Transepithelial Corneal Cross-linking Using an Enhanced Riboflavin Solution

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ABSTRACT

PURPOSE: To assess the efficacy of a modified high concentration riboflavin solution containing benzalkonium chloride 0.01% for transepithelial corneal cross-linking (CXL).

METHODS: In this prospective, interventional multicenter cohort study, 26 eyes of 26 patients with documented progressive keratoconus who underwent transepithelial CXL were included. Follow-up at 6 and 12 months postoperatively included slit-lamp examination, uncorrected and corrected distance visual acuity (logMAR), maximum keratometry (Kmax), and corneal pachymetry (corneal thinnest point) as determined by Scheimpflug imaging. Statistical analysis was performed using repeated measures analysis of variance and the Friedman test for parametric and non-parametric data, respectively. *P* values less than .05 were considered significant.

RESULTS: Kmax did not change significantly at postoperative months 6 and 12. Changes in corneal thinnest point did not change postoperatively over 12 months. Uncorrected and corrected distance visual acuity did not change postoperatively. Progression (defined by an increase in Kmax greater than 1.00 diopter occurred in 46% of eyes at 12 months. Corneal epithelial defects were observed in 46% of the patients and marked punctate corneal epitheliopathy/loose epithelium in 23% of the patients in the immediate postoperative period. No corneal infection, sterile infiltrates, or haze were observed.

CONCLUSIONS: Transepithelial CXL with an enhanced riboflavin solution did not effectively halt progression of keratoconus. Significant epithelium damage was evident in the immediate postoperative period.

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Keratoconus is a progressive, degenerative disease resulting in corneal thinning, impaired biomechanics and conical ectasia, which may lead to visual impairment due to irregular astigmatism.[1-3] Corneal cross-linking (CXL) is a procedure to arrest keratoconus progression.[4] During the standard epithelium-off CXL procedure, also called the "Dresden protocol," the epithelium is removed, the photosensitizer riboflavin instilled, and the stroma subjected to irradiation with ultraviolet light-A (UVA) at 365 nm. The photochemical reaction results in a biomechanical strengthening of the stroma by inducing additional covalent bonds between collagen and the proteoglycans of the extracellular matrix. Numerous clinical studies have documented that CXL may arrest the progression of keratoconus and postoperative ectasia, with documented improvements in visual acuity and corneal shape.[5-17]

Before 2013, two elements were considered essential for successful CXL: the presence of riboflavin in the corneal stroma and induction of cross-links via photoactivation of riboflavin. Because new riboflavin formulations achieve satisfying riboflavin concentrations in the cornea,[18,19] removal of the corneal epithelium seemed to become a less crucial step in the cross-linking procedure.[20,21]

A CXL technique that would keep the epithelium intact while delivering enough riboflavin into the corneal stroma for cross-linking to occur would therefore be advantageous and, moreover, reduce the risk of postoperative infection.[20-23] In 2013, Richoz et al. showed that yet another factor is essen-

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tial for successful cross-linking: the presence of oxygen in the corneal stroma.[12] The corneal epithelium represents a barrier to oxygen diffusion and consumes 10 times more oxygen than a stromal layer of comparable thickness.[24] In consequence, most transepithelial CXL clinical studies published so far have shown a reduced efficacy and high failure rates when compared to standard epithelium-off CXL.[19] However, with a modified high concentration riboflavin solution containing benzlyalkonium chloride, they found an increase in stiffness. Based on previous preclinical work by Raiskup et al.,[19] in this study we tested the efficacy of such a high concentration of modified riboflavin solution containing benzlyalkonium chloride in transepithelial CXL in patients with documented progressive keratoconus.

PATIENTS AND METHODS

This was a prospective, multicenter cohort study, commenced in March 2013 and terminated in August 2014. Three tertiary eye centers participated in the study: the Department of Ophthalmology, Geneva University Hospitals, Geneva, Switzerland; the Department of Ophthalmology, C. G. Carus University Hospital, Dresden, Germany; and the St. Thomas Hospital, London, United Kingdom. The study protocol was approved by the institutional review board of the University Hospitals of Geneva and was in agreement with the tenets of the Declaration of Helsinki.

The study group included 26 eyes from 26 patients with progressive keratoconus. All patients showed clinically evident keratoconus grades 1 to 3 according to the Amsler–Krumeich classification. Progression of keratoconus was defined as an increase of maximum keratometry (Kmax) of the anterior surface of greater than 1.00 diopter (D) within the preceding 12 months, as measured by Scheimpflug imaging (Pentacam; Oculus Optikgeräte, Wetzlar, Germany). All patients were 18 years or older at enrollment, and minimum corneal thickness was greater than 400 µm with the corneal epithelium. Exclusion criteria were: patients younger than 18 years, keratoconus stage 4 according to the Amsler–Krumeich classification, corneal stroma thinner than 350 µm without the epithelium, central corneal scarring/corneal dystrophies, a history of herpetic eye disease, patients previously subjected to any corneal or intraocular surgical procedures, and pregnancy or breast-feeding at the time of CXL treatment.

Transepithelial CXL was performed as follows: proparacaine 0.5% eye drops were instilled 5 minutes before the procedure. After insertion of an eyelid retractor, a modified riboflavin solution (0.25% riboflavin and 0.01% benzlyalkonium chloride) was instilled every minute for 30 minutes. A retention ring was not used. At the end of riboflavin instillation, the remaining solution was washed off using saline and corneal thickness was measured by ultrasound pachymetry. The cornea was irradiated at 9 mW/cm^2 for 10 minutes using the UV-X 2000 device (Peschke Meditrade GmbH, IROC, Zurich, Switzerland). Postoperatively, patients received a bandage contact lens (Focus Night & Day; Ciba Vision, Duluth, GA), preservative-free ofloxacin eye drops four times daily, and preservative-free artificial tears five times daily. The contact lens was removed after 3 days, whereas ofloxacin eye drops were maintained until complete epithelial closure. After complete epithelial closure, dexamethasone preservative-free eye drops were administered twice daily for 2 weeks, followed by a single application in the morning for 2 weeks. Postoperative follow-up was scheduled daily until complete epithelial closure, and at 6 and 12 months postoperatively.

The following examinations were performed preoperatively and at 6 and 12 months postoperatively: slit-lamp examination, uncorrected and corrected distance visual acuity (logMAR), and Kmax and corneal thickness with the aid of Scheimpflug imaging (Pentacam). An increased Kmax reading of greater than 1.00 D at 12 months postoperatively was considered as progressive disease and treatment failure.

Statistical Analysis

For calculation of the mean and subsequent statistical comparisons, decimal units of visual acuity were transformed to the logMAR units. Normality of the data was tested with the Kolmogorov–Smirnov test and quantitative characteristics were compared using the repeated measures analysis of variance (ANOVA) and the Friedman test for parametric and non-parametric data, respectively. A *P* value of less than .05 was used to mark statistical significance. All statistical analyses were performed using MedCalc software (version 14; MedCalc, Oostende, Belgium).

RESULTS

The study included 26 eyes of 26 patients (16 males and 10 females) with progressive keratoconus who underwent transepithelial CXL. The average age was 27.6 ± 6.4 years (range: 15 to 53 years).

Change in Kmax, UDVA, CDVA, and corneal thinnest point (CTP) are shown in **Table 1**. There were no significant variable except CTP.

The treatment failed in 12 of 26 patients (46%), showing an increase of greater than 1.00 D in Kmax readings at 12 months after transepithelial CXL. Corneal epithelial defects were observed in 12 patients (46%) in the immediate postoperative period (**Figure**

TABLE 1
Clinical Outcomes After Transepithelial Corneal Cross-linking at 6 and 12 Months Postoperatively

Parameter	Baseline	6 Months	12 Months	*P*
Kmax (D)	59.5 ± 9.0 (356 to 75.2)	59.4 ± 8.9 (35.6 to 75.2)	59.2 ± 8.8 (35.3 to 74.4)	.86
UCVA (logMAR)	0.97 ± 0.97 (0.52 to 1.30)	1.02 ± 0.24 (0.70 to 1.30)	1.00 ± 0.28 (0.40 to 1.30)	.52
CDVA (logMAR)	0.53 ± 0.54 (0.22 to 1.00)	0.59 ± 0.28 (0.22 to 1.30)	0.57 ± 0.22 (0.22 to 1.00)	.14
CTP (μm)	417 ± 14 (301 to 456)	414 ± 14 (290 to 445)	412 ± 15 (285 to 446)	< .01

Kmax = maximum keratometry; D = diopters; UCVA = uncorrected visual acuity; CDVA = corrected distance visual acuity; CTP = corneal thinnest point

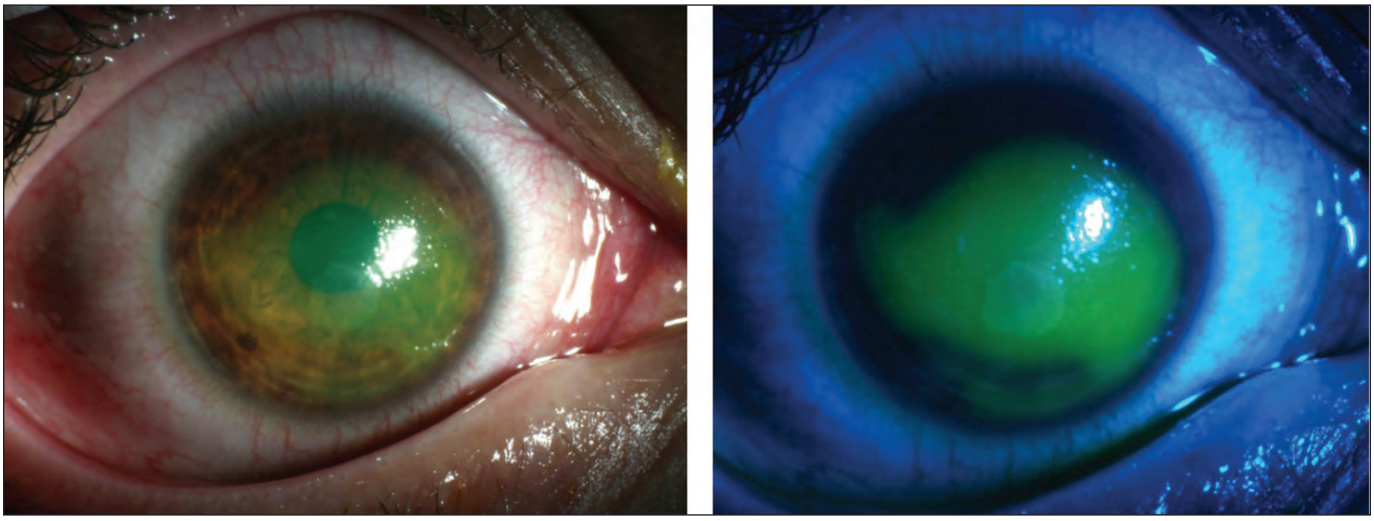

Figure 1. Corneal epithelium on postoperative day 1 after transepithelial corneal cross-linking. A diffuse and large scale erosion is observed (left and right).

1), whereas 6 patients (23%) showed marked punctate corneal epitheliopathy/loose epithelium in the immediate postoperative period. No corneal infection, sterile infiltrates, or haze were observed (**Figure 2**). Data are summarized in **Table 1**.

DISCUSSION

Transepithelial CXL strives to provide corneal biomechanical stability in patients with progressive keratoconus and avoid patient discomfort and complications associated with epithelial removal in the standard epithelium-off technique. A major challenge for every cross-linking technique is to achieve adequate riboflavin penetration into the corneal stroma. Baiocchi et al. were the first to suggest that an adequate stromal riboflavin concentration may only be obtained when the epithelium is removed.[25] Therefore, enhancing the penetration of riboflavin into the corneal stroma remains a key issue for the success of transepithelial CXL.[18,19,26]

Several approaches may be taken to enhance the penetration of riboflavin via an intact epithelium, including iontophoresis,[27-29] ultrasound treatment,[29] and modification of riboflavin composition.[19,30,31] Although these experimental techniques show encouraging results in vitro, further investigation is required before they can be routinely applied in clinical practice.[32-34]

In our study, we used a novel riboflavin solution containing 0.25% riboflavin and 0.01% benzalkonium chloride, similar to a composition we published previously that demonstrated an increase in biomechanical stiffness similar to that obtained in epithelium-off CXL in experimental conditions.[19] Also, unpublished data from in vitro experiments from our group show that this specific composition can achieve satisfactory absorption in the corneal tissue.

We opted to use an accelerated CXL protocol in this study because most clinical data published and in preparation suggest that moderately accelerated CXL (9 mW/cm^2 for 10 minutes) is effective in stabilizing progressive keratoconus,[25,26,35] with an excellent safety profile.[36-38]

Previous clinical studies of epithelium-off CXL have consistently reported a reduction in Kmax and a corresponding increase in visual acuity, both of which support the efficacy of the procedures.[10] In this study, neither Kmax nor visual acuity showed any significant changes at 6 and 12 months postoperatively (**Table 1**).

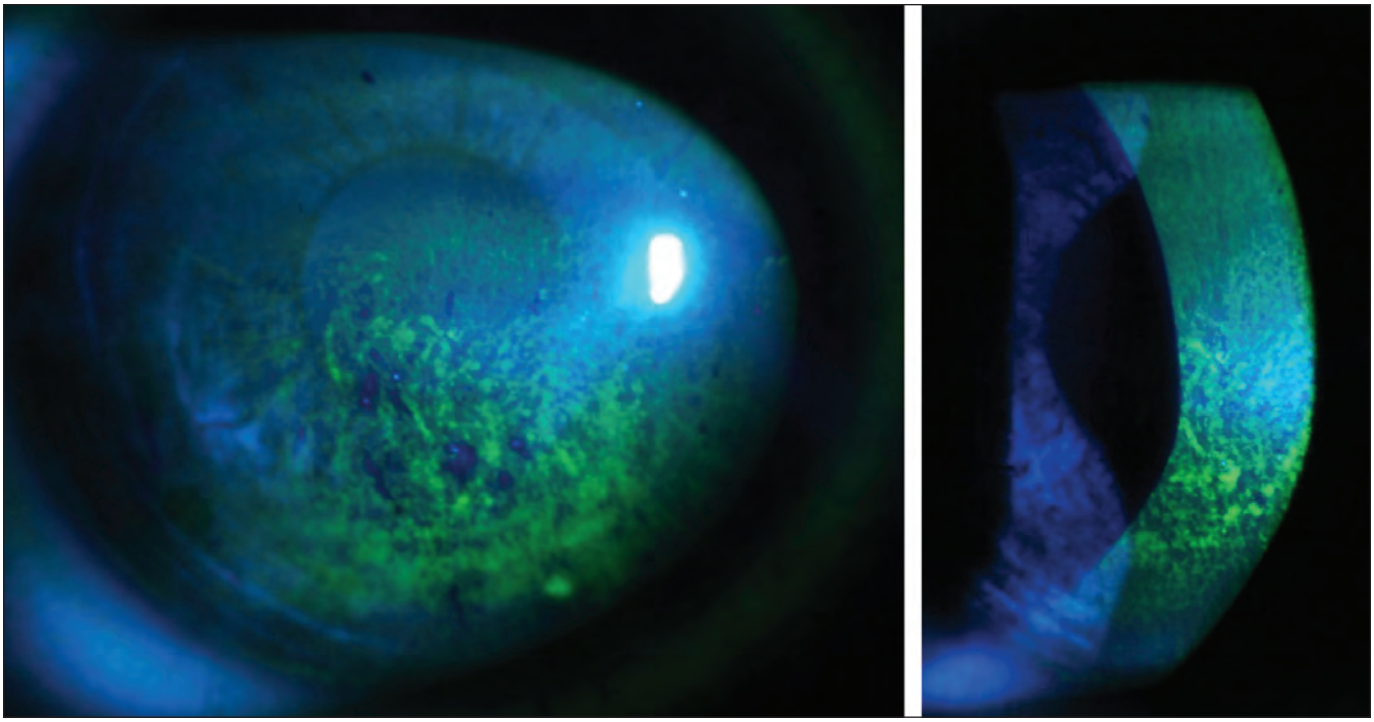

Figure 2. Corneal epithelium on postoperative day 3 after transepithelial corneal cross-linking. The images on the left and the right show a heavily broken down epithelium with positive fluorescein staining.

The most striking finding was that 12 eyes showed an increase of Kmax readings of greater than 1.00 D at 12 months postoperatively. This translates to a treatment failure rate of 46%, compared to 3% to 5% reported in epithelium-off CXL studies.[39] Another finding concerning ocular surface was the high incidence of epithelial defects (46%) and punctate corneal epitheliopathy/loose corneal epithelium (23%), which was observed in the immediate postoperative period. No sterile infiltrates or haze were observed in any of the eyes treated.

The current published data regarding the efficacy of transepithelial CXL are generally disappointing, although there is general consensus that it is a safe procedure. Koppen et al. reported reduced efficacy of benzalkonium chloride-assisted transepithelial CXL compared to standard CXL in stabilizing progressive keratoconus in a cohort of 38 patients (53 eyes).[40] The same conclusion was reported by Kocak et al.,[33] whereas Caporossi et al. found keratoconus instability after transepithelial CXL, in particular in pediatric patients 18 years old and younger, with functional regression in patients between 19 and 26 years old after 24 months of follow-up.[32] Recently, Soeters et al. documented in a randomized controlled trial that transepithelial CXL is less effective than standard epithelium-off CXL, showing a high failure rate (23%) in a cohort of 35 patients with progressive keratoconus.[41] On the contrary, in a study by Salman et al., transepithelial CXL treatment appeared to halt keratoconus progression in a pediatric population over 12 months.[42]

The unsatisfactory results yielded by the current study may be related to two factors. First, it is likely that the stromal uptake of riboflavin was impaired in the study eyes, with the corneal epithelium representing a major barrier prohibiting tissue penetration of the riboflavin molecules even in the presence of prolonged application of a cationic surfactant such as benzlyalkonium chloride.[24] However, the corneal epithelium reduces not only riboflavin absorption, but also oxygen diffusion into the stroma. Freeman performed a series of experiments in the ex vivo rabbit cornea in the early 1970s and showed that the epithelial layer of the cornea consumes as much total oxygen as the entire stroma, and that epithelial oxygen use is 10 times higher than that of the stroma.[43] Similarly, Augsburger and Hill showed that oxygen flux into the rabbit cornea in vivo is significantly higher when the epithelium is denuded.[44] Our group has recently shown that oxygen is essential for the biomechanical effect of CXL.[45] It is therefore likely that limited oxygen availability resulted in impairment of the CXL process in our study eyes.

Transepithelial CXL has to overcome the double disadvantage of limited intrastromal riboflavin concentration and oxygen saturation. Future research should focus on delivering the UVA energy using a

slower setting (ie, 3 mW/cm^2 for 30 minutes) to allow for adequate re-oxygenation of the stroma.

Other limitations of this study are that it was not conducted as a randomized controlled study and that different grades of keratoconus severity were included in the study group. Nevertheless, our results provide further evidence that transepithelial CXL has to address challenging issues to compete with standard epithelium-off CXL in terms of efficacy.

AUTHOR CONTRIBUTIONS

Study concept and design (FR, FH); data collection (ZG, FR, DO, ES); analysis and interpretation of data (ZG, FR, GDP, FH); writing the manuscript (ZG, DO, GDP, FH); critical revision of the manuscript (FR, DO, ES, FH); statistical expertise (ZG, GDP); administrative, technical, or material support (FH); supervision (DO, FH)

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