Corneal Stiffness Affects IOP Elevation during Rapid Volume Change in the Eye

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PURPOSE. To investigate whether corneal stiffness affects the elevation of intraocular pressure after an acute increase in the volume of ocular fluid.

METHODS. Saline (200 μL total) was infused into porcine globes before and after corneal stiffening. Intraocular pressure (IOP) was continuously monitored by a pressure sensor that was cannulated to the vitreous chamber. Corneal stiffening was achieved by immersing the corneas in a 1% or 4% glutaraldehyde solution for 20 minutes. Corneal strips were dissected from the globes, and the stress-strain relationships were measured. The mean secant modulus of each group of corneas at 5% strain was calculated. Control eyes with no corneal stiffening were also tested.

RESULTS. A significantly higher IOP elevation was observed in the globes after the corneas were stiffened (mean ± SD, 14.9 ± 1.9 mm Hg before stiffening vs. 19.1 ± 2.6 mm Hg after 1% glutaraldehyde treatment and 24.3 ± 1.9 mm Hg after 4% glutaraldehyde treatment at 200 μL infusion; P < 0.001). The control group showed no change in IOP elevation. The 5% secant modulus was 0.46 ± 0.24 MPa, 1.63 ± 0.41 MPa, and 2.78 ± 1.04 MPa (mean ± SD), respectively for the original corneal tissue and tissue with 1% or 4% glutaraldehyde treatment.

CONCLUSIONS. This study showed that stiffened corneas induced substantially higher IOP elevations when all other geometrical and material properties of the eye remained essentially the same. The results suggested that corneal stiffness may play an important role in determining IOP elevation caused by an acute increase in the volume of intraocular fluid. (Invest Ophthalmol Vis Sci. 2009;50:2224-2229) DOI:10.1167/iovs.08-2565

Steady state intraocular pressure (IOP) is established to achieve the balance between the inflow and the outflow of the aqueous humor so that the drainage of fluid occurs at the same rate at which it is produced. Several physiological factors determine steady state IOP. These factors are aqueous production (inflow; $F_{in}$), trabecular outflow facility ($C_{trab}$), uveoscleral outflow ($F_{u}$), and pressure in the drainage vessels (episcleral venous pressure; $P_v$). Under normal steady state conditions, the hydraulic equation that governs aqueous humor dynamics can be approximated as follows:

$$F_{in} = F_{out} = C_{trab}(IOP - P_v) + F_u$$

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Supported by the Columbus Foundation Ann Ellis Fund, Columbus, Ohio.

Submitted for publication June 2, 2008; revised August 12, October 13, and November 29, 2008; accepted March 18, 2009.

Disclosure: J. Liu, None; X. He, None

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Changes in any of these factors lead to IOP fluctuation. For example, when there is an increase in aqueous production ($F_{in}$) or a decrease in outflow facility ($C_{trab}$), IOP elevation results, and a new equilibrium between inflow and outflow is established.

Unequal fluctuations in inflow and outflow may affect the net volume of the intraocular fluid. Fluctuations in the volume of intraocular fluid are coupled with IOP fluctuations under many physiological conditions. From a biomechanical point of view, the volume-pressure response is influenced by the geometry and material properties of the corneoscleral shell. The stiffness of the ocular tissue may influence the transient changes in IOP that the eye experiences on a daily basis. However, the nature of this influence is not well understood.

Friedenwald introduced the concept of ocular rigidity to characterize the pressure-volume relationship of the whole eye. This concept was used initially for the purpose of improving the accuracy of tonometry,² and its relevance in glaucoma and other diseases was also investigated (Panagiotoglou TD, et al. JOVS 2005;46:ARVO E-Abstract 2726).³ Ocular rigidity gives a description of the overall resistance or distensibility of the eye; however, it does not relate the pressure change specifically to the material or geometric properties of the ocular tissues because these factors are inseparable in the coefficient of ocular rigidity.

In this study, we used an experimental model to investigate the potential role of corneal stiffness in acute IOP elevation. The stiffness of porcine corneas was experimentally altered by introducing different levels of collagen cross-linking (induced by glutaraldehyde treatment) to simulate the variance in corneal stiffness. Acute IOP elevation was induced by an increase of intraocular fluid through brief saline infusion (within minutes). The saline infusion was an experimental model to simulate increased intraocular volume, which may be associated with increased aqueous humor formation, increased outflow resistance, or increased episcleral venous pressure. Although these events usually occur on a much longer time scale, more acute changes are also observed in the eye. For example, eye blinking/rubbing,⁴ therapeutic intraocular injections,⁵ or a temporary head-down position⁶ may lead to sudden changes that are relevant to the time scale simulated in this study.

IOP elevation was measured and compared before and after corneal stiffening in response to a controlled increase of intraocular fluid through saline infusion. Changes in corneal stiffness were confirmed by tensile testing on corneal strips. The relationship between corneal stiffness and IOP elevation was examined.

METHODS

Fresh, nonscalded porcine globes were obtained and tested within 24 hours of death (24 eyes, nonpaired). Globes were enucleated before the animals were boiled for hair removal. All experiments were performed at room temperature. Eyes were kept immersed in dextran solution (with or without glutaraldehyde), except during the brief saline infusion when they were exposed to air. Each globe was immersed in a 20% dextran solution for deturgescence of the corneas. A 22-gauge needle was inserted from the limbus into the vitreous cham-


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Corneal Stiffness and IOP Elevation

Saline infusion in the porcine globes led to a gradual increase in IOP. In control globes whose corneas were immersed in a solution without collagen cross-linking reagent (0% glutaraldehyde), repeatable IOPs were observed before and after the immersion (25.4 ± 1.9 mm Hg vs. 24.9 ± 1.9 mm Hg; P = 0.42; Fig. 3). Significantly higher IOP increases were seen in globes after their corneas were stiffened by glutaraldehyde cross-linking. At 200 μL infusion, the globes whose corneas were immersed in 1% glutaraldehyde had an IOP of 24.3 ± 2.6 mm Hg before treatment and 29.1 ± 2.6 mm Hg after treatment (P < 0.001; Fig. 4). An even larger IOP elevation was observed in the globes whose corneas were treated with 4% glutaraldehyde. Specifically, the IOP in these globes increased to 34.3 ± 1.9 mm Hg before treatment and 29.1 ± 2.6 mm Hg after treatment (P < 0.001; Fig. 5). IOP elevations were also significantly different at the smaller infusion volumes (50, 100, and 150 μL) before and after 1% or 4% glutaraldehyde treatment (P < 0.001 for all volumes and both treatments). Baseline (before treatment) IOP-volume relationships were consistent in all groups (Figs. 3–5).

At a 200 μL infusion volume, mean IOP elevations were 14.9, 19.1, and 24.3 mm Hg in the globes after their corneas were treated with 0%, 1%, and 4% glutaraldehyde, respectively (Fig. 6). This injection volume (200 μL) amounts to approximately 3% of the total volume of the globe, assuming the globe is a 12-mm sphere.

Tensile tests in corneal strips generated the stress-strain relationships in the control porcine corneas and in those
treated with 1% or 4% glutaraldehyde. At 5% strain, the secant tensile modulus of these three groups was $0.46 \pm 0.24$ MPa, $1.63 \pm 0.41$ MPa, and $2.78 \pm 1.04$ MPa (mean $\pm$ SD). The average stress-strain curves up to 8% strain are shown in Figure 7. The plot of the secant corneal modulus compared with IOP elevation at 200 $\mu$L infusion for all three groups is presented in Figure 8. The overall Pearson’s correlation coefficient between corneal modulus (at 5% strain) and IOP elevation (at 200 $\mu$L infusion) was 0.84. A similar level of correlation was found between IOP elevation at 200 $\mu$L infusion and other levels of strain (Pearson correlation was 0.83–0.85 for strain levels at 1% to 8%). Table 1 gives a summary of the mean IOP elevation and the corresponding mean corneal modulus (5% strain) in the three tested groups.

Ocular rigidity of the porcine globes, calculated as the ratio of change in ln(IOP) to the change in volume according to Friedenwald, was $0.0047 \pm 0.0005$ mm Hg/$\mu$L for the control group (fresh porcine globes), $0.0063 \pm 0.0006$ mm Hg/$\mu$L for globes whose corneas were treated with 1% glutaraldehyde, and $0.0071 \pm 0.0005$ mm Hg/$\mu$L for globes whose corneas were treated with 4% glutaraldehyde.

**DISCUSSION**

The effect of corneal stiffness in the pressure-volume relationship of the eye was investigated. This study demonstrated that corneal modulus may affect the characteristics of IOP elevations in response to rapid volume changes. Our study showed that stiffened corneas induced substantially higher IOP elevations when all other geometric and material properties of the eye remained essentially the same.

The level of IOP elevation was correlated with the extent of corneal stiffening, as measured by the secant modulus of the corneal strips at 5% strain. The average secant modulus for the fresh porcine corneas was $0.46 \pm 0.24$ MPa in our study. This value is comparable to that reported in the literature. Spoerl et al. tested fresh porcine corneal strips and obtained the stress-strain curve, which showed a value of approximately 1 MPa. Pierscionek et al. reported a smaller range of corneal elastic moduli (0.07–0.29 MPa) measured from inflation tests on whole porcine globes. Their calculations of the elastic moduli were based on...
equations applicable to thin-walled pressure vessels (strain levels were not specified). Elsheikh et al.9 compared inflation tests (on dissected porcine corneal buttons) and strip tests and concluded that strip tests could considerably overestimate corneal modulus because of the various assumptions used. They reported the stress-strain relationship obtained from strip tests on fresh porcine corneas, and secant modulus at 5% strain was approximately 0.8 MPa (estimated from their reported stress-strain curve). Kampmeier et al.10 reported a secant modulus of approximately 0.5 MPa at 5% strain. These data suggest that the strip test results in our study are comparable to what is reported in the literature.

Our study found that corneal stiffness differed about five to six times (0.48–2.78 MPa) before and after glutaraldehyde treatment. The reported values for human corneal modulus had a wide span in the literature, where they were obtained under wide-ranging experimental conditions. Elsheikh et al.16 measured cadaveric buttons using inflation tests for subjects between 50 to 64 and 80 to 95 years of age. Under the same experimental conditions, they measured a mean value at 0.5 MPa (4% strain) for the 50- to 64-year-old group and 2.0 MPa (4% strain) for the 80- to 95-year-old group (modulus values were estimated from their reported stress-strain curves). Hamilton and Pye17 reported a range from 0.13 to 0.43 MPa in healthy young subjects (18–30 years of age). They calculated Young’s modulus from theoretical models of tonometry measurement, and no strain rate was specified. The comparison within the same studies should more or less reflect the biological variability. Although in vivo population data are not available, the literature suggests that corneal modulus could vary significantly across human subjects, and the variance in corneal stiffness induced in our experimental model (through glutaraldehyde treatment) could be relevant for understanding its potential effects in human eyes.

Ocular rigidity was also increased after corneal cross-linking. The values of ocular rigidity for the control eyes in this study were higher than (but of the same order of magnitude as) the range reported in the literature for fresh porcine eyes.12 The values were significantly smaller than those reported for cadaveric5 or in vivo18 human eyes. Higher ocular rigidity would predict greater IOP elevations in human eyes for the volume changes simulated in our study.

Our data from corneal strip testing confirmed the changes in corneal modulus after cross-linking and demonstrated the relationship between corneal stiffness and IOP elevation resulting from rapid volume change. Johnson et al.19 studied the pressure-volume relationship of dissected human cadaveric corneas. They found considerable variability in the range of IOP elevations with inflation of corneal buttons clamped to an artificial anterior chamber. They postulated that IOP elevation was affected by the distensibility of the cornea and that a more distensible cornea may protect the eye from sudden pressure spikes caused by volume changes. This is consistent with our finding and provides evidence that the innate variability in human corneal stiffness could be substantial.

Results from this study showed that the same amount of intraocular volume change may result in different levels of IOP elevation, depending on corneal properties. Similarly, different volume changes may be tolerated before the eye reaches the same elevated IOP. More compliant corneas may dampen the fluctuations in the inflow or the outflow, and thus the eyes experience a more stable and smooth profile of IOP. On the other hand, stiffer corneas may be associated with rapid and higher-magnitude IOP fluctuations because small variations of aqueous flow may significantly affect the momentary IOP. Our results showed that the IOP elevations were significantly higher in the stiffened eye even at small volume changes (i.e., 50 µL).

Although the clinical significance of IOP fluctuations are unknown, experimental studies have demonstrated damaging effects of brief IOP spikes on retinal ganglion cells (RGCs).20 Resta et al.20 showed that a 1-minute IOP spike at 50 mm Hg induced the loss of membrane integrity in 29% of RGCs in vitro. Furthermore, it was shown that the damage induced by transient pressure was cumulative. A sequence of seven 1-minute pressure spikes at 50 mm Hg (separated by 1-minute resting

**TABLE 1. IOP Elevation in Three Groups of Porcine Globes after 200 µL Saline Injection and Corresponding 5% Secant Corneal Modulus**

<table>
<thead>
<tr>
<th>Glutaraldehyde Treatment, %</th>
<th>IOP Elevation (mm Hg)</th>
<th>Corneal Modulus (MPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Mean: 14.9</td>
<td>Mean: 0.46</td>
</tr>
<tr>
<td>1</td>
<td>19.1</td>
<td>1.63</td>
</tr>
<tr>
<td>4</td>
<td>24.3</td>
<td>2.78</td>
</tr>
</tbody>
</table>

**FIGURE 7.** Stress-strain relationship of porcine corneas (control and with 1% or 4% glutaraldehyde treatment).

**FIGURE 8.** Scatterplot of corneal modulus versus IOP elevation at 200-µL saline infusion in the three groups (control and with 1% or 4% glutaraldehyde treatment), demonstrating an overall good linear correlation between corneal modulus and IOP elevation ($R = 0.84$).
pressure time periods) induced considerably more RGC damage (loss of membrane integrity in 74% of RGCs). Interestingly, it was also demonstrated that a slowly rising pressure (a slow 50-mm Hg insult that lasted for the same period as the seven spikes) damaged none of the cells, indicating that rapid pressure fluctuations can significantly affect RGCs.

Aging has been shown to be associated with increased stiffness in cornea,16 lamina cribrosa,21 and sclera22 (Girard MJA, et al. IOVS 2008;49:ARVO E-Abstract 4058). This study indicates a potential mechanism underlying age-associated risk for glaucoma. The increased stiffness in the aged eye may lead to larger short-term IOP fluctuations if we assume the intraocular volume changes remain more or less the same during the course of aging. Conversely, to maintain a similar magnitude of IOP fluctuations as the eye ages, smaller intraocular volume changes would be expected because of increased tissue stiffness. The reduced intraocular volume change may not be desirable if we consider the pulsatile ocular blood flow by which an intraocular volume change (caused by blood entering the eye) and an IOP change are coupled. If we assume the older, stiffer eye and the younger, more compliant eye have similar ocular perfusion pressure, the stiffer eye would likely experience smaller intraocular volume change for similar pulse amplitude (i.e., the maximum IOP change during the cardiac cycle). This may indicate reduced pulsatile blood flow in the stiffer eye. These age-related IOP and blood flow changes, if present, could contribute to glaucoma risk. The physiological conditions are likely more complicated, and further modeling and experimental studies are needed to understand the role of ocular biomechanics in age-associated glaucoma risk.

Our study is limited by several considerations. First, we only investigated the effect of corneal stiffness, whereas several other parameters may have significant influence on the biomechanical responses of the eye. Corneal thickness may also play a role. Although efforts were made to keep corneal thickness unaltered in this study, it is possible that it could have changed during glutaraldehyde treatment. In that case, what we observed were the combined effects of the changes in thickness and stiffness. Furthermore, finite element modeling studies suggested a significant influence of scleral stiffness in the stress and deformation status at the optic nerve head in response to acute changes in IOP.23 It is likely that scleral biomechanical properties are also influential in sudden IOP elevation. The cornea is generally more expandable than the sclera because it is thinner and has a lower modulus.13 This would indicate that the cornea may have a substantial role in rapid volume changes, but we also must take into consideration that the total volume the cornea occupies is smaller than that of the sclera. Thorough theoretical modeling and experimental validation are needed to understand the relative roles the cornea and sclera play in IOP and volume changes. Potential correlation between the properties of cornea and sclera may also warrant further investigation because they are both collagenous tissue and may be subject to biomechanical alterations caused by aging or disease.

Second, because of the limited availability of donor eyes, our experiments were performed on porcine globes. Judging from reports in the literature20,24 and our tensile test results, the porcine cornea appears to have a smaller modulus than the human cornea. Our results showed that a higher corneal modulus predicts larger IOP elevation in human globes at the same volume change simulated in this study. This prediction is consistent with the findings reported for in vivo human eyes. Pallikaris et al.18 reported that a 70-μL saline infusion into the anterior chamber induced an IOP elevation of approximately 50 mm Hg in vivo in human subjects. Kotliar et al.5 reported an IOP elevation of 40.6 mm Hg on intravitreal injection of 100 μL fluid during therapeutic intervention in humans. Our measurements on porcine eyes (with or without corneal cross-linking) yielded a range of 15 to 25 mm Hg IOP elevation at 200-μL injections. These data indicate that the effect of corneal stiffness could be consequential for smaller volume changes in human eyes. More accurate prediction about the response of living human eyes will require knowledge of the distribution and range of in vivo corneal and scleral stiffness, which are not yet available.

Third, the nonlinear and viscoelastic responses of the ocular tissues were not considered. Because corneal tissue has nonlinear mechanical properties, stiffness is dependent on the strain level. Future studies are needed to understand how nonlinearity could affect IOP elevation in response to rapid volume changes. Furthermore, during the infusion tests in this study, tissue relaxation could lower the IOP. Because the time frame for our study was short, the viscoelastic effects were likely not significant,25 but future studies are needed for a full understanding of this aspect. In the larger time scale, cells in the living eye are known to respond biochemically to longer term mechanical stimuli, and tissue remodeling may occur. Future studies are needed to characterize the longer-term biomechanical properties of the cornea and how these factors may affect IOP elevation.

In summary, our study showed that corneal stiffness, though not affecting steady state value, may play a role in determining the characteristics of IOP fluctuations. Increased corneal stiffness may predispose the eye to the damaging effects of IOP fluctuations when there are disturbances to the steady state.

Acknowledgments

The authors thank Xuexiang (Jeff) Pan for assistance with the statistical analysis and Paul A. Weber and Mark A. Bullimore for discussions concerning the clinical relevance of the study.

References