Biomechanical changes in the human cornea after transepithelial corneal crosslinking using iontophoresis

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PURPOSE: To evaluate the corneal response to variable intraocular pressure (IOP) in human eye globes after ultraviolet-A (UVA) transepithelial corneal crosslinking using iontophoresis.

SETTING: Fondazione G.B. Bietti IRCCS, Rome, Italy.

DESIGN: Experimental study.

METHODS: Four human donor eye globes were treated with transepithelial crosslinking using iontophoresis and rapid UVA corneal irradiation, and 4 globes had standard crosslinking. Inflation experiments were performed on the globes before and after crosslinking. Topographic maps of the anterior and posterior cornea were acquired using Scheimpflug topography. Images were obtained using a mechanical regimen to analyze corneal strain in response to cyclic stress. Corneal shape changes were analyzed as a function of IOP, and corneal stress–strain curves were generated.

RESULTS: Before crosslinking, instillation of hypotonic riboflavin-5–phosphate sodium 0.1% solution using iontophoresis increased corneal thickness by 5% and instillation of dextran-enriched riboflavin 0.1% solution decreased corneal thickness by 13%. Five minutes after treatment, both crosslinking procedures reduced corneal thickness by 2%. Young’s modulus (E) of the anterior cornea increased by a mean of 1.8 times (from 1.6 to 2.9 MPa) and 1.9 times (from 1.3 to 2.5 MPa) after transepithelial crosslinking using iontophoresis and standard crosslinking, respectively. The E value of the posterior cornea also increased after both procedures (mean 1.7 times versus 3.1 times).

CONCLUSIONS: Transepithelial crosslinking using iontophoresis increased the biomechanical strength of human corneal tissue in inflation testing of donor eye globes. The effect on corneal stiffness was almost comparable to that of standard crosslinking.

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through the intact epithelium and reducing pretreatment time from 30 minutes to 5 minutes before ultraviolet-A (UVA) irradiation of the cornea. It is therefore of interest to understand the effect of this new method of transepithelial crosslinking using iontophoresis on the human cornea before it is used in a clinical setting.

The efficacy of crosslinking (i.e., its ability to increase corneal mechanical strength) has been mainly shown via extensiometry measurements. Limitations of strip extensiometry are that the corneal structure is disrupted and several crucial constraints are ignored, such as meridional and thickness differences, boundary conditions, and excessive corneal edema. Whole-eye globe inflation testing overcomes the limits of extensiometry and may be the most adequate method to mimic the in vivo biomechanical behavior of corneal tissue. A previous study showed the feasibility of using inflation testing to evaluate efficacy of standard crosslinking in porcine eyes. In the present study, we evaluated the topographic response of the cornea to inflation testing of human donor eye globes before and after transepithelial crosslinking using iontophoresis and compared it with the response after standard crosslinking.

MATERIALS AND METHODS

Eight human eye globes not suitable for transplantation were obtained from the Veneto Eye Bank Foundation (Venezia Zelarino, Italy). The globes were explanted between 2.3 hours and 16.0 hours after death and immediately preserved at 4°C in corneal storage medium enriched with 15% dextran. All tissue was used for experiments within 48 hours. All eyes were used in compliance with the guidelines of the Declaration of Helsinki for research involving the use of human tissue, and the local ethics committee approved the experimental protocol.

Four eye globes with intact epithelium had transepithelial crosslinking using iontophoresis (study group) and 4 eye globes that were deepithelialized immediately before experiment were treated using the standard crosslinking protocol (control group). The eyes were allocated to obtain age-matched samples in the 2 groups.

Experimental Apparatus

A purpose-designed experimental apparatus, the Ocular Biomechanics Modulator, was used to perform inflation experiments. The intraocular pressure (IOP) of the eye globe was monitored using a water column and a pressure transducer (DS27C002A1, Valcom srl) and modified by infusing saline solution into the posterior segment of the eye by an automated syringe pumping system (Genie Plus, Kent Scientific Corp.). The humidity and temperature in the moist chamber in which the eye was placed during the measurements were continuously monitored and recorded. Corneal topography maps of the anterior and posterior corneal interfaces were obtained using a rotating Scheimpflug camera (Pentacam HR, Oculus Optikgeräte GmbH). The whole apparatus was computer-controlled by dedicated software written in Labview (National Instruments Corp.).

Testing Procedure

Each eye globe was kept in 15% dextran-enriched storage solution at room temperature for 30 minutes before the experiment began. The globe was then gently mounted in a purpose-designed holder with a known vertical-horizontal orientation to guarantee proper centration during the topography measurements. The eye holder was placed in an acrylate box in which an ultrasound humidifier (JC 380, Life Tool Technologies S.p.A.) provided a constantly humid atmosphere. A commercial air conditioner heat pump was used to maintain a constant room temperature during the testing.

Before the experiment, the pressure of each globe was kept constant at 18 mm Hg for 15 minutes to achieve a unique prestressing reference state of the corneal tissue before testing. Thereafter, a cyclic regimen was applied to each globe. The regimen consisted of an initial measurement taken at 18 mm Hg and then taken at 6 mm Hg step increases continuously controlled by the pressure monitoring system. The pressure was increased to 42 mm Hg and then decreased in 6 mm Hg steps to 18 mm Hg in each globe. Scheimpflug camera measurements were taken 3 times 10 minutes after each IOP step change. In each globe, inflation experiments were performed before crosslinking and 2 hours after crosslinking.

Corneal Crosslinking Techniques

Transepithelial Crosslinking Using Iontophoresis In the study group, the cornea was impregnated with a riboflavin-5-phosphate sodium hypotonic 0.1% solution (Ricrolin+, Sooft Italia S.p.A.) using an iontophoresis device (Iontophor, Sooft Italia S.p.A.). The passive electrode was applied to the sclera of the globe. The active electrode, consisting of a plastic bath tube, was applied to the corneal surface. After the tube was applied to the corneal epithelium using suction, it was filled with riboflavin solution. The current intensity was set at 1.0 mA for 5 minutes according to the manufacturer. Corneal UVA irradiation (mean 370 nm ± 8 [SD], 10 mW/cm², Vega 10 mW, Costruzione...
Strumenti Oftalmici) was then applied at 56.0 mm for 9 minutes. No hypotonic riboflavin drop was instilled over the corneal surface during irradiation.

**Standard Crosslinking** In the control group, standard treatment was performed using common clinical procedures. A solution containing riboflavin 0.1% and dextran 20.0% (Ricrolin, Sooft Italia S.p.A.) was instilled every 3 minutes for 30 minutes before irradiation. The cornea was then irradiated with a UVA lamp (mean 370 ± 8 nm) for 30 minutes while riboflavin was instilled every 3 minutes with an irradiance of 3 mW/cm². The UVA delivery system (Vega 3mW, Costruzione Strumenti Oftalmici) was located 56.0 mm from the cornea.

**All Cases** Both UVA lamps were calibrated with a power meter before corneal irradiation. An irradiation area of 8.0 mm diameter was used in all cases.

**Surface Fitting**

Topographic maps of the anterior and posterior corneal interfaces were exported from the rotating Scheimpflug camera. Technical computing software (Matlab, Mathworks, Inc.) was used for data processing and analysis.

Starting from Navarro et al.’s model, a mathematical procedure was developed to fit the elevation topographic data (ie, the x, y, z points coordinates) with a nonrevolution general ellipsoid. This procedure provides corneal parameters that depend on the shape of the corneal surface only and not on the relative cornea-instrument position, thereby correcting the errors derived from misalignment. A detailed description of the surface-fit model is available online as Supplement A (available at http://jcrsjournal.org). The radii of curvature (R) in millimeters of the horizontal (Rx) and vertical (Ry) meridians for both the anterior and posterior corneal surfaces were computed by fitting the central 6.0 mm of the corneal elevation maps to the model. Thickness data were obtained directly from the commercial rotating Scheimpflug camera software. Difference maps of thickness data acquired during the cyclic regimen before and after crosslinking were calculated.

**Biomechanical Model**

A model for the corneal stress–strain relationship was used. The model is based on IOP variation and related changes in the thickness and radius of curvature and is used to provide estimates of the corneal biomechanical response in untreated corneas and crosslinked corneas.9,14,15 The model assumes that the corneal thickness and the mean radius of curvature are a function of IOP. The corneal thickness decreases because the cornea becomes stretched over a larger area. However, stretching the cornea induces stress, which acts to prevent further corneal extension. Both forces—the tendency of IOP to stretch the cornea and the tendency of stress to prevent it—combine to form a stable equilibrium. The stress of the anterior cornea and posterior cornea was then calculated at equilibrium as a function of IOP, as follows:

\[
\sigma = \frac{R_p}{2d}
\]

(1)

where \(\sigma\) is the stress, \(R\) is the mean radius \([R_x + R_y]/2\) of the anterior or posterior curvature, \(p\) is the IOP, and \(d\) is the corneal thickness. The strain \((\varepsilon)\) on the stress produced by IOP can be expressed by \(\varepsilon = \Delta R/R_0\), where \(\Delta R\) is the difference in the radius of curvature with respect to the initial reference measurement (ie, IOP = 18 mm Hg) and \(R_0\) is the initial radius of curvature. The model does not include potential meridional anisotropy behavior in deformation.

For each sample, the stress was plotted as a function of the induced strain. The modulus of elasticity (\(E,\) Young’s modulus) of the anterior cornea and posterior cornea was calculated by plotting the stress and strain values obtained during the loading phase of the cyclic regimen before crosslinking and after crosslinking. Young’s modulus was estimated as the slope of the curve in the linear region of the loading phase.

**Statistical Analysis**

One-way analysis of variance (ANOVA) was used to statistically compare the differences of the anterior and posterior corneal shape and thickness changes before and after the crosslinking procedures. Differences with a P value of 0.05 or less were considered statistically significant. A commercial software program (Kyplot, Kyenslab, Inc.) was used for statistical testing.

**RESULTS**

Each session of measurements on a sample took a mean of 8 hours. During the experiment, the mean room temperature and mean humidity in the moist chamber were 30.6°C ± 0.8°C and 71% ± 4%, respectively. There were no differences in donor age between the study group (mean 67.50 ± 5.07 years; range 61 to 72 years) and the control group (mean 67.00 ± 5.23 years; range 60 to 71 years).

After application of hypotonic riboflavin-5-phosphate sodium solution through iontophoresis, the mean increase in central corneal thickness (CCT) from baseline was 5% (from 574 ± 160 μm to 605 ± 150 μm). After transepithelial crosslinking, the mean CCT was 595 ± 147 μm, decreasing by a mean of 2% from before UVA irradiation. Corneal thickness further decreased by 15% (mean 506 ± 106 μm) up to 2 hours after transepithelial crosslinking using iontophoresis. After application of riboflavin–dextran solution before standard crosslinking, the mean decrease in CCT from baseline was 13% (from 608 ± 131 μm to 533 ± 46 μm) in deep epithelialized corneas. Five minutes after standard crosslinking, the CCT further decreased by a mean of 2% (mean 520 ± 25 μm) and remained relatively unchanged (mean ≤2% and 513 μm) up to 2 hours after treatment.

Corneal crosslinking influenced the changes in CCT during the cyclic regimen. On average, the pre-crosslinking changes in CCT were significantly higher than after both crosslinking procedures. The differences were statistically significant at all pressure levels (\(P<.001,\) ANOVA). Before treatment, the CCT decreased by 82 μm (from 656 to 574 μm) and 49 μm (from 657 to 608 μm) in samples with an intact
epithelium and samples without an intact epithelium, respectively. It decreased by 8 μm (from 506 to 498 μm) and 5 μm (from 513 to 508 μm) after transepithelial crosslinking using iontophoresis and standard crosslinking, respectively. Figure 1 shows the mean corneal thickness changes during the cyclic regimen for transepithelial crosslinking using iontophoresis and standard crosslinking samples.

Two hours after transepithelial crosslinking using iontophoresis, the mean radius of curvature of the anterior cornea changed by \(0.10 \pm 0.01\) diopter (D); it changed by \(0.35 \pm 0.12\) D in the posterior cornea. After standard crosslinking, the mean radius of curvature changed by \(0.22 \pm 0.01\) mm and \(0.30 \pm 0.11\) mm in the anterior cornea and posterior cornea, respectively. The mean changes in the anterior and posterior radii of curvature to increasing and decreasing IOP levels after both crosslinking treatments were smaller than preoperatively (\(P < .001\), ANOVA). At the end of the cyclic regimen, crosslinked corneas tended to return to their initial anterior and posterior curvature radii, contrary to what was found before treatment. This behavior indicated a more elastic response of the corneal tissue after crosslinking. Figure 2 and Figure 3 show the mean changes in the anterior and posterior corneal radii of curvature with varying IOP in the study group and in the control group, respectively.

After both crosslinking procedures, the stress–strain curves of the anterior cornea showed steeper loading curves than preoperatively. The area inside the loading and unloading curves was smaller than preoperatively. Figure 4 and Figure 5 plot stress as a function of strain in 2 samples before and after transepithelial crosslinking using iontophoresis and standard crosslinking, respectively. The slope of the curves for increased pressure (loading phase) represented a measure of Young's modulus of the cornea, considering that the steeper the curve, the stiffer the cornea. After standard crosslinking, Young's modulus of the anterior cornea increased by a mean of 1.9 times (from 1.3 ± 0.9 mPa to 2.5 ± 1.4 mPa). It increased by a mean of 1.8 times (from 1.6 ± 1.0 mPa to 2.9 ± 1.6 mPa) after transepithelial crosslinking using iontophoresis. Young's modulus of the posterior cornea increased by a mean of 3.1 times (from 0.22 ± 0.12 mPa to 0.71 ± 0.40 mPa) and by 1.7 times (from 0.17 ± 0.09 mPa to 0.29 ± 0.12 mPa) after standard crosslinking and transepithelial crosslinking using iontophoresis, respectively.

Figure 1. A: Change in CCT with IOP before and after transepithelial crosslinking using iontophoresis. B: Change in CCT with IOP pressure change before and after standard crosslinking. The symbols represent mean data across eye globes, and the error bars represent the standard deviation.

Figure 2. Mean changes from the initial value \(R_0\) of the anterior corneal curvature radius with varying IOP before and after transepithelial crosslinking using iontophoresis (A) and standard crosslinking (B). The error bars represent the standard deviation.
DISCUSSION

Over the past decade, corneal crosslinking has become common as a treatment option for individuals with progressive keratoconus. The efficacy of crosslinking was primarily based on laboratory data suggesting that crosslinking using riboflavin and UVA irradiation increases the biomechanical strength of the treated cornea.\textsuperscript{1,6,7,16–29} The effect of crosslinking on the mechanical properties of the cornea has been evaluated using different methods and techniques, which included strip extensiometry, indentation, Brillouin optical microscopy, inflation testing of corneal tissues or whole eye globes. Few studies have used human corneal tissue,\textsuperscript{1,6,7,24,25} and so far none has used human eye globes.

In this study, we present an experimental protocol to perform inflation testing on donor eye globes not suitable for transplantation, under strict monitoring of IOP and environmental parameters. Several factors make inflation testing using the whole eye preferable for biomechanical testing of human corneal tissue. The procedure is minimally invasive of the corneal tissue; the humidity and temperature of the environment, which influence corneal biomechanics, can be monitored; the corneal tissue can be mounted with respect to its correct vertical–horizontal orientation; the applied stress is generated by IOP; corneal topography and tomography allow detailed characterization of the corneal surfaces with micrometric resolution and data can be easily exported for analysis; and native boundary conditions of the cornea are preserved. Limitations include that the experiment and analysis of data are time consuming. In addition, in this study some assumptions have been made when constitutive biomechanical properties were derived from tomography measurements; for example, the stress–strain calculation referred to a homogenous membrane sphere and the same elasticity and thickness were assumed over the entire cornea. Therefore, the influence of a corneal thickness gradient or local elasticity differences could not be detected.

The testing procedure used in the present study involved subjecting the corneas to cyclic variations in IOP within physiologic levels. A 27 to 60 mm Hg pressure range has been shown to produce small viscoelastic deformations and a nearly linear pressure-deformation response,\textsuperscript{11} which suggests that for physiologic pressure ranges, the cornea may be reasonably approximated as a linear viscoelastic or linear pseudoelastic material. On the other hand, the corneal model used in the present study included the assumptions of spherical topography, material homogeneity, and corneal elasticity. In addition, the mechanical properties of the sclera were not considered to contribute to the response of the cornea to IOP changes.

Our scope was to evaluate the effect of transepithelial crosslinking using iontophoresis to increase the corneal mechanical stiffness. An equal number of cases were treated by standard crosslinking for comparison purposes. The major difference between transepithelial crosslinking using iontophoresis and standard crosslinking was the effect of riboflavin 0.1% solution application on corneal thickness. Deepithelialized tissues showed a decrease in corneal thickness after riboflavin instillation; the decrease was caused by the hyperosmolarity of the riboflavin–dextran solution.\textsuperscript{30} Tissue with intact epithelium that were treated with iontophoresis had an increase in corneal thickness caused by the hypoosmolarity of the riboflavin solution. Changes in corneal thickness represent an indirect parameter of effective riboflavin penetration in the stroma. The decreased corneal thickness after standard riboflavin instillation was comparable to that reported in eyes in which corneal pachymetry was monitored with Scheimpflug imaging (mean decrease 18%).\textsuperscript{31} This confirms the reliability of inflation testing on human tissues; indeed, much greater corneal thinning (approximately 50%) was found when measuring the effect of dextran 20%-enriched riboflavin 0.1% solution in inflation testing of porcine
The hypoosmolar riboflavin solution has been shown to increase corneal thickness in patients with thin corneas. Immediately after both crosslinking procedures, the corneal thickness decreased on average by 2%. It was noteworthy to measure an additional mean decrease in thickness by 15% over a period of 2 hours after transepithelial crosslinking using iontophoresis. (A decrease of only 2% was measured 2 hours after standard crosslinking.) It is valuable to understand the source of the different behavior between crosslinking treatments. The factors involved may be related to the stromal hydration before UVA irradiation and the type of lamp used (10 mW versus 3 mW), although the immediate change in thickness (2% after 5 minutes) was comparable between the procedures.

Changes in thickness during mechanical testing were greatly influenced by crosslinking, as previously reported. After both crosslinking procedures, we found minimal changes in the CCT (≤3%), contrary to what was measured before crosslinking, when changes greater than 10% were found. With the changes in corneal thickness limited to this range, it is expected that the effect of swelling on the corneal material properties was small, as previously reported. However, in vivo CCT changes might be smaller than those found here due to a more effective tissue hydration homeostasis. Before starting the experiment, corneal thicknesses were a mean of 15% above the normal physiologic levels, although they were kept in 15% dextran-enriched storage solution at room temperature for 30 minutes and thereafter they were submitted to 15 minutes of prestressing. The procedure produces a unique pretesting reference state and ensures reproducibility of corneal strain measurements. Improving storage and preconditioning protocols of donors may improve the results of mechanical testing of eye globes.

Figure 4. Stress–strain curves of the anterior cornea (A) and posterior cornea (B) derived from the model in a sample with intact epithelium (eye A0120) before transepithelial crosslinking using iontophoresis. Stress–strain curves of the anterior cornea (C) and posterior cornea (D) after transepithelial crosslinking using iontophoresis. The black lines represent the loading phase and the gray lines, the unloading phase of the cyclic regimen. Preoperatively, strain of the posterior cornea was higher than that found in the anterior cornea in all samples. The slope of the loading curve for the anterior cornea and posterior cornea became steeper postoperatively. The area inside the curves was greatly smaller after treatment than preoperatively. Although not a direct measure of hysteresis, the area inside the loading and unloading curves relates to the viscoelastic behavior of the cornea. The smaller the area, the more elastic the corneal tissue.
Corneal crosslinking increases corneal stiffness immediately after treatment. Photochemical-induced stromal crosslinks are generated via the process starting with riboflavin excitation in the presence of oxygen.\textsuperscript{3,23} Theoretically, crosslinking occurs between stromal collagen molecules and between collagen and proteoglycan core proteins (eg, decorin and mimecan).\textsuperscript{37} In this study, we found smaller mean changes in the radii of curvature of the anterior and posterior crosslinked corneas with cyclic pressure variation than were found before treatment; in addition, the stress–strain curves showed steeper loading curves 2 hours after both crosslinking procedures than preoperatively. A mean increase in Young’s modulus of the anterior cornea by 1.9 times and 1.8 times was found after standard crosslinking and transepithelial crosslinking, respectively. A mean increase in stiffness was also measured in the posterior cornea after both crosslinking procedures (by 3.1 times and 1.7 times, respectively). Because we probed intact corneal tissues in whole eye globe inflation experiments, the increase in the anterior $E$ value reflects stiffening of the most anterior layers of the stroma, while the increased $E$ value of the posterior cornea reflects a convolution of the mechanical properties of all above stromal layers and thus may better represent the stiffening effect induced by the crosslinking procedures. Corneal stiffening may also be related to decreased corneal thickness after crosslinking (de-swelling of the posterior cornea), as previously discussed.\textsuperscript{8,12,38} In strip extensiometry testing of porcine corneas,\textsuperscript{6} the stiffening effect was greater in the 200 $\mu$m anterior corneal lenticule than in the 200 $\mu$m posterior-treated lenticule. Using atomic force microscopy (AFM) on human donor corneal tissues, Dias et al.\textsuperscript{25} found that the stiffness of the anterior stroma (mean thickness of 6 anterior lenticules 41 $\pm$ 15 $\mu$m) after crosslinking increased significantly, while the stiffness of the

**Figure 5.** Stress–strain curves of the anterior cornea ($A$) and posterior cornea ($B$) derived from the model in a sample (eye B080) with denuded stroma before standard crosslinking. Stress–strain curves of the anterior cornea ($C$) and posterior cornea ($D$) after standard crosslinking. After treatment, the anterior cornea and posterior cornea tended to recover their initial shape, contrary to the preoperative state in which residual viscoplastic deformation was found. This phenomenon was due to a more elastic behavior of the crosslinked corneal tissue.
posterior stroma did not. We cannot directly compare our data with those obtained from strip extensiometry and AFM measurements in which excised corneal tissues were used. However, it is well accepted that the combined effect of decreasing riboflavin concentration with depth and the Lambert-Beer law contributes to a nonhomogeneous induction of additional crosslinking bonds in the stroma so most of the stiffening occurs in the anterior stromal layers.\(^6,20,25\)

The experimental procedure permitted us to understand the stiffening effect of crosslinking on an individual basis for each corneal sample, thus minimizing bias due to interindividual variability in corneal biomechanics. The results of the crosslinking procedures were not compared because of the presence of epithelium that might influence mechanical measurements of the cornea when data are derived from corneal topography. In addition, the removal of epithelium after transepithelial crosslinking using iontophoresis could have biased the direct comparison between pretreatment and posttreatment data. Previous work has estimated the epithelium as having 10% of the stiffness of a stromal layer of the same thickness\(^39\); thus, it is expected that the epithelium does not contribute significantly to anterior corneal stiffness. It is also plausible that the UVA energy delivered to the stroma in tissues with an intact epithelium might be lower than that delivered with the standard epithelium-off procedure. The epithelium acts as a barrier to UV light transmittance, mainly for wavelengths of 300 nm or less.\(^40\)

Ringvold\(^41\) showed that bovine corneas without epithelium had 15% to 20% lower absorbance spectra than specimens with intact epithelium in the 300 to 400 nm range. Tao et al.\(^42\) found that the Young modulus of the rabbit corneal specimens in the epithelium-off group was only 15% higher than that in the epithelium-on group after crosslinking treatment using distilled water diluted riboflavin solution and a 3 mW/cm\(^2\) lamp. However, the use of a 10 mW/cm\(^2\) lamp was shown to deplete more riboflavin in the mid stroma than lamps of lower energy\(^22,43\); accordingly, the rate and number of stromal crosslinking bonds induced by 10 mW/cm\(^2\) UVA corneal irradiation in tissues with intact epithelium could be higher than those reported by Tao et al.\(^22\) using a 3 mW/cm\(^2\) lamp.

Recently, the efficacy of transepithelial crosslinking using iontophoresis was evaluated in an uncontrolled clinical study in which 22 eyes of 19 patients with grades I and II keratoconus according to the Amsler classification were treated.\(^34\) The authors used a device for iontophoresis (different from that used in the present work) and incremental current intensity from 0.2 to 1.0 mA for 10 minutes to deliver hypotonic riboflavin solution in the stroma.\(^44\) Ultraviolet-A irradiation of the cornea has been performed using a 3 mW/cm\(^2\) lamp for 30 minutes while riboflavin drops were administered every 2 minutes. Six months after the procedure, stable visual acuity and decreased keratometry values (by a mean of 2.3 D) at the apex of the keratoconus were found. Wernli et al.\(^16\) and Schumacher et al.\(^22\) evaluated the increase in the modulus of elasticity in porcine corneas. They found that for a constant energy dose of 5.4 J/cm\(^2\), the standard protocol using 3 mW/cm\(^2\) irradiance and the rapid one using 10 mW/cm\(^2\) irradiance in denuded stromal tissue were equivalent.

In conclusion, we present a method of testing a new rapid transepithelial crosslinking procedure using iontophoresis. Experimental data acquired in donor human eye globes showed that the procedure was effective in increasing corneal stiffness. In general, the results were comparable to those obtained with standard crosslinking.

**WHAT WAS KNOWN**

- Corneal crosslinking performed after deepithelialization achieves better outcomes than transepithelial procedures.

**WHAT THIS PAPER ADDS**

- Inflation experiments on human eye globes showed that transepithelial crosslinking using iontophoresis was effective in increasing the biomechanical strength of the anterior cornea and showed results that were almost comparable to those of standard crosslinking.

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