MINERVA OFTALMOL 2010;52:00-00

Collagen crosslinking without corneal de-epithelialization

M. FILIPPELLO, E. STAGNI

Casa di Cura Di Stefano Velona, Catania, Italy

Aim. **The aim of this paper was to evaluate the effectiveness of transepithelial crosslinking (TE CXL) in patients with bilateral progressive keratoconus.**

Methods. **The study population was patients with a history of bilateral progressive keratoconus. The worse eye was treated; the untreated fellow eye served as control. TE CXL was performed stepwise in six steps of irradiation delivered by a laser source (CBM X-linker VEGA®) and application of Ricrolin TE® (0.1% riboflavin, dextrane T500, and enhancers to facilitate passage of the solution through the corneal epithelium) every 5 minutes, 2 hours before imbibtion with Ricrolin TE® in the worse eye.**

Results. **A gradual improvement in topographic and abberometric values, without postoperative corneal haze or other side effects, was noted in the TE-CXL-treated eyes. Confocal microscopy demonstrated corneal crosslinks starting 5 months after treatment; all indices deteriorated in the control eyes.**

Conclusion. **TE CXL treatment was found to be effective in slowing keratoconus progression, with a statistically significant improvement in parameters. Thanks to transepithelial treatment and absence of side effects, TE CXL permits treatment of keratoconus in cases of corneal thickness <400 micron; "complicated" patients <12 years of age (e.g., trisomy 21 syndrome); patients aged ≥35 years or with a mean K >55. Unlike conventional CXL, TE CXL, because it spares the epithelium, ensures better patient compliance, without postoperative pain or haze or deterioration of vision. TE CXL is an innovative treatment that acts on the anterior third of cornea, without side effects; as it is simple to perform, it can be used in conjunction with conventional CXL, which acts on the posterior third of cornea.**

Key words: **Collagen - Cross-linking reagents - Cornea.**

Received on march 5, 2010.
Accepted for publication on March 9, 2010.

Corresponding author: Dr. Massi,mo Filippello, Casa di Cura Di Stefano, Via Sant'Eupilio 160, 35124 Catania, Italy. E-mail: info@ditefanovelona.com

Corneal collagen crosslinking (CXL) is a surgical procedure that was developed to slow or halt progressive corneal thinning, as occurs in keratoconus, pellucid marginal degeneration (PMD), and refractive surgery-induced corneal ectasia. In the late 1990s, the first studies appeared that demonstrated that CXL augmented stromal rigidity by over 300% by increasing collagen fiber diameter by 12.2% and by inducing the formation of crosslinks in the collagen network.[1-3]

Treatment entails exposure of the corneal stroma to ultraviolet A (UVA) light after saturating the corneal stroma with riboflavin.[4] But since hydrosoluble riboflavin is unable to cross the epithelial barrier, the cornea first

needs to be deepithelialized to a depth of at least 8 mm in diameter for imbibtion of the stroma to be effective.[5]

The early postoperative period after CXL with deepithelialization is extremely painful, at least as painful as photorefractive keratotectomy (PRK). In the first months following treatment, corneal reepithelization leads to an apparent deterioration of topographic indices and of visual acuity in some cases.[6] In addition, a light corneal haze during the first postoperative weeks reduces topographic and initial aberrometric values.[6] Studies have demonstrated that starting from around 6 months posttreatment visual acuity and topographic and aberrometric indices gradually begin to improve.[7, 8]

With the use of transepithelial CXL (TE CXL), however, the occurrence of a painful postoperative course and impaired vision during the first 2 months could be prevented. In addition, patients ineligible for conventional CXL with deepithelialization (*i.e.*, those with low compliance and keratoconus with a corneal thickness <400 micron) could be treated.

With this prospective, non-randomized study we wanted to determine the feasibility of performing TE CXL with the use of an eye drop solution (Ricrolin TE®, Sooft Italia, Montegiorgio [AP], Italy) composed of 0.1% riboflavin combined with enhancers which would facilitate riboflavin penetration into the corneal stroma in an intact epithelium.

We also developed a silicone corneal ring that obviates the need for a blepharostat and delivers the photosensitizing solution directly onto the intact cornea during the entire procedure. The enhancer is composed of tromethamol and sodium EDTA. Tromethamol (tris[hydroxymethyl]aminomethane) is a weakly toxic, biologically inert amino alcohol used in a wide variety of products, including cosmetics, as an industrial buffer solution and a component in drugs because of its intracellular and extracelluar alkalizing action. Although widely used, sensitization to the product has rarely been reported; only in one case was a gel containing tromethamol associated with periorbital dermatitis. Its safety profile is well established. [9] EDTA breaks cell-cell bonds, thus facilitating the penetration of various substances.

Materials and methods

Patients

Twenty eyes from 20 patients (14 males and 6 females; age range, 12-42 years; mean, 27) with bilateral progressive keratoconus were included in the study. In all, 15 right eyes and 5 left eyes were treated; the untreated fellow eye served as the control. All patients presented with a transparent cornea and had not worn contact lenses for at least 4 weeks before treatment. CXL was performed on the eye with steeper curvature and less corneal thickness.

All patients had a mean corneal thickness of 412.9 micron (range, 380-444) and were classified as stage II or III keratoconus as defined by the Amsler-Krumreich classification. Disease progression was defined as an increase in keratoconus apical keratometry of at least 1 diopter (D), topographically measured within the last 6 months, or as a reduction in corneal thickness >2% and an increase in central corneal astigmatism of 1 D in the last 6 months.

Assessment was performed before treatment (baseline), then at 7 days, 15 days, 1 month, 3, 6 and 9 months after treatment.

Visual acuity

Visual acuity was measured under conditions of natural miosis by means of early treatment diabetic retinopathy study charts (logMar/ETDRS). The letter charts and the procedure are described in detail in Ferris *et al.*[10, 11] Uncorrected visual acuity (UCVA) and best-corrected visual acuity (BCVA) were measured at baseline and at control visits in both eyes at a distance of 4 meters.

Diagnostic tests

The cornea in both eyes was evaluated by spilt lamp examination, ultrasound and optic pachymetry, topography and corneal aber-

Figure 1.—Silicon corneal ring.

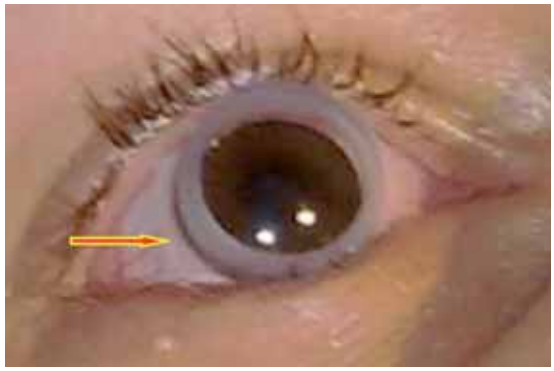

Figure 2.—Corneal ring positioned on the cornea and held in place by the ring flange (arrow) inserted under the eyelid rim.

rometry (Optikon topographer, Keratron Scout software version 4.2, produttrice, città).

Keratoconus progression in the CXL-treated eye and the untreated fellow eye was monitored using axial, curvature, and refractive topographic mapping, and evaluated using software-generated cone location and magnitude indices (CMLI).[12]

Abberometric analysis of the corneal surface was done using a refractive map from which the comatic aberrations, spherical aberrations, root mean square (RMS), and corneal refraction were calculated. All pre- and post-CXL topographic and abberometric tests were performed on a simulated pupil diameter of 8 mm.

Pachymetry was done using an ultrasound pachymeter (Mizar biometer, Optikon 2000, Rome, Italy) and with optical coherence tomography (OCT) of the cornea (Spectral SLO ™ System, OPKO, Miami, FL, USA). The corneal module of the OCT-SKO system was useful for studying stromal variations and the corneal profile.

Endothelial cell count was performed at baseline and at 9 months follow-up using an endothelial microscope (CSO, Florence, Italy).

The study was conducted according to the ethical principles set out in the 2000 Declaration of Helsinki. All patients gave their written informed consent to treatment.

Crosslinking

To facilitate CXL and to improve penetration of the product, a silicone corneal ring (12 mm in diameter, 3 mm in height; flange base 2 mm in width, 0.3 mm in thickness) was developed. When the flange is applied to the eyelid rim, it stabilizes the device over the cornea, thus obviating the need for a blepharostat. The device is elastic yet rigid enough to withstand eyelid pressure, while permitting eyelid movement. The external edge of the corneal ring and the flange protect the limbus corneae from inadvertent UV irradiation, thus protecting the stem cells of the limbus (Figure 1, 2). The source of UVA light was a UV light single-LED emitter for keratoconus treatment (UV-A Vega, CSO, Florence).

Surgical technique

Three days before surgery, norfloxacin (Naflox, Farmigea, Pisa, Italy) was administered at a dose of 1 drop four times a day. Ricrolin TE® (1 drop every 10 minutes) was applied 2 hours before treatment. Twenty minutes before the beginning of treatment, the cornea was anesthetized using an anesthetic eyewash (oxybuprocaine hydrochloride 0.2%) at a dose of 1 drop every 5 minutes four times. To reduce the risk of UV exposure to the ocular structures behind the iris, serrated miosis was induced with pilocarpine 1% (Allergan, Rome, Italy) 30 minutes before treatment.

The UVA light emitter was switched on 2 hours before the start of treatment. All treatments were performed at a power of 2.9-3

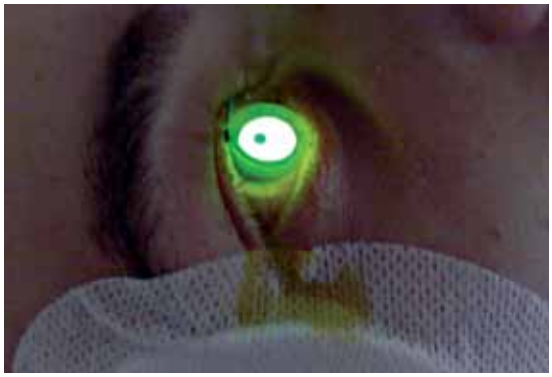

Figure 3.—Treatment with Ricrolin TE®.

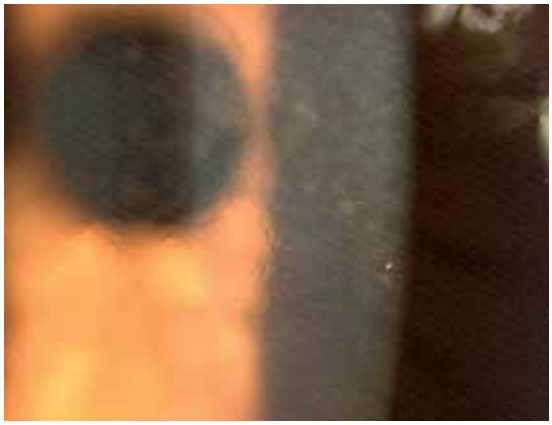

Figure 4.—Corneal epithelium at completion of treatment. Note the slight epithelial fissures without signs of corneal abrasion.

Figure 5.—The same patient on postoperative day 2. Note the perfectly transparent epithelium and the complete absence of corneal damage.

mW/cm^2, as measured with a UV meter onboard the machine. The cornea was irradiated at a point measuring 8 mm in diameter.

Treatment was performed in an eye clinic. The patient was seated in a reclining chair and the periocular skin was disinfected with 10% iodioprovidone (Betadine ophthalmic solution, Med Pharma, Milan, Italy). Surgical drapes were not used.

The corneal ring was positioned over the cornea, kept in place by eyelid pressure on the flange and filled with 2 drops of Ricrolin TE® directly in contact with the corneal epithelium until the entire corneal apex was covered. If eye blinking caused some of the product to spill out, it was re-administered to ensure that the corneal epithelium was uniformly covered with riboflavin.

To ensure good saturation of the corneal stroma through the intact cornea, the corneal ring was left in direct contact with the cornea for 15 minutes.

Following these preparations, CXL was performed with irradiation for 30 minutes, according to the treatment protocol designed by Prof. Caporossi *et al.*[13] During irradiation, the level of Ricrolin TE® was kept uniform, adding a drop every 3-5 minutes on average (Figures 1-3). A simple sterile adhesive bandage was applied alongside the eye to absorb any Ricrolin TE® that spilled out during treatment.

At the end of the procedure, the silicone device was removed and the eye was irrigated with BSS to rinse away any residual Ricrolin TE®.

The eye was then treated with 1 drop of norfloxacin and eye drops (0.15% hyaluronic acid plus amino acids [BLUyal A, Sooft Italia, Montegiorgio [AP], Italy). The patient felt only a mild foreign body sensation that was easily managed with eye drops.

Assessment was performed using split lamp examination to assess epithelial integrity (Figure 4, 5), and the patient was discharged with a prescribed therapy of norfloxacin (1 drop/3 times daily), 0.15% sodium hyalronate plus amino acids (1 drop, 3 times a day for 20 days), and a liposome-based spray containing vitamins A and C (Lacrisek spray, Bioos,

TABLE I.—*Uncorrected visual acuity and best-corrected visual acuity.*

	Pre CXL		1 month		3 months		6 months		9 months	
	CXL	Controls	CXL	Controls	CXL	Controls	CXL	Controls	CXL	Controls
UCVA	0.71±0.12	0.84±0.23	0.49±0.12	0.81±0.18	0.40±0.15	0.80±0.09	0.36±0.19	0.85±0.10	0.36±0.07	0.88±0.13
BCVA	0.35±0.23	0.46±0.21	0.26±0.10	0.48±0.29	0.22±0.08	0.50±0.06	0.18±0.16	0.62±0.08	0.16±0.10	0.66±0.11

PRE CXL denotes visual acuity before TE CXL treatment; UCVA uncorrected visual acuity; BCVA best-corrected visual acuity; LogMar logarithm of minimum angle of resolution; P>0.05.

TABLE II.—*Changes in central keratometric values (3 mm).*

	Pre CXL		1 month		3 months		6 months		9 months	
	CXL	Controls	CXL	Controls	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	47,82±0,78	51,40±0,92	47,85±0,71	51,32±1.13
Sim kF	45,13±0.97	46,12±0.99	44,46±1.03	46,12±0.65	44,13±0.89	46,52±0.91	44,57±1,11	46,74±0.71	47,85±0.84	46,23±0.50

SimkS denotes steeper meridian keratometry; SimkF flatter meridian keratometry; Sim cyl corneal cylinder (K1 – K2); P<0.05.

TABLE III.—*Keratoconus apical keratometry (KcAK) and CLMI (Ma, Mc).*

	Pre CXL		1 month		3 months		6 months		9 months	
	CXL	Controls	CXL	Controls	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	57,31±0,78	59,86±2.45	57,56±1.21	60,06±1.13
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	55,49±1,11	57,59±2.02	55,51±1.23	58,18±0.93
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	20,01±2.02	23,21±0.67	20,12±1.22	23,81±0.88

KcAK denotes keratoconus apical keratometry; CLMI cone location and magnitude indices; Ma axial magnitude; Mc apical magnitude; P<0.05, *P>0.05.

Italia, Montegiorgio [AP], Italy), 1 application with eyes closed, 3 times a day for 20 days.

Statistical analysis

Statistical analysis was performed using the Statistica software package, version 8 (Statsoft Inc, Tulsa, OK, USA). The data were analyzed as the mean and the standard deviation in the treated eyes and the untreated eyes as controls. Statistical significance was set at P<0.05.

Results

Visual acuity

Table I reports the pre- and post-treatment values for visual acuity up to and including 9 months of follow-up. Postoperative UCVA and BCVA values began to improve from post-treatment month 1 and continued up to month 6. BCVA continued to improve up to month 9. In the untreated eye, a progressive deterioration of both UCVA and BCVA values was noted, especially after month 6, which marked a sign in disease progression. UCVA appeared to worsen significantly less than BCVA (P<0.05).

Table II reports 3-mm keratometric data as measured on topography (simulated keratometry) at baseline and at 1, 3, and 6 months post-treatment. Simulated keratometry (simKS, simKF) and corneal astigmatism (sim k cyl) are also reported. The data were calculated by the topographer in a central area measuring 3 mm.

TABLE IV.—*Changes in corneal aberrations, RMS, comatic and spherical aberrations.*

	Pre CXL		1 month		3 months		6 months		9 months	
	CXL	Controls	CXL	Controls	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	3,01±0,38	4,56±2.45	3,21±0.45	4,71±1.02
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	1,65±1,01	2,41±1.88	1,59±1.23	2,39±0.93
S.A.	0,98±0.15	1,12±.052	0.77±0.42	1,08±0.67	0,45±0.59	1,26±0.72	0,45±0.39	1,26±0.47	0,35±0.64	1,31±0.98

RMS denotes root mean square; SA spherical aberration; P<0.05.

Figure 6.—OCT SLO image of the cornea after treatment.

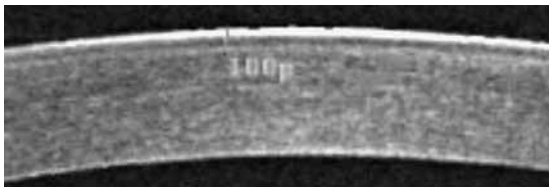

Figure 7.—OCT SLO image of the cornea after treatment.

Table III reports changes in keratoconus apical keratometry (KcAK) and CMLI data generated by the software for studying keratoconus progression.

Table IV reports changes in corneal aberration. The root mean square (RMS) together with the corneal and spherical aberrations gradually improved starting from month 1 to month 6, after which they appear to have stabilized. In the untreated eyes, aberrometric values continued to deteriorate over the entire follow-up period.

Corneal OCT

Comparison of corneal OCT values before and after treatment showed a linear thickening of the corneal stroma at approximately 100 micron from the corneal epithelium (Figure 6).

This alteration in the corneal structure after TE CXL treatment was visible at about 1 month post-treatment. Thickening was observed just under Bowman's membrane (Figure 7).

Pachymetry

The minimal corneal thickness of the treated eye at baseline was 412.9±21.5 mm and 423.3±12.2 mm in the untreated eye. At the end of follow-up, the minimal corneal thickness was 410.3±15.3 mm in the treated eye and 409±16.5 mm in the untreated eye. The difference was not statistically significant (P<0.5).

Endothelial cell count

Mean baseline endothelial cell count was 2427±236.4 cells/mm^2 in the worse eye and 2523±198.2 cells/mm^2 in the fellow eye; the difference was not statistically significant (P<0.5).

Corneal transparency

The cornea remained perfectly transparent over the entire follow-up period; no signs of post-treatment haze or subedema typically following deepithelialization were observed.

Postoperative side effects

In the first 24 hours posttreatment, 8 patients reported conjunctival hyperemia and a foreign body sensation, which was resolved with artificial tear eye drops. Of these 8 patients, 2 also referred experiencing slight photophobia which spontaneously resolved 4 days later.

Postoperative complications

No treatment-related complications were observed during the entire follow-up period.

Conclusions

Within the limitations of this small patient sample, it can be stated that TE CXL with Ricrolin TE® proved effective in slowing keratoconus progression over the mid-term. No side effects occurred during the intervention or the follow-up period.

Treatment without deepithelilization and with the use of a silicon corneal ring was extremely simple to perform, offering distinct advantages for both patient and surgeon alike:

— Corneal crosslinking with Ricrolin TE® need not be performed in an operating room.

— Cases of keratoconus with a minimum corneal thickness of 380 micron, and probably even less, may be treated. Pre-operative visual acuity is maintained. Patients under age 10 years may be treated.

— No pain during the posttreatment period. No deepithelialization-related complications.

— The silicon corneal ring obviates the need for a blepharostat and improves patient compliance owing to minimal eyelid blinking.

— The corneal ring holds the Ricrolin TE® and increases the contact time with the corneal epithelium. The corneal ring protects the limbus corneae and stem cells against exposure to UVA radiation.

— Optimal patient compliance. Treatment with an intact epithelium prevents postoperative pain due to reepithelialization.

Our patients were able to return to work or school the same day several hours after the procedure. Analysis of results at one month follow-up showed a net improvement in topographic indices, which was statistically significant with respect to the control eye. Improvement continued, though to a lesser extent, from months 3 to 6. A reduction in keratoconus apical keratometry (KcAK) and a general improvement in the other topographic indices, particularly in CLMI were observed (Figures 8, 9).

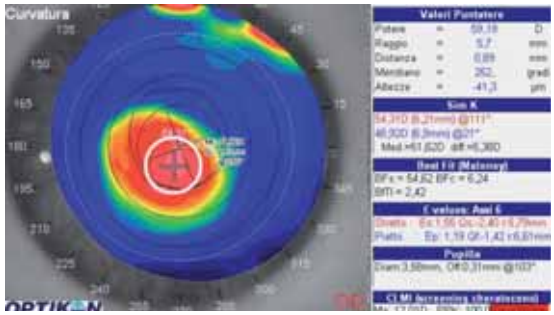

Figure 8.—Corneal topography before TE CXL treatment.

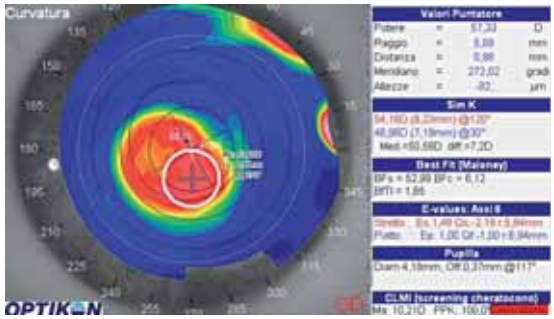

Figure 9.—The same patient at 9 month-follow-up assessment. Note the net reduction in keratoconus apical keratometry (KaCK) from 59.19 to 57.33 D and the reduction in the MA index from 12.01 to 10.21 D.

Corneal stroma analysis by OCT showed a linear formation of stroma thickening approximately 80-100 microns from the epithelial surface. This change in corneal stroma differs from outcomes after CXL with deepithelialization. Anatomically, the two types of CXL treatment do not produce the same results but may be considered complementary to one another.

With this innovative technique, crosslinks are induced in the upper third of the corneal stroma, 20-30 microns below Bowman's membrane, whereas the crosslinking effect of the technique with deepithelialization occurs at approximately 240 microns.

Our results require further study; even so, they open a new perspective in the treatment of keratoconus insofar as the aim of TE CXL is not to replace the conventional technique but rather to complement it. Thanks to the possibility to treat without deepithelization, and without related postoperative seque-

lae, the effect of conventional CXL can be strengthened in cases in which progression of keratoconus cannot be completely halted.

Furthermore, treatment eligibility may be extended with TE CXL as it allows greater patient compliance, even in younger children (our series included 2 patients aged 12 years), so as to intervene at an earlier stage of the disease, slowing its progression in adolescence (14-15 years of age), when CXL with deepithelialization may be contemplated in the attempt to definitively halt keratoconus progression.

Of special note is that one of our patients was a young Down patient (trisomy 21 syndrome), demonstrating that TE CXL may also be performed in "complicated" patients who would be otherwise ineligible for a more invasive technique like that of CXL with deepithelialization.

Riassunto

Cross-Linking del collagene corneale senza disepitelizzazione

Obiettivo. Obiettivo del presente lavor è stato quello di studiare l'efficacia del Cross Linking Trans Epiteliale in pazienti con cheratocono evolutivo.

Metodi. Sono stati arruolati pazienti con storia documentata di cheratocono evolutivo; è stato trattato l'occhio peggiore, mentre l'occhio adelfo è stato utilizzato come controllo. L'intervento di Cross-linking transepiteliale (CXL TE) è stato effettuato attraverso 6 steps di irraggiamento mediante sorgente laser CBM X-linker VEGA® e instillazione di Ricrolin TE® (Riboflavina 0.1%, destrano T500 ed enhancer per aumentare il passaggio del composto attraverso l'epitelio corneale) ogni 5 minuti, previa due ore di imbibizione con Ricrolin TE dell'occhio da trattare.

Risultati. Nel gruppo trattato con CXL TE, si è evidenziato un miglioramento progressivo dei valori topografici ed aberrometrici senza haze ne altri effetti collaterali. I ponti di cross-linking visibili con la microscopia confocale a partire da cinque mesi dopo il trattamento. Viceversa, nel gruppo di controllo si è evidenziato un peggioramento di tutti gli indici.

Conclusioni. Il CXL Trans Epiteliale si è dimostrato pienamente efficace nel rallentare l'evoluzione del cheratocono, migliorando in modo statisticamente significativo l'evoluzione della malattia. Grazie al trattamento trans epiteliale e all'assenza di effetti collaterali, il CXL TE permette il trattamento di cheratoconi con spessore corneale inferiore a 400 micron, pazienti sotto i 12 anni e "complicati" (es. Trisomia 21), soggetti con più di 35 anni o con un K medio superiore a 55. A differenza del CXL tradizionale, grazie alla conservazione dell'epitelio, il CXL TE garantisce una miglior compliance del paziente con assenza di dolore post-trattamento e senza peggioramento del visus ne haze post operatorio. In conclusione il CXL TE è un nuovo tipo di trattamento che agisce sul terzo anteriore della cornea e che grazie all'assenza di effetti collaterali e alla facilità di esecuzione si affianca al trattamento di CXL tradizionale che, viceversa, agisce nel terzo posteriore della cornea.

Parole chiave: Collagene - Reagenti per crosslinking - Cornea.

References

1. Spoerl E, Huhle M, Seiler T. Induction of cross-links in corneal tissue. Exp Eye Res 1998;66:97-103.
2. Spörl E, Huhle M, Kasper M, Seiler T. Increased rigidity of the cornea caused by intrastromal cross-linking. Ophthalmologe 1997;94:902-6.
3. Wollensak G, Spoerl E, Seiler T. Stress-strain measurements of human and porcine corneas after riboflavin-ultraviolet-A induced cross-linking. J Cataract Refract Surg 2003;29:1780-5.
4. Wollensak G, Spoerl E, Seiler T. Riboflavin/ultraviolet-A induced collagen crosslinking for the treatment of keratoconus. Am J Ophthalmol 2003;135:620-7.
5. 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;35:893-9.
6. Mazzotta C, Balestrazzi A, Baiocchi S, Traversi C, Caporossi A. Stromal haze after combined riboflavin-UVA corneal collagen cross-linking in keratoconus: in vivo confocal microscopic evaluation. Clin Experiment Ophthalmol 2007;35:580-2.
7. Vinciguerra P, Albè E, Trazza S, Rosetta P, Vinciguerra R, Seiler T *et al*. Refractive, topographic, tomographic, and aberrometric analysis of keratoconic eyes undergoing corneal cross-linking. Ophthalmology 2009;116: 369-78.
8. Caporossi A, Baiocchi S, Mazzotta C, Traversi C, Caporossi T. Parasurgical therapy for keratoconus by riboflavin-ultraviolet type A induced cross-linking of corneal collagen: preliminary refractive results in an Italian study. J Cataract Refract Surg 2006;32:837-45.
9. Nahas G G, Sutin K M, Fermon C, Streat S, Wiklund L, Wahlander S *et al*. Guidelines for the treatment of acidaemia with THAM. Drugs 1998;55:191-224.
10. Bailey I, Lovie J. New design principles for visual acuity letter charts. Am J Optom Physiol Opt 1976;53:740-5.
11. Ferris FL III, Kassoff A, Bresnick GH, Bailey I. New visual acuity charts for clinical research. Am J Ophthalmol 1982;94:91-6.
12. Mahmoud AM, Roberts CJ, Lembach RG, Twa MD, Herderick EE, McMahon TT. CLMI: the cone location and magnitude index. CLEK Study Group. Cornea 2008;27:480-7.
13. Mazzotta C, Traversi C, Baiocchi S, Sergio P, Caporossi T, Caporossi A. Conservative treatment of keratoconus by riboflavin-uva-induced cross-linking of corneal collagen: qualitative investigation. Eur J Ophthalmol 2006;16:530-5.