.001$) and after ($P = 0.017$) cross-linking (Table 1).

Group 3, like group 2, involved focal GTA-mediated cross-linking of the PS region, but incorporated a much larger IOP range. Under preinflation IOP conditions (2–4 mm Hg), ON/LC stiffness increased significantly (from 25.7 ± 1.6 to 29.5 ± 2.9 m/s) with PS cross-linking ($P = 0.005$), a phenomenon that may relate to the nonlinearity of ocular compliance over this extended range of loads. Similar to the results in the other groups at a maximum IOP of 30 mm Hg, localized PS cross-linking had no effect on ON/LC stiffness at 80 mm Hg ($P > 0.05$). PS stiffness was significantly increased by cross-linking at the preinflation pressures (54.8 ± 3.6 to 149.9 ± 35.7 m/s, $P < 0.001$) and at 80 mm Hg (114.0 ± 19.0 to 190.2 ± 45.8 m/s, $P = 0.003$). The PS stiffened with increasing IOP both before ($P < 0.001$) and after annular PS cross-linking ($P = 0.029$). However, regarding the laminar impact of increased IOP, the ON/LC stiffened significantly with IOP loading before PS cross-linking ($P = 0.003$) but not after ($P = 0.87$).

Finally, in group 3, a photographic strain analysis during IOP elevation (Table 2) revealed significant centripetal PS strain both horizontally (6.5% ± 4.2%) and vertically (8.1% ± 4.9%) when measured before annular cross-linking. By a similar analysis of strain across the ON in the same eyes, the pressure-induced change in ON diameter was 4.2% across the ON/LC region alone ($P = 0.012$) and nearly immeasurable in both the horizontal and vertical directions (Table 2).

**Sonic Wave Velocity Results**

Uniaxial tensile test results from supplemental experiments to assess the validity of SWV as a measure of scleral stiffness are shown in Figure 3. In Figure 3, the average stress–strain values for the three groups, untreated, riboflavin/UVA irradiation, and GTA were plotted. The stress increased nonlinearly with strain among all the groups. Further stress increased with cross-linking at similar strain, and the greatest effect was observed in the GTA group.

Figure 4 plots the SWV measurements obtained in scleral strips against the square root of the modulus ($E$) evaluated at 4% strain, from tensile tests in the same strips. On average, the elastic modulus of the sclera increased by 210% and 1540% after treatment with riboflavin/UVA irradiation and GTA, respectively. A similar magnitude of increase in $E$ was obtained after treatment with riboflavin/UVA irradiation and GTA when evaluated at 8% and 15% strain. Theoretically, the speed of sound in a homogenous medium is directly proportional to the square root of modulus. An increase in the elastic modulus of the sclera leads to an increase in the square root of modulus. The speed of sound increases with the square root of modulus.

**DISCUSSION**

Glucomatous optic atrophy is a common disorder that has multiple risk factors and no widely accepted therapy, except that of lowering the IOP. The primary pathologic feature of glaucoma, however, is not elevated IOP, but rather a progressive loss of optic nerve fiber layer within the ON/LC complex. Although elevated IOP is a prominent risk factor for glaucomatous damage, resilience of the optic nerve against abnormally high pressure is noted in some eyes with ocular hypertension, while other eyes show focal, progressive damage even with normal IOP.

It is believed that the connective tissue structures within (i.e., lamina cribrosa) and around the optic nerve (i.e., PS) provide structural support to the ganglion cell axons as they traverse the optic nerve head. The anatomic relationship of the LC, being contiguous with and anchored to the PS, has been understood for sometime; however, their biomechanical properties and relevance to glaucoma have only more recently been investigated experimentally. Major contributions have been made characterizing the biomechanical properties of the ON/LC and PS in monkey eyes with early induced glaucoma. Confocal scanning laser tomography (CSLT) has demonstrated that normal compliance of the lamina cribrosa is acutely altered when IOP is increased as it becomes hypercompliant and displaces posteriorly. With chronic IOP elevation, the lamina not only further displaces posteriorly (eight times more than with high IOP only), but its thickness along with the scleral

**TABLE 2. Horizontal and Vertical Circumferential Strain Percentage of the Optic Nerve/Lamina Cribrosa Complex and Peripapillary Sclera with IOP Elevation from before Inflation (2–4 mm Hg) to 80 mm Hg before and after Cross-Linking**

<table>
<thead>
<tr>
<th>Strain (%)</th>
<th>Pre-cross-linking</th>
<th>Post-cross-linking</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>ON/LC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizontal</td>
<td>10.5 ± 3.9</td>
<td>0.0 ± 0.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Vertical</td>
<td>9.4 ± 6.5</td>
<td>0.0 ± 0.0</td>
<td>0.012</td>
</tr>
<tr>
<td>Average</td>
<td>10.2 ± 4.9</td>
<td>0.0 ± 0.0</td>
<td></td>
</tr>
<tr>
<td>PS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horizontal</td>
<td>6.4 ± 4.2</td>
<td>0.0 ± 0.0</td>
<td>0.01</td>
</tr>
<tr>
<td>Vertical</td>
<td>8.1 ± 4.9</td>
<td>0.7 ± 1.9</td>
<td>0.011</td>
</tr>
<tr>
<td>Average</td>
<td>7.3 ± 4.4</td>
<td>0.4 ± 1.3</td>
<td></td>
</tr>
</tbody>
</table>

**SWV**

$SWV = 0.0337 \times \sqrt{E} + 22.166 \quad (R^2 = 0.89).$

**FIGURE 3.** Uniaxial tensile testing results of untreated sclera, riboflavin/UVA irradiation cross-linking, and GTA from supplemental experiments, to assess the validity of sonic wave velocity as a measure of scleral stiffness.
canal opening diameter increases as well (hypercompliant and plastic deformation). These deformities in glaucomatous monkey eyes have been verified by three-dimensional reconstructions of serially sectioned optic nerve head samples embedded in paraffin.22 In subsequent viscoelastic tensile testing, the PS within and around the scleral canal wall revealed higher equilibrium moduli and thus greater stiffness in eyes with early induced glaucoma (7.46 ± 1.58 MPa) than in normal eyes (4.94 ± 1.22 MPa).23 Because these were studies of induced glaucoma and not of disease predisposition, stiffening of the PS is likely to represent a compensatory response involving an attempt by the extracellular matrix to resist the increased stress and strain imposed by elevated IOP. Within a conceptual framework in which IOP-related connective tissue stress and strain mediate and predict the onset and progression of glaucoma and not of disease predisposition, stiffening of the PS can be achieved using therapeutic collagen cross-linking to enhance the natural compensatory stiffness associated with glaucoma. Although advancing age is a known risk factor for glaucoma,24 natural cross-linking of ocular collagen with age25 does not necessarily implicate collagen stiffening as a risk factor. On the contrary, current models of biomechanical injury support a decrease in the predisposition for glaucomatous optic neuropathy by enhancing the biomechanical resilience of the PS and lamina cribrosa. Diabetes mellitus without vasculopathy has also been associated with a protective effect in the onset and progression of glaucoma in several studies, and this further supports the contention that tissue stiffening can have protective effects.25 Further research on the specific localization of cross-linking effects in ocular tissues during aging and diabetic nonenzymatic glycation will be important for better understanding these relationships.

This study provides the first nondestructive measurements of stiffness along the posterior globe using surface wave elastometry.18 It suggests the PS is stiffer than the ON/LC, which is an observation consistent with prior studies in excised porcine sclera.11 Although one might criticize the surface wave elastometry technique as new and unproven in the sclera (an inhomogeneous and poorly organized collagen structure) in comparison to the cornea (lamellar orientation), we believe this method of testing should yield even better accuracy on the sclera because of the greater degree of randomness in fiber directionality.

Our additional experiment in excised porcine scleral strips aimed at testing the validity of SWV as an indicator of scleral stiffness suggests that SWV measurements and measurements of elastic modulus from uniaxial extensometry are affected similarly by cross-linking and are capable of discerning differences in the efficacy of riboflavin/UVA irradiation and diadhydro-mediated cross-linking mechanisms. The strong linear correlation between SWV and E indicates that SWV measurements can be used as a reliable indicator of the stiffness of the tissue before and after cross-linking in this study. Furthermore, the pre- cross-linking and post- cross-linking measurements are within the same regions and with the same probe orientation, so that the metric of stiffness is less dependent on the actual values than on the changes induced by cross-linking. Consistency in orientation of the sonic wave device may be particularly important in the PS, where fibers oriented along the plane of the scleral shell can turn posteriorly to form the dural sheath.

Despite our confidence in measuring the PS, we acknowledge the weakness of our measurements across the LC. Since the position of the lamina within the scleral canal varies considerably and the PS fibers run along the margin of the lamina near where the probe makes its measurements, we qualify our laminar values as those representatives of the ON/LC complex. Although these values are less representative of the actual collagen of the LC alone, they do reflect the region within the PS ring, which when stiffened reduces the ON/LC stiffness.

Furthermore, we acknowledge our limitations in the stain measurements (Table 2) in group 3 due to pixel resolution. The pixel size in the study is equivalent to 120 micrometers/pixel (0.012 cm/pixel). Therefore, our images lack sufficient resolution to provide accurate digital image-based strain calculations and the zero strain values post- cross-linking in Table 2 could be explained by undetectable expansions from insufficient resolution. We believe, however, that the resolution is sufficient for a crude strain measurement and demonstration of a decrease in strain of the optic nerve/PS complex after cross-linking.

In our study, the IOP elevation measurably increased the in situ stiffness and circumferential strain of the ON/LC and PS, which is important in the pathogenesis and progression of glaucomatous optic neuropathy. Furthermore, we believe that collagen cross-linking stiffens the PS and shields the ON/LC from further stiffening and circumferential strain during IOP elevation.

Stiffening of the ON/LC with an increase in IOP was unique to group 3 and is probably related to that fact that SWV was lower at the subphysiologic pressures (2–4 mm Hg) of this group in comparison to the groups tested at 10 mm Hg and higher (Table 1). A shift from very high compliance to lower compliance as the collagen fibrils of the ocular coat become taut with the stress of inflation will result in a higher effective elastic modulus (approximated by the local slope of the curve). For similar reasons, the ON/LC may be more heavily affected by stiffening of the surrounding scleral ring, which may reduce the flaccidity of the entire region in this very low pressure range and increase the effective stiffness of adjacent structures.

![Figure 4. Sonic wave velocity measurements obtained in scleral strips against the square root of elastic moduli (E) from subsequent tensile tests in the same strips.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932958/)
These observations suggest that the range of pressures investigated is an important consideration for future work and that the effects of PS stiffening on ON/LC stiffness and strain behavior should be explored over several different ranges of IOP.

In studying the relative efficacy of therapeutic cross-linking in human and porcine sclera, Wollensak and Spoerl have determined the three most effective agents to be GTA, glyceraldehydes, and riboflavin/UVA irradiation with an increase in porcine scleral stress (rigidity) of 8, 5, and 1.5 times and human scleral stress (rigidity) of 122%, 34%, and 29%, respectively. The length of time for cross-linking of parallel scleral strips also varied considerably. As with group 1 in our study, the riboflavin/UVA method, although rapid, had the smallest effect in scleral stiffening because of the poor penetration of UVA through scleral tissues. Blue light at 436 nm is also effective in cross-linking with riboflavin and may be preferable due to its greater penetration in sclera.

Although GTA is known to be cytotoxic, and riboflavin/UVA has been shown to cause retinal damage in rabbit eyes, these cross-linking therapies can be dose adjusted to reduce their penetration and consequent cytotoxic effect. Alternatively, a nonenzymatic glycation-induced cross-linking (0.5 M glyceraldehyde and 0.5 M methylglyoxal over 5 days) can be implemented over a longer exposure period with good efficacy and without the potential loss of cell viability. These two agents may be excellent choices if prolonged exposure can be efficiently delivered.

In our study of whole porcine globes, measuring the SWV of PS and LC under both low and raised IOP, we chose to use only riboflavin/UVA and GTA as our cross-linking agents because of the their ready availability and shorter exposure time requirements. Extrapolating from histomorphologic observations of early induced glaucoma in primates and computational models, increasing PS stiffness may provide a protective effect against ON/LC deformation and radial strain. Our efforts in cross-linking of the PS verify this biomechanical benefit, both with surface wave elastometry, and in particular with a notable reduction of ON/LC and PS strain.

Because this work represents a first attempt to explore the mechanical effects on the ON/LC of modifying PS stiffness, it has several limitations. The effect of PS cross-linking on PS and ON/LC deformation was assessed with a simple optical approach that only considered radial deformation. Posterior deformation of the LC is an important morphologic feature of glaucoma that may contribute to axonal injury, and this study was not designed to measure potential consequences of peripapillary stiffening on posterior laminar strain. In contrast, higher levels of IOP-induced stress and strain within the LC, for a given IOP, has recently been demonstrated by 3-D finite element modeling in both thinner and flatter laminar geometries. This finding suggests a larger role of circumferential than posterior stress and strain, due to the higher values among the flatter and thinner lamina (Sigal IA, et al. J OVS 2008;ARVO E-Abstract 3668). The protective effect of PS cross-linking on both laminar stiffness and strain were also observed over nonphysiologically high pressures in porcine tissue and should be explored in human tissue over a range of pressure changes more likely to be encountered in the setting of primary open-angle glaucoma. The demonstration of any efficacy in prevention or treatment of clinical glaucoma would require extensive additional work in living animal models, and the safety and secondary effects of various peripapillary cross-linking approaches have yet to be explored in vivo.

CONCLUSIONS

IOP elevation measurably increases the in situ stiffness of the ON/LC and PS and is accompanied by circumferential strain in both regions. Collagen cross-linking of the PS measurably stiffens the PS and buffers the ON/LC from stiffening and circumferential strain during IOP elevation. These observations may have implications for modifying stress-and-strain–based mechanisms of injury in glaucomatous optic neuropathy.

Acknowledgments

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References