ORIGINAL ARTICLE

Corneal collagen cross-linking in keratoconus — long-term prospective study

Cross-linking du collagène corneen dans le traitement du kératocône — étude prospective

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KEYWORDS
Keratoconus; Cross-linking therapy; Refractometry; Corneal collagen

Summary
Purpose. — To determine the refractometric, keratometric and functional results 3 years after cross-linking therapy in patients with keratoconus.
Patients and methods. — Eighty-one patients in various stages of keratoconus were studied, all meeting eligibility criteria for the cross-linking technique.
Results. — The results showed a decrease both in the K-values, of about 1.5 D, and in the refraction. Visual acuity improved by 1, 2 or 3 Snellen lines in 70\% of the cases.
Conclusions. — The cross-linking technique is a modern method for stopping the progression of keratoconus.

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MOTS CLÉS
Kératocône ; Cross-linking technique

Résumé
But. — Notre but est de mettre en évidence à la fois les résultats réfractométriques, kératométriques et fonctionnels, 3 ans après l’application du traitement de cross-linking technique chez les patients ayant un keratocône.

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Introduction

Over the past decade, corneal collagen cross-linking (CCL) has been introduced as a treatment that has been reported to slow or halt the progression of the keratoconus.

Corneal collagen cross-linking consist of the photopolymerization of the stromal fibrillar tissue in order to increase their stiffness and resistance to the keratectasia. This is possible through the combined action of the photosensibilising substance (riboflavin–B2) with the irradiation of the UV light type A (UVA) performed with an illuminator.

UVA acts upon the riboflavin which results in loss of internal chemical balance of the riboflavin molecule. Oxygen free radicals are being released causing instability of the molecule. This creates a stable-link between two collagen fibrils, strengthening the cornea.

Cross-linking has been reported to produce some effects on the cornea. It results in early apoptosis of keratocytes to a depth of 300 μm [1]. Confocal microscopy has demonstrated stromal oedema, loss of sub-epithelial nerve plexus and mid-stromal nerve fibres and increase reflectivity in the mid-stroma [2,3]. Between 3 and 12 months, after treatment, keratocytic repopulation is seen with a regeneration of the nerve plexus and lamellar compaction [4]. An increase in collagen fibre diameter has been demonstrated by electrophoretic studies [5], especially in the anterior stroma. Another effect is an increase in resistance to enzymatic degradation, which needs to increase a biomechanical stability of the cornea [6].

Purpose

Our purpose is to evaluate the functional results, keratometric data, spherical and cylindrical equivalent of the patients with keratoconus treated with collagen cross-linking therapy, at 1, 3, 6, 12, 24, 36 months after the procedure.

Material and method

This is a prospective study. The study group was composed of 100 eyes from 81 patients, eligible for inclusion criteria between January 2008–December 2013. The inclusion criteria were: patients between 15–54 years of age, both genders, diagnosed with different stages of keratoconus (stage I, II, III, III/IV), corneal thickness of at least 400 μm and transparent cornea.

The non-inclusion criteria were: patients with an average corneal thickness inferior to 400 μm, with Vogt striae, herpetic keratitis or/and other active ocular infection, patients with severe dry eye or aphakia.

The ocular exam before CCL consisted of:

- uncorrected visual acuity (UCVA) and best corrected visual acuity (BCVA);
- ocular refraction and keratometry;
- slit lamp examination;
- intraocular pressure measurement;
- pachymetry;
- corneal topography—Pentacam;
- endothelial corneal cell count.

CCL technique was the same of the SIENA Protocol ("Epi-off") and consisted of the following steps:

- opening the ophthalmic solution of riboflavin 0.1%—dextran 20% and after the verification of the power of the illuminator UVA array in a solid state CBMx linker with a UVA power meter;
- topical anesthesia: 3–4 drops, 15–20 min before CCL;
- removal of the corneal epithelium about a diameter of 9 mm and instillation of a drop of benoxi;
- instillation of riboflavin 0.1% every 3 min for 30 min before the irradiation;
- irradiation of the central cornea through the CBMx linker and instillation of riboflavin 0.1% every 3 to 30 min;
- instillation of antibiotic and anti-inflammatory eye drops;
- therapeutic contact lenses for 3–4 days after the procedure.

The follow-up was after 24, 48, 72 h and the treatment consisting of instillation of antibiotics, steroids and artificial tears for 2 and 3 months. The check-up was at 1, 3, 6, 12, 24, and 36 months regarding the visual acuity, keratometric data, cylinder and spherical equivalent.

For the statistical analysis, we used Student’s t-test for comparing the means of two samples. In simple terms, the t-test compares the actual difference between two means in relation to the variation in the data (expressed as the standard deviation of the difference between the means). We considered statistically significant as P value < 0.05 and statistically highly significant as P value < 0.001.
**Results**

The average age was between 20–40 years old (75%). The majority were males (60%). The most frequent stage was the second one.

The difference between the preoperative and postoperative spherical equivalent at 3 years was 1.04 D. The P value was statistically relevant after 1 month (P value at 1 month: 0.0102) (**Table 1**, **Fig. 1**).

The difference between the preoperative and postoperative cylinder at 3 years was 0.52 D. The P value was statistically relevant after 3 months (P value at 3 months: 0.0106) (**Table 1**, **Fig. 1b**).

The difference between the preoperative and postoperative keratometric values at 3 years was 0.76. The P value was statistically relevant at 3 months (P value at 3 months: 0.0498) (**Table 1**, **Fig. 1c**). The visual acuity, with and without correction, was improved at 12 months after the procedure and gained also at 36 months (**Figs. 2–4**).

Patients complained in the first 3 or 4 months after cross-linking of haze, which disappeared at 6 months postoperative. Visual acuity gained 1 or 2 Snellen lines after 6 months postoperative and at 1, 2 and 3 years BCVA doubled (P value was statistically relevant at 6 months after the procedure).

**Discussion**

The advantages of cross-linking are multiple. This is a parasurgical procedure, which produces a slowdown of the Kc progression, prevents or delays the need of corneal graft, is less invasive and easy to perform; produces lack of scaring and has easy availability of riboflavin. Of course, there are known risks, as following: no side effects for the corneal endothelium, lens and retina; the post-surgery pain last about 24–48 h, can cause transitory corneal oedema and

**Table 1** The P value at 1, 3, 6, 12, 24, 36 months for spherical equivalent, cylinder and K-values.

<table>
<thead>
<tr>
<th>Months</th>
<th>ES</th>
<th>Cylinder</th>
<th>Media k</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>0.010273875</td>
<td>0.16642564</td>
<td>0.466462424</td>
</tr>
<tr>
<td>3</td>
<td>0.000613521</td>
<td>0.010628458</td>
<td>0.049872401</td>
</tr>
<tr>
<td>6</td>
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<td>0.009564375</td>
<td>0.004203433</td>
</tr>
<tr>
<td>12</td>
<td>0.0019395034</td>
<td>0.002795211</td>
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</tr>
<tr>
<td>24</td>
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<td>0.003878305</td>
<td>0.0001876689</td>
</tr>
<tr>
<td>36</td>
<td>0.0030754409</td>
<td>0.003799381</td>
<td>0.000286241</td>
</tr>
</tbody>
</table>

ES: spherical equivalent; media K: keratometric media. In italics P value < 0.05 - statistically significant.

**Figure 1.** a: distribution of the cases regarding the spherical equivalents; b: distribution of the cases regarding the cylinder; c: distribution of the cases regarding the keratometry.
visual haze. The technique does not exclude the possibility of keratoplasty.

The main indication for the use of collagen cross-linking is to inhibit the progression of corneal ectasias, such as keratoconus and pellucid marginal degeneration [7–9]. Collagen cross-linking also is effective in the treatment and prophylaxis of iatrogenic keratectasia resulting from Lasik [10].

Riboflavin has a good stromal penetration, absorption and concentration of UV radiation. It is a photosensitizing agent for the production of reactive oxygen radicals and it has endothelial protection. Cross-linking technique can be combined with intrastromal corneal rings (ICR), Intacs and limited photo refractive keratectomy (PRK) topoguided.

In vitro studies showed that the UVA light arriving to an intact cornea is absorbed within its lamellae by approximately 30%, while an additional 50% of the UVA absorption occurs in the lens [11]. On the other hand, in the presence of riboflavin acting as a photosensitizer, the cornea absorbs a considerable amount of UV light. Thus, using the irradiance of 3 mW/cm² of UVA and 0.1% riboflavin, as much as 95% of UVA light will be absorbed within the cornea. This results in a 20-fold reduction of the original irradiance from 3 mW/cm² of UVA (at the corneal surface) to 0.15 mW/cm² (at the endothelial level), which is below the cytotoxic threshold for endothelium of 0.36 mW/cm² [7–9]. For the sake of comparison, the same UVA irradiance at the corneal surface, as used in this study, can be measured at noon during an average sunny summer day in the tropics (23° latitude and 800 m above sea level). Even with the expected reduction of irradiance from the corneal surface towards deeper layers of corneal stroma, as described above, the irradiation levels still exceed the threshold to a depth of approximately 300 μm. Keratocyte apoptosis in the anterior segment of the corneal stroma has therefore been described, and a demarcation line between the treated and untreated cornea has been clearly shown in in vitro studies [7–9]. Confocal microscopy studies also show that repopulation of keratocytes is visible 1 month after treatment, reaching its preoperative quantity and quality in terms of functional morphology within 6 months after treatment [12].

The first in vivo controlled clinical study, which included 23 eyes with moderate or advanced progressive keratoconus, showed that collagen cross-linking was effective in halting the progression of keratoconus over a period of up to 4 years.

Figure 2. a: UCVA preoperative and at 1 month; b: BCVA preoperative and at 1 month; c: UCVA preoperative and at 3 months; d: BCVA preoperative and at 3 months.
In this study, a mean preoperative progression of keratometry (max K) by 1.42 D in 52% of eyes over a 6-month period immediately prior to treatment was followed by a postoperative decrease in 70% of eyes. The statistics also revealed a reduction of max K by 2.01 D, whereas the postoperative spherical equivalent refraction was reduced by an average of 1.14 D. Also during this time, 22% of the untreated fellow control eyes had average postoperative progression of keratectasia of 1.48 D. Our results confirm previous findings: the group treated with collagen cross-linking showed a similar mean decrease in spherical equivalent refraction.

Tan et al. [14] described a new approach for treating keratoconus—intra-lamellar keratoplasty, where the donor tissue is tuck-folded as a 250 μm lamella through a 3.0 mm incision into a corneal pocket previously prepared by femtosecond laser. This method produced a comparable reduction of spherical equivalent refraction, cylinder, and max K by 1.13 D, 1.82 D, and 1.14 D, respectively. Compared to the study by Tan et al. [14], Miranda et al. [15] reported a higher reduction of spherical equivalent refraction and max K by more than 2.50 D and 6.00 D, respectively, using Ferrara rings. Their study included 26 eyes diagnosed with keratoconus grades III and IV. The Ferrara rings flattened the central and peripheral cornea, thus, displacing the corneal apex to its physiological position in front of the pupil by reducing the paracentral ectasia commonly seen in keratoconic corneas.

Colin [16] compared two groups, one using corneal collagen cross-linking with ICR and the other one using just ICR. He had greater reduction in cylinder and keratometry than ICR alone, which testifies the effect of cross-linking. The study of Henriquez et al. [17], who used cross-linking before ICR, had better results than our study. It demonstrates the additional effect of ICR, as following: UCVA preoperatively was 1.11 logMAR, after CXL 0.75 logMAR P = 0.03, and 0.23 logMAR after 6 months after ICR implantation P < 0.001; BCVA at 1 year was 0.05; spherical equivalent had a P value < 0.001; keratometric values at 6 months after ICR implantation were statistically decreased P < 0.01. Aylin et al. [18] used riboflavin injection into the corneal channel for combined collagen cross-linking and ICR segment implantation; both UCVA and BCVA had a P value < 0.05; SE decreased with 2.5 D; cylinder decreased with 2 D; mean K with 4.51 D. All the results were better than ours, which proves the positive simultaneous effect that riboflavin has when ICR is implanted.
When compared to other treatment methods for keratoconus, such as penetrating keratoplasty, deep lamellar keratoplasty, or intracorneal rings, collagen cross-linking shows only a modest reduction in spherical equivalent refraction, cylinder and max K. However, the collagen cross-linking method used in the present study stops or slows down, rather than reverts, the progression of keratoconus. The small regression that occurred may be explained as an effect of the rearrangement of corneal lamellae and the surrounding matrix [11]. Due to an increased number of cross-linking sites within the collagen molecule after collagen cross-linking, stiffer fibrils and lamellae are likely generated. This process produces a rearrangement of corneal lamellae and the consequent relocation of the surrounding matrix, which, in turn, results in the reduction of the central corneal curvature.

Although collagen cross-linking resulted in a decrease of spherical equivalent refraction, astigmatism, and max K, UCVA and BSCVA increased modestly up to one line. Other studies with alternative treatment methods showed more than two-line increase in BSCVA after implantation of Ferrara rings INTACS and deep lamellar penetrating keratoplasty [19]. This leads us to the following hypothesis: if collagen cross-linking treatment stops or slows the progression of keratoconus, whereas other methods reshape the cornea, a logical solution would be to combine the two treatment methods to synergize their effect. Pretreatment with an alternative method would significantly reshape the cornea by flattening and regularizing it, which would be followed by collagen cross-linking to stabilize the cornea in this newly achieved state. Alternatively, the collagen cross-linking procedure could be done first, followed by a reshaping procedure.

Collagen cross-linking has also been used successfully in stopping the advancement of iatrogenic ectasia in eyes after excimer laser ablation. In a recently published study [20], collagen cross-linking was performed in 10 patients with a formerly undiagnosed keratoconus or pellucid marginal corneal degeneration who underwent LASIK for myopic astigmatism and subsequently developed iatrogenic keratectasia. Collagen cross-linking led to an arrest and/or partial reversal of keratectasia over a postoperative follow-up period of up to 25 months as demonstrated by pre- and postoperative corneal topography and reduction of maximal K-readings.

Riboflavin/UVA collagen cross-linking appears to be an efficacious procedure in inhibiting the progression of irregular astigmatism due to keratoconus while reducing
the corneal curvature, spherical equivalent refraction, and refractive cylinder in eyes with corneal instability due to progressive keratoconus.

Conclusions

Cross-linking represents a true progress in the treatment of keratoconus. The procedure slows or stops the progression of keratoconus. Our results showed a decrease in the k-values, spherical equivalent and cylindrical values, 6 months after the procedure. Regarding the visual acuity, our patients gained 1–2 lines Snellen charts 1 year after the surgery. Long-term results are necessary to evaluate the duration of the stiffening effect and to exclude the long-term side effects.

Disclosure of interest

The authors declare that they have no conflict of interest concerning this article.

References