Long-term follow-up of riboflavin/ultraviolet A (370 nm) corneal collagen cross-linking to halt the progression of keratoconus

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ABSTRACT

Aims To determine long-term efficacy of riboflavin/ultraviolet A corneal cross-linking (CXL).

Methods Thirty patients (30 eyes) who had undergone CXL following epithelial removal 4–6 years previously were examined.

Results At 1-year mean, spherical equivalent error (SEQ) increased by +0.72 dioptres (D) ($p<0.002$), corrected distance visual acuity (CDVA) improved ($p<0.005$), mean simulated keratometry (Sim K) reduced by 0.27D ($p<0.04$), cone apex power (CAP) reduced by 0.4D ($p<0.02$), and secondary astigmatism improved ($p<0.03$) compared with preoperative values. At 4–6 years, mean SEQ increased by +0.82D ($p<0.001$), CDVA improved ($p<0.03$), mean Sim K reduced by 0.84D ($p<0.00001$), CAP reduced by 1.16D ($p<0.0005$), and root mean square (RMS) ($p<0.0001$), coma ($p<0.0001$), secondary astigmatism ($p<0.005$) and pentafoil ($p<0.05$) decreased compared with preoperative values. At 4–6 years, mean Sim K reduced by 0.59D ($p<0.0005$), CAP reduced by 0.76D ($p<0.02$), RMS ($p<0.001$), coma ($p<0.002$) and secondary astigmatism ($p<0.02$) reduced and central pachymetry increased ($p<0.05$) compared with 1 year. No treated eyes progressed. None lost >1 line of CDVA. Seven untreated fellow eyes progressed.

Conclusions CXL is an effective and safe treatment with up to 4–6 years follow-up. Improvements in topographic and wave-front parameters evident at 1 year continue to improve at 4–6 years.

INTRODUCTION

Riboflavin/ultraviolet corneal collagen cross-linking (CXL) is the first treatment that halts the progression of keratoconus.[1] Ex vivo studies have shown it to increase stromal stress-strain measurements and its resistance to enzymatic digestion, thermal damage and hydration.[2–6] Two initial uncontrolled, prospective studies reported stabilisation of keratoconus with no alteration in transparency and a reduction in keratometry measurements.[7 8] Subsequent prospective studies demonstrated similar outcomes with reductions in corneal power and improvements in visual performance.[9 10] In a randomised prospective bilateral study with 18 month follow-up, we reported CXL to be effective and safe, halting the progression of keratoconus in treated eyes and improving visual and topographic indices and wave-front parameters, while in 14% of untreated eyes progression was documented.[11]

While clinical studies support the efficacy of CXL, there is a paucity of long-term data. Keratoconus typically progresses at a variable rate, for up to two decades following presentation.[12] The need to repeat CXL is uncertain. Within the literature, there are two groups that have presented data with over 3–4 years' follow-up. Raiskup–Wolf reported 33 eyes with over 3 years' follow-up, and found stabilisation of keratoconus with reduction of keratometry and improvements in vision with time.[13] Caporossi *et al* detected keratoconus stability in 44 eyes after 48 months, with a reduction in keratometry and coma and improvements in vision.[14] The aims of this study were to present further data on the long-term efficacy. We report 29 eyes of 29 patients, who underwent epithelium-off CXL with 4–6 years' follow-up.

METHODS

Subjects

All subjects, with the inclusion and exclusion criteria listed below, who underwent CXL at our institution 4–6 years previously were invited to return for an ophthalmic examination. Thirty patients from a cohort of 36 subjects were examined (four patients were lost to follow-up, one did not wish to attend due to work commitments, one had died from an unrelated neoplastic illness, and one patient who had achieved refractive and topographic stability after CXL elected to undergo intrastromal ring segment insertion at 24 months due to poor spectacle corrected vision). Mean age at time of CXL was 26.3 years (range 12–40, median 25.5). Mean time from CXL was 53.3 months (range 48–72 months, median 48.5 months). Twenty-five patients were men, five were women. One eye of each patient was selected for analysis. In 22, only one eye had been treated (19 subjects had participated in our previous randomised prospective bilateral study; in two cases, the fellow eye was unsuitable because of advanced disease and underwent deep anterior lamellar keratoplasty, and in one case, the fellow eye had undergone intrastromal ring segment insertion). In two patients, bilateral simultaneous CXL had been performed. In these individuals, one eye was selected randomly for analysis, using an independent observer with a shuffled envelope system. In the remaining six patients, CXL was performed in the contralateral eye 18–48 months after treatment of the first eye. In these individuals, the first eye treated was selected for analysis.

All treated eyes had early to moderate keratoconus (grade I to II according to Amsler–Krumeich's classification, with mean central keratometry

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readings of less than 53 dioptres (D), absence of corneal scarring and minimum corneal thickness of greater than 400 μm) with documented evidence of progression with reduced uncorrected distance (UDVA) or corrected distance visual acuity (CDVA) by >1 line and/or worsening of refractive or corneal astigmatism, keratometry or cone apex power (CAP) by 0.75D over the 12–24 months prior to CXL.

Exclusion criteria included advanced keratoconus where corneal irregularity/scarring prevented acquisition of accurate refractive and topographic data, central corneal thickness less than 400 μm (with epithelium on), other ocular pathology, previous anterior segment surgery and diabetes.

Prior to CXL, subjects were counselled as to the nature of the procedure. An ophthalmic and medical history was taken, including refractive and contact lens history, past ophthalmic and family history. Patients were asked to refrain from rigid lens wear for 3 weeks and soft lens wear for 1 week prior to ophthalmic examinations.

Objective and subjective refraction, Snellen decimal equivalent UDVA and CDVA, scanning-slit corneal topography (Orbscan II, Bausch and Lomb, Germany) and Placido-disc video-keratography (Keraton Scout Corneal Analyzer, Optikon 2000, Rome, Italy), ultrasonic central corneal pachymetry (Pachmate DGH55, DGH Technology Inc, Pennsylvania, USA), slit-lamp biomicroscopy, tonometry and mydriatic fundoscopy were performed. For Orbscan examinations, two scans for each eye were taken and the average of values calculated. For Keraton Scout examinations, four scans for each eye were taken and the highest quality scan closest to the average keratometry values was selected for wave-front analysis and CAP measurement. CAP was determined using the Cone Location and Magnitude Index.[15]

Surgical procedure

We have described this procedure previously,[11] which was based on the original protocol described by Wollensak *et al*.[7] Following informed consent, tetracaine 1% and chloramphenicol 0.5% were instilled. A 9.00 mm area of central epithelium was removed using a disposable corneal epithelial spatula (Malosa Medical, Elland, UK). Five drops of riboflavin 0.1%, in dextran 20% were instilled. A period of 5–10 min elapsed before UVA exposure. UVA exposure was for 30 min and utilised 370 nm UVA radiation at 3 mW/cm^2, with a beam diameter of 8.00 mm. During UVA exposure, riboflavin 0.1% drops were administered every 3–5 min and tetracaine 1% drops if the patient reported discomfort. Intraoperative pachymetry was not performed in these initially treated cases.

Postoperative treatment and assessment

Following surgery, ofloxacin 0.3% and chloramphenicol 1% were administered and the eye padded. Oral analgesics, ibuprofen 400 mg PRN tds (400 milligrams three times a day if required) and codeine phosphate 30–60 mg PRN qds (four times a day if required), were prescribed. Three vials of benoxinate 0.4% were given, with instructions to be administered if the postoperative pain was severe, and with a maximum dosage of one drop only every 2 h for a maximum of 48 h. Ofloxacin 0.3% eye was administered four times a day for 1 week, and chloramphenicol 1% ointment at night for 2 weeks.

Postoperative examinations were conducted at regular intervals including at 1 week and at 1, 3, 6, 12 and 48–72 months. Patients were questioned concerning ophthalmic symptoms and an examination was performed, including UDVA, CDVA, refraction, Placido-disc and scanning-slit topography, corneal pachymetry, slit-lamp biomicroscopy, tonometry and mydriatic fundoscopy. Endothelial counts were not performed in this study.

Vector analysis

To investigate astigmatic change in the manifest refraction, vector analysis was performed according to the system described by Retzlaff *et al*.[16]

Statistical methods

Paired Student t tests were used to compare pre- and postoperative outcomes within the treated and untreated groups. Visual acuity results were converted into logmar values before averaging and statistical calculation. Results $p<0.05$ were considered significant.

RESULTS

Spherical equivalent refractive error

Preoperatively, the mean spherical equivalent error (SEQ) in the study eyes was −1.61D. At 12 months, it reduced to −0.89D ($p<0.002$). At 48–72 months, it reduced to −0.79D ($p<0.001$). There was no statistically significant difference in SEQ at 12 months and 48–72 months (table 1).

In 25 fellow eyes that were suitable for CXL but initially remained untreated, the mean SEQ was −2.09D at first examination, and −2.06D at 18–65 months ($p=0.9$).

Table 1 Pre- and 1 and 4–6 year postoperative results (±1 SD) in 30 study eyes

Parameter	Preoperative	12 months	4–6 years	p Value*	p Value**
SEQ	−1.61±1.97D	−0.89±1.79D	−0.79±1.7D	<0.001	0.5
Refractive cylinder	−3.39±2.5DC	−3.41±2.6DC	−3.11±2.4DC	0.6	0.1
UDVA (Snellen decimal equivalent)	0.27±0.29	0.29±0.33	0..286±0.31	0.6	0.9
CDVA (Snellen decimal equivalent)	0.8±0.27	0.905±0.24	0.905±0.2	<0.04	1.0
Pachymetry	488±34 μm	483±35 μm	491±35 μm	0.5	<0.04
Mean Sim K	46.44±3.4D	46.18±3.38D	45.6±3.3D	<0.001	<0.001
Corneal topographic astigmatism	3.75±2.52D	3.7±2.58D	3.31±1.87D	0.2	0.2
Cone apex power	51.69±4.63D	51.29±4.27D	50.53±4.58D	<0.001	<0.02

*p Values at 4–6 years compared with preoperative values.
**p Values at 4–6 years compared with 1 year values.
DC, diopters cylinder; SEQ, spherical equivalent error; UVA, ultraviolet A.

Refractive cylindrical correction

Preoperatively, the mean cylindrical error in study eyes was −3.39 diopters cylinder (DC) at 82°. At 12 months, it was −3.41DC at 85° (p=0.7). At 48–72 months, it was −3.11DC at 77°, which was not different to preoperative (p=0.6) or 12 month values (p=0.2) (table 1). Vector analysis at 48–72 months demonstrated a mean 2.0D change.[16]

In 25 fellow eyes that were suitable for CXL but remained initially untreated, the mean cylindrical error was −2.78D at 104° at the first examination, and −3.55D at 94° at 18–65 months (p<0.0005). Vector analysis demonstrated a mean 2.2D change.

VISUAL PERFORMANCE

Uncorrected distance visual acuity

Preoperatively, the mean Snellen decimal equivalent UDVA in the study eyes was 0.27. At 12 months, it was 0.29 (p=0.6). At 48–72 months, it was 0.286 (p=0.6) (table 1).

At first examination, the mean Snellen decimal equivalent UDVA in 25 untreated eyes was 0.22. At 18–65 months, it was 0.22 (p=0.9).

Corrected distance visual acuity

Preoperatively, the mean Snellen decimal equivalent CDVA in the study eyes was 0.8. At 12 months, it had increased to 0.905 (p=0.02). At 48–72 months, it was 0.905, which was increased compared with preoperative values (p<0.04), but unchanged from 12 months (p=1) (table 1).

At first examination, the mean Snellen decimal equivalent CDVA in the 25 untreated eyes was 0.86. At 18–65 months, it was 0.85 (p=0.9).

Corneal pachymetry

Preoperatively, the mean pachymetric measurement in the study eyes was 488 µm. At 12 months, it was unchanged at 483 µm (p=0.2). At 48–72 months, it was 491 µm, which was not significantly changed from preoperative values (p=0.5), but increased from 12-month measurements (p<0.04) (table 1).

At first examination, the mean pachymetric measurement in the 25 untreated eyes was 490 µm. At 18–65 months, it was unchanged at 490 µm (p=0.9).

Corneal topography

Preoperatively, the mean simulated keratometry (Sim K) in study eyes was 46.44D. At 12 months, it had reduced to 46.18D (p<0.04) (range −1.53 to +1.05D, median −0.175D). At 48–72 months, it was reduced to 45.6D which was less than preoperative values (p<0.00001) (range −2.45 to +0.85, median −0.68) and 12 month measurements (p<0.0005) (range −1.69 to +0.6D, median −0.51D) (table 1).

Preoperatively, the mean simulated corneal topographic astigmatism in study eyes was 3.75D. At 12 months, it was 3.7D (p=0.7). At 48–72 months it was 3.31D (p=0.2) (table 1).

Preoperatively, mean CAP in the study eyes was 51.69D. At 1 year, it was reduced to 51.29D (p<0.02) (range −2.78 to 1.35D, median −0.475D). At 4–6 years, it was reduced to 50.53D, which was less than preoperative values (p<0.0005) (range −4.14 to 0.56D, median −1.035D) and those at 12 months (p<0.02) (range −2.44 to 0.55D, median −0.57D) (table 1).

In 25 untreated eyes at first examination, the mean Sim K in 24 untreated eyes was 45.960D. At 18–65 months, it was 46.49D (p=0.05). At first examination, the mean simulated astigmatism was 3.03D. At 18–48 months, it was 3.34D (p=0.1). At first examination, the CAP was 52.15D. At 18–48 months, it was 52.92D (p=0.09).

Corneal wave-front measurements

Higher-order aberrations of the anterior corneal surface for a 6.0 mm pupil diameter preoperatively, at 12 months and 4–6 years for the 29 study eyes are shown in table 2. Secondary astigmatism was reduced at 12 months compared with preoperative values (p<0.03). At 4–6 years, root mean square values (RMS) (p<0.0001), coma (p<0.0001), secondary astigmatism (p<0.002) and pentafoil (p<0.05) were reduced compared with preoperative values. RMS (p<0.001), coma (p<0.001) and secondary astigmatism (p<0.02) were reduced at 4–6 years compared with 1-year values.

In the 25 initially untreated eyes, spherical aberration became more negative over the follow-up period of 18–48 months (p=0.002).

Improvement/progression of ectasia

Ten study eyes (34%) showed evidence of reduction of ectasia at 48–72 months, identified on the basis of both a decrease in Sim K and CAP of >0.75D and improvements in other refractive, visual, topographical and keratometric parameters. Seven of 25 initially untreated eyes (28%) demonstrated evidence of progression, with an increase in both Sim K and CAP of >0.75D, and consistent worsening of other measurements at 18–65 month follow-up, and underwent, or have been scheduled for, CXL. No study eyes appeared to show evidence of progression, with

Table 2 Pre- and 1 and 4–6 year postoperative results (±1 SD) in 30 study eyes

Parameter	Pre-operative	12 months	4–6 years	p Value*	p Value**
Root mean square	2.45±1.22 µm	2.45±1.13 µm	2.19±1.08 µm	<0.001	<0.001
Coma	2.09±1.23 µm	2.12±1.21 µm	1.82±1.13 µm	<0.001	<0.001
Trefoil	0.78±0.4 µm	0.79±0.33 µm	0.81±0.33 µm	0.7	1.0
Spherical aberration	0.2±0.45 µm	0.18±0.44 µm	0.21±0.37 µm	0.3	0.4
Secondary astigmatism	0.59±0.35 µm	0.51±0.28 µm	0.46±0.26 µm	<0.002	<0.02
Quatrefoil	0.21±0.11 µm	0.22±0.13 µm	0.21±0.11 µm	0.8	1.0
Secondary coma	0.27±0.2 µm	0.26±0.2 µm	0.24±0.16 µm	0.1	0.4
Secondary trefoil	0.15±0.1 µm	0.13±0.1 µm	0.14±0.09 µm	0.3	1.0
Pentafoil	0.08±0.05 µm	0.07±0.05 µm	0.07±0.04 µm	<0.05	0.6

*p Values at 4–6 years compared with preoperative values.
**p Values at 4–6 years compared with 1 year values.

none showing an increase in either Sim K or CAP of >0.75D at 4–6 years.

Complications

There were no changes in transparency of the cornea or lens. All retinal examinations were normal. At 48–72 months, in study eyes, CDVA was increased by two lines in six eyes (20%), one line in six eyes (20%), was unchanged in 13 eyes (43%), and reduced by one line in five eyes (17%). No eyes lost more than one line. One untreated eye lost two lines of BSCVA.

DISCUSSION

CXL is the first modality that halts the progression of keratoconus and postrefractive surgery ectasia. Multiple prospective clinical studies support its efficacy.[7–11 13–14] However, while the mid-term efficacy of riboflavin/UVA CXL has been confirmed, its long-term efficiency is still undetermined. Keratoconus typically presents at puberty and progresses for 10–20 years when it tends to stabilise, presumably as physiological age-related CXL occurs.[1] It is a heterogeneous condition, with variable morphological characteristics and unpredictable and inconsistent rates of progression.[1] The turnover rate of corneal collagen and the extracellular matrix (ECM) is unknown. Taking these factors into consideration, the length of efficacy of CXL, and the need to repeat the procedure is uncertain, and long-term follow-up is necessary.

Raiskup–Wolf presented 33 eyes with over 3-years' follow-up, and reported stabilisation of keratoconus in 98% with continuing reduction of keratometry and improvements in BSCVA with time.[13] Similarly, Caporossi *et al* reported keratoconus stability in all 44 eyes after 48 months, with reduction in keratometry, coma and improvements in visual performance.[14] In our study we found stabilisation of keratoconus in all eyes at 4–6 years. Similar to Raiskup–Wolf, we found significant reductions in topographic and corneal wave-front values at 4–6 years compared with 1 year, suggesting stability and some continuation of improvement of the condition. It is important to note that while no treated eyes appeared to progress, 25% of initially untreated fellow eyes demonstrated evidence of progression. Such findings further support the efficacy of CXL, and indicate that improvements in measured parameters with time are not due to physiological age-related CXL changes.

Our results, and those of Raiskup–Wolf and Caparossi, indicate that CXL is effective in halting the progression of keratoconus for at least 4–6 years. Further follow-up will determine long-term efficacy and the need, if any, to repeat the procedure, and will elucidate how long and to what degree eyes might continue with improvement in visual and topographic parameters. The recurrence of keratoconus following keratoplasty which, typically, albeit rarely, occurs 10–20 years following surgery,[17] suggests that turnover of corneal collagen and ECM may be measured in decades, and that CXL might be effective for at least this length of time, if not longer, given physiological CXL changes with age.

Although most eyes are stabilised after CXL, failure of treatment with progression of ectasia can occur. Koller in 177 eyes described progression in eight eyes (7.6%). They identified eyes with advanced keratoconus with maximum keratometry values >58D being at greatest danger of progression.[18] In our study, no treated eyes progressed with 4–6 year follow-up. However, only eyes with grade I to II, according to Amsler–Krumeich's classification, were included, and no eyes had mean simulated keratometry values greater than 54D. Our study cannot, therefore, elucidate the long-term stability of CXL in advanced keratoconus where given the greater biomechanical weakness and more aggressive nature of the disease, efficacy and stability of CXL might not be so promising.

A most interesting finding of our study was the continuation of improvement of visual and topographic parameters with time (table 1). An increased activity of proteinase enzymes and reduced activity of proteinase inhibitors has been identified in keratoconic corneas,[19] with the increased stromal protein digestion perhaps being responsible for reduced biomechanical stability and corneal thinning.[20] It may be postulated that CXL of corneal collagen and ECM reduces the efficacy of these proteinase enzymes, reducing stromal protein digestion, while the laying down of new collagen and ECM continues as part of normal physiological processes, resulting in improvement in corneal biomechanics and shape, and an increase in corneal thickness, as identified in our study. The observations by Speorl, identifying increased resistance of cross-linked corneal tissue against enzymatic digestion would support this hypothesis.[4]

There were no long-term adverse events in our study. No eyes lost more than one line of BSCVA. Given the limited total UVA dosage required for CXL, especially in relation to internal ocular structures, such as the endothelium, lens and retina, such findings are not surprising.

This study demonstrates that CXL appears to be an effective and safe modality to halt the progression of keratoconus with up to 4–6 years' follow-up. Improvements in topographic and wave-front parameters evident at 1 year continue to improve at 4–6 years. Further follow-up is indicated given the chronic nature of Keratoconic progression.

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