

## Original Article

# Effects of topical travoprost 0.004% on intraocular pressure and corneal biomechanical properties in an animal model

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## Abstract

**Purpose**—To determine whether topical application of travoprost 0.004% induces changes in corneal biomechanical properties affecting intraocular pressure (IOP) values in rabbits.

**Methods**—Both eyes of 10 New Zealand rabbits were measured 3 times with the Ocular Response Analyzer (ORA) before treatment. Each measurement included corneal hysteresis (CH), corneal resistance factor (CRF), corneal-corrected IOP (IOPcc), and Goldmann equivalent IOP (IOPg). A drop of travoprost 0.004% was applied once daily in right eyes for 3 months; left eyes received no treatments. After 3 months of treatment both eyes of all rabbits were again measured 3 times. After complete keratectomy of both eyes, tissues prepared with hematoxylin-eosin stain were analyzed by means of light microscopy.

**Results**—The mean pre- and post-treatment IOPg, respectively, for right eyes was  $9.92 \pm 5.64$  mm Hg and  $7.62 \pm 2.99$  mm Hg ( $P = 0.027$ ); IOPcc,  $19.81 \pm 5.25$  mm Hg and  $17.79 \pm 4.09$  mm Hg ( $P = 0.063$ ); CRF,  $1.65 \pm 1.63$  mm Hg and  $2.18 \pm 2.50$  mm Hg ( $P = 0.266$ ); and CH,  $2.79 \pm 1.74$  mm Hg and  $2.64 \pm 2.08$  mm Hg ( $P = 0.72$ ). Mean post-treatment right and left eye IOPg values were, respectively,  $7.62 \pm 2.99$  and  $10.30 \pm 4.40$  ( $P = 0.002$ ); IOPcc,  $17.79 \pm 4.09$  mm Hg and  $20.37 \pm 4.32$  mm Hg ( $P = 0.009$ ); CRF,  $1.65 \pm 1.63$  mm Hg and  $2.17 \pm 2.47$  mm Hg ( $P = 0.274$ ); and CH,  $2.79 \pm 1.74$  mm Hg and  $2.54 \pm 2.08$  mm Hg ( $P = 0.575$ ). No difference in CH and CRF was observed between treated and untreated eyes.

**Conclusions**—Post-treatment reduction of IOP in treated eyes was a direct hypotensive effect of travoprost 0.004% and was not affected by changes in corneal biomechanical properties (CH and CRF), resulting in real lower IOP values.

Prostaglandin analogues (PGAs) have become the first-line therapy for patients with glaucoma.<sup>1</sup> PGAs promote aqueous humor outflow by the uveoscleral pathway and, to a lesser extent, through the trabecular meshwork and Schlemm's canal, which are part of the conventional pathway.<sup>2</sup> This effect is due to connective tissue remodeling by active metalloproteinases in the ciliary body and trabecular meshwork.<sup>3</sup> Recent studies suggest that corneal biomechanical properties may provide important information about glaucoma progression, such as increased deformation of the optic nerve surface.<sup>4</sup> Corneal hysteresis (CH) is considered to be a direct meas-

urement of corneal biomechanical properties, with central corneal thickness (CCT) being one of the factors directly related to it.<sup>5</sup> Corneal resistance factor (CRF) is also believed to be useful in predicting glaucoma progression and may be correlated with CCT.<sup>6,7</sup>

Recently Tsikripis et al<sup>8</sup> prospectively analyzed the effect of latanoprost and latanoprost/timolol combination on corneal biomechanical properties and CCT and concluded that low CH and a thin cornea may lead to underestimation of intraocular pressure (IOP); they suggested that the Ocular Response Analyzer (ORA; Reich-

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ert Ophthalmic Instruments, Depew, NY) be used in combination with Goldmann applanation tonometry (GAT) to obtain more accurate and reliable IOP values. Multiple articles have analyzed the effects on corneal biomechanics of specific PGAs, including latanoprost or bimatoprost.<sup>9</sup> Agarwal et al<sup>10</sup> retrospectively compared the relationship between CH and the magnitude of IOP reduction with PGA, and they found that baseline CH is independently associated with the magnitude of IOP reduction with PGA therapy.

Experimental studies have demonstrated that PGAs alter corneal collagen structure and affect CCT.<sup>10</sup> These changes imply that results of GAT could be affected by PGA treatment.<sup>11–13</sup> In a study of 136 human eyes, Schlote et al<sup>12</sup> found that topical therapy with travoprost was accompanied by a significant reduction of CCT within 1 year of treatment. In a study with F2 $\alpha$  prostaglandin, Viestenz et al found that CCT in PGA-treated eyes decreased significantly, suggesting that these changes might be attributed to effects of F2 $\alpha$  PGA on the extracellular matrix of corneal stroma via upregulation of matrix metalloproteinases.<sup>13</sup>

It is not known whether PGAs induce changes in corneal biomechanical structure or, subsequently, affect GAT measures, which assume that there is minimal variation due to the biomechanical properties of the cornea and to CCT. However, in clinical practice, corneal thinning under local effect of PGA treatment could result in underestimation of IOP levels as measured by applanation tonometry.<sup>14,15</sup> Bergonzi et al<sup>16</sup> used A-scan laser confocal microscopy to analyze 52 eyes that had only been on PGA therapy for 3 years and concluded that PGA therapy increased the keratocyte density in each layer of corneal stroma but more significantly in the anterior stroma than in the midstroma or the posterior stroma.

The increased keratocyte density might be the result of diminished extracellular matrix, possibly owing to the activation of matrix metalloproteinases and inhibition of their tissue inhibitors.<sup>17</sup> The current interest in improving methods of IOP measurement has led to study of the influence of corneal biomechanical properties on the development of glaucoma.

The Ocular Response Analyzer performs measurements of corneal properties such as corneal hysteresis (CH) and corneal resistance factor (CRF) and incorporates both Goldmann-correlated IOP (IOPg) and corneal-corrected IOP (IOPcc), a measurement that is minimally influenced by corneal properties.<sup>17</sup> CH is defined as the difference between the air-jet pressure at inward and

outward applanation and is considered to be a measure of corneal viscous damping and thus the cornea's resistance to deformation.<sup>18–20</sup> Lower CH values have been linked with progression of visual fields in primary open-angle glaucoma (POAG); lower CH values, with worse visual field damage in patients with asymmetric POAG.<sup>11</sup> The purpose of the present study was to determine whether topical application of travoprost 0.004% induces changes in corneal structure and biomechanical properties affecting intraocular pressure measures in rabbits.

## Materials and Methods

The prospective longitudinal and experimental study was reviewed and approved by the local internal review board of the Asociación para Evitar la Ceguera en Mexico I.A.P. Dr. Luis Sanchez Bulnes and conforms to all guidelines of the "Statement for the Use of Animals in Ophthalmic and Visual Research" (Association for Research in Vision and Ophthalmology, [http://www.arvo.org/about\\_arvo/policies/statement\\_for\\_the\\_use\\_of\\_animals\\_in\\_ophthalmic\\_and\\_visual\\_research/#investigators](http://www.arvo.org/about_arvo/policies/statement_for_the_use_of_animals_in_ophthalmic_and_visual_research/#investigators)). The study was sponsored by the Asociación para Evitar la Ceguera en Mexico I.A.P. Research funding was also provided by the Glaucoma Department of the same institution.

Ten 6-month-old female New Zealand rabbits were utilized. Each rabbit weighed approximately 3 kg. All the rabbits were anesthetized intravenously with pentobarbital (25 mg/kg dose). A drop of topical tetracaine 0.5% was applied to both eyes, and each eye was measured 3 times with the ORA when the rabbit was completely anesthetized (approximately 5 minutes after pentobarbital was administered).

Baseline and post-treatment measurements were obtained in the same fashion. To obtain measurements, a small eyelid speculum was used to keep the rabbit's eyes open. Each rabbit was held gently by its ears, we centered each eye in front of the automatic "air puff" system at approximately 4 cm until an automatic measurement was performed. This procedure was repeated 3 times for each eye by the same tester. Mean values of CH, CRF, IOPg, and IOPcc were calculated using the 3 measurements for each eye.

One drop of travoprost 0.004% was applied daily at 9:00 a.m. to the right eye of all rabbits for 3 consecutive months. After 3 months of treatment both eyes were again measured 3 times each. After measuring corneal properties and IOP with ORA, both eyes were enucleated using a scalpel and corneal scissors. A complete ker-

**Table 1.** Pre- and post-treatment values of intraocular pressure (IOP), corneal resistance factor (CRF), and corneal hysteresis (CH)

Eye		IOPg	IOPcc	CRF	CH
Right eye <sup>a</sup>	Pre-treatment	9.92 ± 5.64	19.81 ± 5.25	1.65 ± 1.63	2.79 ± 1.74
	Post-treatment	7.62 ± 2.99	17.79 ± 4.09	2.18 ± 2.50	2.64 ± 2.08
	<i>P</i> value	0.027	0.063	0.266	0.729
Left eye <sup>b</sup>	Pre-treatment	10.22 ± 6.79	20.41 ± 7.25	2.17 ± 2.44	2.69 ± 1.62
	Post-treatment	10.30 ± 4.40	20.37 ± 4.32	2.17 ± 2.47	2.54 ± 2.08
	<i>P</i> value	0.951	0.981	0.998	0.724
Right vs left post-treatment <i>P</i> value		0.002	0.009	0.274	0.575

*IOPcc*, corneal corrected IOP; *IOPg*, Goldmann equivalent IOP.

<sup>a</sup>Treated with travoprost 0.004%.

<sup>b</sup>Untreated.

atectomy in both eyes was performed. Before enucleation of both eyes, 50 mg/kg of intravenous pentobarbital (for a total dose of 75 mg/kg) was administered to each rabbit to achieve euthanasia. Corneal tissue was fixed in formaldehyde to be analyzed by light microscopy. An experienced ophthalmic pathologist compared the 20 corneas using hematoxylin-eosin stain. Data on each slide depicting treated or control group was not masked for the pathologist. Any histological difference between eyes was reported. ORA data was collected in a spreadsheet and values were analyzed using a one-sided *t* test for paired samples. A *P* value of <0.05% was considered statistically significant.

## Results

Pre- and post-treatment measurements (IOPg, IOPcc, CRF and CH) of both eyes are provided in Table 1. We found no histological differences in endothelium, stromal, or epithelial cells between treated and untreated eyes. Corneal epithelial hydropic degeneration was observed in all eyes (Figure 1).

## Discussion

The present study shows that PGAs decrease IOP by increasing uveoscleral outflow of aqueous humor and not by changing the biomechanical properties of the cornea, which could result in falsely lower values of IOP with GAT. IOP values decreased only in the right eyes of rabbits after treatment with travoprost 0.004%. IOPg was the only value that had a statistically significant decrease. Although not statistically significant, a decrease in IOPcc values was observed. When comparing post-treatment values between right and left eyes, an important difference was observed in IOPg and IOPcc values; this was due to the hypotensive effect of travoprost 0.004% on the right eyes.

Tsikripis et al<sup>8</sup> observed that the magnitude of IOP reduction with PGA was significantly associated with

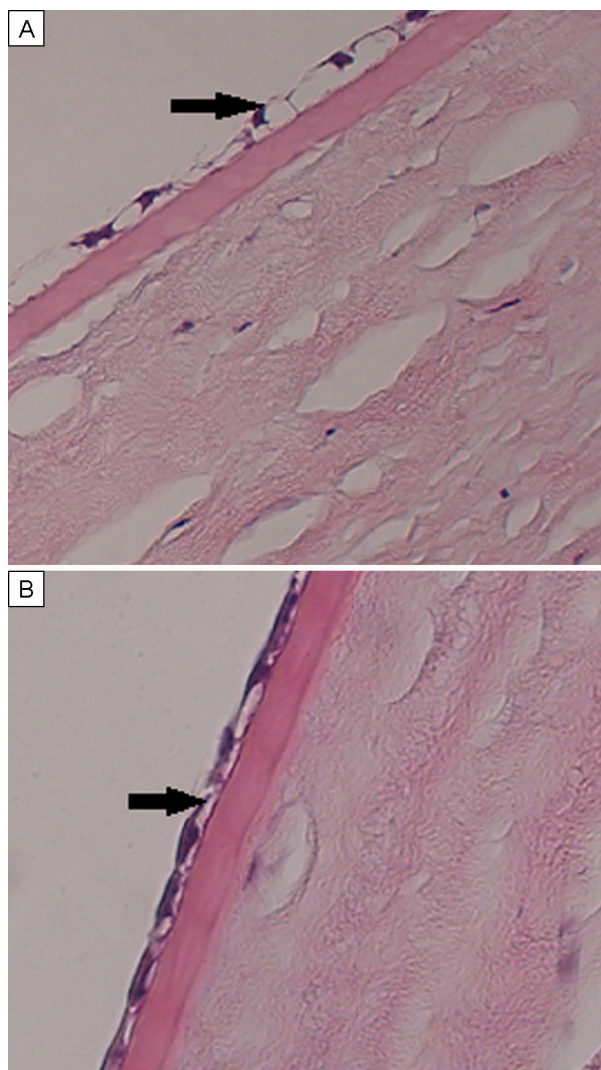
CH and that there was a negative statistically significant correlation, particularly in the first semester. In our study CFR and CH values were not affected after travoprost treatment; this became evident when comparing pre- and post-treatment values of right eyes and post-treatment values of right and left eyes. This could be explained by the short duration of our 3-month experiment versus the changes found by Tsikripis et al in the first 6 months. In our study CRF did not significantly change in both groups. These results correlate with those of Tsikripis et al.

Although GAT remains the gold standard for IOP control, ORA has been shown to be a reliable instrument for IOP monitoring, not only by analyzing IOP values but also by measuring corneal biomechanical properties that could be affected by corneoscleral pathologies but not by PGAs, as demonstrated by this study.

PGAs have been shown to produce changes in CCT by diminishing stromal keratocytes. This effect is secondary to the activation of matrix metalloproteinases and degradation of extracellular matrix. The present study did not analyze CCT, although CH is directly related to CCT.

There are several limitations in our study. The small sample number and the use of pentobarbital as an anesthetic in rabbits are the most important. It is well known that anesthetics may increase intraocular pressure, thus affecting the IOP values in this study. We observed that the only parameter that was significantly affected by PGA treatment was IOPg; the biomechanical properties of the cornea (CH, CRH) were not affected. The reduction in the IOP in right eyes was due to a direct hypotensive effect of travoprost 0.004% and was not affected by changes in corneal biomechanical properties.

Although this study was performed using an animal model and the population size is small, reliable results about the behavior of the pressure and corneal properties



**Figure 1.** Light microscopy of corneal tissue (hematoxylin-eosin, original magnification  $\times 40$ ). A, Travoprost-treated right eye. B, Untreated left eye. Evidence of hydropic degeneration of the corneal epithelium with no difference in the arrangement or structure of stromal collagen fibers in both eyes is illustrated with the arrows.

in animals were observed. More studies in animals or humans are needed to corroborate our findings.

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