A Randomized, Controlled Trial of Corneal Collagen Cross-Linking in Progressive Keratoconus

Three-Year Results

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Purpose: To report the refractive, topographic, and clinical outcomes 3 years after corneal collagen cross-linking (CXL) in eyes with progressive keratoconus.

Design: Prospective, randomized controlled trial.

Participants: One hundred eyes with progressive keratoconus were randomized into the CXL treatment or control groups.

Methods: Cross-linking was performed by instilling riboflavin 0.1% solution containing 20% dextran for 15 minutes before and during the 30 minutes of ultraviolet A irradiation (3 mW/cm^2). Follow-up examinations were arranged at 3, 6, 12, 24, and 36 months.

Main Outcome Measures: The primary outcome measure was the maximum simulated keratometry value (K_{max}). Other outcome measures were uncorrected visual acuity (UCVA; measured in logarithm of the minimum angle of resolution [logMAR] units), best spectacle-corrected visual acuity (BSCVA; measured in logMAR units), sphere and cylinder on subjective refraction, spherical equivalent, minimum simulated keratometry value, corneal thickness at the thinnest point, endothelial cell density, and intraocular pressure.

Results: The results from 48 control and 46 treated eyes are reported. In control eyes, K_{max} increased by a mean of 1.20±0.28 diopters (D), 1.70±0.36 D, and 1.75±0.38 D at 12, 24, and 36 months, respectively (all $P < 0.001$). In treated eyes, K_{max} flattened by −0.72±0.15 D, −0.96±0.16 D, and −1.03±0.19 D at 12, 24, and 36 months, respectively (all $P < 0.001$). The mean change in UCVA in the control group was +0.10±0.04 logMAR ($P = 0.034$) at 36 months. In the treatment group, both UCVA (−0.15±0.06 logMAR; $P = 0.009$) and BSCVA (−0.09±0.03 logMAR; $P = 0.006$) improved at 36 months. There was a significant reduction in corneal thickness measured using computerized videokeratography in both groups at 36 months (control group: −17.01±3.63 μm, $P < 0.001$; treatment group: −19.52±5.06 μm, $P < 0.001$) that was not observed in the treatment group using the manual pachymeter (treatment group: +5.86±4.30 μm, $P = 0.181$). The manifest cylinder increased by 1.17±0.49 D ($P = 0.020$) in the control group at 36 months. There were 2 eyes with minor complications that did not affect the final visual acuity.

Conclusions: At 36 months, there was a sustained improvement in K_{max}, UCVA, and BSCVA after CXL, whereas eyes in the control group demonstrated further progression. *Ophthalmology 2014;121:812-821 © 2014 by the American Academy of Ophthalmology.*

Keratoconus is a bilateral, noninflammatory, progressive corneal ectasia.[1] It is characterized by corneal thinning and protrusion, progressive myopia, and irregular astigmatism. Although only a small percentage of individuals with keratoconus progress to require corneal transplantation for visual rehabilitation,[2] keratoconus remains the most common indication for corneal transplantation surgery.[3]

The use of corneal collagen cross-linking (CXL) to strengthen corneal tissue is based on the theory that a relative paucity of covalent bonds within and between collagen molecules leads to the decreased biomechanical strength of the keratoconic cornea. Corneal collagen cross-linking treatment is believed to induce photochemically triggered cross-links within the collagen network by using a combination of vitamin B_2 (riboflavin) and longer-wavelength ultraviolet A radiation (370 nm). In vitro studies have shown that CXL leads to biochemical and biomechanical changes in both rabbit and human corneal tissue, suggesting that CXL may have a similar effect on the keratoconic cornea and thereby modify the natural course of the disease.[4–9]

Since the first clinical study was published in 2003,[10] there has been an increasing number of case series published reporting the safety and efficacy of the treatment in slowing down or halting the progression of keratoconus. These studies, however, are limited by their lack of a control group and relatively short-term follow-up, particularly considering the inherent variability in the course of keratoconus and the limited reproducibility of the measurement of outcome parameters. There are only 3

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published randomized controlled trials of CXL in keratoconus to date.[11–13] Two of these trials demonstrated statistically significant flattening of the corneal curvature in a small number of treated eyes compared with a control group.[11,12]

This trial was designed to test the hypothesis that CXL slows or halts the progression of keratoconus. Our previous publication reported the findings for the first 20 eyes to reach 1 year of follow-up in a randomized controlled trial of CXL for progressive keratoconus.[11] In this article, we present the outcomes from the complete cohort of 100 eyes with the results from 48 control eyes and 46 treated eyes analyzed after 3 years.

Methods

Study Design

This was a prospective, unmasked, randomized controlled trial conducted at the Royal Victorian Eye & Ear Hospital and the Centre for Eye Research Australia, Melbourne, Australia, commencing in 2006. The aim of the study was to assess the efficacy and safety of CXL in the treatment of progressive keratoconus. Approval was obtained from the hospital's Human Research and Ethics Committee, and the conduct of this study adhered to the tenets of the Declaration of Helsinki. The trial is registered with the Australian and New Zealand Clinical Trials Registry (ACTRN1261 3000143729). Written informed consent was obtained from all participants before enrollment in the study.

The primary outcome measure was the maximum simulated keratometry (K_{max}) value of the steepest axis on corneal topography. The secondary outcome measures were uncorrected visual acuity (UCVA), best-spectacle corrected visual acuity (BSCVA), spherical and cylindrical error on subjective refraction, spherical equivalent, minimum simulated keratometry value, corneal thickness at the thinnest point, endothelial cell density, and intraocular pressure.

Participants

Patients attending the Cornea Clinic at the Royal Victorian Eye & Ear Hospital with a confirmed diagnosis of progressive keratoconus were invited to participate in this study. Keratoconus was deemed to be progressive if there was a subjective deterioration in vision and at least 1 of the following criteria were met over the preceding 12 months: an increase of at least 1 diopter (D) in the steepest simulated keratometry value derived from computerized videokeratography or in the steepest meridian measured by manual keratometry, an increase in astigmatism as determined by manifest subjective refraction of at least 1.0 D or a 0.1 mm or more decrease in the back optic zone radius of the best-fitting contact lens. Exclusion criteria included a minimum corneal thickness less than 400 μm, axial corneal scarring, previous refractive or other corneal surgery, a history of chemical burns, severe infections, and other corneal or ocular surface disorders. Patients who were pregnant or breastfeeding at the time of enrollment also were excluded. Only patients aged between 16 and 50 years were included in the study. The upper age limit of 50 years was chosen because progressive keratoconus typically is observed in younger patients. Furthermore, changes in visual acuity, subjective refraction, and corneal measurements in the older age group may result from other corneal pathology or cataract development.

Eligible patients were randomized after enrollment into either the treatment or control groups using a computer-generated randomization plan with block randomization in groups of 10. If both eyes of 1 patient qualified for participation in the study, each eye was randomized independently. The randomization plan was maintained in a secure location by a staff member in another hospital department not involved with the recruitment or conduct of the study.

Assessments

At baseline and postoperative visits at 3, 6, 12, 24, and 36 months, the following assessments were performed: UCVA (expressed in logarithm of the minimum angle of resolution [logMAR] units), BSCVA (expressed in logMAR units), subjective refraction (manifest sphere, cylinder, and spherical equivalent), slit-lamp examination, computerized videokeratography (Orbscan II; Bausch and Lomb Surgical, Salt Lake City, UT), ultrasound pachymetry of the thinnest point (Pachy Meter SP3000; Tomey, Nagoya, Japan), confocal microscopy (Confoscan 4; NIDEK Co. Ltd, Gamagori, Japan), intraocular pressure measurement using the Tono-Pen XL (Medtronic, Jacksonville, FL) and Goldmann applanation tonometer (Haag-Streit AG, Koeniz, Switzerland), endothelial cell evaluation using the SP 2000 Specular Microscope (Topcon Corp., Tokyo, Japan), and slit-lamp photography (Slit Lamp BX900; Haag-Streit AG). All images were acquired and analyzed in an unmasked manner.

To improve the reliability of the computerized videokeratography imaging measurements, a minimum of 3 consecutive scans were performed for each eye. If the value for the primary outcome measure (K_{max}) varied by more than 1 D between the scans, then a further 2 scans were obtained. From all scans obtained at each appointment, the scan with the median K_{max} value was selected for analysis.

Treatment

Cross-linking was performed within 4 weeks of the baseline examination using a modification of the Dresden protocol.[10] Topical anesthetic (oxybuprocaine hydrochloride 0.4%; Bausch & Lomb Pty Ltd, Macquarie Park, NSW, Australia) was instilled 3 times over a 10-minute period followed by 2 drops of topical antibiotic (chloramphenicol 0.5%; Chlorsig, Sigma Pharmaceuticals Pty Ltd, Clayton, Victoria, Australia). The corneal epithelium was removed to a diameter of 8.5 mm using a no. 57 Beaver blade with handle (BD Medical Ophthalmic Systems, Waltham, MA). Riboflavin 0.1% eye drops (Streuli Pharmaceuticals, Uznach, Switzerland) containing 20% dextran (500 000 Dalton; Sigma Aldrich, Pty Ltd, Holbaek, Denmark) prepared on the day of the procedure by the Royal Victorian Eye & Ear Hospital Pharmacy Department were applied to the cornea every 1 to 3 minutes over a 15-minute period. Riboflavin application was continued every 1 to 3 minutes during the 30 minutes of ultraviolet A exposure together with topical anesthetic as required. The UV-X device (UV-X 1000; IROC, Zurich, Switzerland) was used to deliver ultraviolet A radiation of 370-nm wavelength with an aperture of 9 mm at a distance of 50 mm from the apex of the cornea. The UV-X device output parameters were verified by an independent government authority (Australian Radiation Protection and Nuclear Safety Agency). In addition, the ultraviolet source output was confirmed to be 3.0 mW/cm^2 (range, 2.74−3.1 mW/cm^2) before and after every treatment using the UV Light Meter (Model YK-34UV; Lutron Electronic Enterprise Co. Ltd, Taipei, Taiwan).

After 30 minutes of irradiation, the eye was rinsed with a sterile saline solution, 1 drop of chloramphenicol 0.5% was applied, and a bandage contact lens was inserted (PureVision; Bausch & Lomb, Rochester, NY). After surgery, chloramphenicol 0.5% was continued 4 times daily for 1 week and the contact lens remained in place until epithelial closure was confirmed, usually on day 3. At this time, the contact lens was removed and fluorometholone

acetate 0.1% (Flarex; Alcon Laboratories, Frenchs Forest, NSW, Australia) was commenced 4 times daily for 1 week and twice daily for a second week. In addition to the follow-up schedule outlined previously, eyes in the treatment group also were examined 1, 3, 7, and 30 days after CXL to document healing and exclude possible early postoperative complications.

Control Group

Eyes randomized to the control group did not receive a sham treatment and were monitored in accordance with the follow-up schedule. Participants in the control group were offered compassionate CXL treatment if continuing and significant disease progression was noted during the course of the study. Compassionate treatment was performed no earlier than 6 months after enrollment and randomization. Data collection for the purpose of this study was terminated for compassionately treated eyes at the time of the procedure (Fig 1).

Statistical Analysis

Sample size calculation was performed to detect a difference of 1.0 D between the mean K_{max} for the control and treatment groups, at a significance level of 0.05 and a power of 80%, assuming a standard deviation of 1.5 D. A discontinuation rate of 25% was anticipated, to give a sample size of 49 per group. A total of 100 eyes therefore were recruited for this trial.

Where CXL treatment was performed for an eye with progressive keratoconus in the control group, the eye was withdrawn from the trial. Data collected before the date of compassionate treatment were used for analysis. For data that were missing on a particular visit, the last observation carried forward (LOCF) method was used.

The difference from baseline for each parameter was calculated at each time point (3, 6, 12, 24, and 36 months) for each eye. The differences within each group were compared using one-sample *t* tests. These changes also were compared between the control and treatment groups using independent-sample *t* tests. To report the trend over time, the general linear model was used by applying the polynomial option where 6 time points (baseline, 3, 6, 12, 24, and 36 months) were used as categorical variables to report the trend. A *P* value of ≤ 0.050 was considered as statistically significant.

Additional analyses of the change in the primary outcome parameter (K_{max}) from baseline to 36 months and the repeated measure analysis to investigate the trend were performed without the LOCF data to reconfirm the results. The relationship between the change in K_{max} at 36 months and baseline parameters was assessed using Pearson's correlation analysis. Where a significant relationship was observed, subgroup analysis was performed. Data analysis for each outcome parameter was performed using SPSS software (IBM Corp, NY).

Results

Recruitment for the trial was completed in 2009 with 100 eyes randomized to control (50 eyes) and treatment (50 eyes) groups. Six eyes were excluded from analysis: 4 eyes (3 in the treatment group, 1 in the control group) withdrew from the trial before any follow-up data were obtained; 1 patient was pregnant at the time of her 3-year follow-up appointment, and 1 eye had a delay in treatment date, with only 2-year follow-up data available at the time of preparation of this manuscript.

This article reports the results of 46 treated eyes and 48 control eyes after 36 months of follow-up. Included in this analysis are a total of 26 eyes for which data collection was discontinued before the end of the third year and the LOCF method was used to complete follow-up data: 12 eyes from the control group elected to undergo compassionate CXL for progressive disease, 5 eyes from the control group underwent corneal transplantation during the follow-up period, and 9 patients (5 treated and 4 control eyes) withdrew from the trial for personal reasons (Fig 1).

Baseline

At baseline, there was no significant difference between the 2 groups in any of the baseline demographic or clinical parameters, apart from the intraocular pressure measured using the Tono-Pen, which was 2 mmHg higher in the control group (15.6±3.7 mmHg) compared with the treatment group (13.8±3.1 mmHg; $P = 0.026$). The difference in baseline K_{max} between groups almost reached statistical significance (control group: mean, 51.2 D; CXL group: mean, 52.9 D; $P = 0.052$). The baseline parameters are summarized in Table 1.

Topographic Results

On average, there was a notable improvement in treated eyes with a flattening of K_{max} by −1.03±0.19 D at 36 months. Six eyes improved at least −2.00 D between baseline and 36 months, with a maximum improvement of −2.90 D observed in 2 eyes. Only 1 eye in the treatment group progressed more than 2.00 D (4.10 D) during the same period. Conversely, in the control group, no eyes improved by 2.00 D or more, whereas 19 eyes had documented progression of 2.00 D or more, with 7 eyes in this group progressing by 4.00 D or more over 36 months. The maximum progression recorded in the control group was 9.60 D. The average increase in K_{max} for control eyes was 1.75±0.38 D at 36 months. Comparing the changes between control and treatment groups revealed statistically significant differences for all evaluated time points ($P < 0.001$). Similarly, the trends of the 2 groups over time were significantly different ($P = 0.001$). In both groups, most of the change in K_{max} occurred during the first 24 months, whereas changes were less marked during the third year (Table 2; Fig 2).

Repeat analyses using only recorded measurements (excluding LOCF for missing data values) were performed comparing K_{max} at 36 months with baseline and the trends in K_{max} over time. These results confirmed a significant change between baseline and 36 months in the treatment group (−1.13±0.19 D; $P < 0.001$; n = 41), but not in the control group (0.78±0.29 D; $P = 0.01$; n = 27). The trends observed in each group over time were also confirmed to be significantly different ($P < 0.001$).

Using the Pearson's correlation assessment, a negative correlation was found for the treatment group between the baseline K_{max} and the change in K_{max} after 36 months ($r = -0.314$, $P = 0.033$). Stratified analysis dividing the baseline K_{max} into 3 groups (<50.0 D, 50.0−53.9 D, and ≥54.0 D) supported this finding, with a trend suggesting greater improvement in eyes with a baseline K_{max} of 54.0 D or more than in eyes with baseline K_{max} of either less than 50.0 D or 50.0 to 53.9 D ($P = 0.055$). In addition, in the control group, a negative correlation was found between the patient's age at enrollment and the observed change in K_{max} at 3 years ($r = -0.417$, $P = 0.003$). No other correlations were found to be significant between the baseline parameters (age, gender, baseline K_{max}, and corneal thickness) and the change in the K_{max} at 3 years in either control or treatment groups.

Changes in minimum simulated keratometry reflected the changes in K_{max}, with a significant flattening observed in the treatment group at each time point, in contrast to a steepening in control eyes (Table 2).

Visual Acuity and Refractive Outcomes

The UCVA improved in the treatment group compared with baseline at 12 ($P = 0.008$), 24 ($P = 0.020$), and 36 ($P = 0.009$)

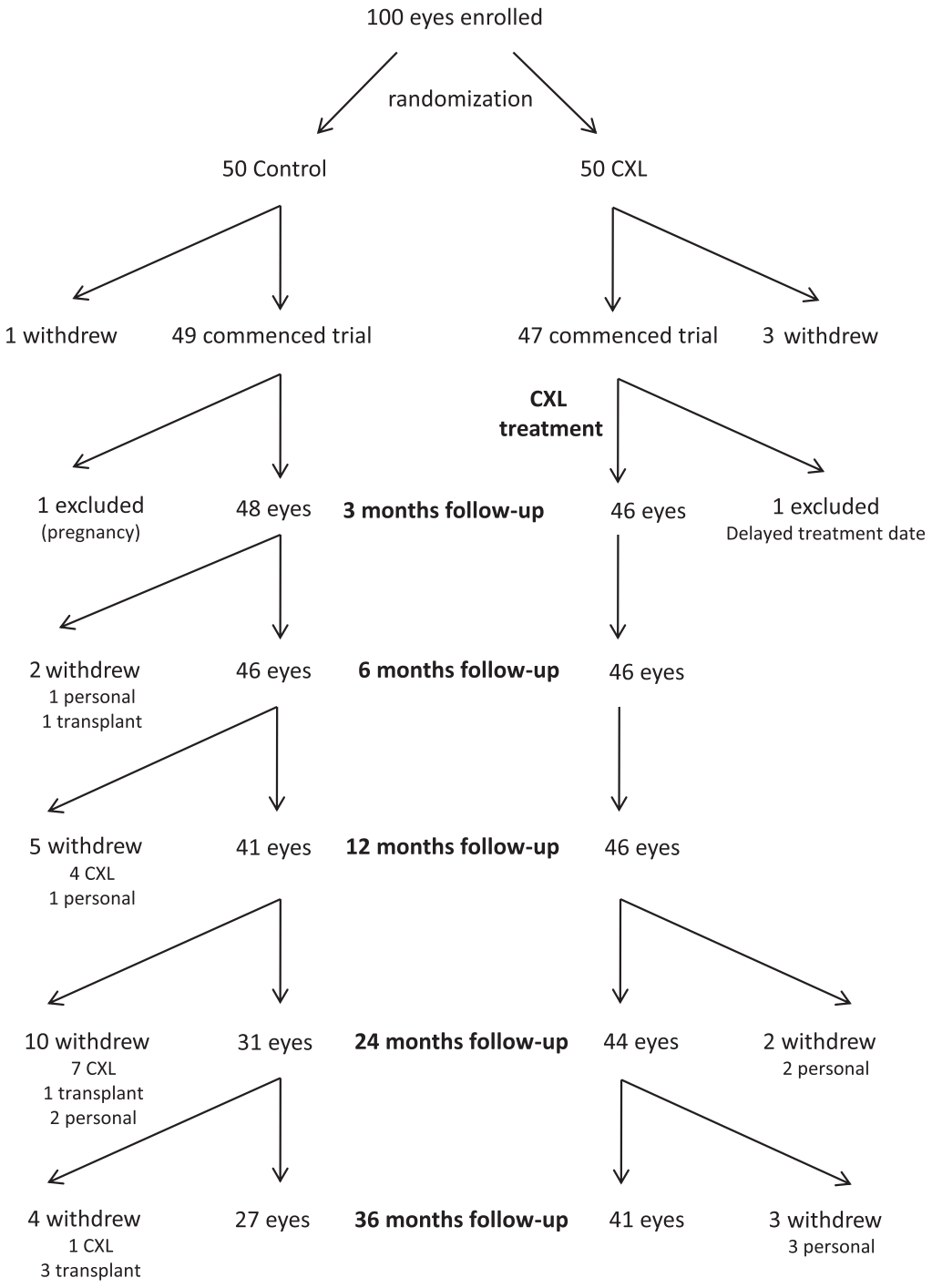

Figure 1. Flow chart demonstrating randomization, number of eyes examined at each follow-up visit, and reasons for patients withdrawing from the trial. CXL = corneal collagen cross-linking.

months, respectively (Table 2; Fig 3). In contrast, control eyes on average demonstrated deterioration of UCVA that was statistically significant at 36 months ($P = 0.034$). The difference between the changes in both groups also was significant at each analyzed time point ($P < 0.001$). Similarly, the trends observed in both groups over time were significantly different ($P < 0.001$).

Treated eyes significantly improved in BSCVA at 12 ($P = 0.007$), 24 ($P = 0.002$), and 36 ($P = 0.006$) months compared with baseline values. In contrast, the mean change in the control group was not significant at 36 months ($P = 0.101$). There was no significant difference between the 2 groups at any time point nor between the trends over time.

There was also no statistically significant difference in manifest spherical error on subjective refraction at any time point between groups or within groups. There was no difference in the change in the manifest cylindrical error from baseline in the treatment group,

Table 1. Baseline Demographic and Clinical Characteristics of Eyes in the Treatment and Control Groups

	Control (n = 48)	Corneal Collagen Cross-Linking (n = 46)	P Value
Age (yrs)	25.8±6.4	25.6±6.2	0.888
Male gender (%)	26 (54.2)	28 (60.9)	0.511
Right eye laterality (%)	24 (50)	23 (50)	1.00
K_{max} (D)	51.18±4.03	52.87±4.31	0.052
K_{min} (D)	46.62±3.27	47.47±3.68	0.237
UCVA (logMAR)	0.81±0.40	0.93±0.39	0.157
BSCVA (logMAR)	0.28±0.26	0.33±0.26	0.395
Thinnest point on ultrasound pachymetry (μm)	454±30	444±34	0.153
Thinnest point on Orbscan (μm)	424±47	429±43	0.652
Spherical error (DS)	−0.84±4.06	−1.40±4.35	0.520
Cylindrical error (DC)	−4.52±2.64	−4.62±2.36	0.841
Spherical equivalent	−3.10±4.06	−3.71±4.38	0.485
IOP (mmHg)			
Tono-Pen	15.40±3.63	13.82±3.09	0.026
Goldmann	14.25±2.58	13.76±2.44	0.360
Endothelial cell density (cells/mm^2)	2556±312	2491±295	0.311

BSCVA = best spectacle-corrected visual acuity; D = diopters; DC = diopter cylinder; DS = diopter sphere; IOP = intraocular pressure; K_{max} = maximum simulated keratometry; K_{min} = minimum simulated keratometry; logMAR = logarithm of the minimum angle of resolution; UCVA = uncorrected visual acuity.
Data are mean ± standard deviation, unless otherwise indicated.

whereas in the control group, the manifest cylinder increased significantly at 36 months ($P = 0.020$). The changes at 12 and 24 months were not significant. The change in the spherical equivalent was not significant at any point for either group.

Corneal Thickness Measurements

Corneal thickness measurements using Orbscan computerized videokeratography (CVK) and manual ultrasound pachymetry are shown in Table 2 and Figure 4. Using ultrasound pachymetry, measurements from treated eyes revealed no significant difference at any time point, whereas control eyes exhibited a small reduction in thickness at the thinnest point that reached statistical significance at 36 months (−9.60±4.25 μm; $P = 0.029$). The repeated-measures analysis of variance showed a significant difference in the trends over time between the 2 groups ($P = 0.003$; Table 2).

In contrast, measurements of corneal thickness in the treatment group using Orbscan computerized videokeratography revealed a highly significant decrease in the measured values at the thinnest point compared with baseline readings that was most marked at 3 months (−93.00±7.98 μm; $P < 0.001$). The measured thinning reversed over the follow-up period to −19.52±5.06 μm at 36 months. However, the thinnest point on Orbscan computerized videokeratography remained significantly lower than at baseline ($P < 0.001$) at all time points. A different pattern of change was observed in control eyes with a statistically significant, progressive decrease in the measurement at the thinnest point at 12, 24, and 36 months. The trends over time were significantly different between the groups ($P < 0.001$).

Intraocular Pressure

Using the Tono-Pen, no significant difference was detected for the mean change in intraocular pressure from baseline to 36 months in either group. Using the Goldmann applanation tonometer, a significant decrease from baseline was observed in both groups at 36 months, but this was not significantly different between the groups. There was also no significant difference observed comparing the trends between the groups with either device (Table 2).

Endothelial Cell Density

At no point was a significant difference in endothelial cell density found when compared with baseline for either group. Similarly, there was no significant difference between the 2 groups (Table 2).

Adverse Events

Two eyes with adverse events after CXL treatment were initially reported in an earlier publication.[11] In one case, there was mild, diffuse corneal edema and a small paracentral infiltrate 1 week after treatment. This was attributed to the premature (day 3) resumption of rigid contact lens wear and was treated with a prolonged course of fluorometholone acetate 0.1%. The BSCVA was not adversely affected, with an improvement observed from 0.30 logMAR at baseline to 0.00 logMAR at 3 months. By 12 months, there was only a faint corneal scar, with BSCVA stable at 0.00 logMAR (Fig 5).

In a second case, subepithelial infiltrates and anterior chamber inflammation were observed 2 days after treatment in a patient with a history of severe atopy. This was treated as possible microbial keratitis with removal of the bandage soft contact lens and the frequent application of ofloxacin eye drops (Ocuflox; Allergan, Sydney, Australia). Fluorometholone acetate 0.1% was initiated 1 week later. There were no organisms identified on either Gram stain or culture from corneal scrapings. The clinical signs had resolved by 3 months after CXL. The BSCVA improved from 0.6 logMAR at baseline to 0.3 logMAR at 6 months in this eye.

A third patient was noted to have peripheral corneal vascularization 3 years after CXL treatment. However, there was evidence of acne rosacea and vascularization in both the treated eye and the untreated fellow eye. This change was thought to be unrelated to the CXL treatment. There were no other treatment-related adverse events.

Discussion

Corneal collagen cross-linking is often described as the most promising innovation in the treatment of progressive

Table 2. Clinical Characteristics of Eyes in the Treatment and Control Groups after 12, 24, and 36 Months Compared with Baseline Measurements

Parameter	Group	12 Months	*P* Value*	24 Months	*P* Value*	36 Months	*P* Value*	*P*‡ Value
ΔK_{max} (D)	Control	1.20±0.28	<0.001	1.70±0.36	<0.001	1.75±0.38	<0.001	<0.001
	CXL	−0.72±0.15	<0.001	−0.96±0.16	<0.001	−1.03±0.19	<0.001	
	P value†	<0.001		<0.001		<0.001		
ΔK_{min} (D)	Control	0.66±0.22	0.005	1.31±0.32	<0.001	1.35±0.34	<0.001	<0.001
	CXL	−0.42±0.12	0.001	−0.52±0.14	<0.001	−0.73±0.15	<0.001	
	P value†	<0.001		<0.001		<0.001		
ΔUCVA (logMAR)	Control	0.06±0.03	0.094	0.07±0.04	0.069	0.10±0.04	0.034	<0.001
	CXL	−0.14±0.05	0.008	−0.13±0.05	0.020	−0.15±0.06	0.009	
	P value†	0.001		0.003		0.001		
ΔBSCVA (logMAR)	Control	−0.02±0.03	0.534	−0.04±0.03	0.138	−0.05±0.03	0.101	0.32
	CXL	−0.09±0.03	0.007	−0.09±0.03	0.002	−0.09±0.03	0.006	
	P value†	0.094		0.224		0.347		
ΔSphere (DS)	Control	−0.41±0.45	0.366	−0.06±0.46	0.893	−0.20±0.53	0.704	0.64
	CXL	0.52±0.45	0.253	0.50±0.49	0.310	−0.16±0.45	0.727	
	P value†	0.147		0.404		0.948		
ΔCylinder (DC)	Control	−0.27±0.43	0.532	−0.71±0.48	0.147	−1.17±0.49	0.020	0.73
	CXL	−0.85±0.44	0.060	−0.81±0.50	0.115	−0.90±0.50	0.081	
	P value†	0.351		0.884		0.690		
ΔSE (D)	Control	−0.55±0.35	0.126	−0.42±0.36	0.251	−0.79±0.42	0.065	0.40
	CXL	0.10±0.38	0.798	0.10±0.42	0.82	−0.61±0.41	0.146	
	P value†	0.215		0.353		0.752		
ΔThinnest point (μm)								
USP	Control	−5.40±3.38	0.117	−4.30±4.19	0.313	−9.60±4.25	0.029	0.003
	CXL	3.53±3.50	0.318	4.14±4.63	0.376	5.86±4.30	0.181	
	P value†	0.07		0.18		0.013		
Orbscan	Control	−10.08±3.42	0.005	−12.84±3.58	0.001	−17.01±3.63	<0.001	<0.001
	CXL	−33.69±4.18	<0.001	−23.16±5.16	<0.001	−19.52±5.06	<0.001	
	P value†	<0.001		0.101		0.686		
ΔIOP (mmHg)								
Tono-Pen	Control	−0.48±0.57	0.398	−0.42±0.58	0.474	−1.15±0.70	0.107	0.42
	CXL	0.84±0.51	0.105	0.58±0.58	0.322	0.16±0.50	0.757	
	P value†	0.087		0.227		0.136		
GAT	Control	−0.75±0.46	0.11	−1.42±0.56	0.014	−1.92±0.48	<0.001	0.38
	CXL	−0.62±0.45	0.175	−0.60±0.52	0.262	−1.50±0.44	0.001	
	P value†	0.84		0.298		0.526		
ΔECD (cells/mm^2)	Control	−65±49	0.194	17±57	0.767	−30±49	0.540	0.88
	CXL	28±53	0.596	13±65	0.844	−35±50	0.490	
	P value†	0.201		0.963		0.941		

BSCVA = best spectacle-corrected visual acuity; CXL = corneal collagen cross-linking; D = diopters; DC = diopter cylinder; DS = diopter sphere; ECD = endothelial cell density; GAT = Goldmann applanation tonometry; IOP = intraocular pressure; K_{max} = maximum simulated keratometry; K_{min} = minimum simulated keratometry; logMAR = logarithm of the minimum angle of resolution; SE = spherical equivalent; UCVA = uncorrected visual acuity; USP = ultrasound pachymetry; Δ = change from baseline.
Data are mean±standard error unless otherwise indicated.
*Comparing changes at 12, 24, and 36 months from baseline.
†Comparing control and treatment groups at the same time point.
‡Comparison of the trends over time.

keratoconus in recent years. The growing interest in CXL is reflected in the rapid increase in publications since the first report by Spoerl et al[4] in 1998. A keyword search using PubMed (http://www.ncbi.nlm.nih.gov/pubmed) accessed on September 6, 2013, using the terms *collagen cross-linking* and *keratoconus* yielded 323 citations, 90% of which were published in the past 4 years. Despite the growing body of literature and continuing efforts to optimize the treatment protocol, there remains a lack of randomized controlled studies with longer-term follow-up to support the widespread clinical use of CXL for keratoconus. This article reports our 36-month results from a 5-year randomized controlled clinical trial of CXL for progressive keratoconus. The results continue to demonstrate a statistically significant improvement in K_{max}, UCVA, and BSCVA in treated eyes. In contrast, the K_{max} in control eyes increased and the UCVA deteriorated, suggesting continuing progression of the disease.

A number of published case series similarly suggest a therapeutic effect of CXL in slowing the progression of keratoconus. However, the level of evidence they can provide is limited by their lack of control data.[10,14–17] There have only been 3 published randomized controlled trials to date. In 2008, we reported the initial results of our trial in

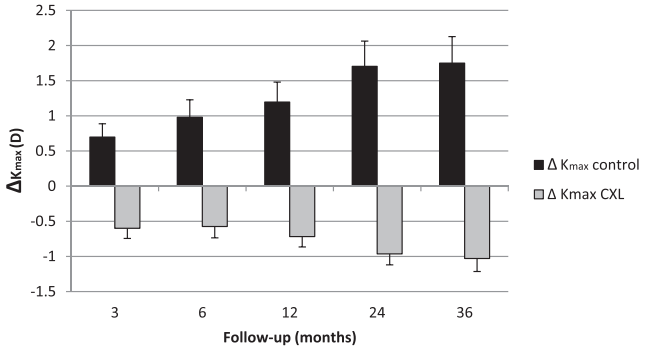

Figure 2. Bar graph showing the mean change in maximum simulated keratometry value (K_{max}) between baseline and 3, 6, 12, 24, and 36 months after treatment for the control and treatment groups. In the control group, there was a significant increase in K_{max} compared with continued flattening observed in the treatment group. The columns represent the mean change in K_{max} from baseline (ΔK_{max}) in diopters (D) and the error bars represent the standard error. CXL = corneal collagen cross-linking.

which we observed an improvement in K_{max} by a mean of −0.74 D after 12 months in the first 9 treated eyes, whereas 11 control eyes progressed by a mean of +1.28 D. In 2011, O'Brart et al[12] reported an improvement in average Orbscan simulated keratometry values by −0.62 D after 18 months in the treatment group ($P < 0.001$), whereas the control group progressed by +0.14 D ($P = 0.3$). Hersh et al[13] reported a randomized controlled trial with shorter follow-up (3 months) for control eyes that were treated subsequently. In this study, K_{max} improved by a mean of −2.0 D from baseline in treated keratoconic eyes at 1 year, whereas no change in K_{max} was documented in control eyes at 3 months.

Only 4 case series report results extending to 36 months. Caparossi et al[14] reported a decrease in the mean K value by −2.24 D in 44 eyes, Raiskup-Wolf et al[16] documented a decrease in K_{max} by an average of −2.57 D in 33 eyes, Vinciguerra et al[18] observed a decrease in K_{max} of −1.68 D in 12 eyes at 36 months, and O'Brart et al[19] reported a decrease in cone apex power of −1.16 D in 30 eyes after 4 to 6 years. We observed an improvement in K_{max} that was maintained at 3 years, with some eyes showing an ongoing improvement between 24 and 36 months. This apparent continuing flattening effect of CXL needs to be taken into account when combining CXL with refractive procedures such as photorefractive keratectomy and LASIK. A continued improvement over time may be the result of altered expression of enzymes, including transglutaminase and other keratocyte-derived markers[20] (Covre JL, et al. Evaluation of the riboflavin and ultraviolet light effect on keratocytes cultured in vivo. Abstract presented at: ARVO Annual Meeting, May 6, 2013; Seattle).

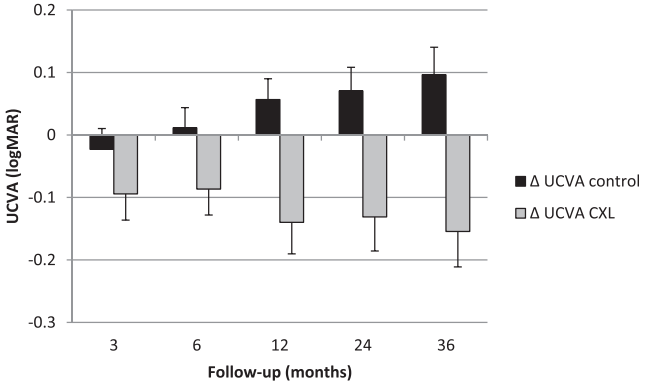

Figure 3. Bar graph showing the mean change in uncorrected visual acuity (UCVA) between baseline and 3, 6, 12, 24, and 36 months after treatment for the control and treatment groups. In the control group, the deterioration from baseline was significant only after 36 months ($P = 0.034$). In the treatment group, the improvement was statistically significant at 12, 24, and 36 months. The columns represent the mean change in UCVA from baseline (ΔUCVA) and the error bars represent the standard error. CXL = corneal collagen cross-linking; logMAR = logarithm of the minimum angle of resolution.

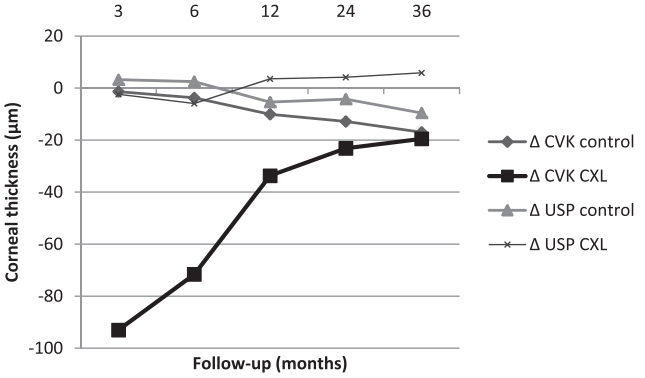

Figure 4. Graph showing corneal thickness measurements compared with baseline at 3, 6, 12, 24, and 36 months after treatment. The lines represent the mean change in the thinnest point from baseline (Δ) in micrometers (μm). The diamonds and squares represent measurements using computerized videokeratography (CVK) in the control group and treatment groups, respectively; the triangles and crosses represent measurements using ultrasound pachymetry (USP) in the control and treatment groups, respectively. CXL = corneal collagen cross-linking.

Several authors suggest a possible correlation between preoperative parameters and the degree of postoperative corneal flattening. We found that a baseline K_{max} of more than 54.0 D was associated with a greater degree of flattening. Although of borderline statistical significance, this observation is consistent with the report by Koller et al.[21] Conversely, Asri et al[15] suggested that progression of disease after CXL was more common in cases with a preoperative K value of more than 58.0 D. They also reported that age of more than 35 years and female gender were further risk factors for postoperative progression. Vinciguerra et al[17] reported the most promising results in patients 18 to 39 years of age. We did not observe any correlation between either age or gender with the change in K_{max} in the treatment group. The negative correlation between age and change in K_{max} ($r = -0.314$) in the control group of our study is consistent with the more rapidly progressive disease observed in patients diagnosed with keratoconus at a younger age.[2]

After CXL, UCVA has been reported to improve by 2.8 Snellen lines at 36 months.[14] Similarly, BSCVA has been shown to improve by 2 Snellen lines[14] and −0.15

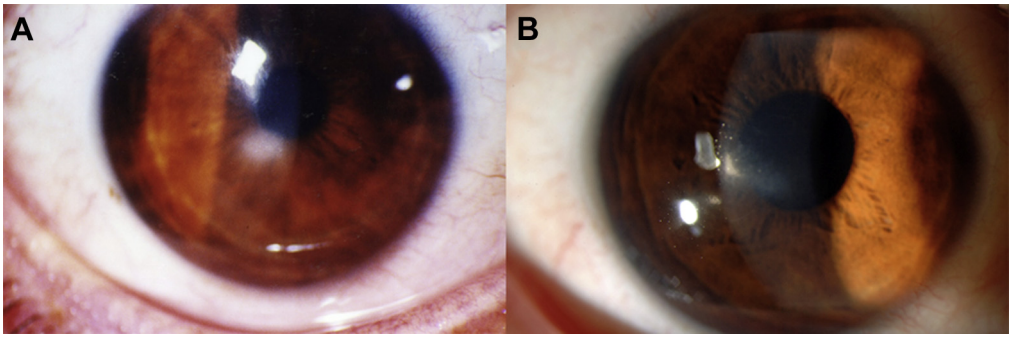

Figure 5. Slit-lamp photographs of a patient with mild, diffuse, corneal edema and a small paracentral infiltrate obtained (**A**) 1 week after corneal collagen cross-linking (CXL) and (**B**) 12 months after CXL demonstrating residual corneal scarring.

to −0.23 logMAR.[16,18] Our results demonstrate a more modest but significant improvement of −0.15 logMAR in UCVA and −0.09 logMAR in BSCVA at 36 months. We did not detect any change in spherical equivalent or the spherical or cylindrical component of the subjective refraction. However, a change in spherical equivalent (reduction in myopia) of between +1.39 and +2.13 D has been reported previously.[14,18] In the control group, the UCVA deteriorated at all follow-up visits, but this change reached statistical significance only at 36 months. These results are consistent with the continued progression of keratoconus in this group. The BSCVA unexpectedly improved by −0.05 logMAR in the control group, but this was not statistically significant. This observation may be explained by the inherent variability and poor reproducibility of subjective refraction and visual acuity measurement in keratoconic eyes. The withdrawal of eyes with progressive disease from the control group (after their CXL treatment on compassionate grounds or corneal transplantation surgery) also would reduce the ability to detect deterioration in BSCVA.

The effect of CXL on corneal thickness has so far been less clear. Thinning immediately after CXL has been reported and is thought to be the result of several factors, including treatment-related effects from stromal compaction, postoperative dehydration, and alterations in epithelial healing and distribution.[22,23] It also may represent a measurement artefact after treatment.[24] Longer-term observations vary from no change in corneal thickness[25] to a decrease at 12 months[26] and an increase at 24 months.[16] In our study, 2 different devices were used to assess corneal thickness. The marked reduction in the corneal thickness measurements at the thinnest point using Orbscan computerized videokeratography 3 months after treatment was not observed with ultrasound pachymetry. Caporossi et al[14] similarly reported an underestimation of corneal thickness measurements using the Orbscan compared with ultrasound pachymetry and confocal microscopy. This may be due to alterations in light transmission as the result of postoperative corneal haze when using optical methods for measuring corneal thickness.[14] Postoperative haze is less evident after 6 to 12 months,[11] which is consistent with the gradual return of corneal thickness measurements to near-baseline levels after 24 months. The small but persistent difference at 36 months using the Orbscan device may be the result of subclinical changes in optical properties that can be observed using confocal microscopy.[27] In contrast, the corneal thickness in control eyes determined with both Orbscan and ultrasound pachymetry was thinner after 36 months, again consistent with progressive disease.

A number of adverse effects have been reported. Most commonly, a transient stromal edema may occur in up to 70% of patients. Caparossi et al[14] observed stromal haze in 9.8% of eyes after CXL. In contrast, we observed a mild degree of haze in all patients undergoing CXL that resolved with time. The risk of haze may be greater in corneas that are steeper and thinner at baseline.[28] Sterile infiltrates have also been reported in up to 7.6% of treated eyes.[29] We observed 1 case of clinically evident postoperative edema associated with a paracentral infiltrate and a second eye in which subepithelial infiltrates developed. The mechanism underlying sterile infiltration in this setting is unknown, but may relate to an altered immune response to antigens or may be a direct result of the phototoxic effect of CXL.[30] In our patient, a contributing factor may have been the premature resumption of contact lens wear. Several cases of proven infectious keratitis have been reported by others.[31–33] In the 1 treated eye in which we initially suspected the diagnosis of microbial keratitis, microbiologic cultures did not demonstrate any growth. This infiltrate may have been sterile, occurring in a patient with a marked atopic predisposition.

Cases of endothelial irregularity and damage associated with CXL have been published by other authors.[34–36] There was no evidence of endothelial cell damage in any of our study patients, including the single eye with early postoperative edema. The endothelial cell density at baseline in our study population seemed to be slightly lower than that of the normal population, but this is consistent with the findings of Niederer et al,[37] who reported a reduction in cell density in all layers of the keratoconic cornea. Progression of keratoconus after CXL by +1.00 D over 12 months has been described in up to 7.6% of treated eyes.[29] We identified only 1 treatment failure after CXL, with progression of +4.10 D over 36 months in a patient who demonstrated evidence of rosacea keratitis during the follow-up period.

A limitation of this study was the decision to offer CXL to patients in the control group after a minimum of 6 months of follow-up, provided that continuous significant progression was documented. This could lead to masking of progression in the control group and an underestimation of the treatment effect demonstrated in this study. It also may explain the lack of deterioration in BSCVA at 36 months in the control group. It is also notable that the baseline mean corneal curvature (K_{max}) was steeper by 1.65 D in the treatment group compared with the control group, with this difference almost reaching statistical significance ($P = 0.052$).

The course of keratoconus is typically variable in its severity and rate of progression. There are also difficulties in reliably and reproducibly measuring outcome parameters.[38] For this reason, randomized controlled clinical trials are essential to assess the efficacy of CXL properly. Long-term studies also are necessary given the natural history of the disease and to monitor the persistence of the CXL effect.

Overall, the results of this randomized controlled trial of CXL continue to support the efficacy of this treatment in progressive keratoconus, with an improvement in K_{max}, UCVA, and BSCVA 36 months after CXL and progression of these parameters in the control eyes over the same period. Furthermore, the risks associated with the procedure seem to be minor relative to the morbidity of advanced disease. The findings of this study suggest that CXL should continue to be considered as a treatment option for patients with progressive keratoconus.

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Footnotes and Financial Disclosures

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