ORIGINAL ARTICLE

Corneal collagen cross-linking for the treatment of progressive keratoconus: 3-year prospective outcome

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ABSTRACT • RÉSUMÉ

Objective: To assess the long-term effects of treatment of progressive keratoconus with ultraviolet A-riboflavin collagen cross-linking (CXL).

Design: This was a prospective clinical study.

Participants: Seventeen eyes of 17 patients with progressive keratoconus were treated with CXL.

Methods: Patients were examined preoperatively, at week 1, months 1, 3, 6, 9, 12, 24, and 36 after treatment. We assessed uncorrected visual acuity (UCVA) and best spectacle-corrected visual acuity (BSCVA), refraction, biomicroscopy and fundus appearance, intraocular pressure, endothelial cell density (ECD), corneal topography, minimal corneal thickness (MCT), macular optical coherence tomography, axial length, and corneal biomechanics with the ocular response analyzer.

Results: Comparing the 36-month time point results with pretreatment values, we found that UCVA and BSCVA were unchanged. Steepest meridian keratometry (D) and mean cylinder (D) did not show significant change compared with pretreatment values but showed a slight increase as compared with the 24-month time point (53.9 vs 51.7 vs 52.5, and 10.5 vs 8.1 vs 9.2 before, at 24 months, and at 36 months, respectively). Axial length (mm) showed an elongation trend throughout the follow-up period (24.56 vs 24.61 [$p = 0.04$] vs 24.71 [$p = 0.05$], before, at 24 months, and at 36 months, respectively). No significant change was observed in ECD, corneal hysteresis and corneal resistance factor, MCT, or foveal thickness.

Conclusions: Three-year results after CXL show stable visual acuity, stable corneal thickness, and stable corneal biomechanical parameters. The decreasing trend in keratometry values that was observed during the first 2 years after CXL was no longer evident. Longer follow-up is needed to decide whether it is a first sign of loss of achieved stability and resumption of keratoconus progression.

Objet : Évaluation des effets à long terme du traitement du kératocône progressif avec la réticulation du collagène par Riboflavine UVA. (RC)

Méthodes : Étude clinique prospective. Dix-sept yeux de 17 patients ayant un kératocône progressif ont été soignés avec la RC. Ils ont été examinés avant l'opération, puis 1 semaine, 1, 3, 6, 9, 12, 24 et 36 mois après le traitement. Nous avons évalué l'AVSC et la MAVC, la réfraction, la biomicroscopie et l'apparence du fond d'oeil, la TIO, la densité cellulaire endothéliale (DCE), la topographie cornéenne, l'épaisseur minimale de la cornée (EMC), la TCO maculaire, la longueur axiale et les biomécaniques de la cornée avec l'analyseur de la réaction oculaire.

Résultats : La comparaison des résultats de point après 36 mois avec les valeurs préopératoires nous a indiqué que l'AVSC et la MAVC n'avaient pas changé. Kmax (D) et Kcyl (D) ne présentaient pas de changement significatif comparativement aux valeurs prétraitement, mais montraient une légère augmentation comparativement aux points du 24^{e} mois. (53,9vs51, 7vs52,5, et 10,5vs8,1vs9,2; avant, à 24 mois et à 36 mois respectivement). La longueur axiale (mm) montrait une tendance à l'élongation pendant la période de suivi (24,56vs24,61 (P = 0,04) vs 24,71 (P = 0.05); avant, à 24 mois et à 36 mois respectivement). Aucun changement modificatif n'a été observé concernant la DCE, l'hystérésis de la cornée, le facteur de résistance cornéenne, l'EMC ou l'épaisseur de la fovéa.

Conclusions : Les résultats des trois années de suivi de la réticulation du collagène ont démontré une triple stabilité, soit l'acuité visuelle, l'épaisseur de la cornée et les paramètres biomécaniques de la cornée. La tendance à la baisse des valeurs kératométriques observée pendant les deux premières années suivant le traitement n'était plus évidente. Il faudra un suivi plus long pour décider s'il s'agit d'un premier signe de perte de stabilité réalisée et de reprise de la progression du kératocône.

Keratoconus is a bilateral, progressive, noninflammatory corneal degeneration.[1] Corneal deformation and thinning causes irregular astigmatism and leads to visual impairment.[2] Treatment modalities are based on refractive correction with spectacles, contact lenses, and intrastromal corneal rings to correct astigmatism and restore visual acuity.[3] Such modalities do not stop ectatic progression and further visual deterioration, which ultimately necessitate corneal transplantation in 10% to 20% of patients.[4]

Corneal collagen cross-linking (CXL) using ultraviolet A light (UVA) and riboflavin was introduced by Wollensak et al.[5] as a method to halt the progression of keratoconus. Recent clinical studies evaluated the efficacy of this new treatment modality.[6–10] The aim of this report is to

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Originally received Feb. 8, 2013. Accepted Sep. 13, 2013

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Can J Ophthalmol 2014;49:54–59
0008-4182/14/$-see front matter
http://dx.doi.org/10.1016/j.jcjo.2013.09.002

Table 1—Visual acuity and refractive error before and after CXL

Parameter	Before CXL	12 mo after CXL	24 mo after CXL	36 mo after CXL
Mean BSCVA ± SD (logMAR)	0.18 ± 0.1	0.13 ± 0.1	0.14 ± 0.1	0.14± 0.1 ($p = 0.14$)
Mean UCVA ± SD (logMAR)	0.65 ± 0.4	0.79 ± 0.5	0.82 ± 0.5	0.74 ± 0.4 ($p = 0.73$)
Mean SE ± SD (D)	–4.4 ± 3.4	–4.0 ± 3.3	–4.0 ± 3.3	–2.7 ± 2.6 ($p = 0.01$)

CXL, corneal collagen cross-linking; BSCVA, best corrected visual acuity with glasses; UCVA, uncorrected visual acuity; SE, spherical equivalent.

describe the observed changes in keratoconic eyes after 3 years of close follow-up after CXL treatment.

Methods

Patients with keratoconus were prospectively recruited from the cornea outpatient clinic of the Assaf Harofeh Medical Center. Included were subjects with progressive keratoconus confirmed by an increase of at least 1.5 D in astigmatic refraction and/or maximum curvature documented by corneal topography at 3 time points within the past 12 months. Other inclusion criteria were age older than 18 years, no previous ocular surgery, no corneal opacities, minimal corneal thickness (MCT) of 400 μm, and avoidance of contact lens wear for 1 month before initial evaluation and treatment. Patients were treated with UVA-riboflavin CXL under aseptic conditions using topical preoperative anesthaesia with oxybuprocaine hydrochloride 0.4% drops (Localin; Fisher Pharmaceutical Labs). Treatment included 7-mm diameter corneal de-epithelization, instillation of riboflavin 0.1% in dextran 20% solution (Peschke Meditrade GmbH, Switzerland) every 5 minutes for 40 minutes and corneal irradiation with UVA 3 mW/cm^2 (UV-X; Peschke Meditrade GmbH, Switzerland) for 30 minutes, 5 cm from the cornea with persistent repeated application of 0.1% riboflavin in 20% dextran solution drops. After the procedure, patients were treated with a topical antibiotic (ofloxacin 0.3% [Oflox]; Allergan) 4 times a day for 7 days and a topical corticosteroid (dexamethasone 0.1% [Sterodex]; Fisher Pharmaceutical Labs) 4 times a day for 1 month, and the eye was dressed with a soft therapeutic contact lens (Ocular Sciences Ltd, Southampton, U.K.) for 3 days. UV irradiance was checked preoperatively in each case using a UV meter.

Patients were assessed preoperatively and at week 1, months 1, 3, 6, 12, 24, and 36 after treatment. Each examination included measurement of uncorrected visual acuity (UCVA), best spectacle-corrected visual acuity (BSCVA), and slit-lamp and dilated fundus examination. Corneal topography, pachymetry, endothelial cell density (ECD), intraocular pressure by Goldmann applanation tonometry (GAT-IOP), central foveal thickness (CFT), and corneal biomechanical parameters according to the ocular response analyzer (ORA; Reichert Inc, Buffalo, NY) were assessed as follows. Corneal topography and pachymetry were assessed preoperatively and at months 6, 9, 12, 24, and 36 with Orbscan II (Bausch & Lomb, Claremont, Calif.). ECD was assessed preoperatively and at months 1, 6, 12, 24, and 36 with the Konan Noncon Robo SP 6000 noncontact specular microscope (Konan Medical Inc, Hyogo, Japan). CFT was assessed preoperatively and at months 3, 6, 9, 12, 24, and 36 with Stratus optical coherence tomography (OCT; Zeiss Humphrey Instruments, Dublin, Calif.). Axial length was assessed preoperatively and at months 12, 24, and 36 with IOL Master (Carl Zeiss Meditec AG, Jena, Germany). Users of contact lenses were asked to remove them 14 days before each follow-up examination.

The study was approved by the institutional ethics committee of Assaf Harofeh Medical Center, and a written informed consent was obtained from each subject after the nature and intent of the study had been fully explained. The study protocol was consistent with the tenets of the Declaration of Helsinki.

The data are presented as mean ± SD. Paired 2-tailed Student *t* test was used to assess differences in respective parameters. The distributions of values within each data set were evaluated graphically. A p value of 0.05 was selected for the threshold of statistical significance. Analyses were performed using Excel (Microsoft Corp, Redmond, WA).

Results

Seventeen eyes of 17 patients (12 males, 5 females) aged 27.3 ± 5.1 years were included. The UCVA, BSCVA, and subjective spherical equivalent refraction (SE) data are summarized in Table 1. BSCVA was statistically significantly better at 12 months compared with the preoperative data ($p = 0.04$) and remained better than preoperatively at 24 and 36 months, although without statistical significance (Fig. 1). UCVA did not show a statistically significant change throughout the follow-up period as compared with baseline. Mean SE decreased continuously and significantly during the 36-month follow-up ($p = 0.01$).

The steepest meridian keratometry (Kmax) and mean cylinder (Kcyl = Kmax – Kmin) showed an initial tendency to decrease continuously over the first 24 months with changes being statistically significantly different from baseline (Kmax at 12 months: $p = 0.002$, at 24 months: $p = 0.007$; Kcyl at 12 months: $p = 0.018$, at 24 months: $p = 0.007$). Evaluation after 36

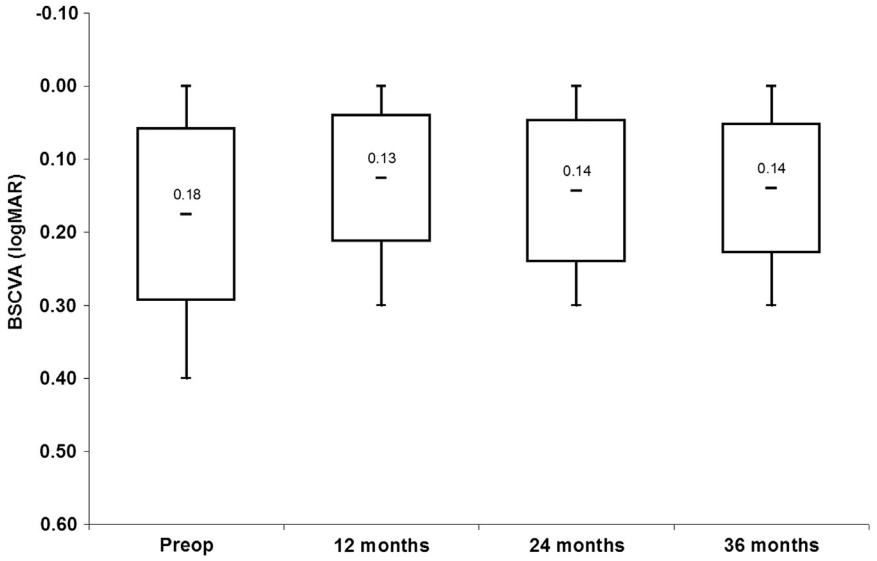

Fig. 1—Box (mean ± SD) and whisker (smallest and largest values) plots showing best corrected visual acuity with glasses (logMAR) before treatment (Preop) and on 12, 24, and 36 months thereafter.

months showed that such a decreasing tendency has stopped and even reversed (Figs. 2 and 3, Table 2). In 3 patients, Kmax increased at 36 months as compared with baseline (49.3–52.6 D, 56.1–56.7 D, and 51.9–52.2 D). Mean simulated keratometry (simK) did not show statistically significant changes throughout the follow-up period.

Both biomechanical parameters, corneal hysteresis (CH) and CRF, did not show statistically significant changes throughout the study period (Fig. 4, Table 2).

Axial length measurement increased continuously during the 36-month follow-up, and differences from pretreatment values showed borderline statistical significance (24 months: $p = 0.048$, 36 months: $p = 0.053$; Table 2).

Mean GAT-IOP was 10.35 ± 1.4 mm Hg before cross-linking, 11.06 ± 0.9 mm Hg 1 year later, 10.79 ± 0.8 mm Hg 2 years after treatment, and 11.07 ± 1.4 mm Hg 3 years after treatment ($p = 0.1$). There were no statistically significant differences between preoperative and postoperative values of ECD, MCT, and CFT at any time point during follow-up (Table 2).

Discussion

In this study, after 3 years of continuous follow-up after cross-linking treatment, we observed stability in visual acuity and corneal thickness, and decrease in previous beneficial effects of CXL on corneal curvature.

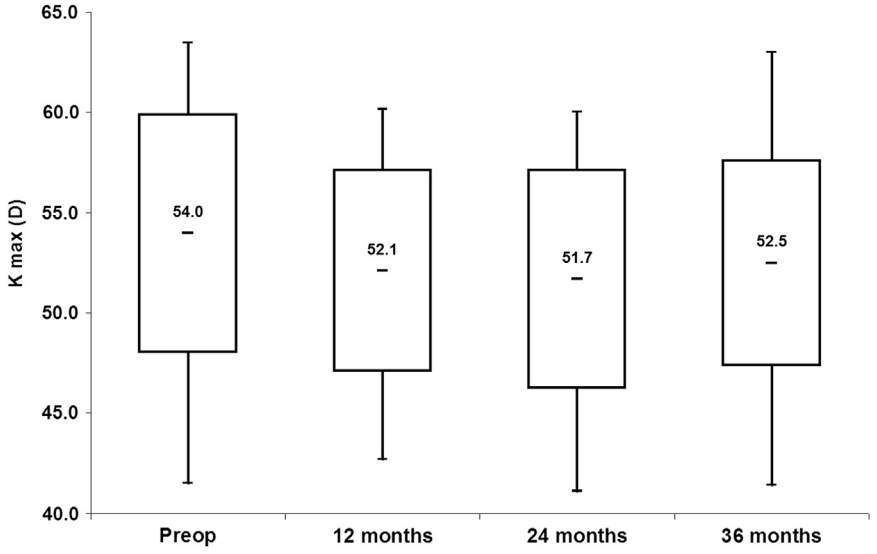

Fig. 2—Box (mean ± SD) and whisker (smallest and largest values) plots showing steepest meridian keratometry (Kmax) (D) before treatment (Preop) and 12, 24, and 36 months later.

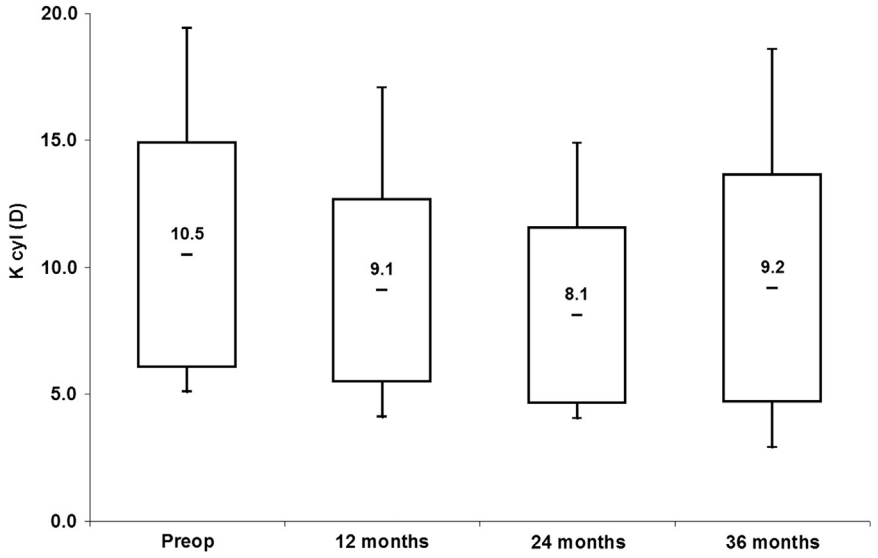

Fig. 3—Box (mean ± SD) and whisker (smallest and largest values) plots showing mean cylinder (Kcyl) (D) before treatment (Preop) and on 12, 24, and 36 months thereafter.

Currently, the CXL treatment is the only conservative modality aiming to reduce keratoconic progression. Various studies reported not just halting of progression, but also some continuous flattening of corneal curvature.[5–7,11] The average reported flattening of the steepest meridian ranged from 1.3 to 2.0 D for the first 2 years. In our study group, the mean reduction in Kmax was statistically significant during the first 2 years at 2.2 D. However, at the 36-month time point, the mean Kmax reduction from baseline was only 1.4 D and not significant statistically. The behaviour of Kcyl was similar, with initial 2 years of continuous significant flattening and reversal of such tendency compared with preoperative values at the 3-year examination time point. The nature of the initial continuous flattening still remains unclear, but plausibly at the longer term, inherent pathological stromal remodeling leads to reversal of achieved cross-linking stabilization effects and renewal of KC progression.

Table 2—Study parameters and their mean change after cross-linking

Parameter	Before CXL	12 Months after CXL	24 Months after CXL	36 Months after CXL
CH (mm Hg)	7.8 ± 1.7	7.3 ± 1.6	7.3 ± 1.5	7.6 ± 1.5
CRF (mm Hg)	6.6 ± 1.7	6.2 ± 1.3	6.6 ± 0.8	6.6 ± 1.1
CFT (μm)	208 ± 19	207 ± 22	204 ± 20	210 ± 21
ECD (cells/mm^2)	2730 ± 261	2640 ± 266	2541 ± 344	2526 ± 470
MCT (μm)	463 ± 38	476 ± 50	462 ± 43	466 ± 50
AL (mm)	24.56 ± 1.9	24.59 ± 1.9	24.61 ± 1.8*	24.71 ± 1.9*
Kmax (D)	53.9 ± 5.9	52.1 ± 5.0*	51.7 ± 5.5*	52.5 ± 5.1
Kcyl (D)	10.5 ± 4.4	9.1 ± 3.6*	8.1 ± 3.5*	9.2 ± 4.5
Mean SimK (D)	45.9 ± 2.7	45.2 ± 2.8	45.6 ± 3.7	45.7 ± 3.4

Values are mean ± SD.

CXL, corneal collagen cross-linking; CH, corneal hysteresis; CRF, corneal resistance factor; CFT, central foveal thickness; ECD, endothelial cell density; MCT, minimal corneal thickness; AL, axial length; Kmax, maximal keratometry; Kcyl, mean cylinder; mean SimK, average simulated keratometry.

*$p \leq 05$.

In terms of visual acuity, we observed 3 years of stability of BSCVA and UCVA. Similarly, other studies reported stabilization or even improvement in visual acuity after CXL.[6,8,9,11] It was theorized that corneal flattening together with reduction in total wavefront higher-order aberrations contribute to improved visual function.[6]

Considering long-term safety of CXL, we did not observe significant change in ECD as was assessed throughout the 3-year study period. We did notice some statistically nonsignificant reduction in ECD, averaged 2.4% a year. Similar reduction, although similar to others, as reported by Vinciguerra et al.[11] (2.4% per year) and Caporossi[6] (2.0% per year), is still somehow higher than the reported physiologic ECD reduction (0.6% per year); therefore, we would recommend further close long-term evaluation of corneal endothelium.[6,11,12]

Another safety parameter we assessed was central foveal thickness as measured by OCT. Being an indicator for anatomic stability of the retina, it did not show significant change during the 3-year follow-up, similar to previous reports.[6,8,13]

CXL supposedly works through increasing corneal stiffening. Intuitively, we would expect to observe a change in corneal biomechanics, but several in vivo studies did not show recordable changes in CH and CRF as measured with ORA.[14–16] Similarly, in this study, during 3-year follow-up, we did not observe significant change in corneal biomechanical parameters as presented by CH and CRF. Whether the theorized biomechanical changes are too subtle to be measured by ORA or have characteristics not measured well by ORA remains unclear and requires further study. Rehnman et al.[17] suggested that cross-linking effect is stronger at the corneal centre and diminishes toward the corneal periphery. Using Scheimpflug photography, they

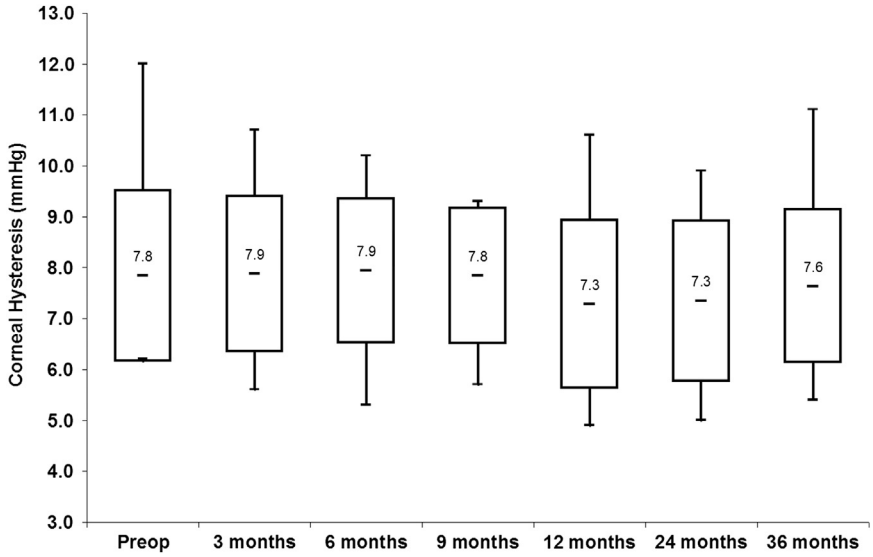

Fig. 4—Box (mean ± SD) and whisker (smallest and largest values) plots showing corneal hysteresis (mm Hg) before treatment (Preop) and on 3, 6, 9, 12, 24, and 36 months thereafter.

reported increased light scattering at the anterior central corneal stroma after CXL. This may explain an absence of increase in measurable corneal rigidity, despite the positive clinical effect on the disease course.[17]

Whether CXL actually leads to an increase in the number of interfibrillar and intrafibrillar covalent collagen bonds, as often claimed, remains unclear. Currently, there is no direct evidence for formation of new cross-links between collagen molecules. Several studies reported increased collagen fibre diameter after CXL. Wollensak and Redl[18] studied the electrophoretic pattern of corneal collagen type I after CXL treatment in ex vivo porcine corneas. They reported a strong band of high-molecular-weight collagen polymers that complies well with the morphologic correlate of an increased fibre diameter after cross-linking treatment.[18] Hayes et al.[19] measured collagen D-periodicity, fibril diameter, and interfibrillar spacing using small-angle X-ray scattering. They reported no change in collagen D-periodicity after CXL treatment and concluded that observed increase in collagen interfibrillar spacing and increase in fibril diameter is a consequence of treatment-induced changes in tissue hydration rather than cross-linking. Mencucci et al.[20] used immunohistochemical analysis for the morphologic evaluation of collagen fibres diameter after CXL and also observed an increase in collagen fibres diameter.

In conclusion, our observations 3 years after UVA-riboflavin CXL for keratoconus demonstrate stable visual acuity, stable corneal thickness, and stable corneal biomechanical parameters. The trend of decreasing keratometry values observed during the first 2 years was no longer evident. Longer follow-up is needed to determine whether this is a first sign of loss of achieved stability and resuming of keratoconus progression.

Disclosure: The authors have no proprietary or commercial interest in any materials discussed in this article.

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