Safety Profile of High-Fluence Corneal Collagen Cross-Linking for Progressive Keratoconus: Preliminary Results From a Prospective Cohort Study

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ABSTRACT

PURPOSE: To investigate the effect of high-fluence corneal collagen cross-linking (CXL) with riboflavin and ultraviolet-A in the management of progressive keratoconus.

METHODS: Preliminary results from a prospective cohort study. Seven eyes from 7 patients with progressive keratoconus subjected to CXL were included. CXL was performed as a standard epithelium-off procedure, irradiating with high-fluence settings (18 mW/cm^2 for 5 minutes). Endothelial cell density (ECD), speed of postoperative epithelial healing, maximal and average keratometric readings (Kmax and Kmean, respectively) of the anterior corneal surface, and corrected distance visual acuity (CDVA) were evaluated preoperatively and at 1 and 6 months after CXL. One-way analysis of variance was applied for statistical analysis. *P* values less than .05 were considered significant.

RESULTS: ECD did not change significantly postoperatively and complete epithelial healing occurred in all eyes within 96 hours postoperatively. No morphological alterations in the corneal limbus were observed. Kmax, Kmean, and CDVA showed no significant changes at 1 and 6 months postoperatively. No complications were observed postoperatively.

CONCLUSIONS: Although the preliminary results are not sufficient for a valid evaluation of the biomechanical effect and the overall safety profile of high-fluence CXL in vivo, they demonstrate that CXL at 18 mW/cm^2 for 5 minutes affects neither endothelial cell density nor the speed of epithelial healing, an indirect indicator of limbal stem cell function.

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Corneal collagen cross-linking (CXL) with riboflavin and ultraviolet-A has become a standard treatment in the management of progressive keratoconus and postoperative ectasia.[1-4] CXL increases the biomechanical stability of the corneal stroma, arrests keratoconus progression, and, in distinct patients, increases corrected distance visual acuity (CDVA), improving the quality of life in patients with keratoconus.[5]

Conventional CXL, as described by the Dresden protocol, is an epithelium-off procedure using standard settings: irradiation with ultraviolet-A light at 365 nm with a fluence of 3 mW/cm^2 for 30 minutes, corresponding to a total energy dose of 5.4 J/cm^2. The efficacy and safety of the Dresden protocol for the treatment of progressive keratoconus and ectasia after LASIK and photorefractive keratectomy have been extensively documented.[2,3]

In an attempt to improve the method by shortening irradiation time, considerations were made based on one of the fundamental laws of photochemistry, the Bunson-Roscoe law of reciprocity. It states that a photochemical effect should be similar when changing irradiation time and intensity while maintaining the total amount of energy. In consequence, several new commercially available CXL devices offer high-fluence settings, with only limited experimental and clinical evidence.

We started a prospective cohort study to investigate the efficacy and safety of high-fluence CXL in the management of progressive keratoconus, exploring its potential benefits and limitations. In this preliminary report, we present the short-term results of the first eyes treated.

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Drs. Richoz and Hafezi hold a patent on a UV light source (PCT/CH 2012/000090). The remaining authors have no financial or proprietary interest in the materials presented herein.

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PATIENTS AND METHODS

Clinical Setting and Patients

This was a prospective cohort study, initiated in May 2012 in the Department of Ophthalmology, Geneva University Hospitals, Geneva, Switzerland. The study protocol foresees inclusion of 22 eyes with progressive keratoconus undergoing high-fluence epithelium-off CXL. Currently, 7 patients are included with a follow-up of 6 months. When compared to the original Dresden protocol,[4] only fluence (18 mW/cm^2) and irradiation time (5 minutes) were varied. Inclusion criteria were: age older than 18 years, presence of clinically evident keratoconus grade 1-3 according to the Amsler-Krumeich classification, documentation of keratoconus progression by corneal topography (mean of three consecutive measurements, showing an increase of Kmax > 1 dpt within 12 months) and minimal stromal thickness of at least 400 µm after removal of the epithelium. Exclusion criteria included: presence of central corneal scarring, history of herpetic eye disease, previous corneal or intraocular surgical procedures, and pregnancy or breast-feeding at the time of the procedure.

The local Institutional Review Board of the Geneva University Hospitals approved the treatment protocol and a written consent was obtained from all patients. All procedures and interventions were performed in accordance with the tenets of the Declaration of Helsinki.

High-Fluence CXL Protocol

All patients were subjected to epithelium-off CXL according to a modified Dresden protocol.[4] Briefly, proparacaine 0.5% eye drops were instilled every 5 minutes for a total of 15 minutes, alternated with 0.2% oxybuprocaine eye drops. A speculum was placed and the epithelium was removed using a blunt knife in a radius of 9 mm. Iso-osmolaric riboflavin solution with dextran 20% was instilled every 3 minutes for 30 minutes prior to ultraviolet-A irradiation. No retention ring was used. At the end of riboflavin instillation, corneal thickness was measured by ultrasound pachymetry (SP-100; Tomey, Nagoya, Japan). Finally, riboflavin was washed off, followed by ultraviolet-A irradiation at 18 mW/cm^2 for 5 minutes (CXL-365 Vario; Schwind eye-tech solutions, Kleinostheim, Germany).

Postoperatively, patients received ofloxacin ointment and a bandage contact lens (Focus Night & Day; Ciba Vision, Atlanta, GA), which was removed on postoperative day 1. Patients were advised to apply ofloxacin ointment every 2 hours and were seen every day until complete healing of the epithelium, followed by application of fluorometholone eye drops for 4 weeks.

Postoperative follow-up was daily until complete corneal epithelial healing and at 1 and 6 months postoperatively. The following parameters were evaluated during each follow-up examination: CDVA in logarithmic expression (logMAR), Kmax and Kmean in diopters (D) (Pentacam; Oculus Instruments, Wetzlar, Germany), and endothelial cell density (ECD) (cells/mm^2) (HRT Rostock Cornea Module; Heidelberg Engineering GmbH, Heidelberg, Germany).

Statistical analysis was performed using SPSS for Mac (version 17.0; SPSS, Inc., Chicago, IL). Data showed normal distribution (Saphiro-Wilk test). One-way analysis of variance with pairwise multiple comparisons (Bonferoni *t* test) was applied for statistical analysis. *P* values less than .05 were considered statistically significant. All parameters are expressed in mean ± standard deviation.

RESULTS

Seven eyes from seven patients (4 males/3 females) with an average age of 29.3 ± 4.6 years have been included so far in the study. All treatments were uneventful. No corneal edema or other irregularities were observed in the early postoperative period.

There was no significant difference between preoperative (2,943 ± 162 cells/mm^2) and postoperative (2,918 ± 189 cells/mm^2) ECD at 1 month postoperatively (P = .64). All eyes showed complete healing of the epithelium within 96 hours. Slit-lamp examination did not reveal any biomicroscopic alterations of the corneal limbus. Kmax decreased from 55.6 ± 3.8 D preoperatively to 54.7 ± 3.4 D at 4 weeks postoperatively (P = 0.4), whereas CDVA increased from 0.41 ± 0.34 to 0.51 ± 0.29 at 4 weeks postoperatively (P = .062). Kmean decreased from 46.2 ± 2.8 D preoperatively to 45.7 ± 3.4 D at 4 weeks postoperatively (P = .51). At 6 months postoperatively, Kmax was 52.9 ± 2.7 D and CDVA was 0.58 ± 0.37 D with no significant differences compared to preoperative values (P = .42 and .055, respectively). Kmean decreased to 45.3 ± 3.6 D (P = .44), whereas ECD did not change significantly at 6 months postoperatively (2,906 ± 174 D). No complications occurred during the 6-month postoperative follow-up period.

DISCUSSION

Recently, CXL technology focuses on modifying the technical characteristics of the procedure to shorten treatment time. The theory behind this concept is based on the photochemical law of reciprocity (Bunson-Roscoe law), stating that the biological effect of irradiation on a tissue should stay similar when the total energy dose is maintained. In other words, one achieves the same photochemical effect with reduced irradiation time and correspondingly increased

irradiation intensity, meaning that a 5-minute irradiation at 18 mW/cm^2 should provide the same effect as the standard 30 minutes at 3 mW/cm^2.

Whereas devices providing high-fluence treatments have been commercially available since 2011, there is only limited experimental evidence regarding the biomechanical effect of high-fluence CXL. Wernli et al. documented in vitro that the Bunsen-Roscoe reciprocity law is only valid for illumination intensities up to 50 mW/cm^2 and illumination times of more than 2 minutes; intensities higher than 50 mW/cm^2 do not provide significant corneal biomechanical effects.[6] Our own experiments in vitro indicate that intensities higher than 3 mW/cm^2 are associated with a proportional reduction in the corneal biomechanical effects.[7]

Currently available systems offer a wide intensity spectrum from 3 to 43 mW/cm^2, which did not yield satisfying biomechanical results in vitro.[7] The biomechanical effects of high-fluence CXL in vivo and, most important, its safety profile have not yet been investigated.

In our prospective cohort study, the first seven patients with progressive keratoconus were subjected to high-fluence epithelium-off CXL. No complications were observed during the 6-month postoperative period. Measurement of ECD did not reveal any significant differences postoperatively. The speed of postoperative epithelial healing, an indirect indicator of limbal stem cell function,[8] was comparable to CXL using the standard settings (3 mW/cm^2 for 30 minutes) and complete healing occurred within 96 hours in all cases.[9] Kmax, Kmean, and CDVA showed no significant changes postoperatively.

Our preliminary data suggest that high-fluence CXL at 18 mW/cm^2 does not seem to have a negative impact on ECD or the corneal limbus. Until the total number of eyes is treated in this cohort study, the low number of eyes and the short follow-up period do not allow for a valid evaluation of the biomechanical effect and the safety profile of high-fluence CXL in vivo.

AUTHOR CONTRIBUTIONS

Study concept and design (FH); data collection (EB, ZG, OR); analysis and interpretation of data (FH, ZG, OR); drafting of the manuscript (FH); critical revision of the manuscript (EB, ZG, OR); statistical expertise (ZG); administrative, technical, or material support (FH); supervision (FH)

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