Experimental evaluation of travoprost-induced changes in biomechanical behavior of ex-vivo rabbit corneas

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Abbreviated title

Experimental evaluation of travoprost-induced biomechanical change of rabbit cornea

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PRECIS:

Prostaglandin F2α analogue travoprost causes a stiffness reduction effect on corneal biomechanical properties under low applied stresses

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Abstract

Purpose: To assess the effects of prostaglandin F2α analogues travoprost on the biomechanical behavior of ex-vivo rabbit cornea.

Methods: 18 Japanese white rabbits were included in the study. The left eye (treated group, Tr) of each rabbit was preserved for 10 days in storage medium Eusol-C solution with 1:10 travoprost diluent, while the contralateral eye (control group, Co) was preserved in a similar but travoprost-free medium. Strips of corneal tissue were dissected and tested under cyclic load conditions with up to 0.1 N uniaxial tension force. The resulting load-elongation data were used to derive the stress-strain behavior and the tangent modulus (Et) of the tissue. Differences in Et between the treated ($Et\text{-}_{Tr}$) and control group ($Et\text{-}_{Co}$) were assessed statistically to determine the biomechanical effects of travoprost on the cornea.

Results: Central corneal thickness remained similar in the two groups before ($p= 0.073$) and after storage ($p= 0.303$), although it became significantly thicker in both groups after storage ($P< 0.01$). Compared with the control group, the travoprost treated corneas exhibited lower Et values but the differences reduced and became insignificant with rises in stress to which the tissue was subjected ($1 - Et\text{-}_{Tr}/Et\text{-}_{Co} = -11.7\pm41.8\%$, $p< 0.05$ at 10 kPa stress, $-9.2\pm36.1\%$, $p> 0.05$ at 20 kPa, $-7.3\pm35.4\%$, $p>0.05$ at 30 kPa).

Conclusions: Significant reductions in corneal stiffness, that are associated with the use of travoprost, were observed experimentally under low applied stresses. This stiffness-reduction effect should be considered in clinical management, especially in primary open angle glaucoma treatment.

Keywords: prostaglandin; cornea; tangent modulus, tensile test

Introduction

Glaucoma, the second leading cause of blindness worldwide [1], is a form of optic neuropathy associated with progressive degeneration of retinal ganglion cells and irreversible vision loss [2]. Raised intraocular pressure (IOP) remains the most important risk factor, and reduction of IOP can slow the progression of optic neuropathy [3] and is reportedly the most effective management method for glaucoma. Pharmacologic therapy is the initial treatment for glaucoma, and the most commonly prescribed classes of topical hypotensive agents are prostaglandin analogs (PGAs), especially for primary open angle glaucoma (POAG) [4]. Prostaglandin F2α analogues (PGF 2α) upregulates the activity of matrix metalloproteinase (MMP) and downregulates the tissue inhibitor of metalloproteinase (TIMP) [5-7], which results in remodeling of the extracellular matrix, increasing the space between the bundles of smooth muscle cells, allowing better outflow and leading to lowering of IOP.

However, in addition to the effect of PGAs-induced IOP reduction, PGF 2α has been shown to decrease the collagen fibril density and corneal thickness [8, 9]. Fibroblasts and extracellular matrix are the main structural components of the cornea, responsible to a large extent for determining corneal biomechanical properties. Collagen degradation caused by long-term topical prostaglandin therapy could influence corneal biomechanical behavior and induce reductions in corneal hysteresis (CH), corneal resistance factor (CRF) – both measured by the Ocular Response Analyzer, ORA) [10] – and the deformation amplitude (DA) provided by the Corvis ST (CVS) [11]. Changes in corneal biomechanical properties after long-term topical prostaglandin therapy possibly introduces inaccuracies in IOP measurement and in other applications that require knowledge of corneal biomechanics such as planning of surgical procedures, assessment of stiffness deterioration associated with keratoconus and optimization of corneal cross-linking treatment [12].

Corneal biomechanical metrics provided by the ORA and CVS have been widely used

to assess the general biomechanical response of the cornea. Nevertheless, these metrics may be influenced by corneal shape and the intraocular pressure (IOP), and their links to standard mechanical properties, such as the tangent modulus of tissue (Et), have not been established [12-14]. This study aims to address this shortfall through an experimental investigation of whether the usage of PGF 2α (in particular travoprost) influences the biomechanics of corneal tissue.

Materials and methods

2.1. Experimental animals

Eighteen Japanese white rabbits (2-3 kg) from the Animal Breeding Unit at Wenzhou Medical University were included in this study. All animals were treated in agreement with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research, and every effort was made to minimize suffering. This study was approved by the Animal Care and Ethics Committee of the University's Eye Hospital.

2.2. Experimental design

After being euthanized by intravenous injection of high concentrations of pentobarbital sodium (Merok, Germany), bilateral eyes of each rabbit were immediately enucleated. The entire cornea, with the adjacent 3 mm wide scleral strip, was extracted from each ocular globe while all other ocular components were removed. The left eyes of the 18 rabbits, which form the treated group (Tr), were placed in storage medium of Eusol-C solution (Alchimia S.r.l, Ponte S. Nicolo`, Italy) with 0.0004% travoprost (Travatan; Alcon Laboratories, Inc., Fort Worth, TX) diluent (1:10 dilution of stock solution). The corresponding 18 right eyes constituted the control group (Co), and were placed in the same medium but without travoprost. Based on other studies [15, 16], the 1/10 dilution was selected to take into account the relatively short duration of storage adopted in the study compared to the long-term exposure in clinical usage. All corneas were incubated in standard incubator conditions (37°C, 5% CO_2) for 10 days as described in a previous study [17].

2.3. Biomechanical Tensile Testing

A 2-mm-wide corneoscleral strip centered on the cornea was excised from each specimen using two parallel surgical blades along the inferior-superior direction. The strips were connected to a pair of mechanical clamps, leaving a distance of 10mm in between. An electronic caliper (Exploit 033004, Exploit Tools Group, Yiwu, China) was used to measure the thickness (t) and width (w) of the strip in 5 equally-spaced locations along this length. Mechanical tests were conducted using a material testing machine (EZ-Test, Shimadzu, Kyoto, Japan) equipped with a 50 N capacity load cell at a room temperature of 22°C, Figure 1. The initial distance between the clamps was measured by a vernier caliper and recorded as Lo. The specimens were conditioned by four cycles of loading and unloading with 1mm/min elongation rate and 0.10N max load, and the behavior recorded in the fourth cycle was considered representative of specimens' stable behavior [18]. The order of testing paired specimens, obtained from the same animal, was randomized and recorded. Strips were covered with gauze soaked with Phosphate buffered saline (PBS, Maixin, China) to keep them moist during the test procedure.

The load–displacement (F–ΔL) data obtained from the fourth cycle were used to calculate the stress under each load, F, as $\sigma = \frac{F}{w \cdot t}$, where t was the average corneal thickness and w the average specimen width. The related strain was obtained as $\varepsilon = \Delta L / Lo$. The stress-strain results were fitting to an exponential function $\sigma = A \cdot (e^{B \cdot \varepsilon} - 1)$, where A and B were constants, and the tangent modulus (E_t) was calculated as $E_t = \frac{d\sigma}{d\varepsilon} = A \cdot B \cdot e^{B \cdot \varepsilon} = B \cdot (\sigma + A)$.

2.6 Statistical analysis

All analyses were performed using the PASW Statistics 20.0 (SPSS Inc., Chicago, USA). Comparisons of biomechanical and geometrical parameters in the two specimen groups were performed using the paired T-test. P values less than 0.05 were considered indicative of statistical significance.

Results

3.1. Corneal thickness

After 10 days of incubation in culture medium with 0.0004% travoprost diluent, the central corneal thickness (CCT) of the treated group increased from 369.4±22.5 μm to 658.9±184.4 μm ($p<0.01$), and in control group from 363.8±19.3 μm to 602.4±208.1 μm ($p<0.01$). There were no significant differences in corneal thickness between the two groups before storage ($p= 0.073$), and the difference in corneal thickness between the groups remained statistically non-significant ($p= 0.303$) after incubation.

3.2 Biomechanical behavior

There was a clear difference in the load-displacement behavior observed for the two specimen groups as shown in Figure 2. With material parameters A and B determined (Table 1), the stress-strain (σ-ε) relationships (Figure 3), and hence the tangent modulus ($Et = d\sigma/d\varepsilon$) at any stress level can be obtained. For statistical evaluation purposes, the Et values were compared at 10, 20 and 30 kPa stresses, the first two of which were within the tissue's nonlinear stage, while the third was within the later linear part. At 10 kPa stress, Et was significantly lower in the treated group ($Et\text{-}_{Tr}$), compared to the control groups ($Et\text{-}_{Co}$), but this difference reduced in value and became insignificant under 20 and 30 kPa (Table 2).

Discussion

Topical medication is commonly used in the primary management of glaucoma. Among the several anti-glaucoma eye drops developed, PGF 2α are considered highly effective first-line agents because of their significant success in lowering IOP levels [19], that is in spite of reported side effects including eyelid skin darkening [20], iris pigmentation [21], conjunctival hyperemia [22] and ocular irritation [23]. While these biological side effects have been considered previously [15, 24], little attention has been given to the effect of PGF 2α on corneal biomechanics. PGF 2α have previously been found to accelerate

collagen degradation [25], decrease fibronectin protein content [26], stimulate collagen gel contraction [27] and change collagen distribution in corneal stroma [26]. Since collagen fibrils are the main load carrying components of the cornea , these effects may lead to material stiffness reduction. This study, which attempted to address this point, showed that travoprost eye drops significantly reduced the mechanical stiffness (as measured by the tangent modulus, Et) of the ex-vivo rabbit cornea.

Earlier studies that relied on the ORA and CVS to provide indications of the biomechanical effects of PGAs produced inconsistent results. While some studies reported increases in corneal hysteresis parameter (CH – a measure of corneal viscoelasticity) with PGA treatment [28-33], others reported decreases [10]. Also, there was no agreement on the effect of PGAs on the corneal resistance factor (CRF – a measure of corneal stiffness) with reported increases [31], decreases [10, 33], and no significant change [28,32]. However, after adjusting for IOP, CCT and other factors, which may influence corneal behavior, a significant reduction in the CVS's deformation amplitude (DA – a measure of corneal stiffness) was detected after PGA therapy [11]. Nevertheless, since the biomechanical metrics provided by the ORA and CVS cannot be linked directly to the traditional measures of tissue stiffness (primarily Et), and could be influenced by factors such as IOP and CCT [12-14], the present study relied instead of the classic tensile test in quantifying the effect of PGAs on corneal biomechanics.

The uniaxial tension test is a simple and well-accepted experimental technique for characterizing the mechanical behavior of tissue [34]. In spite of the limitations caused by the initially curved form of specimens and the termination of fibrils along the specimen sides [35], the test method remains viable for comparative studies, such as the present research, where the focus is on the variation in tissue behavior due to different treatment regimes. All specimens exhibited clear nonlinear behavior, as indicated in a previous study [36], with an initial low stiffness increasing gradually until a stage of constant stiffness was reached at stresses slightly below 30 kPa. In order to ensure the test results were repeatable, three loading-unloading cycles were carried out before using the

results of the forth cycle as representative of specimens' stable behavior [18]. The results showed significant decreases in Et in the treated group, by -11.7±41.8% ($p< 0.05$) at a stress of 10 kPa compared to the control group. However, these differences decreased and became insignificant with higher stress levels (-9.2±36.1%, $p> 0.05$ at 20kPa and -7.3±35.4%, $p> 0.05$ at 30 kPa).

The changes in tissue stiffness reported in this study may well influence the accuracy of IOP measurement – needed for glaucoma management [37]. Most tonometry techniques, contact or non-contact, depend on applying a mechanical force and correlating corneal resistance to deformation under this force to the value of IOP. While simple and easy to implement, this measurement concept makes the estimation of IOP dependent on corneal biomechanical properties [38]. With the application of PGAs leading to reductions in corneal stiffness, and hence underestimations of true IOP, the result may be an overestimation of the effect of PGAs in lowering IOP, which can have significant implications for glaucoma management.

There was a significant thickness increase observed during the storage period – due to tissue swelling – which correlated with the anaerobic state and increased lacate concentration [39] caused by the storage medium. The increase in corneal thickness, which affected both treated and control groups, may have masked the thickness reduction effect caused by PGAs usage as reported in earlier studies [8, 9], possibly as PGF 2α can induce excessive production of MMPs and inhibit the production of TIMP, both of which lead to an accelerated matrix degradation and decrease in CCT.

The present study relied on rabbit eyes due to their similarity to human eyes in biomechanical behavior [40, 41], and the difficulty in obtaining human donor eyes in sufficient numbers for research. The tests were also done ex vivo, and while concerted efforts were made to preserve the tissue and test it within 2 hours post-mortem, there may have some degradation, which can affect the results obtained.

To the best of our knowledge, this is the first study to investigate the effect of PGAs hypotensive medications on corneal biomechanical property changes measured in standard biomechanical experiments. Corneal material stiffness reduced significantly with the use of PGF 2α (travoprost diluent, 0.0004%), causing concern over the accuracy of IOP measurement in patients undergoing chronic PGA therapy. This finding warrants caution when evaluating IOP measurements and the results of patient follow-up. Further investigation is required to quantify the effect of the stiffness reduction reported herein on the IOP measurements with commonly used tonometers, and hence the management of glaucoma.

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Figure Captions:

Figure 1 Corneal specimen and experimental platform

Figure 2 Mean load-displacement behavior in treated and control groups

Figure 3 Mean stress-strain behavior of corneas in each specimen group – error bars represent standard deviation of strain values

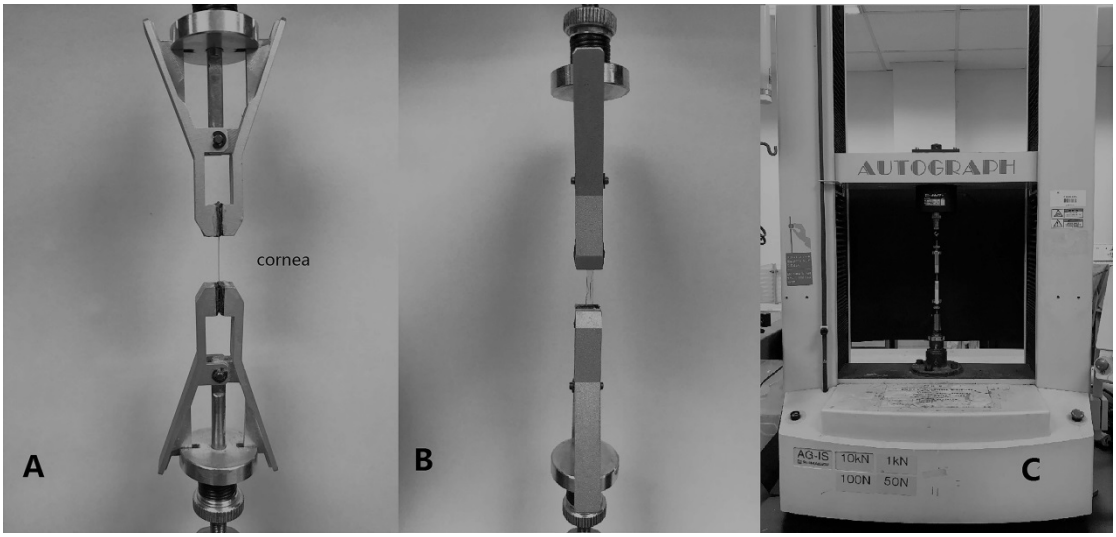
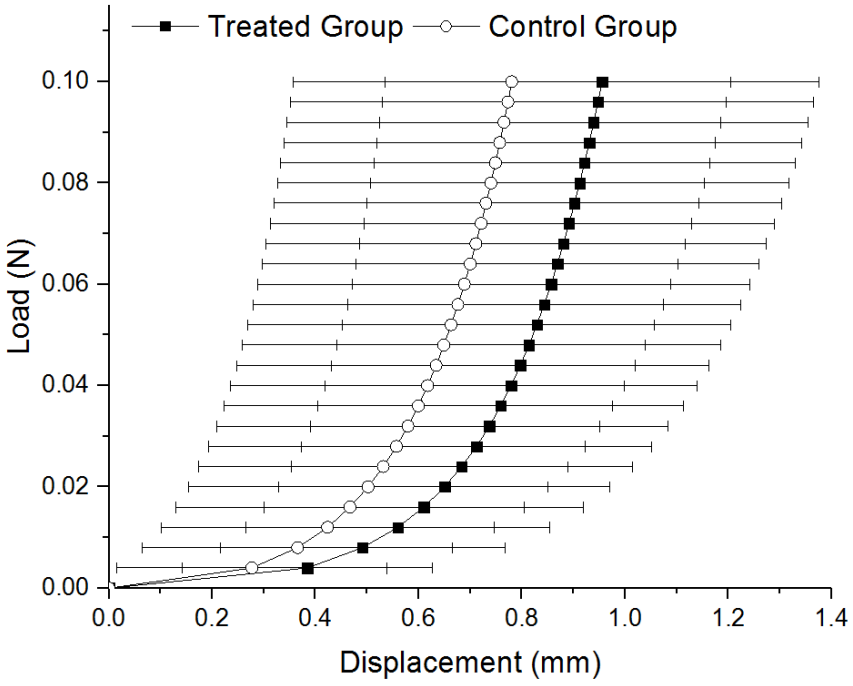


Figure1 Corneal specimen and experimental platform, A. front view of testing strip specimens after assembly, B. side view of testing strip specimens after assembly, C. material testing machine



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13 **Figure 2** Mean load-displacement behavior in treated and control groups

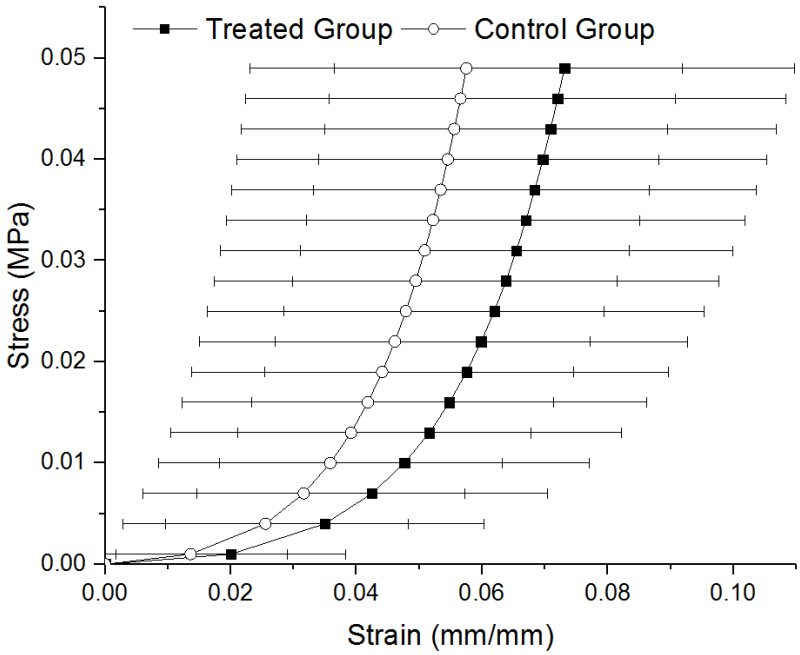

Figure 3 Mean stress-strain behavior of corneas in the treated and control groups – error bars represent the standard deviation of strain values

Table Captions:

Table 1 Mean and standard deviation of constitutive parameters A and B in two specimen groups

Table 1 Mean and standard deviation of constitutive parameters A and B in the two specimen groups

Group	A	B	RMS, mm
Treated group	0.002 ± 0.003	66.481 ± 23.706	0.0017 ± 0.0014
Control group	0.004 ± 0.006	71.495 ± 26.946	0.0012 ± 0.0008

Table 2 Average and standard deviation values of tangent modulus (MPa) in treated and control groups at three stress levels

Stress (kPa)	Tangent Modulus, Et (MPa)		p	$Et\text{-}_{Tr}/Et\text{-}_{Co}$, %
	Tr	Co		
10	0.78 ± 0.27	1.00 ± 0.45	0.025	88.3 ± 41.8
20	1.45 ± 0.49	1.71 ± 0.64	0.059	90.8 ± 36.1
30	2.11 ± 0.72	2.43 ± 0.88	0.119	92.7 ± 35.4

Tr = treated group, Co = control group; $Et\text{-}_{Tr}/Et\text{-}_{Co}$ = ratio between tangent modulus in treated group ($Et\text{-}_{Tr}$) and control group ($Et\text{-}_{Co}$)