Transepithelial Iontophoresis Corneal Collagen Cross-linking for Progressive Keratoconus: Initial Clinical Outcomes

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ABSTRACT

PURPOSE: To report initial clinical results of transepithelial corneal collagen cross-linking with iontophoresis (I-CXL).

METHODS: Twenty eyes of 20 patients diagnosed as having progressive keratoconus who underwent I-CXL were included in this prospective non-randomized clinical study. Corrected distance visual acuity (CDVA), spherical equivalent and cylinder refraction, various corneal topography and Scheimpflug tomography parameters, aberrometry, anterior segment optical coherence tomography, and endothelial cell count were assessed at baseline and at 1, 3, 6, and 12 months postoperatively.

RESULTS: CDVA improved significantly at 3, 6, and 12 months postoperatively (logMAR difference of -0.07 ± 0.01, -0.09 ± 0.03, and -0.12 ± 0.06, respectively; $P < .05$). Aberrometry remained stable during follow-up and a trend toward improvement was noted. All topographic parameters (including maximum keratometry) were stable during the follow-up, but exhibited a positive non-significant trend toward improvement. Minimum corneal thickness values were stable for up to 12 months postoperatively. None of the patients showed a progression of keratoconus. Endothelial cell counts did not change significantly ($P > .05$).

CONCLUSIONS: Preliminary results up to 1 year postoperatively indicate the efficacy of I-CXL in stabilizing the progression of this degenerative disease combined with significant improvement of CDVA. I-CXL, which spares the corneal epithelium, has the potential to become a valid alternative for halting the progression of keratoconus while reducing postoperative patient pain, risk of infection, and treatment time in select patients; however, the relative efficacy of this technique compared to standard epithelium-off techniques remains to be determined.

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Corneal collagen cross-linking (CXL) is able to change the biomechanical properties of corneas and is currently the only treatment that can potentially slow or block the progression of ectatic disease.[1,2] Long-term follow-up studies on CXL mostly refer to the standard technique, which entails epithelial debridement to allow riboflavin penetration in the corneal stroma.[1,3] Epithelial removal causes pain[4] and a higher risk of corneal infection,[5] as well as visual loss for the first few months after treatment.[2,3] To avoid these drawbacks, transepithelial corneal collagen cross-linking (TE-CXL) was developed. The transepithelial protocol currently used employs a specially formulated riboflavin solution (Ricrolin TE; SOOFT, Montegiorgio, Italy) in which two enhancers (ie, trometamol and sodium ethylenediaminetetraacetic acid) are added to help riboflavin penetration in the corneal stroma.[6] However, results of TE-CXL are limited and have not achieved the same efficacy as standard CXL, frequently due to inadequate riboflavin penetration.[7-10]

The use of enhancers may not be the only way to increase riboflavin penetration through the epithelium. In other specialties (ie, dermatology), iontophoresis has been adopted for a long time. It is a non-invasive technique in which a small electric current is applied to enhance an ionized drug's penetration.

Preclinical results have shown that CXL with iontophoresis (I-CXL) is able to increase the concentration of riboflavin in the corneal stroma when compared to TE-CXL[11,12] with

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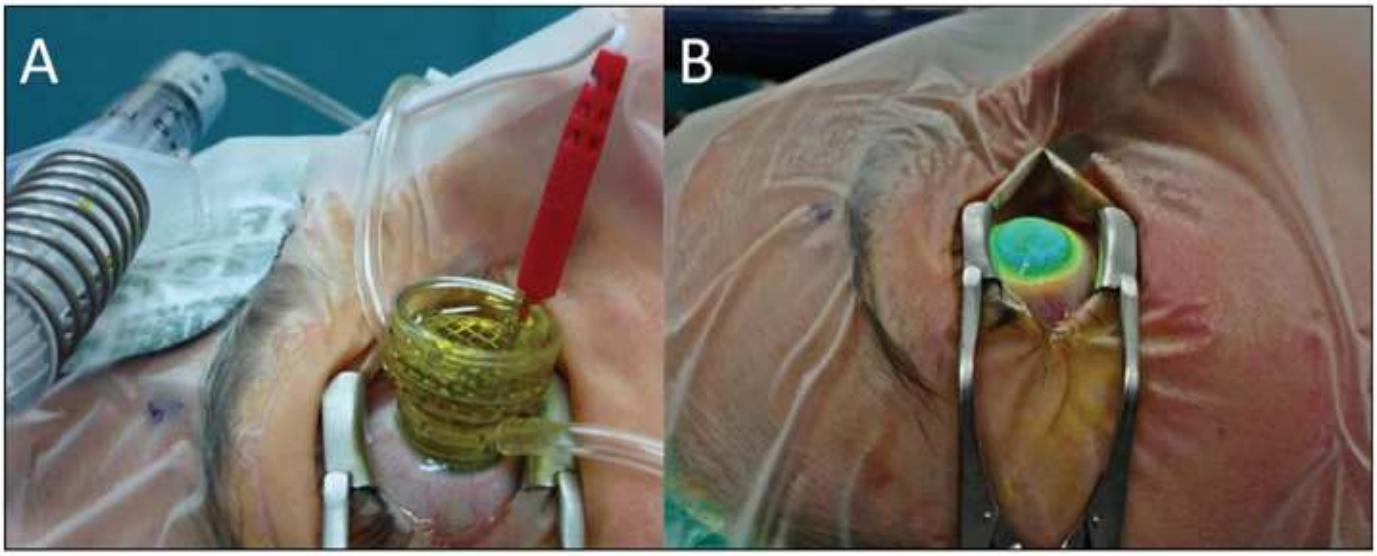

Figure 1. The iontophoresis cross-linking procedure. (A) The iontophoresis corneal applicator is placed on the cornea, filled with riboflavin, and connected to the constant current generator. (B) The irradiation phase: even after a complete wash with balanced salt solution, an intense fluorescence is noted inside corneal stroma, confirming effective penetrance of riboflavin through the intact epithelium.

demonstrable histological changes.[13] In the current study, we present the preliminary results of a prospective non-randomized clinical trial of I-CXL for progressive keratoconus.

PATIENTS AND METHODS

Patients diagnosed as having progressive keratoconus who underwent I-CXL at the Eye Center of the Humanitas Clinical and Research Center (Rozzano, Italy) were evaluated. Inclusion criteria were documentation of the progression of keratoconus in patients older than 18 years and signed informed consent. Keratoconus progression was demonstrated by at least two differential Scheimpflug optical corneal thicknesses and corneal topographies obtained a minimum of 3 months apart. The parameters indicating keratoconus progression were always proved with differential maps (ie, change in curvature in the cone area of at least 1 diopter obtained with instantaneous map, or thinning of more than 20 µm in minimal Scheimpflug corneal thickness). Exclusion criteria were a history of herpetic keratitis, dry eye, severe corneal infection and concomitant ocular or systemic autoimmune disease, pregnancy or breastfeeding, the presence of central or paracentral opacities, a history of poor compliance, and the use of rigid contact lenses for more than 4 weeks before the baseline evaluation.

The study received Institutional Review Board approval from the ethical committee of Humanitas Clinical and Research Center and was conducted according to the tenets of the Declaration of Helsinki. All patients provided informed consent.

In the preoperative and postoperative examinations (1, 3, 6, and 12 months), the following parameters were assessed: corrected distance visual acuity (CDVA), slit-lamp biomicroscopy, corneal topography, corneal aberrometry for the evaluation of lower- and higher-order aberrations (Costruzione Strumenti Oftalmici, Florence, Italy), optical coherence tomography and corneal thickness with the Pentacam (Oculus Optikgeräte GmbH, Wetzlar, Germany), anterior segment optical coherence tomography (Cirrus HD-OCT; Carl Zeiss Meditec, Dublin, CA), and endothelial biomicroscopy (Konan Specular Microscope; Konan Medical, Inc., Hyogo, Japan). All preoperative and postoperative functional and morphological tests were performed in an identical manner to a previously published clinical study,[3] and postoperative complications were recorded.

I-CXL was performed as a same-day surgery. It was conducted under topical anesthesia with two applications of 4% lidocaine drops and 0.2% oxybuprocaine hydrochloride under sterile conditions. Before the procedure, pain medication was administered (1 pill of ketorolac, 10 mg) and 2% pilocarpine drops were instilled in the eye to be treated to reduce the amount of ultraviolet light reaching the retina.[2] After the eyelid speculum was applied, the iontophoresis device for corneal application was placed on the cornea using an annular suction ring. The riboflavin solution used was specifically designed for I-CXL, consisting of 0.1% riboflavin, no dextran or sodium chloride, and the addition of two enhancers: ethylenediaminetetraacetic acid and trometamol (Ricrolin +; SOOFT). The corneal iontophoresis electrode was filled with approximately 0.5 mL of riboflavin solution from the open proximal side until it (stainless steel mesh) was covered. The

device was then connected (**Figure 1A**) to a constant current generator (**AQ1**I-ON XL) set at 1 mA (the total dose of 5 mA/5 minutes is monitored by the generator). Subsequently, the cornea was irradiated at a working distance of 45 mm with an ultraviolet lamp of 10 mW (UV-X 2000; IROC Innocross AG, Zug, Switzerland) for 9 minutes (**Figure 1B**). A calibrated ultraviolet-A meter (LaserMate-Q; Laser 2000, Wessling, Germany) was used before treatment to check the irradiance at a 1.0-cm distance. Both topical anesthetics were added as needed during irradiation.

After surgery, a soft therapeutic contact lens was applied even if the corneal epithelium was not removed because the high ultraviolet intensity applied during the procedure can partly damage the epithelium, causing discomfort during the first postoperative days.[14] The postoperative pain management protocol entailed taking 10 mg of ketorolac (1 pill) every 8 hours. An ophthalmic gel containing 0.15% sodium hyaluronate, 1% xanthan gum, and 0.3% netilmicin (Xanternet; SIFI S.p.A., Catania, Italy) was prescribed four times per day until no epithelial damage was observed (epithelial integrity was evaluated with fluorescein staining every day postoperatively). After removal of the contact lens, dexamethasone 21-phosphate 0.15% drops (Etacortilen; SIFI S.p.A.) to be used twice daily for 10 days and 0.15% sodium hyaluronate drops (BluYal; SOOFT) to be used six times daily for 45 days were prescribed. In addition, all patients received oral amino acid supplements (Aminoftal; SOOFT) for 7 days.[15]

Statistical Analysis

Statistical analysis was performed using the SPSS statistics software version 20.0 (IBM Corp., Armonk, NY). Data are described as mean ± standard deviation. All data samples were first checked using the Shapiro–Wilk's test. The Student's *t* test for paired data was applied to assess the significance of differences between preoperative and postoperative data, using the same level of significance ($P < .05$) in all cases.

RESULTS

Twenty eyes of 20 patients (12 male and 8 female) were evaluated. Patient demographics are listed in **Table 1**. All patients attended the 1-month follow-up visit; however, only 85% (17 of 20), 55% (11 of 20), and 40% (8 of 20) of patients attended the 3-month, 6-month, and 1-year follow-up visits, respectively. No patients were lost to follow-up at the time of submission. Keratoconus was classified on the basis of the modified Amsler–Muckenhirn classification provided by the Pentacam: 1 patient (5%) with grade I, 5 patients (25%) with grade II, 13 patients (65%) with grade III, and 1 patient (5%) with grade IV. No patients had atopy.

TABLE 1
Patient Demographics (n = 20)

Variable	Mean ± Standard Deviation
Age	27.7 ± 6
Male/female	12/8
Corrected distance visual acuity (logMAR)	0.26 ± 0.15
Spherical equivalent	-0.045 ± 2.756
Higher-order aberrations	1.04 ± 0.39
Comatic aberrations	2.40 ± 1.03
Spherical aberrations	0.14 ± 0.44
Maximum keratometry	59.07 ± 3.90
Surface asymmetry index	7.79 ± 3.86
Index of surface variance	106.6 ± 31.5
Index of vertical asymmetry	1.20 ± 0.44
Keratoconus index	1.29 ± 0.11
Central keratoconus index	1.06 ± 0.04
Index of height asymmetry	31.6 ± 22.5
Index of height decentration	0.11 ± 0.04
Minimum radius of curvature	5.88 ± 0.49
Minimum corneal thickness	434.3 ± 37.8
Endothelial cell density	2,446 ± 209

Comparative analyses at 1, 3, 6, and 12 months of follow-up showed the findings reported in **Table 2**. CDVA showed a significant improvement at 3, 6, and 12 months ($P = .03$, .03, and .01, respectively), starting from 0.26 ± 0.15 logMAR. At the 1-month follow-up visit, CDVA improvement was not statistically significant (**Figure 2**).

Topographic analysis included the evaluation of maximum keratometry and the surface asymmetry index provided by the CSO topographer. Initially, maximum keratometry and the surface asymmetry index showed a significant increase at 1 month followed by a continuous decrease over time that did not reach statistical significance (**Figure 3**). All topometric values evaluated with the Pentacam showed stability during follow-up (**Table 2**), whereas **AQ2**IHA showed a positive trend of improvement.

Overall, comparative analysis of higher-order aberrations showed no significant trend. Comatic aberration values showed a positive, although non-significant, trend toward improvement (**Figure 4**). Spherical aberration values showed a slight non-significant increase. Minimum preoperative corneal thickness val-

TABLE 2

Clinical Outcomes of Transepithelial Iontophoresis Corneal Collagen Cross-linking[a]

	Follow-up Time							
	Difference at Month 1 (n = 20)		Difference at Month 3 (n = 17)		Difference at Month 6 (n = 11)		Difference at Month 12 (n = 8)	
Parameters	**Mean ± SD**	***P***	**Mean ± SD**	***P***	**Mean ± SD**	***P***	**Mean ± SD**	***P***
Refractive parameters								
Corrected distance visual acuity	-0.05 ± 0.03	.1	-0.07 ±0.01	.03	-0.09 ±0.03	.03	-0.12 ±0.06	.01
Spherical equivalent	-2.487 ± 4.218	.57	0.0±0.0	.20	1.091 ± 1.233	.40	1.117 ± 3.783	.20
Cylinder	-0.025 ± 0.66	.8	-0.103 ± 0.712	.50	-0.409 ±0.957	.10	0.656 ± 1.01	.10
Cylinder axis	5.5 ± 36.8	.5	-0.5 ± 27.8	.90	2.2 ± 26.6	.70	3.7 ± 42.5	.80
Aberrometric parameters								
Higher-order aberrations	0.075 ± 0.131	.01	0.004 ± 0.139	.90	0.017 ± 0.186	.70	-0.019 ± 0.182	.70
Comatic aberrations	0.16 ± 0.17	.0006	0.005 ± 0.211	.92	0.033 ± 0.237	.60	0.006 ±0.258	.90
Spherical aberrations	0.23 ± 0.46	.03	-0.108 ± 0.725	.50	0.091 ± 0.163	.09	0.101± 0.140	.08
Morphological parameters								
Maximum keratometry	0.5 ± 1.2	.05	0.166 ± 2.103	.80	-0.083 ± 1.897	.70	-0.549 ± 2.344	.40
Surface asymmetry index	0.47 ± 0.77	.01	-0.154 ± 1.716	.70	-0.301 ± 1.861	.60	-0.888 ± 2.017	.20
Index of surface variance	7.8 ± 14.4	.001	0.1 ± 5.1	.90	1.0 ± 8.5	.70	2.6 ± 10.2	.40
Index of vertical asymmetry	0.023 ± 0.09	.20	-0.016 ± 0.09	.40	-0.019 ± 0.13	.60	-0.08 ± 0.16	.90
Keratoconus index	0.01 ± 0.02	.30	-0.004 ± 0.03	.60	0.007 ± 0.03	.50	0.014 ± 0.02	.10
Central keratoconus index	0.017 ± 0.02	.002	0.011 ± 0.02	.05	0.006 ± 0.01	.10	0.008 ± 0.01	.20
Index of height asymmetry	-0.9 ± 28.3	.80	-1.8 ± 17.4	.67	-4.3 ± 20.1	.50	-5.3 ± 17.5	.40
Index of height decentration	0.003 ± 0.01	.40	0.02 ± 0.1	.40	-0.001 ± 0.01	.80	0.07 ± 0.1	.30
Minimum radius of curvature	-0.07 ± 0.2	.10	-0.04 ± 0.2	.30	0.08 ± 0.4	.50	-0.03 ± 0.29	.70
Minimum corneal thickness	-0.4 ± 9.01	.80	2.1 ± 10.4	.40	5.5 ± 10.4	.10	1.2 ± 7.9	.60

SD = standard deviation

[a]Differences are calculated from baseline (ie, difference at month 6 signifies changes between month 6 values and baseline value).

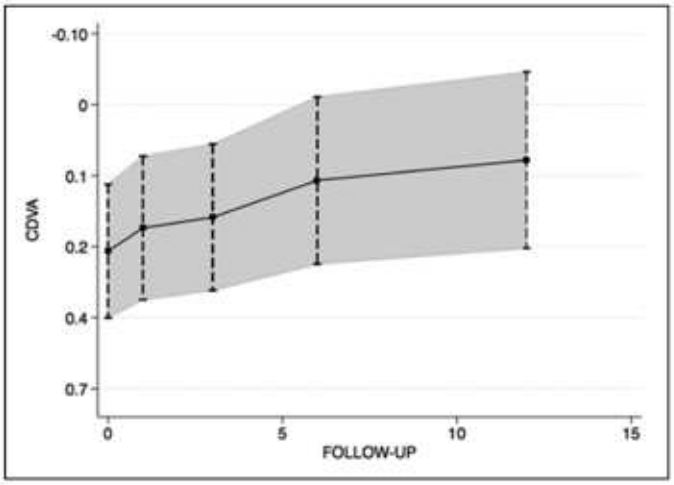

Figure 2. Change in corrected distance visual acuity (CDVA) during 1 year of follow-up. CDVA showed a significant improvement at 3, 6, and 12 months together, with a non-significant improvement at 1 month.

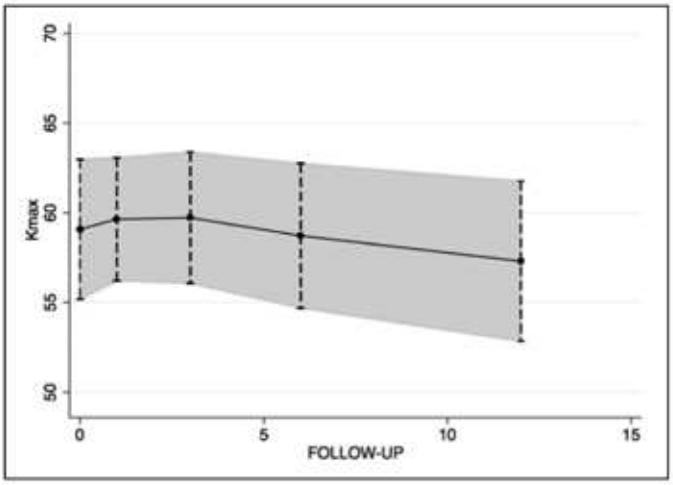

Figure 3. Change in maximum keratometry (Kmax) during 1 year of follow-up. Kmax showed an initial significant increase at 1 month followed by a continuous decrease over time; however, it did not reach statistical significance.

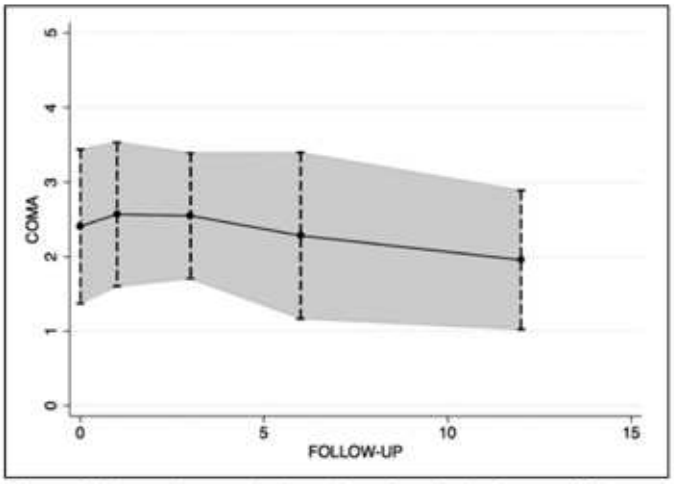

Figure 4. Change in comatic aberration values during 1 year of follow-up. Comatic aberration values showed a positive, although non-significant, trend toward improvement.

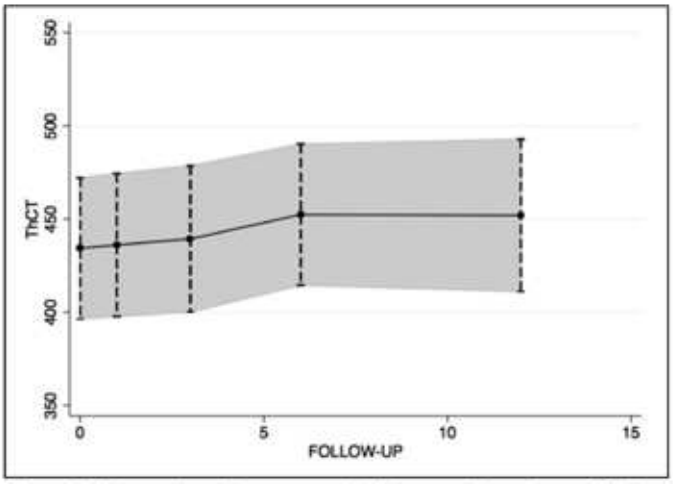

Figure 5. Change in minimum corneal thickness during 1 year of follow-up. Preoperative values (434.3 ± 37.8 μm) remained stable during the entire follow-up period, with a trend toward increase.

ues (434.3 ± 37.8 μm) were stable after 12 months of follow-up (**Figure 5**). None of the other evaluated morphological and functional parameters showed significant worsening during the follow-up period, excluding month 1, which indicated stabilization of keratoconus. No clear demarcation line was measurable with anterior segment optical coherence tomography after I-CXL; however, an increase of anterior stroma reflectance was noted (**Figure 6**).

The preoperative mean endothelial cell count was 2,436.90 ± 132.32 cells/mm^2 and did not significantly change during the follow-up period ($P > .05$). None of the patients developed infection. No significant haze was found after I-CXL. One patient developed epithelial whitening with pain in the early postoperative period that healed with medical therapy.

DISCUSSION

These preliminary results support the concept that I-CXL may be effective in halting the progression of keratoconus in adult patients. All measured parameters were either stable or improved within 1 year of follow-up and no repeat CXL was necessary for any patient. Patients had improved CDVA and no change in endothelial cell density or any other significant complications.

CXL with epithelial removal using the standard protocol reduces or halts the progression of keratoconus, inducing significant improvements in morphological and functional parameters within 4 or more years of follow-up.[1,3,16] However, due to consequent discomfort,[4] temporary vision loss, and increased risk of infection[5] related to epithelial removal, a CXL procedure

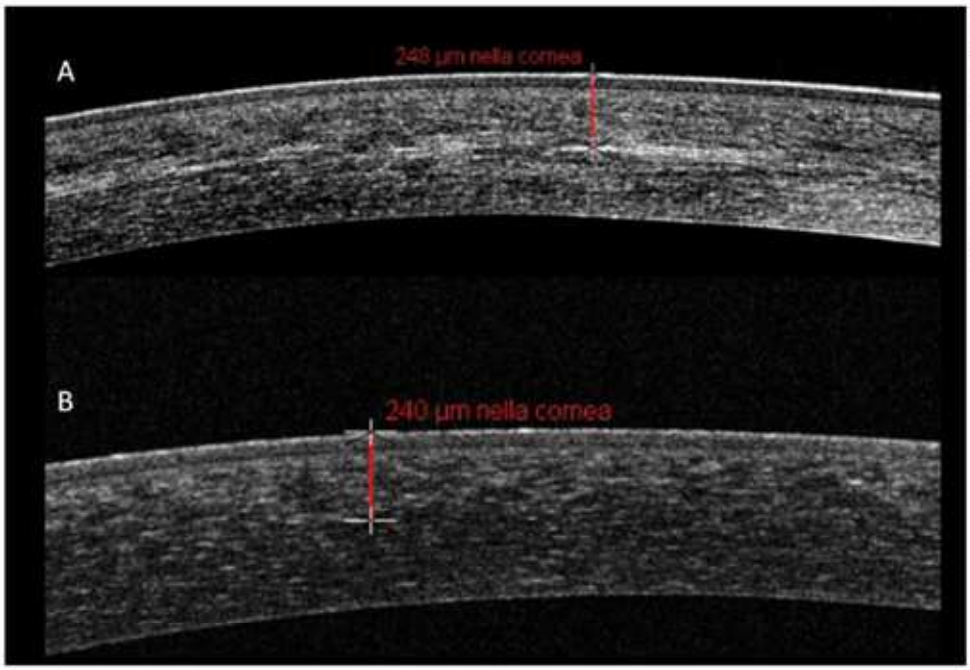

Figure 6. Difference in anterior segment optical coherence tomography in a patient who underwent (A) standard epithelium-off cross-linking (S-CXL) in one eye and (B) transepithelial iontophoresis cross-linking (I-CXL) in the other. In S-CXL, an increase of reflectance with a white line (demarcation line) is normally visible. In I-CXL, it is possible to detect a similar increase of reflectance; however, no white line is visible.

that spares the epithelium while retaining maximal efficacy has been constantly sought after. Clinical studies of conventional TE-CXL remain controversial, with evidence showing that treated patients continue to progress,[7-10] whereas other reports show positive results.[6,17]

Both corneal epithelium-blocking ultraviolet penetration and blockage of riboflavin penetration may be important factors for the reduced effect of TE-CXL.[18,19] However, Kolozsvari et al. reported that the corneal epithelium and Bowman's layer mostly absorb ultraviolet-B light (up to 300 nm) and allow penetration of ultraviolet-A light.[20] Bottos et al. showed that the reduced effect of TE-CXL compared to standard CXL is principally due to the limited penetration of riboflavin through the epithelium and confirmed that it is not a barrier to ultraviolet-A transmittance.[21] Thus, riboflavin penetration appears to be the primary factor limiting transepithelial approaches.

I-CXL could minimize this problem by enhancing the diffusion of the photosensitizer. Riboflavin is theoretically a good candidate for ocular iontophoresis due to its negatively charged structure and low molecular weight. Recent preclinical reports have shown the ability of I-CXL to enhance the penetration of riboflavin through the intact epithelium, as well as to induce an effective CXL.[11,12,22] Our preclinical results that compared the biomechanical effect, riboflavin penetration, and distribution of I-CXL are in agreement with the literature, showing that iontophoresis imbibition is able to increase the stromal amount of riboflavin when compared to standard TE-CXL.[11,12] Nevertheless, it reached a lower concentration when compared to the conventional epithelium-off protocol.[11,12] In particular, I-CXL was able to increase the stromal amount of riboflavin when compared to standard TE-CXL.[11,12] Similarly, stress-strain measurements demonstrated a significant increase in corneal stiffness after I-CXL when compared to controls, but still lower than that of standard CXL.[12] Other studies are needed to understand if this stiffening effect will be enough to halt the ectatic disease process in the long-term even if reduced.

To the best of our knowledge, this is the first prospective clinical study in which preoperative and postoperative refractive, topographic, tomographic, and aberrometric outcomes have been analyzed in eyes with progressive keratoconus treated with a commercial ocular iontophoresis device. One-year functional results showed significant improvement in CDVA after 3, 6, and 12 months of follow-up. Comatic, spherical, and higher-order aberrations remained stable during follow-up after an initial worsening. This positive functional improvement was concomitant, with no significant worsening of morphological and functional parameters at the 1-month follow-up visit (typical of standard CXL).[1,3,16] It is therefore possible that I-CXL may allow a faster visual recovery compared to standard CXL, in which patients show a significant decrease in visual functions and corneal shape in the first few months after treatment.[1,3,16] Larger study populations will be necessary to reach this conclusion.

No signs of ectasia progression were noted. Minimum corneal thickness values were stable at up to 12 months of follow-up. It has been reported in patients

with thin corneas that a permanent stromal scar tends to develop after CXL.[3,23] After I-CXL, no one developed deep stromal opacities, even in some patients who had a preoperative minimum corneal thickness slightly below 400 µm.

Anterior segment optical coherence tomography after I-CXL shows a completely different image compared to that after standard CXL. In standard CXL, an increase of reflectance with a white line (demarcation line) (**Figure 6A**) is normally visible.[24] In I-CXL, it is possible to detect a similar increase of reflectance; however, no white line is visible (**Figure 6B**). This finding could be explained by either the different concentration gradient induced by iontophoresis or a reduced CXL effect.

Our findings are in partial agreement with the 1-year follow-up report by Bikbova and Bikbov[25] of 19 patients treated with I-CXL. Their results showed that I-CXL is able to halt the progression of keratoconus. They did not report any significant change in functional parameters, but showed a significant decrease in corneal thickness after 6 months of follow-up. There are some differences between the current study and that of Bikbova and Bikbov (eg, the device used was not specifically designed for ocular use). The same device, which does not have a corneal ring, has already been used in other specializations such as gynecology[26] and dermatology.[27] Additionally, the patients in their study presented at an early keratoconic stage (Amsler I-II) and with a lower average keratometry value. Conversely, we included patients with advanced keratoconus with a maximum keratometry value of up to 64 diopters (**Table 2**). Even in these patients, I-CXL was safe and effective in arresting the ectatic disease, as well as inducing improvement in visual acuity from the 3-month follow-up visit.

The principal limits of our study are the relatively low number of patients, short follow-up, and lack of comparison with standard CXL. The energy dose used may be a further limitation. The total energy dose of 5.4 J/cm^2 was applied to be comparable to all other historical standard CXL[1,2] and TE-CXL[6] studies that chose this dose. However, Zhang et al. showed that the epithelial cells are not enriched with riboflavin.[28] For that reason, only a small part of the ultraviolet light should be absorbed by the epithelium (ie, approximately 15% to 20%).[29] Therefore, it could be advisable to increase the energy dose in I-CXL by 20% (ie, to 6.5 J/cm^2).

I-CXL has the potential to become a valid alternative to halt the progression of keratoconus while reducing postoperative patient pain, risk of infection, and treatment time in select patients. Further studies are in progress to assess its long-term safety and efficacy when compared to standard CXL, which entails epithelial removal.

AUTHOR CONTRIBUTIONS

Study concept and design (CA, RP, PV); data collection (RV, EFL, PR); analysis and interpretation of data (FIC, VR, JBR); drafting of the manuscript (RV, RP, VR, JBR); critical revision of the manuscript (CA, FIC, EFL, PR, PV, JBR); statistical expertise (VR); administrative, technical, or material support (RV, PR, JBR); supervision (PV, JBR)

REFERENCES

1. Raiskup-Wolf F, Hoyer A, Spoerl E, Pillunat LE. Collagen crosslinking with riboflavin and ultraviolet-A light in keratoconus: long-term results. *J Cataract Refract Surg.* 2008;34:796-801.
2. Vinciguerra P, Albe E, Trazza S, et al. Refractive, topographic, tomographic, and aberrometric analysis of keratoconic eyes undergoing corneal cross-linking. *Ophthalmology.* 2009;116:369-378.
3. Vinciguerra R, Romano MR, Camesasca FI, et al. Corneal cross-linking as a treatment for keratoconus: four-year morphologic and clinical outcomes with respect to patient age. *Ophthalmology.* 2013;120:908-916.
4. Ghanem VC, Ghanem RC, de Oliveira R. Postoperative pain after corneal collagen cross-linking. *Cornea.* 2013;32:20-24.
5. Dhawan S, Rao K, Natrajan S. Complications of corneal collagen cross-linking. *J Ophthalmol.* 2011;2011:869015.
6. Filippello M, Stagni E, O'Brart D. Transepithelial corneal collagen crosslinking: bilateral study. *J Cataract Refract Surg.* 2012;38:283-291.
7. Malhotra C, Shetty R, Kumar RS, Veluri H, Nagaraj H, Shetty KB. In vivo imaging of riboflavin penetration during collagen cross-linking with hand-held spectral domain optical coherence tomography. *J Refract Surg.* 2012;28:776-780.
8. Touboul D, Efron N, Smadja D, Praud D, Malet F, Colin J. Corneal confocal microscopy following conventional, transepithelial, and accelerated corneal collagen cross-linking procedures for keratoconus. *J Refract Surg.* 2012;28:769-776.
9. Caporossi A, Mazzotta C, Paradiso AL, Baiocchi S, Marigliani D, Caporossi T. Transepithelial corneal collagen crosslinking for progressive keratoconus: 24-month clinical results. *J Cataract Refract Surg.* 2013;39:1157-1163.
10. Buzzonetti L, Petrocelli G. Transepithelial corneal cross-linking in pediatric patients: early results. *J Refract Surg.* 2012;28:763-767.
11. Mastropasqua L, Nubile M, Calienno R, et al. Corneal cross-linking: intrastromal riboflavin concentration in iontophoresis-assisted imbibition versus traditional and transepithelial techniques. *Am J Ophthalmol.* 2014;157:623-630.e1.
12. Cassagne M, Laurent C, Rodrigues M, et al. Iontophoresis transcorneal delivery technique for transepithelial corneal collagen crosslinking with riboflavin in a rabbit model [published online ahead of print March 18, 2014]. *Invest Ophthalmol Vis Sci.*
13. Mastropasqua L, Lanzini M, Curcio C, et al. Structural modifications and tissue response after standard epi-off and iontophoretic corneal Crosslinking with different irradiation procedures. *Invest Ophthalmol Vis Sci.* 2014;55:2526-2533.
14. Golu A, Gheorghisor I, Balasoiu AT, et al. The effect of ultraviolet radiation on the cornea: experimental study. *Rom J Morphol Embryol.* 2013;54:1115-1120.
15. Torres Munoz I, Grizzi F, Russo C, Camesasca FI, Dioguardi N,

Vinciguerra P. The role of amino acids in corneal stromal healing: a method for evaluating cellular density and extracellular matrix distribution. *J Refract Surg*. 2003;19:S227-S230.

16. Caporossi A, Mazzotta C, Baiocchi S, Caporossi T. Long-term results of riboflavin ultraviolet a corneal collagen cross-linking for keratoconus in Italy: the Siena eye cross study. *Am J Ophthalmol*. 2010;149:585-593.
17. Salman AG. Transepithelial corneal collagen crosslinking for progressive keratoconus in a pediatric age group. *J Cataract Refract Surg*. 2013;39:1164-1170.
18. Baiocchi S, Mazzotta C, Cerretani D, Caporossi T, Caporossi A. Corneal crosslinking: riboflavin concentration in corneal stroma exposed with and without epithelium. *J Cataract Refract Surg*. 2009;35:893-899.
19. Wollensak G, Iomdina E. Biomechanical and histological changes after corneal crosslinking with and without epithelial debridement. *J Cataract Refract Surg*. 2009;35:540-546.
20. Kolozsvari L, Nogradi A, Hopp B, Bor Z. UV absorbance of the human cornea in the 240- to 400-nm range. *Invest Ophthalmol Vis Sci*. 2002;43:2165-2168.
21. Bottos KM, Schor P, Dreyfuss JL, Nader HB, Chamon W. Effect of corneal epithelium on ultraviolet-A and riboflavin absorption. *Arq Bras Oftalmol*. 2011;74:348-351.
22. Vinciguerra P, Rechichi M, Rosetta P, et al. High fluence iontophoretic corneal collagen cross-linking: in vivo OCT imaging of riboflavin penetration. *J Refract Surg*. 2013;29:376-377.
23. Raiskup F, Hoyer A, Spoerl E. Permanent corneal haze after riboflavin-UVA-induced cross-linking in keratoconus. *J Refract Surg*. 2009;25:S824-S828.
24. Doors M, Tahzib NG, Eggink FA, Berendschot TT, Webers CA, Nuijts RM. Use of anterior segment optical coherence tomography to study corneal changes after collagen cross-linking. *Am J Ophthalmol*. 2009;148:844-851.
25. Bikbova G, Bikbov M. Transepithelial corneal collagen crosslinking by iontophoresis of riboflavin. *Acta Ophthalmol*. 2014;92:e30-e34.
26. Strugatskii VM. Gynecologic electrode assembly for the Potok-1 galvanization apparatus [article in Russian]. *Nov Med Priborostr*. 1969;3:46-51.
27. Ragelis S. Tetracycline penetration into tissue by modified electro- and phonophoretic methods [article in Russian]. *Antibiotiki*. 1981;26:699-703.
28. Zhang Y, Sukthankar P, Tomich JM, Conrad GW. Effect of the synthetic NC-1059 peptide on diffusion of riboflavin across an intact corneal epithelium. *Invest Ophthalmol Vis Sci*. 2012;53:2620-2629.
29. Spoerl E. Corneal collagen cross-linking epithelium-on versus epithelium-off treatments. In: Hafezi F, Randleman JB, eds. *Corneal Collagen Cross-Linking*. Thorofare, NJ: SLACK Incorporated; 2013:139-142.