Collagen crosslinking without corneal de-epithelialization

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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 imbibition with Ricrolin TE® in the worse eye.

Results. A gradual improvement in topographic and aberrometric 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.
needs to be deepithelialized to a depth of at least 8 mm in diameter for imbition of the stroma to be effective.\(^5\)

The early postoperative period after CXL with deepithelialization is extremely painful, at least as painful as photorefractive keratectomy (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\textsuperscript{c}, 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 extracellular alkalizing action. Although widely used, sensitization to the product has rarely been reported; only in one case was a gel containing tromethamol associated with peribital 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 slit lamp examination, ultrasound and optic pachymetry, topography and corneal aber-
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
mW/cm², as measured with a UV meter on board 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.

<table>
<thead>
<tr>
<th></th>
<th>Pre CXL</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CXL</td>
<td>Controls</td>
<td>CXL</td>
<td>Controls</td>
<td>CXL</td>
</tr>
<tr>
<td>UCVA</td>
<td>0.71±0.12</td>
<td>0.84±0.23</td>
<td>0.49±0.12</td>
<td>0.81±0.18</td>
<td>0.40±0.15</td>
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<tr>
<td>BCVA</td>
<td>0.35±0.23</td>
<td>0.46±0.21</td>
<td>0.26±0.10</td>
<td>0.48±0.29</td>
<td>0.22±0.08</td>
</tr>
</tbody>
</table>

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).

<table>
<thead>
<tr>
<th></th>
<th>Pre CXL</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CXL</td>
<td>Controls</td>
<td>CXL</td>
<td>Controls</td>
<td>CXL</td>
</tr>
<tr>
<td>Sim kS</td>
<td>51.02±1.10</td>
<td>51.12±1.02</td>
<td>49.05±0.92</td>
<td>51.0±0.92</td>
<td>48.65±0.89</td>
</tr>
<tr>
<td>Sim kF</td>
<td>45.13±0.97</td>
<td>46.12±0.99</td>
<td>44.46±1.03</td>
<td>46.12±0.65</td>
<td>44.13±0.98</td>
</tr>
</tbody>
</table>

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

### Table III.— Keratoconus apical keratometry (KcAK) and CLMI (Ma, Mc).

<table>
<thead>
<tr>
<th></th>
<th>Pre CXL</th>
<th>1 month</th>
<th>3 months</th>
<th>6 months</th>
<th>9 months</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CXL</td>
<td>Controls</td>
<td>CXL</td>
<td>Controls</td>
<td>CXL</td>
</tr>
<tr>
<td>KcAK</td>
<td>59.12±1.10</td>
<td>58.89±2.02</td>
<td>58.01±0.92</td>
<td>58.92±2.34</td>
<td>57.42±0.89</td>
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<tr>
<td>Mc</td>
<td>56.46±0.97</td>
<td>56.31±1.93</td>
<td>55.73±1.41</td>
<td>56.29±2.18</td>
<td>55.52±0.89</td>
</tr>
<tr>
<td>Ma</td>
<td>23.89±0.75</td>
<td>21.91±0.05</td>
<td>20.07±2.42</td>
<td>21.98±1.67*</td>
<td>20.09±2.50</td>
</tr>
</tbody>
</table>

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 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/mm² in the worse eye and 2523±198.2 cells/mm² 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 deep epithelialization 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.

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

<table>
<thead>
<tr>
<th></th>
<th>CXL</th>
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<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMS</td>
<td>4.66±0.27</td>
<td>4.43±0.75</td>
<td>4.21±0.66</td>
<td>4.12±0.83</td>
<td>3.75±0.59</td>
<td>4.39±1.47</td>
<td>3.01±0.38</td>
<td>4.56±2.45</td>
<td>3.21±0.45</td>
<td>4.71±1.02</td>
</tr>
<tr>
<td>Coma</td>
<td>2.21±0.97</td>
<td>2.28±1.93</td>
<td>2.19±1.09</td>
<td>2.10±1.74</td>
<td>1.72±0.32</td>
<td>2.23±1.05</td>
<td>1.65±1.01</td>
<td>2.41±1.88</td>
<td>1.59±1.23</td>
<td>2.39±0.93</td>
</tr>
<tr>
<td>S.A.</td>
<td>0.98±0.15</td>
<td>1.12±0.02</td>
<td>0.77±0.42</td>
<td>1.08±0.67</td>
<td>0.45±0.59</td>
<td>1.26±0.72</td>
<td>0.45±0.39</td>
<td>1.26±0.47</td>
<td>0.35±0.64</td>
<td>1.31±0.98</td>
</tr>
</tbody>
</table>

RMS denotes root mean square; SA spherical aberration; P<0.05.
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 deepithelialization 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 microns, 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).

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 deepithelialization, and without related postoperative seque-
il 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 disepiteliolazione

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

**Metodi.** Sono stati arruolati patienti con storia documentata di cheratocono evolutivo; è stato trattato l’occhio peggiori, mentre l’occhio adelfo è stato utilizzato come controllo. L’intervento di Cross-linking non è stato effettuato attraverso 6 steps di irraggiamento mediante sorgente laser CBM X-linker VEGA® e installazione di Ricrolin TE® (Riboflavina 0.1%, destrano T500 ed enhancer per l’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 aberometrici 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 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 maggiore probabilità di successo nel trattamento di keratocono evolutivo.

**References**