The Influence of Various Substances on the Biomechanical Behavior of Lamina Cribrosa and Peripapillary Sclera

Eberhard Spoerl, Andreas G. Boebm, and Lutz E. Pillunat

PURPOSE. Changes in the biomechanical properties of the lamina cribrosa (LC) and of the peripapillary sclera (ppSc) may play a role in the pathogenesis of glaucoma. The purpose of this study was to assess the influence of glyceraldehyde, methylglyoxal, and collagenase A on the mechanical properties of the LC and ppSc.

METHODS. Two strips of 1-mm width were cut from each of 80 porcine eyes and 24 pairs of enucleated human eyes. One strip contained the LC and the other the adjacent superior ppSc. One half of the strips was divided into groups and treated with 0.5 M glyceraldehyde, 0.5 M methylglyoxal, and 0.1% collagenase A. The other strips served as the control. The stress strain relation was measured in the stress range of 0.02 to 6.0 MPa by a biomaterial tester.

RESULTS. Stress values at 20% strain of the human LC changed from 1.97 ± 1.48 to 3.40 ± 1.60 MPa after incubation with glyceraldehyde (P = 0.029), from 2.42 ± 2.22 to 5.46 ± 1.91 MPa (P = 0.014) after incubation with methylglyoxal, and from 2.45 ± 1.3 to 1.35 ± 0.19 MPa after incubation with collagenase A. The stress values of human ppSc without glyceraldehyde were 3.40 ± 2.59 and 7.45 ± 4.46 MPa after incubation with glyceraldehyde (P = 0.047), 4.80 ± 3.05 MPa without methylglyoxal and 16.10 ± 5.53 MPa (P = 0.001) after incubation with methylglyoxal, 4.14 ± 2.56 MPa without collagenase A, and 1.97 ± 0.55 MPa after incubation with collagenase A. At a 20% strain, Young’s moduli of the untreated LC were in the range of E = 11.8 to 15.6 MPa and E = 28.5 to 36.0 MPa of the untreated ppSc.

CONCLUSIONS. Glyceraldehyde and methylglyoxal increase the stiffness of the LC and of the ppSc in human and in porcine eyes. These substances induce changes in the extracellular matrix according to the Maillard reaction as it occurs during the ageing process or in case of high blood glucose levels. Collagenase reduces the stiffness of the tissues. (Invest Ophthalmol Vis Sci. 2005;46:1286–1290) DOI:10.1167/iovs.04-0978

The structure of the lamina cribrosa (LC) and of the peripapillary sclera (ppSc) appears particularly relevant to glaucomatous optic nerve damage. Cupping of the optic nerve head is a hallmark of glaucomatous optic nerve damage, and actually precedes field loss. Optic nerve cupping seems to be caused primarily by increased intraocular pressure (IOP) or by a vascular response. However, the optic nerve head reacts differently to equal IOP-levels in different subgroups of patients (e.g., in normal-tension glaucoma or ocular hypertension).1

These differences in susceptibility to glaucomatous damage may be at least partially caused by different biomechanical properties of the LC and ppSc. These differences may be induced by changes in the extracellular matrix, which consists of elastin, collagen, and proteoglycans. One factor that affects the biomechanical behavior of the optic nerve head tissue is a different elasticity (e.g., in infantile glaucoma optic nerve cupping is reversible after reduction of IOP, whereas it is usually irreversible in adult eyes).2

Differences in elasticity are probably due to a different degree of collagen and elastin cross-linking.3 Another possible factor is a reduction in the supportive matrix of the LC, which is caused by tissue remodeling due to collagenolytic activities of activated astrocytes.4 Therefore, in the present in vitro study, the influence of cross-linking and collagenolytic activity on the biomechanics of the LC and of the ppSc of human donor and porcine eyes was investigated.

MATERIAL AND METHODS

Eighty porcine eyes and 24 pairs of enucleated human eyes (n = 48) were examined. The eyes were cleaned of all extraocular structures. At the equator, the eyes were halved horizontally, and vitreous, retina, and choroid were removed. Two strips of 1-mm width and 8-mm length were cut from each eye. One strip contained the LC (Fig. 1) and the other one the adjacent superior ppSc. These strips were incubated in Dulbecco’s modified Eagle’s medium (DMEM), with different substances.

Porcine Eyes

Each 10 strips of LC and the superior ppSc were incubated in DMEM plus 0.5 M glyceraldehyde for 6 days, DMEM plus 0.5 M methylglyoxal for 6 days, DMEM plus 0.1% glutaraldehyde for 30 minutes, and DMEM plus 0.1% collagenase A for 1 day. For each experiment, 10 untreated LC strips and 10 untreated peripapillary strips were used as the control and were placed in DMEM for the same amount of time.

Human Eyes

Each eight strips of LC and the superior ppSc (one of each pair) were incubated in DMEM plus 0.5 M glyceraldehyde for 6 days, DMEM plus 0.5 M methylglyoxal for 6 days, DMEM plus 0.1% glutaraldehyde for 30 minutes, and DMEM plus 0.1% collagenase A for 1 day. For each experiment, the strips of the other eye served as the control and were placed in DMEM for the same amount of time.

Thickness Measurements

The thickness of each specimen was determined with the help of ultrasound pachymetry (Pach-Pen XL; Mentor, Norwell, MA).

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Stress–Strain Measurements

Both the human and porcine strips were clamped horizontally with a distance of 3 mm between the clamps of the microcomputer-controlled biomaterial tester (MiniMat; Rheometric Scientific GmbH, Bensheim, Germany; Fig. 2). To tighten the strips, stress–strain measurements were performed with a prestress of 0.02 MPa. Then, the strain was increased linearly at a strain rate of 2 mm/min. The stress

Statistical Evaluation

The stress data necessary for a strain of 20% were compared between treated and untreated specimens of the porcine eyes by using ANOVA followed by the Sidak post hoc test and between treated and untreated specimens of human eyes by using Student’s t-test for paired samples. All statistical analyses were performed on computer (SPSS GmbH).

RESULTS

Thickness Measurements

The thickness of the human ppSc was 0.86 ± 0.17 mm and of the LC, 0.44 ± 0.15 mm. The thickness of the porcine ppSc was 0.93 ± 0.23 and of the ppSc, 0.58 ± 0.21 mm. None of the tissues showed a statistically significant change after any of the treatments (P > 0.05).

Stress–Strain Measurements

The results are shown in Table 1 for the porcine and in Table 2 for the human specimens.

Porcine Eyes. In the porcine eyes (Figs. 3, 4) with a strain of 20%, the stress was approximately 1.6 times higher in the ppSc than in the LC (P = 0.047). Glyceraldehyde, methylglyoxal, and glutaraldehyde led to an increase in stiffness (by factors 1.5, 2.0, and 2.1, respectively). Collagenase A reduced the stiffness of the LC significantly (by a factor of 0.4) and led to increased extensibility. The effect of collagenase A on the ppSc was similar (a factor of 0.2). Young’s moduli of the untreated porcine LC was 17.1 MPa and of the ppSc was 29.3 MPa.

Human Eyes. In the human specimens (Figs. 5, 6) glyceraldehyde and methylglyoxal led to an increase in stiffness of the LC by a factor of 1.7 and 2.2, respectively. The effect of collagenase A on the stiffness of the LC significantly (by a factor of 0.4) and led to increased extensibility. The effect of collagenase A on the ppSc was similar (a factor of 0.2). Young’s moduli of the untreated porcine LC was 17.1 MPa and of the ppSc was 29.3 MPa.

### Table 1. Stress at 20% Strain of Porcine LC and Peripapillary Sclera and the Young’s Modulus

<table>
<thead>
<tr>
<th>Stress at 20% Strain (MPa)</th>
<th>Young’s Modulus at 20% Strain (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC control</td>
<td>2.2 ± 1.5</td>
</tr>
<tr>
<td>LC + glyceraldehyde</td>
<td>3.2 ± 1.5</td>
</tr>
<tr>
<td>LC + methylglyoxal</td>
<td>4.5 ± 0.8</td>
</tr>
<tr>
<td>LC + glutaraldehyde</td>
<td>4.7 ± 1.3</td>
</tr>
<tr>
<td>LC + collagenase</td>
<td>0.9 ± 0.4</td>
</tr>
<tr>
<td>ppSc control</td>
<td>3.6 ± 2.0</td>
</tr>
<tr>
<td>ppSc + glyceraldehyde</td>
<td>5.7 ± 2.8</td>
</tr>
<tr>
<td>ppSc + methylglyoxal</td>
<td>6.5 ± 2.6</td>
</tr>
<tr>
<td>ppSc + glutaraldehyde</td>
<td>8.9 ± 4.8</td>
</tr>
<tr>
<td>ppSc + collagenase</td>
<td>0.6 ± 0.4</td>
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</table>

n = 10 in each group. ppSc, peripapillary sclera.

### Table 2. Stress at 20% Strain of Human LC and Peripapillary Sclera and the Young’s Modulus

<table>
<thead>
<tr>
<th>Stress at 20% Strain (MPa)</th>
<th>Young’s Modulus at 20% Strain (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC control</td>
<td>1.97 ± 1.48</td>
</tr>
<tr>
<td>LC + glyceraldehyde</td>
<td>3.40 ± 1.60</td>
</tr>
<tr>
<td>LC control</td>
<td>2.42 ± 2.22</td>
</tr>
<tr>
<td>LC + methylglyoxal</td>
<td>5.46 ± 1.91</td>
</tr>
<tr>
<td>LC control</td>
<td>2.43 ± 1.30</td>
</tr>
<tr>
<td>LC + collagenase</td>
<td>1.35 ± 0.19</td>
</tr>
<tr>
<td>ppSc control</td>
<td>3.40 ± 2.59</td>
</tr>
<tr>
<td>ppSc + glyceraldehyde</td>
<td>7.45 ± 4.46</td>
</tr>
<tr>
<td>ppSc control</td>
<td>4.80 ± 3.05</td>
</tr>
<tr>
<td>ppSc + methylglyoxal</td>
<td>16.10 ± 5.53</td>
</tr>
<tr>
<td>ppSc control</td>
<td>4.14 ± 2.56</td>
</tr>
<tr>
<td>ppSc + collagenase</td>
<td>1.97 ± 0.55</td>
</tr>
</tbody>
</table>

n = 8 in each group. ppSc, peripapillary sclera.
was higher by factors of 2.2 and 3.3, respectively. Collagenase reduced the stiffness of the LC by a factor of 0.7 and of the ppSc by a factor of 0.6. At 20% strain, Young’s moduli of the untreated LC and ppSc were $E = 11.8$ to $15.6$ MPa and $E = 28.5$ to 36.0 MPa, respectively. Young’s modulus of the untreated LC was half that of the ppSc ($P = 0.02$).

**DISCUSSION**

In porcine and human eyes, significant increases in stiffness of the LC and the ppSc were found after application of aldehyde sugars (e.g., glyceraldehyde, methylglyoxal, and glutaraldehyde [porcine only]) by ~50% to 200%. Bailey et al.\(^5\) showed that with increasing age, collagenous tissue becomes stiffer and more inflexible. Advanced glycation end products (AGEs) are the molecular reason for these alterations. Nonenzymatic glycation, the reaction of reducing sugars with amino groups of collagen, and the formation of AGEs and stable cross-links lead to increased stiffness of the collagen-containing tissues. This gradual process occurs with aging, especially in the presence of high blood glucose levels (diabetes).\(^5\) In long-lived collagens, this molecular process leads to cumulative changes of the physicochemical properties (increased stiffness and resistance to collagenases). In addition, because of the cross-linking, collagen becomes undigestible for matrix metalloproteinases (MMPs), which results in an increased amount of total collagen. This molecular aging process affects all connective tissues, including the LC and ppSc, both of which play an important role in the development of glaucoma. Instead of the common slow-reacting aldehyde sugar glucose, we used glyceraldehyde and methylglyoxal in our study, because of the higher reaction rate and shorter time needed to reach a stiffening effect.\(^6\)

Two mechanisms seem to contribute to the change of the condition of the LC: mechanical and cellular. It is known that the biomechanical stiffness of the LC increases and the mechanical compliance decreases with age.\(^3,7\) The adult LC is more rigid because of the abundance of collagen stabilized by mature cross-links.\(^8\) In addition, the reversibility of the LC cupping decreases due to loss of tissue elasticity with age. Each time the pressure fluctuates beyond the yield point, the extracellular matrix of the LC undergoes plastic changes that result in permanent deformation and irreversible cupping.\(^8\) Consistent with these findings, an age-related increase in collagen cross-links including hydroxyllysyl-pyridinoline and pentosidine, a Maillard product, has been demonstrated.\(^3\) The increase in pentosidine induces a stiffening or increased rigidity of the lattice-like connective tissue structure of the LC. In addition, the decrease in type III collagen and increased elastin cross-linking (desmosine and isodesmosine) cause a reduction in the mechanical compliance of the aging LC.\(^3\) Also, the incidence rate of POAG increases with age.\(^10\) At the same IOP, higher stress acts on a stiffer LC than in a more elastic one. The stiffer LC is not able to change its curvature radius to reduce the stress according to the Laplace law as an elastic LC. This elevated mechanical stress leads to activation of astrocytes, which produce collagenases to reduce such stress.\(^4\)

Consistent with these findings, many investigators have demonstrated a reversal of optic disc cupping after reduction of intraocular pressure in infantile and congenital glaucoma, in which the connective tissue is more elastic. In adult glaucoma, however, with the stiffer cross-linked connective tissue matrix, this phenomenon is observed rarely.\(^11\) A possible explanation may be the lower stress in an elastic LC compared with that in stiffer tissue at the same IOP. In a young LC, the lower stress possibly does not lead to an activation of astrocytes, the pro-
cess of remodeling and plastic deformation is not initiated, and a reversion of the cupping can occur when IOP is decreased. The elastic limit of the LC decreases with age, especially in glaucoma, suggesting an increased susceptibility to plastic flow and permanent deformation. These biomechanical changes may explain the absence of reversibility of cupping in adults. However, in children, the reversibility of disc cupping is possible, as the number of cross-links is very low. Reversibility of cupping in the infant after normalization of IOP has become a major criterion for successful therapy in congenital glaucoma. 

Diabetes mellitus is also reported to be a risk factor for glaucoma in several studies. However, reports about the association between diabetes and glaucoma have been contradictory. A possible reason for the contradiction is the different definition of diabetes ranging from patient history during an interview to laboratory confirmation. From the analysis of several trials an association between elevated blood glucose level (hyperglycemia) and glaucoma seems to be likely. The diagnosis of diabetes alone without any specification seems to be an imprecise parameter because the glucose level seems to be the crucial parameter. Good glycemic control or intensive insulin treatment reduces the accumulation of AGEs in collagen and may diminish the risk of glaucoma in diabetes.

This theory is also supported by the fact that only elevated glucose levels accelerate the formation of AGEs according to an exponential correlation. So-called AGEs have been identified in the optic nerve head in diabetic eyes immunohistochemically. Accumulation of AGEs induces collagen cross-linking and decreases the elasticity of the LC. These changes are similar to changes in the LC due to aging.

The fact that the ppSc may also deform under pressure is ignored in several studies. The sclera and especially the ppSc play a role in the pathomechanism of glaucomatous optic nerve head excavation as a self-perpetuating process. A decrease in the density of collagen types IV, V, and VI has been observed after treatment with aldehyde sugars in our study. The stiffened edge of the sclera canal due to cross-linking because of aging or increased sclera collagen density in collagen-mutated mice contributes to increased susceptibility of the optic nerve to damage by elevated IOP.

In the present study we found a significant decrease in biomechanical stiffness of the LC and the ppSc after collagenase treatment. The extracellular matrix of the LC contains primarily collagen types I and III and in much smaller quantities collagen types IV, V, and VI. From other studies it is known that reactive astrocytes, the major glial cell type in the optic nerve head, undergo stress-induced migration accompanied by collagenolytic degradation. Reactive astrocytes can be induced by increased IOP, and in contrast to mature quiescent astrocytes, they migrate within the axonal tissue and release proteolytic enzymes such as MMPs, degrading and cleaving extracellular matrix components such as collagen, proteoglycans, and elastins. A decrease in the density of collagen fibers and marked elastotic degeneration have been reported with increased IOP. These reactive changes due to IOP-induced axonal damage may further contribute to optic nerve head excavaation as a self-perpetuating process. A decrease in the biomechanical stability accompanied by plastic deformation of LC in patients with normal-tension glaucoma may also be explained by the increased expression of MMPs.

The biomechanical changes observed after treatment with aldehyde sugars and collagenase might be considered a simulation of the conditions that increase the risk of the development of glaucoma and its progression. Generally, there is a stiffening of the mechanical support of the optic nerve head in early stages of glaucoma. Apart from cross-linking, the loss of elasticity is also due to elastosis, with curled elastic microfibers in the glaucomatous LC and a degeneration of elastic elements similar to the conditions in an aortic aneurysm. Compression of the LC sheets is the earliest abnormality in glaucomatous optic nerve damage. Backward (posterior) bowing of the LC with cupping occurs as a later change involving preferably the upper and lower poles of the optic nerve head. The increased IOP may act directly on the nerve fiber bundles or blood vessels or by compression of the stiffened LC.

IOP-related damage to the load-bearing connective tissue with biomechanical changes in the support matrix occurs within 2 to 4 weeks of experimental glaucoma. The elastic reversibility of the glaucomatous cupping is reduced with chronic plastic posterior deformation of the disc at 3 to 8 weeks after onset of glaucoma, possibly because of the collagenolytic action of reactive astrocytes.

There are some limitations to our study. Our measurements represent only a uniaxial stress–strain relation, not biaxial. Therefore, with our model we can show only biomechanical changes, which also would be reflected qualitatively in biaxial stress–strain or in compliance. However, in our model, we can investigate whether the biomechanical properties of a tissue change and evaluate the effect of various substances on the biomechanical behavior of the tissue. Hence, the model used in our study seems to simulate adequately the biomechanical changes that would probably occur in pathologic conditions.

In the present study, tangential stress was measured, whereas in glaucoma a bending deformation of the LC occurs, and the stress is partly tangential but also radial. Edwards and Good reported that stress in the tangential direction exhibits the same trend as the stress in the radial direction. Therefore, not only increased IOP is essential for damage of axons and remodeling of LC but also tangential stress in the tissue, which may be calculated by the Laplace law. The tangential stress seems to be a sufficient parameter for description of the biomechanical conditions to characterize the tissue after treatment with various substances.

In summary, the present study demonstrated in vitro a significant stiffening effect of sugar aldehyde treatment (most likely due to collagen cross-linking) on the biomechanical properties of the LC and ppSc in both human and porcine eyes and a significant decrease in biomechanical stiffness after collagenase treatment. These findings support the clinical observations about the changes in optic nerve head compliance due to aging or glucose-related cross-linking or tissue weakening by reactive astrocytes, which might be at least partially responsible for the increased risk of glaucoma in older or diabetic patients. The relevance of the investigation lies in the simulation of the molecular conditions for higher risk of the development of glaucoma with an increased LC and ppSc stiffness and the progression of glaucoma with a deep excavation of the optic nerve head due to the collagenolytic action of reactive astrocytes.

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