·Clinical Research·

A short-term study of corneal collagen cross-linking with hypo-osmolar riboflavin solution in keratoconic corneas

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Received: 2014-03-02 Accepted: 2014-05-13

Abstract

• AIM: To report the 3mo outcomes of collagen cross-linking (CXL) with a hypo-osmolar riboflavin in thin corneas with the thinnest thickness less than 400 μm without epithelium.

• METHODS: Eight eyes in 6 patients with age 26.2±4.8y were included in the study. All patients underwent CXL using a hypo-osmolar riboflavin solution after its de-epithelization. Best corrected visual acuity, manifest refraction, the thinnest corneal thickness, and endothelial cell density were evaluated before and 3mo after the procedure.

• RESULTS: The mean thinnest thickness of the cornea was 408.5±29.0 μm before treatment and reduced to 369.8±24.8 μm after the removal of epithelium. With the application of the hypo-osmolar riboflavin solution, the thickness increased to 445.0±26.5 μm before CXL and recover to 412.5±22.7 μm at 3mo after treatment, P=0.659). Before surgery, the mean K-value of the apex of the keratoconus corneas was 57.6±4.0 diopters, and slightly decreased (54.7±4.9 diopters) after surgery (P=0.085). Mean best-corrected visual acuity was 0.55±0.23 logarithm of the minimal angle of resolution, and increased to 0.53±0.26 logarithm after surgery (P=0.879). The endothelial cell density was 2706.4±201.6 cells/mm² before treatment, and slightly decreased (2641.2±218.2 cells/mm²) at last fellow up (P=0.002).

• CONCLUSION: Corneal collagen cross-linking with a hypo-osmolar riboflavin in thin corneas seems to be a promising treatment. Further study should be done to evaluate the safety and efficiency of CXL in thin corneas for the long-term.

• **KEYWORDS:** corneal collagen cross-linking; keratoconus; hypo-osmolar riboflavin; thin corneas

DOI:10.3980/j.issn.2222-3959.2015.01.17

Gu SF, Fan ZS, Wang LH, Tao XC, Zhang Y, Wang CQ, Wang Y, Mu GY. A short-term study of corneal collagen cross-linking with hypo-osmolar riboflavin solution in keratoconic corneas. *Int J Ophthalmol* 2015;8(1):94-97

INTRODUCTION

Corneal collagen cross-linking (CXL) is a novel and minimally invasive technique to strengthen the cornea. This method was first described by Spoerl in 1998 [1]. With the Ultraviolet A (UVA 365 nm) and riboflavin (as photosensitizer), new chemical bonds are induced between collagen molecules, fibers, and micro-fibrils by photosensitized oxidation[2]. It has been proved that CXL can result in an increase of the biomechanical stability of the corneal tissue, halting the progression of the ectasia. And many studies have demonstrated that CXL was effective in halting the progression of keratoconus [3,4]. For the current protocol of CXL, corneal thickness below 400 μm is the most important risk factor for complications [5-7]. To prevent UVA from reaching the deep stroma, the endothelium and crystalline lens, this method requires a minimum stromal thickness of 400 μm after epithelial removal for safety purpose. Unfortunately, there are many cases of keratoconus and post-LASIK ectasia with corneal stromal thickness less than 400 μm. To avoid this limitation, Hafezi *et al* [8] have proposed an alternative treatment protocol by using hypo-osmolar riboflavin solution to swell the corneal stroma. However, there is one study reported the failure with this modified method for progressive keratoconus in an extremely thin cornea[9].

To further evaluate this method, we observed the early change of corneas and complications of patients with thin corneas who were treated with hypo-osmolar riboflavin solution.

SUBJECTS AND METHODS

Subjects Patients with keratoconus were prospectively recruited from the Cornea Outpatient Clinic of Shandong Provincial Hospital. Inclusion criteria were progressive

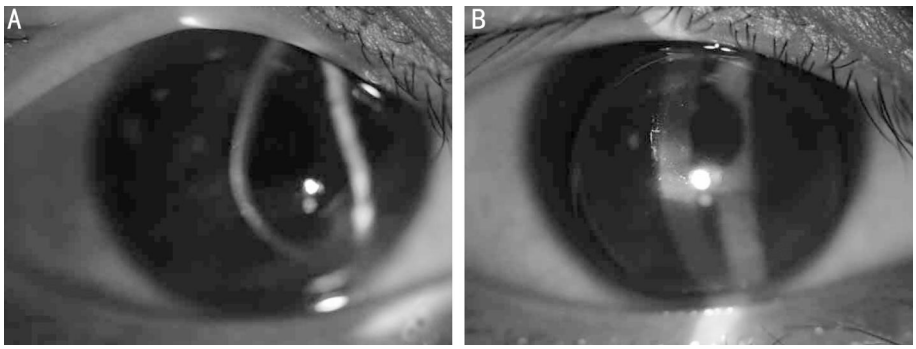

Figure 1 Photographs of slit-lamp biomicroscopy of one cornea A: Pre-CXL; B: Post-CXL at 3mo.

keratoconus documented within the past 12mo as evident by astigmatic refraction and/or topography, no previous ocular surgery, the thinnest corneal thickness of less than 400 μm (without epithelium), and no wearing of contact lenses before initial evaluation and treatment.

The study was approved by the ethics committee of Shandong Provincial Hospital under the principles of the Helsinki Declaration. Informed consent was obtained from all study participants before the initiation of crosslink treatment.

Methods Hypo-osmolar riboflavin solution (0.1%) was generated by diluting vitamin B_2-riboflavin-5-phosphate 0.5% (Shandong Fangming pharmaceutical Limited by Share Ltd, Shandong, China) in physiological salt solution (sodium chloride 0.9% solution; 310 mOsmol/L; Sichuan Kelun pharmaceutical Limited by Share Ltd, Sichuan Province, China). The procedure was performed under sterile conditions. After topical anesthesia using proparacaine hydrochloride 0.5% (Alcaine; Alcon Laboratories, Inc., Fort Worth, Texas, USA) eye drops, corneal epithelium was swelled by contact of filter paper (the diameter was 9 mm) soaked with 20% alcohol for 60s followed by removal using a rotating brush. De-epithelialization was followed by measuring the corneal thickness with optical coherenece tomography (OCT, Cirrus HD-OCT 4000; Carl Zeiss Meditec Inc, Hacienda Drive, Dublin, USA) to validate the thinnest thickness was less than 400 μm. Hypo-osmolar riboflavin solution (0.1%) was applied to the cornea every 3min for 30min. The corneal thickness was checked by OCT and hypo-osmolar riboflavin solution was again administered until corneal thickness was more than 400 μm at the thinnest point. A digital slit-lamp photograph (True Digital Slit Lamp SL DC-3; Topcon Corporation, Hasunuma-cho, Itabashi-ku, Tokyo, Japan) was performed to ascertain that the riboflavin penetrated into the cornea stroma.

Then the eye was irradiated with UVA of 370-nm wavelength (UV-X illumination system version 1000, UVXTM, IROC AG, Zurich, Switzerland) at a working distance of 5 cm. An area with 9 mm diameter in the center of the cornea was irradiated with an energy density of 3.0 mW/cm^2. During the 30min of irradiation, hypo-osmolar riboflavin solution was applied every 3min to maintain the riboflavin saturation in cornea stroma. At the end of the procedure, a combination of dexthamesone 0.1% and tobramycin 0.3% (Tobradex, Alcon Co. Ltd., USA) was administered 4 times daily in all patients and a bandage soft contact lens was applied until healing of the corneal epithelium was completed.

Examinations Patients were assessed before and at 3mo after the procedure. Each examination included the measurement of best corrected visual acuity (BCVA) with glasses or contact, manifest refraction (diopters; D), corneal topography (Orbscan Ⅱ; Bausch & Lomb Incorporated, Rochester, New York, USA), mean thinnest corneal thickness (MTCT) by OCT device, digital slit-lamp photograph and fundus examinations. Endothelial cell density was acquired with a Specular Microscope (Specular Microscope SP-3000P; Topcon Corporation, Hasunuma-cho, Itabashi-ku, Tokyo, Japan).

Evaluation Statistical evaluation of values before and 3mo after corneal CXL was performed using the Student's *t*-test with SPSS software version 17 (SPSS GmbH Software, Munich, Germany).

RESULTS

The analysis included 8 eyes of 6 patients (5 males and 1 female) with a mean age of 26.2±4.8y and stage 1 to 3 keratoconus, according to the Krumeich classification. All eyes had transparent corneas before the procedure, and preoperative Vogt striae were observed in 1 case. Figure 1 showed a preoperative slit lamp photograph of a cornea with a minimal thickness of 372 μm after removal of the epithelium, in which obvious Vogt striae in the stroma can be observed (Figure 1A). In this case, the corneal thickness increased to 412 μm at 3mo after procedure and an apical scar was showed in the stroma (Figure 1B).

Before surgery, the MTCT was 408.5±29.0 μm and 369.8±24.8 μm with and without epithelium. After the application of hypo-osmolar riboflavin solution, the thickness increased to 445.0±26.5 μm, then returned to 412.5±22.7 μm at 3mo after operation, which has no difference with the values before surgery (P=0.659, Figure 2).

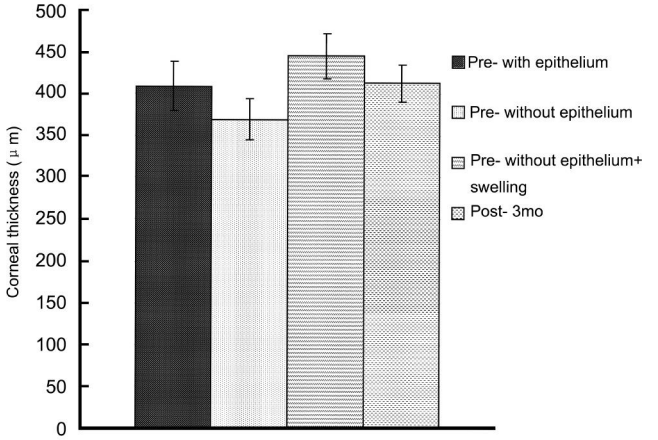

Figure 2 Bar graph showing the mean thinnest corneal thickness with epithelium and without epithelium, after swelling and 3mo after CXL.

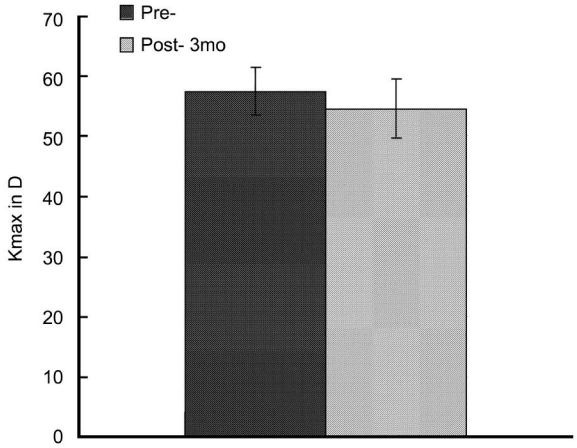

Figure 3 Bar graph showing the Kmax values at the apex of keratoconus pre-CXL and post-CXL at 3mo.

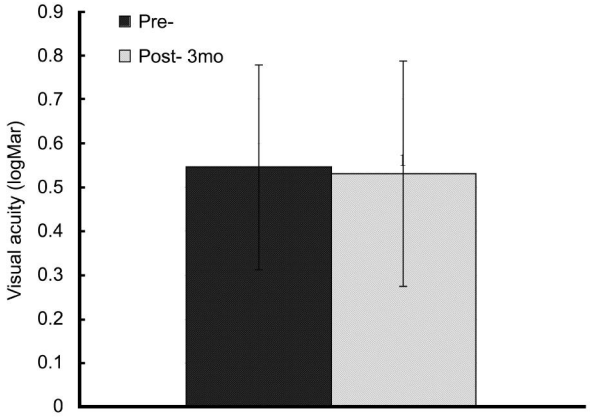

Figure 4 Bar graph showing the mean best-corrected visual acuity (BCVA) pre-CXL and post-CXL at 3mo.

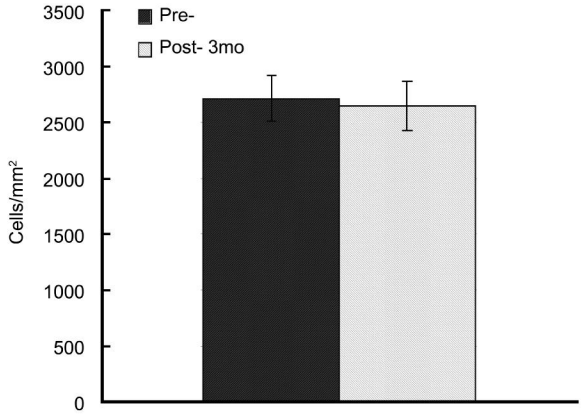

Figure 5 Bar graph showing the mean endothelial cell density (ECD) before and 3mo after CXL.

The mean K-value from the apex of the keratoconus was 57.6±4.0 diopters before surgery, and decreased to 54.7±4.9 diopters at 3mo after surgery (P=0.085; Figure 3).

Mean BCVA at the time of the treatment was 0.55 ±0.23 logarithm of the minimal angle of resolution, and increased to 0.53±0.26 logarithm of the minimal angle of resolution at 3mo after treatment (P=0.879; Figure 4).

Mean endothelial cell density (ECD) was 2706.4±201.6 cells/mm^2 before preoperative, and decreased to 2641.2±218.2 cells/mm^2 at 3mo after treatment (P=0.002; Figure 5).

No corneal stroma infections and side effects were observed after surgery.

DISCUSSION

CXL is a minimally invasive surgical technique, which stabilizes the progression of corneal ectasia and postpones the need of lamellar or penetrating keratoplasty [10,11]. Studies have proved that patients with progressing keratoconus the pattern of increasing mean keratometry values was not only halted but also reversed and flattened after CXL [12-15]. Wollensak *et al* [16] proposed a corneal thickness of at least 400 μm as a minimum safety limit for CXL in order to avoid irreparable tissue damage. In order to overcome this limitation, Hafezi *et al* [8] have proposed an alternative treatment protocol by using hypo-osmolar riboflavin solution to swell the corneal stroma to more than 400 μm. By using this modified protocol, Raiskup and Spoerl [17] followed 32 eyes up to one year and showed stable visual acuity without any scarring lesions in the stroma. However, the endothelial cell account and the thickness cornea after operation were not shown in the article, which could verify the safety and efficacy of the method.

To further evaluate this method, we observed earlier change in thin corneas. We followed 8 eyes in which corneas are thinner than 400 μm after abrasion of the epithelium up to 3mo. It was shown that the corneal stroma thickness of all these eyes was more than 400 μm after the swelling process. As evident by the observation at the last follow-up examination, 3mo after procedure, the mean thickness returned to the preoperative level (412.5±22.7 μm).

The improvements in BCVA, refractive error, and keratometry readings were found at 3mo after surgery. It seems logical that the reductions in refractive error and keratometry measurements in our study led to the improvements in BCVA. But there was no statistically significant difference between pre- and postoperative data.

We thought a longer follow-up would be required to achieve the effectiveness of this modified treatment and to obtain stable refractive and keratometric values.

In current study, we increased corneal thickness before the CXL procedure by swelling the cornea stroma to meet the criteria. However, a significant decrease of endothelial cell density was observed between pre- and postoperative data at 3mo.

We looked into the possible factors for this decrease of endothelial cell density, and we thought the improper focusing of the UV light source maybe was the reason for this. The working distance for CXL between the eye and the light source of UV-A was 5 cm. If the eye is closer than recommended, it may deliver energies above the cytotoxic threshold to the corneal endothelium, causing damage. Wollensak *et al* [18] have proved that CXL can cause significant endothelial cell necrosis and a complete loss of endothelial cells by using higher energy in rabbit corneas with an average thickness of 400 μm. And we noticed that some patients could not hold the position of eyes and heads during the treatment. The change in position of eyes may cause the change in the working distance, which may lead to the improper focusing of the UV light source.

Despite its decrease of endothelial cell density, no adverse endothelial reaction and endothelial cell-related complications such as corneal edema was observed postoperatively. And we noticed that this decrease of endothelial cell density also observed in some study at 3mo after CXL (although, the decreased is not significant), and the authors observed an increase at 6mo and 1y fellow-up [19,20]. For our results are preliminary and shorter follow-up, there may be an increase in longer follow-up.

The cornea thickness of one patient with particular thin corneal thickness of 372 μm after epithelial removal before treatment increased to 412 μm at 3mo. We hypothesized the increase of diameter of the collagen fibers maybe the reason. Thought a scar was observed in the apical stroma, BCVA was remained at last follow-up postoperative.

In essence, our result shown CXL with hypo-osmolar riboflavin solution seems to be is a promising treatment. Further study should be done to evaluate the safety and efficiency of CXL in thin cornea for the long-term.

ACKNOWLEDGEMENTS

The authors thank Feng-Jiao Li, Hai-Qun Yu, Hui Li, Zhi-Wei Li for their help.

Conflicts of Interest: Gu SF, None; **Fan ZS,** None; **Wang LH,** None; **Tao XC,** None; **Zhang Y,** None; **Wang CQ,** None; **Wang Y,** None; **Mu GY,** None.

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