Transepithelial corneal collagen crosslinking: Bilateral study

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PURPOSE: To evaluate the efficacy of transepithelial collagen crosslinking (CXL) in patients with bilateral progressive keratoconus.

SETTING: Outpatient ophthalmic clinic.

DESIGN: Cohort study.

METHODS: Patients with a history of bilateral progressive keratoconus were recruited. The worst eye was treated with transepithelial CXL, while the fellow eye was left untreated as a control. Transepithelial CXL was performed by applying an enhanced riboflavin solution (riboflavin 0.1%, dextrane T500 with trometamol [Tris-hydroxymethyl aminomethane] and EDTA [ethylenediaminetetraacetic] sodium salt) on the intact corneal epithelium for 30 minutes before irradiation with ultraviolet A (370 nm at 3 mW/cm^2) for 30 minutes. Follow-up was 18 months in all eyes.

RESULTS: The study enrolled 20 patients. Transient hyperemia and mild foreign-body sensation occurred in 8 eyes (40%) after treatment; both resolved after 24 hours. In treated eyes, there were statistically significant improvements in uncorrected and corrected visual acuity and topography-derived keratometry, cone apex power, and higher-order aberrations (P<.05). In untreated control eyes, there was a general trend toward worsening of these parameters. No complications were reported.

CONCLUSIONS: Transepithelial CXL treatment appeared to halt keratoconus progression, with a statistically significant improvement in visual and topographic parameters. The treatment was safe and well tolerated. Its noninvasive nature makes it potentially useful in cases in which epithelial debridement is ideally avoided, such as pediatric cases, uncooperative patients, and thin corneas with thicknesses nearing 380 μm.

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Corneal collagen crosslinking (CXL) is the first surgical procedure that appears to halt the progression of corneal ectatic disorders such as keratoconus, pellucid marginal degeneration, and ectasia after refractive surgery.[1–4] In the current classic CXL treatment method, the corneal stroma is infused with a riboflavin solution and then exposed to ultraviolet-A (UVA) radiation.[4] Riboflavin is a hydrophilic compound and cannot easily cross the intact epithelial barrier. To efficiently permeate the corneal stroma, the central corneal epithelium must be debrided, typically in a diameter of 8.0 mm.[5] The epithelial debridement can cause severe pain and visual loss during the first few days after treatment until complete regrowth of the epithelium occurs.[6] In addition, stromal haze typically develops during the first few weeks and months after surgery, which can result in transient deterioration of an already compromised visual performance[6]; however, visual, topographic, and aberrometric indices have been shown to improve by 3 to 6 months.[7,8]

Transepithelial CXL was designed to avoid the early postoperative pain and temporary worsening of vision associated with the classic CXL technique. It is based on the use of a specially formulated riboflavin solution, Ricrolin TE (Sooft Italia SpA). To this formulation, 2 enhancers were added; that is, trometamol and sodium ethylenediaminetetraacetic acid (EDTA). The enhancers help riboflavin penetrate the corneal stroma through an intact epithelium, thereby avoiding the need for epithelial debridement. In addition, to improve permeation and avoid the need for an eyelid speculum, we designed a flat silicone ring

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that is applied around the corneoscleral limbus to contain the riboflavin solution on the intact corneal surface.

This prospective study was designed to evaluate the efficacy of transepithelial CXL with the enhanced riboflavin solution in patients with bilateral progressive keratoconus. The more affected eye was treated; the other eye was untreated and served as a control.

PATIENTS AND METHODS

The study was performed in accordance with the ethical standards described in the 2000 revision of the 1964 Declaration of Helsinki. All patients signed an informed consent form.

Inclusion criteria included corneal thickness greater than 380 μm, keratoconus grades II or III according to the Krumeich classification,[9] evidence of bilateral disease progression documented by an increase in the maximum cone apex curvature of at least 1.00 diopter (D), a reduction in the corneal thickness of more than 2% and/or an increase in central corneal astigmatism of at least 1.00 D over the previous 6 months, and no corneal scarring or other anterior segment pathology.

Preoperative Examinations

Before treatment, patients had a full ophthalmic examination and were counseled about the experimental nature of the procedure. Patients agreed to not wear contact lenses for at least 4 weeks before the examinations. Uncorrected (UDVA) and corrected (CDVA) distance visual acuities were tested under natural miosis using a logMAR Early Treatment Diabetic Retinopathy Study chart at 4 m.[10,11] Biomicroscopic slitlamp examination, ultrasound and optic pachymetry, and corneal topography and aberrometry were performed. For topography and aberrometry examinations (Optikon Keratron Scout, Optikon 2000 SpA), 4 scans were taken of each eye; the highest quality scan closest to the average keratometry (K) value was selected for wavefront analysis (performed with a simulated pupillary diameter of 8.0 mm) and cone apex power measurement. The cone apex power was determined using the cone location and magnitude index (CLMI).[12] The CLMI software locates the cone center and magnitude by finding the steepest curvature with a 2.0 mm circle within the central 8.0 mm zone. The area-corrected mean value of all points outside the circle is subtracted from the area-corrected mean of the points in the 2.0 mm circle. The area-corrected mean of the points in the 2.0 mm circle is compared with that of a 2.0 mm circle 180 degrees away. Results were used to determine whether the steep area represented a cone.[12] Pachymetry was performed with a Mizar ultrasound pachymeter (Optikon) and corneal optical coherence tomography (OCT) with a Spectral OCT/SLO device (OPKO Health, Inc.). Endothelial cell counts (ECC) were performed using an endothelial microscope (Costruzione Strumenti Oftalmici).

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Surgical Technique

The CXL treatment was performed in the eye with the highest apical keratometry. The untreated eye served as a control.

Three days before the procedure, patients were administered single-dose norfloxacin eyedrops (Naflox), 1 drop 4 times a day, for prophylactic purposes. The enhanced riboflavin solution was instilled 30 minutes before UVA exposure, with 1 drop being instilled every 10 minutes thereafter.

The enhanced riboflavin solution is an aqueous solution of 0.1% riboflavin containing trometamol (Tris-hydroxymethyl aminomethane) and sodium EDTA as excipients and epithelial penetration enhancers. The association of sodium EDTA and trometamol may weaken epithelial intercellular junctions. Trometamol is a biologically inert low-toxicity amino alcohol present as buffering solution in a wide range of cosmetic products and as an alkalinizing agent in pharmaceutical drugs.[13] Sodium EDTA is a well-known chelator of calcium and magnesium ions known to be important in keeping the integrity of tissue, including the epithelial tissue.[14,15]

To reduce the risk for UV exposure of retroirideal eye structures, miosis was induced with pilocarpine 1.0% 30 minutes before the procedure. Twenty minutes before UV radiation, the cornea was anesthetized with single-dose anesthetic eyedrops (oxybuprocaine hydrochloride 0.2%), 1 drop every 5 minutes. The UV source (Vega, Costruzione Strumenti Oftalmici) was switched on 2 hours before the procedure. All treatments were performed with the power set at 2.9 to 3.0 mW/cm^2 and a circular spot diameter of 8.0 mm in an outpatient setting. The patient was asked to lie down in a reclining armchair, and the periocular skin was disinfected for 5 minutes with povidone–iodine diluted to 10.0%. No surgical drapes were used. To avoid the need for a blepharostat and to improve riboflavin penetration, a ring-shaped silicone container was designed and used. The ring container was 12.0 mm in diameter and 3.0 mm high with a flange 2.0 mm wide and 0.3 mm thick at its base (Figure 1, *A*). Once covered by the eyelid, the flange stabilizes the ring on the cornea. The elasticity of the cylinder, which is rigid enough to resist the pressure of the eyelid, allows minimum lid adjustments. The outer edge of the cylinder and the flange also protect the corneal limbus and its stem cells from incidental UV irradiation (Figure 1, *B*).The silicone ring was filled with 2 drops of the enhanced riboflavin solution in direct contact with the corneal epithelium to cover the corneal apex entirely. If an adjustment of the eyelid caused part of the product to spill from the ring, more enhanced riboflavin solution was instilled until riboflavin was homogeneously dispersed over the corneal apex and its epithelial layer. The silicone ring filled with enhanced riboflavin solution was left in direct contact with the cornea for 30 minutes before the cornea was irradiated with UVA for 30 minutes.[14] During the irradiation, a homogeneous level of the enhanced riboflavin solution was constantly maintained by adding 1

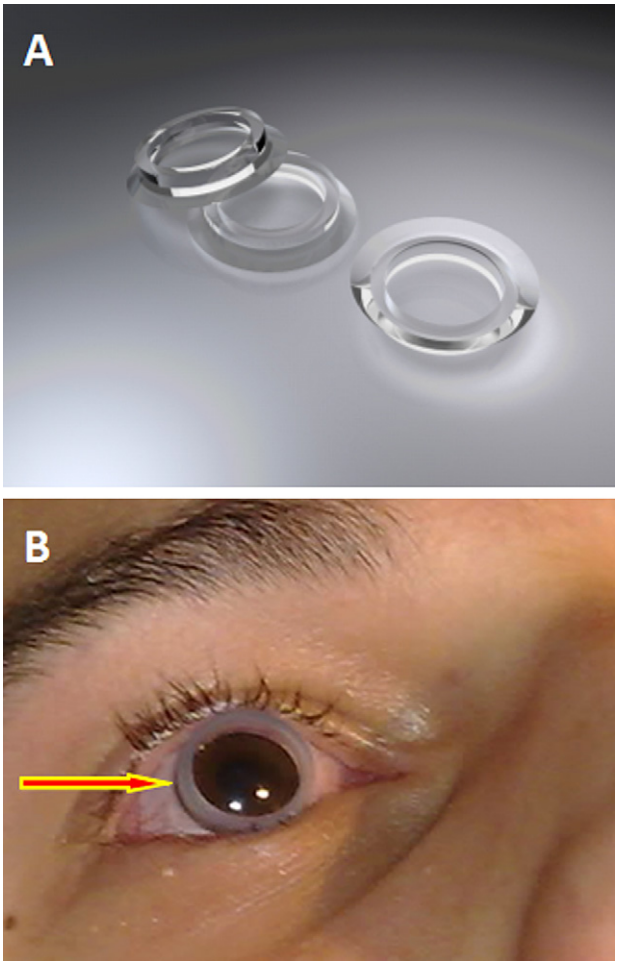

Figure 1. *A*: Silicone corneal ring. *B*: Silicone corneal ring positioned on the cornea and kept in place by its flanges (*arrow*) made to slide under the edges of the upper and lower lids.

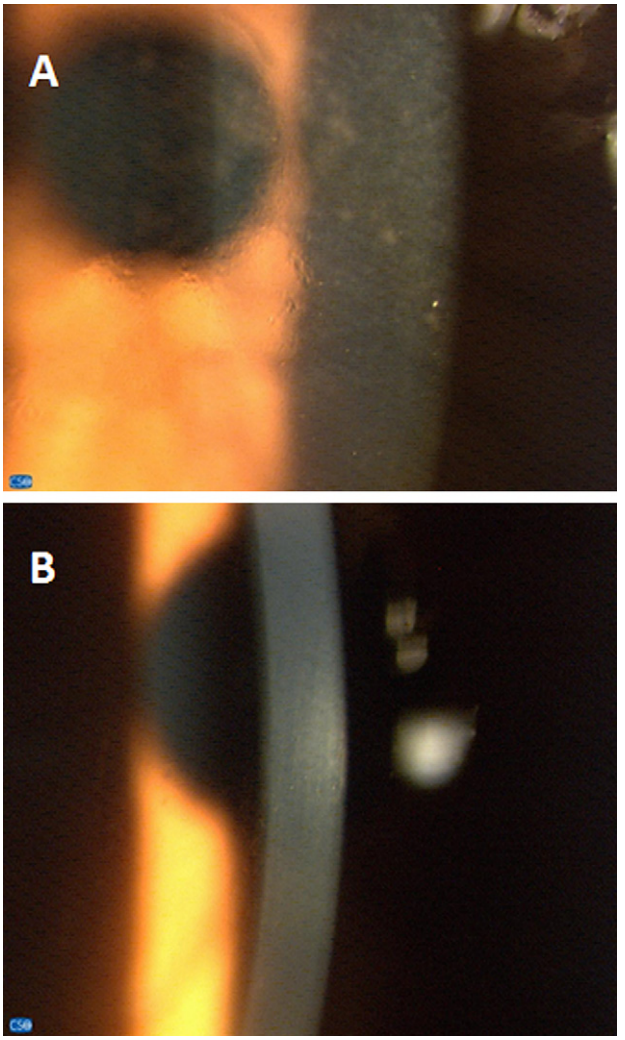

Figure 2. *A*: Corneal epithelial layer at the end of transepithelial CXL treatment. Note the discreet epithelial fissures at the slitlamp examination but with no signs of corneal abrasion. *B*: Same patient 2 days after the procedure. Note how the epithelial layer is transparent and the total absence of signs of corneal suffering.

drop every 3 to 5 minutes. In contrast to the initial permeation phase, during the irradiation phase, riboflavin formed only a thin layer over the epithelium without building up. Thus, the UV rays were not prevented from penetrating the stroma, which was shielded by the corneal epithelium. A simple adhesive sterile plaster was positioned on the outer edge to collect any drops of the enhanced riboflavin solution leaking from the corneal ring during the procedure. At the end of the procedure, the silicone ring was removed and all residue of the enhanced riboflavin solution was rinsed away with a sterile physiologic salt solution.

Immediately postoperatively, the treated eye was medicated with 1 drop of single-dose norfloxacin. The cornea was examined with a slitlamp to assess the integrity of the epithelial layer, and the patient was given a prescription for single-dose norfloxacin, 1 drop 3 times a day; sodium hyaluronate 0.15% with amino acids (BluYal-A), 1 drop 3 times a day for 20 days; and a liposome spray with vitamins A and E (Lacrisek spray), 1 spray over closed lids 3 times a day for 20 days.

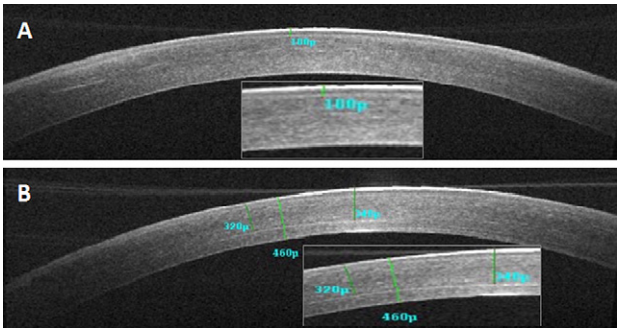

Figure 3. Corneas after CXL with transepithelial riboflavin (*A*) and after epithelium removal (*B*). The dense stromal line can be observed at 100 µm in the former case and at 320 to 340 µm in the latter case. *Insets*: Close up of the dense stromal line (OCT images).

Table 1. Visual acuity over time.

	Mean (LogMAR) ± SD					
			Postoperative			
	Before CXL		1 Month		3 Months	
Parameter	CXL	Controls	CXL	Controls	CXL	Controls
UDVA	0.71 ± 0.12	0.84 ± 0.23	0.49 ± 0.12*	0.81 ± 0.18	0.40 ± 0.15*	0.80 ± 0.09
CDVA	0.35 ± 0.23	0.46 ± 0.21	0.26 ± 0.10*	0.48 ± 0.29	0.22 ± 0.08*	0.50 ± 0.06

CDVA = corrected distance visual acuity; CXL = collagen crosslinking; UDVA = uncorrected distance visual acuity
*$P < .05$ versus controls

Table 2. Central keratometry at 3.0 mm over time.

	Mean (D) ± SD					
			Postoperative			
	Before CXL		1 Month		3 Months	
Parameter	CXL	Controls	CXL	Controls	CXL	Controls
Sim kS	51.02 ± 1.10	51.12 ± 1.02	49.05 ± 0.92*	51.10 ± 1.04	48.65 ± 0.89*	51.42 ± 0.96
Sim kF	45.13 ± 0.97	46.05 ± 0.99	44.46 ± 1.03*	46.12 ± 0.65	44.13 ± 0.89*	46.52 ± 0.91
Sim Cl	5.89	5.07	4.59*	4.98	4.52*	4.90

CXL = collagen crosslinking; Sim Cl = corneal cylinder (K1 – K2); Sim kF = keratometry of the flattest meridian; Sim kS = keratometry of the most curved meridian
*$P < .05$

Postoperative Examinations

Postoperatively, patients were examined weekly for 4 weeks and then at 3, 6, 12, and 18 months. The examinations were the same as preoperatively.

Statistical Analysis

Statistical analysis was performed using the Student *t* test with Statistica software (Version 8, Statsoft, Inc.). Results with 2-sided *P* values less than 0.05 were considered statistically significant.

RESULTS

Twenty patients were recruited in the study. The mean age of the 14 men and 6 women was 27 years (range 12 to 42 years). Fifteen right eyes and 5 left eyes were treated.

Immediate Postoperative Period

In the first 24 hours after CXL, 8 patients (40%) showed signs of conjunctival hyperemia and

Table 3. Topographic analysis over time.

	Mean (D) ± SD					
			Postoperative			
	Before CXL		1 Month		3 Months	
Parameter	CXL	Controls	CXL	Controls	CXL	Controls
KcAK	59.12 ± 1.10	58.89 ± 2.02	58.01 ± 0.92*	58.92 ± 2.34	57.42 ± 0.89*	59.43 ± 1.87
Mc	56.46 ± 0.97	56.31 ± 1.93	55.73 ± 1.41*	56.29 ± 2.18	55.52 ± 0.89*	57.02 ± 0.91
Ma	23.89 ± 0.75	21.91 ± 2.05	20.07 ± 2.42	21.98 ± 1.67	20.09 ± 2.50*	23.06 ± 1.4

CXL = collagen crosslinking; KcAK = Keratoconus apical keratometry; Ma = axial magnitude; Mc = apex magnitude
*$P < .05$

Table 1. (Cont.)

Mean (LogMAR) ± SD					
Postoperative					
6 Months		12 Months		18 Months	
CXL	Controls	CXL	Controls	CXL	Controls
0.38 ± 0.45*	0.86 ± 0.19	0.37 ± 0.12*	0.91 ± 0.23	0.48 ± 0.34*	0.98 ± 0.41
0.18 ± 0.16*	0.62 ± 0.08	0.24 ± 0.31*	0.66 ± 0.11	0.24 ± 0.77*	0.64 ± 0.39

Table 2. (Cont.)

Mean (D) ± SD					
Postoperative					
6 Months		12 Months		18 Months	
CXL	Controls	CXL	Controls	CXL	Controls
47.82 ± 0.78*	51.40 ± 0.92	47.55 ± 0.71*	51.63 ± 1.13	48.05 ± 0.21*	52.12 ± 0.47
44.57 ± 1.11*	46.74 ± 0.71	44.42 ± 0.84*	46.95 ± 0.50	44.43 ± 0.35*	46.88 ± 0.22
3.25*	4.66	3.13*	4.68	3.62*	5.24

reported a mild foreign-body sensation. These symptoms resolved spontaneously. Two patients (10%) reported photophobia, which spontaneously resolved after 4 days. No patient reported significant ocular pain.

Corneal Transparency

All corneas remained transparent on slitlamp examination throughout the follow-up period, with no signs of haze, as typically occurs with classic CXL with epithelial debridement. Figure 2, *A*, shows the corneal epithelial layer immediately at the end of transepithelial CXL treatment. Discreet epithelial fissures can be seen on slitlamp examination, but with no signs of corneal abrasion. Figure 2, *B*, shows the same eye 2 days postoperatively. The epithelial layer is transparent with no signs of corneal trauma.

Table 3. (Cont.)

Mean (D) ± SD					
Postoperative					
6 Months		12 Months		18 Months	
CXL	Controls	CXL	Controls	CXL	Controls
57.31 ± 0.78*	59.86 ± 2.12	57.86 ± 1.08*	60.34 ± 1.13	57.95 ± 0.87*	60.93 ± 1.21
55.49 ± 1.11*	57.59 ± 2.02	55.69 ± 1.02*	58.34 ± 0.93	55.81 ± 0.93*	59.04 ± 1.27
20.01 ± 2.02*	23.21 ± 0.67	20.34 ± 1.05*	24.07 ± 1.16	20.92 ± 1.21*	25.08 ± 0.51

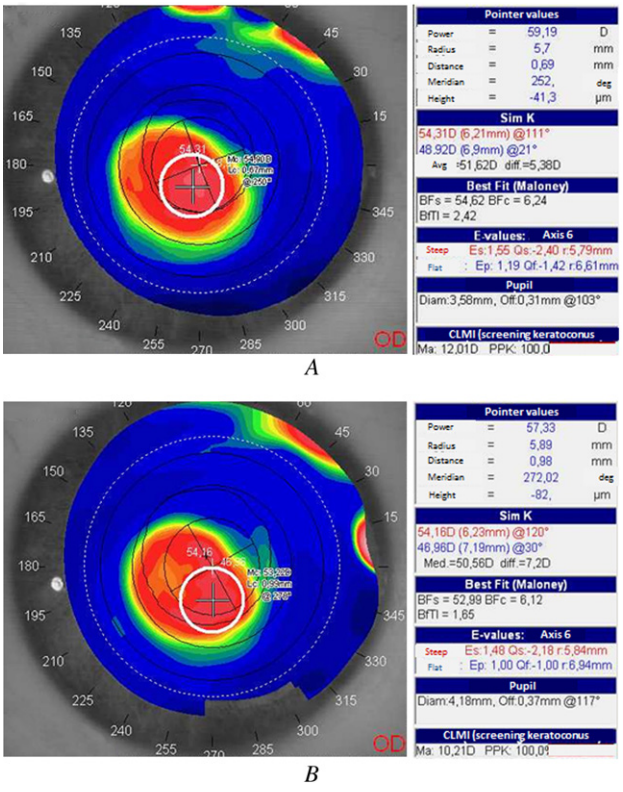

Figure 4. Corneal topography printouts of a keratoconus-affected eye. *A*: Corneal topography before transepithelial CXL. *B*: The same case after 9 months of follow-up. Note the marked reduction in the maximum curve at the apex of the cone (KaCK), which fell from 59.19 D to 57.33 D, and the reduction in the magnitude apex (Ma) from 12.01 to 10.21 D.

Corneal Optical Coherence Tomography

The OCT analysis after CXL treatment showed a dense area in the corneal stroma (not present before CXL) that was linear in shape and positioned approximately 100 µm from the corneal epithelial layer (Figure 3, *A*), slightly beneath Bowman membrane (Figure 3, *A*, *inset*). This was present within 2 weeks postoperatively and was in contrast to that seen after classic CXL performed with epithelial removal, in which the dense line forms deeper, between 320 µm and 340 µm (Figure 3, *B*, and *inset*).

Pachymetry

The mean corneal thickness measured before CXL was 412 µm ± 21 (SD) in treated eyes and 423 ± 12.2 µm in control eyes. At 18 months, treated eyes had a mean CCT of 408 ± 16 µm and control eyes, of 398 ± 24 µm. Although the changes did not reach statistical significance ($P<.5$), there appeared to be an apparent trend toward stabilization of pachymetry in treated eyes.

Endothelial Cell Count

The mean ECC preoperatively was 2398 ± 23 cells/mm^2 in treated eyes and 2414 ± 12 cells/mm^2 in control eyes. At 18 months, it was unchanged (2364 ± 30 cells/mm^2 and 2375 ± 18 cells/mm^2, respectively).

Visual Acuity

Table 1 shows the visual acuity measurements preoperatively and after transepithelial CXL. The UDVA and CDVA improved significantly by the first postoperative month ($P<.05$). Improvements continued throughout the 18-month follow-up. In contrast, untreated control eyes showed a trend toward progressive worsening of UDVA and CDVA.

Topography

Table 2 shows the 3.00 mm simulated keratometry and corneal astigmatism results. Table 3 shows the maximum cone apex curvature and CLMI indexes. All topographic-derived values decreased (improved) in treated eyes over preoperative values; the improvement occurred by 1 month, appeared to be maximum at 6 to 12 months, and continued to the 18-month follow-up ($P<.05$) (Tables 2 and 3). Figure 4 shows an example of the topographic improvement. In

Table 4. Corneal aberrometry over time.

	Mean (D) ± SD					
			Postoperative			
	Before CXL		1 Month		3 Months	
Parameter	CXL	Controls	CXL	Controls	CXL	Controls
RMS	4.68 ± 0.27	4.43 ± 0.75	4.21 ± 0.66*	4.12 ± 0.83	3.75 ± 0.59*	4.39 ± 1.47
Coma	2.21 ± 0.97	2.28 ± 1.93	2.19 ± 1.04*	2.10 ± 1.74	1.72 ± 0.32*	2.23 ± 1.05
SA	0.98 ± 0.15	1.12 ± .052	0.77 ± 0.42*	1.08 ± 0.67	0.65 ± 0.62*	1.26 ± 0.72

CXL = collagen crosslinking; RMS = root mean square; SA = spherical aberration
*$P<.05$

control eyes, there was a trend toward an increase (worsening) in all topography-derived parameters.

Corneal Wavefront Analysis

Table 4 shows the corneal aberration changes. The root-mean-square, coma, and spherical aberration values decreased (improved) significantly from the first month postoperatively; the reductions were greatest at 6 months and continued to improve throughout the 18-month follow-up ($P < .05$). In contrast, the aberrations showed a trend toward worsening in control eyes throughout the follow-up.

Complications

With the exception of transient hyperemia, foreign-body sensation, and photophobia during the first few hours and days after treatment, there were no complications associated with the transepithelial CXL treatment over the 18-month follow-up.

DISCUSSION

Although this study was nonrandomized and the number of patients small, the results of transepithelial CXL with the enhanced riboflavin solution (Ricrolin TE) are encouraging. The patients recruited to the study showed evidence of keratoconus progression in the 6 months before treatment. Two patients were older than 40 years. It is generally accepted that during advanced adulthood, the risk for keratoconus progression is much lower. However, these patients had frank bilateral keratoconus progression (not marginal pellucid degeneration) in the absence of another pathologic condition. Not only did the transepithelial CXL treatment appear to halt the progression of keratoconus in all treated eyes over the 18-month follow-up, it also yielded statistically significant improvements in all visual and topographic outcome measures ($P < .05$). In contrast, in untreated eyes there was a general trend toward worsening of all parameters. These results support the ability of transepithelial CXL to halt the progression of keratoconus. Further long-term observation of these patients is required to determine for how long disease progression is arrested.

After transepithelial CXL in our patients, OCT analysis of the corneal stroma showed a density increase presenting as a linear shape approximately 90 to 110 μm from the epithelial surface (Figure 3, *A*). A similar line is also observed after classic CXL with epithelial debridement, although in this situation it occurs at a depth of about 250 μm. Although the precise nature and significance of these lines of increased optical density in relation to the crosslinking process are unknown, the finding appears to suggest that transepithelial CXL and classic CXL work at different stromal depths. In an unpublished study,[A] we found that with the transepithelial CXL technique, the formation of crosslinks appears to take place in the upper third of the corneal stroma, 20 to 30 μm beneath Bowman membrane. With the classic debridement technique, collagen crosslinks form much deeper in the stroma.[1,2,14] These observations appear to highlight the possible role of the epithelium (also containing riboflavin) in shielding UV light. We previously observed that when experimental corneas were permeated with the enhanced riboflavin solution with the epithelium in situ and then irradiated after epithelium debridement, the dense line in the stroma formed at 250 μm, indicating that the difference in depth does not follow different permeations but rather the presence or the absence of the epithelium.[A] Whatever the level of this demarcation line, the improvements in keratometry (approximate 2.00 D reduction) in our patients treated with transepithelial CXL are comparable to those published for classic CXL.[4,7,8,16,17] Because the density of collagen fibers in corneal stroma is higher in the anterior portion, where most of the collagen crosslinks happen,[18,19] our clinical data may suggest that an anterior CXL is as good as a more posterior one (as obtained after epithelium debridement). Moreover,

Table 4. (Cont.)

Mean (D) ± SD					
Postoperative					
6 Months		12 Months		18 Months	
CXL	Controls	CXL	Controls	CXL	Controls
3.34 ± 0.43*	4.56 ± 2.45	3.46 ± 0.48*	4.86 ± 1.22	3.93 ± 0.73*	4.97 ± 0.78
1.93 ± 0.71*	2.41 ± 1.88	1.98 ± 1.13*	2.72 ± 0.56	2.11 ± 0.68*	2.81 ± 0.91
0.51 ± 0.37*	1.29 ± 0.45	0.61 ± 0.29*	1.33 ± 0.90	0.73 ± 0.49*	1.37 ± 0.92

occurring at different depths, the 2 techniques might be complementary; that is, transepithelial CXL could be performed when the classic procedure does not halt keratoconus progression. Being less invasive, transepithelial CXL might be a retreatment option after different types of keratoconus surgical procedures.

Further studies directly comparing the efficacy of the 2 procedures (classic and transepithelial) are indicated and are being planned. Should transepithelial CXL prove to be as efficacious as classic CXL, its noninvasive nature and simplicity would make it the procedure of choice for corneal collagen CXL.

We believe that our technique of transepithelial CXL performed without the need for epithelial debridement and with the application of the silicone corneal ring is efficacious and comparable with classic CXL. It is also simple and has several advantages for both ophthalmologist and patient. Namely, the procedure, because of the preservation of the epithelial layer, does not require a sterile environment and can be performed in the office setting, especially given that the UV emission reduces any bacterial load on the cornea. The technique does not require an operating microscope because epithelial debridement is not required. The silicone ring obviates the need for an eyelid speculum and protects the limbal stem cells from inadvertent UV radiation. The technique can be used to safely treat keratoconus with a minimum total corneal thickness approaching 380 μm (and maybe less) without the risk for endothelial damage because the epithelium remains intact, is filled with riboflavin, and contributes to UVA shielding. The technique can be used on patients who are normally not wholly cooperative, (eg, children and patients with mental impairment) for whom epithelial debridement may be very distressing. The postoperative treatment regimen is simple. The preoperative vision is maintained and not impaired during the first days and weeks after treatment. There is no significant postoperative pain, and complications associated with epithelial debridement, such as infection and recurrent erosions, are negated.

In conclusion, transepithelial CXL treatment appeared to halt keratoconus progression with a statistically significant improvement in measured visual and topographic parameters. The treatments were safe and well tolerated. Its noninvasive nature makes it a potentially useful treatment in cases in which epithelial debridement is ideally avoided, such as pediatric cases, uncooperative patients, and in eyes with thin corneas (thicknesses nearing 380 μm).

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