

# Corneal collagen cross-linking effects on pseudophakic bullous keratopathy

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## 角膜胶原蛋白交联疗法对人工晶状体大泡性角膜病变的作用

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### 摘要

**目的:**研究核黄素和紫外线(UVA)交联疗法对晚期大泡性角膜病的疗效。

**方法:**15例人工晶状体大泡性角膜病变(PBK)患者参与研究。分别于角膜交联前和干预后1,6mo,对患者行裂隙灯检查、视敏度、异物调查表、角膜透明度分级、眼部疼痛度范围、分析仪角膜厚度分析及超声波角膜厚度测量。氯化钠溶液处理1wk后,去除8mm直径范围的中央角膜上皮,在角膜上频繁点滴核黄素30min,后行3min紫外线(UVA)照射交联疗法。

**结果:**15例患者(5男,10女),平均年龄为66±13岁。平均随访时间6.2mo。治疗后1mo全眼角膜透明度统计结果明显优于术前( $P<0.05$ )。6mo时8眼角膜透明度较术前更佳,5眼持平,2眼低于术前( $P=0.218$ )。70%病患异物感减弱。处理后1mo平均中央角膜厚度(CCT)减少( $P<0.05$ )。6mo时,除3眼外均出现渐进性肿胀,但统计显示CCT明显薄于术前( $P=0.006$ )。统计显示术后1mo平均矫正远视力(CDVA)明显优于术前( $P=0.010$ )。6mo后无

显著差异( $P=0.130$ )。1mo痛值统计结果明显优于术前( $P=0.007$ )。但6mo平均痛值较1mo高,较术前无明显差异( $P=0.070$ )。

**结论:**术后1mo结果显示角膜胶原交联疗法可显著改善角膜透明度、角膜厚度和眼痛。但是,它对减少PBK患者疼痛和维持角膜透明度方面似乎并无长期持久的效果。这种方法延长了角膜移植的时间间隔,并提高了角膜内皮移植术(DSAEK)过程的可视化。

**关键词:**大泡性角膜病变;角膜胶原交联;角膜水肿;测厚仪

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### Abstract

• **AIM:** To evaluate the efficacy of riboflavin administration and ultraviolet A (UVA) cross-linking on advanced symptomatic bullous keratopathy.

• **METHODS:** Fifteen patients with symptomatic pseudophakic bullous keratopathy (PBK) were included. Slit-lamp examination, visual acuity, foreign body sensation (FBS) questionnaire, corneal clarity grading, ocular pain intensity scale and corneal thickness measures with Pentacam and ultrasound pachymetry (UP), were performed before corneal cross-linking and 1 and 6mo thereafter. After using sodium chloride solution, for one week, the central 8mm (diameter) of the corneal epithelium was removed, and cross-linking, with riboflavin instillation every 3min for 30min, and UVA irradiation for 30min was performed.

• **RESULTS:** Five males and 10 females with mean age of 66±13y were included. Mean follow up time was 6.2mo. Corneal transparency in all eyes was statistically significantly better 1 month after treatment than preoperatively ( $P<0.05$ ). At 6mo, however, corneal transparency was better in 8 eyes, the same in 5 eyes, and worse in 2 eyes compared with preoperative levels ( $P=0.218$ ). Foreign body sensation subsided in 70% of patients. The average CCT decreased within 1mo after the procedure ( $P<0.05$ ). At 6mo, all but 3 eyes had progressive swelling, and the CCT increased; however, the CCT was still statistically significantly thinner than preoperatively ( $P=0.006$ ). The improvement in mean CDVA from preoperatively to 1mo postoperatively was statistically significant ( $P=0.010$ ). At 6mo, no significant

differences were observed ( $P=0.130$ ). The pain scores at 1mo were statistically significantly better than preoperatively ( $P=0.007$ ). At 6mo, however the mean pain score was higher than at 1mo and not statistically significantly different from the preoperative score ( $P=0.070$ ).

• **CONCLUSION:** Corneal CXL significantly improved corneal transparency, corneal thickness, and ocular pain 1 month postoperatively. However, it did not seem to have a long-lasting effect in decreasing pain and maintaining corneal transparency in patients with PBK. This procedure extends the time interval for corneal transplantation and increases visualization at DSAEK procedure.

• **KEYWORDS:** bullous keratopathy; corneal collagen cross-linking; corneal edema; pachymetry

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## INTRODUCTION

The cornea is a transparent and avascular tissue exposed to the external environment, and it is responsible for approximately two-thirds of the total refractive power of the eye. Corneal optical properties are determined by its transparency, surface smoothness, contour, and refractive index. Several clinical conditions, especially because of endothelial dysfunction, can disturb the balance among factors in the cornea responsible for its transparency resulting in visual impairment and painful bullous keratopathy<sup>[1]</sup>

Specially pseudophakic bullous keratopathy (PBK) is considered a serious complication of intraocular procedures and is a leading indication for keratoplasty worldwide. The corneal endothelium controls fluid and solute transport across the posterior surface of the cornea and actively maintains the cornea in the slightly dehydrated state that is required for optical transparency. Bullous keratopathy is caused by changes in the corneal endothelium, resulting in an abnormal state of hydration. Excessive corneal hydration can result in edema of the corneal epithelial layer, creating irregularity at the optically critical air-tear film interface. In addition, rupture of epithelial bullae exposes the corneal nerve endings and results in significant pain, tearing, and conjunctival hyperemia<sup>[2]</sup>.

Corneal collagen cross-linking (CXL) was recently introduced for the treatment of progressive keratoconus. The technique consists of photopolymerization of the stromal fibers by the combined action of a photosensitizing substance (riboflavin, vitamin B<sub>2</sub>) and UV light from a solid-state UV-A source. Photopolymerization increases the rigidity of the corneal collagen and its resistance to keratectasia by inducing additional cross-links between or within collagen fibers.

Wollensaket *al* and Bottós *et al*<sup>[3-5]</sup> showed that the degree of swelling of the corneal collagenous matrix was inversely related to the degree of cross-linking. Treated corneas were more

transparent after cross-linking and moist chamber incubation. Recent studies<sup>[1,4,6-8]</sup> suggest that CXL with riboflavin 0.1% and ultraviolet-A (UVA) can strengthen interfiber attachments, reducing the potential space for fluid accumulation in edematous corneas. Theoretically, stromal compaction and improvement in optical function are expected in edematous corneas after CXL. Because of its potential benefits, CXL has been suggested as an alternative for managing bullous keratopathy to minimize ocular discomfort, improve visual acuity, and possibly postpone the need for keratoplasty<sup>[5-8]</sup>. The aim of this study is to evaluate the safety, efficacy and consequences of UVA CXL in patients with painful PBK.

## SUBJECTS AND METHODS

**Subjects** Fifteen eyes of 15 consecutive patients diagnosed with PBK were enrolled in this prospective study. All patients had standard CXL as an alternative method to minimize corneal symptoms and postpone keratoplasty. The study was approved by the internal review boards of the University of Tabriz and was performed according to the principles of the Declaration of Helsinki. All patients provided informed consent before enrollment in the study. All patients had a complete ophthalmologic examination that included assessment of corrected distance visual acuity (CDVA), foreign body sensation (FBS), slit-lamp examination, ultrasound pachymetry (Corneoscan; Sonogauge, Cleveland, Ohio), and Scheimpflug corneal tomography (Pentacam; Oculus Optikgeräte GmbH, Wetzlar, Germany) were performed to assess corneal clarity and central corneal thickness both before and 6mo after the procedure. For precise determination of corneal thickness we used of mean pachymetry of CCT and 4 points in 4 quadrant at 4mm central optical zone. Corneal clarity was graded by visual slit-lamp inspection according to an arbitrary scale, referred to as corneal clarity score, ranging 0 to 4 as follows: 0 = no edema, totally transparent; 1+ = slight corneal edema, slight loss of transparency; 2+ = moderate edema, iris details seen; 3+ = intense edema, some iris details seen; and 4+ = very opaque, no iris details seen. Ocular pain intensity was assessed by a visual analog scale as suggested by the National Pain Education Council. The scale ranged from 0 to 10, with 10 being the most painful scenario. Foreign body sensation (FBS) also evaluated with a questionnaire. Patients included in the study had central corneal edema for at least 4mo with a minimum central corneal thickness (CCT) of 400 $\mu$ m and were in the eye bank list waiting for keratoplasty. Exclusion criteria were corneal scarring, IOL or vitreous touch to the cornea.

## Methods

**Cross-linking procedure after corneal deturgescence** Using 0.65% sodium chloride solution every 4 to 6 time/d for 1wk, corneal treatment was conducted under sterile conditions in the operating room. Tetracaine 0.5%, eyedrops were administered for anesthesia every 15min. The central 8mm (diameter) of the corneal epithelium were cautiously removed using a blunt knife. Riboflavin 0.1% solution (10mg of riboflavin-

**Table 1 Pre- and post-corneal cross-linking cct measurements by up and pentacam**

Variable	UP CCT			Pentacam CCT		
	Pre CXL	1mo	6mo	Pre CXL	1mo	6mo
Mean±SD	709±98.7	608.3±108.0	650.5±112.5	765.3±121.0	708.6±129.1	838.6±196.7
Median(range)	710(509-928)	621(445-830)	655(446-903)	775(547-1104)	679(556-1004)	768(600-1144)

CCT; Central corneal thickness; UP; Ultrasound pachymetry.

5-phosphate in 10mL of dextran-T-500 20% solution) was applied every 3min for 30min before irradiation and every 5min during irradiation. The surgeon had to ascertain that riboflavin was detected in the anterior chamber, by slit-lamp inspection using blue light, before the initiation of UV irradiation. Corneal irradiation was performed according to the manufacturer's instructions (IROC UV - X; IROC A6, Zurich, Switzerland). UV-A wavelength was set at 365nm, and an irradiance of 3mW/cm<sup>2</sup> was applied for 30min, which corresponds to a dose of 5.4J/cm<sup>2</sup><sup>[1,9,10]</sup>. After treatment, the ocular surface was washed with 20/mL balanced salt solution, medicated with 2 drops of cyclopentolate, and protected with a soft contact lens. Topical therapy included betamethasone eyedrops (6 times per day, tapered after the fifth postoperative day), chloramphenicol eyedrops (4 times per day until epithelial healing), and lubricants. Postoperative antibiotic treatment was discontinued after complete epithelial healing and removal of the contact lens. CXL treatment was performed only once in each patient.

**Statistical Analysis** Mean and median central corneal thickness were calculated for the 2 pachymetry instruments used in our study. Preoperative CCT was compared with 1- and 6-month postoperative measurements in both measured with the ultrasound pachymetry (UP) and Pentacam using Wilcoxon sign rank test. Spearman correlation coefficients were also calculated to investigate the correlation between measurements taken by the 2 different equipments. *P* values less than 0.05 were considered statistically significant, and all data were analyzed with SPSS version17.

## RESULTS

Fifteen consecutive patients with PBK were included in the study. Ten patients were female, and 5 were male. The mean age of participants was 66±13y (41-83y). In addition, all participants submitted to either corneal transplant or any other intraocular surgery had history of corneal edema for at least 4mo. Table 1 shows the pre and post-cross-linking CCT values from participants. The average CCT decreased within 1 month after the procedure and these values were statistically significantly different from the preoperative CCT values (*P*<0.05). This phenomenon was observed regardless of the instrument used to measure the corneal thickness. Complete corneal healing, measured as total corneal epithelization, varied from 7d to 1mo.

At 6mo, all but 3 eyes had progressive swelling, and the CCT increased; however, the central cornea was still statistically significantly thinner than preoperatively (*P*=0.006). At 6mo, 2 eyes had increased CCT over preoperative levels. 6 eyes had a recurrence of microbullae, and 3 had macrobullae. Eleven patients remained asymptomatic until their last study

visit. Four participants complained about foreign body sensation at the 6-mo follow-up. No postoperative complications such as persistent corneal scarring, haze, severe inflammation, or lenticular opacification were observed until the 6-mo follow-up visit in any other participant. We observed a high statistically significant correlation between the UP and Pentacam instruments measured by the Spearman correlation coefficients: 0.80 for preoperative, 0.89 for 1-mo postoperative, and 0.89 for 6-mo postoperative (*P*<0.05 for all values).

Corneal transparency in all eyes was statistically significantly better 1 month after treatment than preoperatively (*P*<0.05). At 6mo corneal transparency was better in 8 eyes, the same in 5 eyes, and worse in 2 eyes compared with preoperative levels. The decrease in the transparency was not statistically significant, however (*P*=0.218). The CDVA was better in 9 eyes at 1mo and in 5 eyes at 6mo. The improvement in mean CDVA from preoperatively to 1mo postoperatively was statistically significant (*P*=0.010). At 6mo, no significant differences were observed (*P*=0.130). The pain scores at 1mo were statistically significantly better than preoperatively (*P*=0.007). At 6mo, however, 6 of 15 patients required a therapeutic contact lens to control pain and the mean pain score was higher than at 1mo and not statistically significantly different from the preoperative score (*P*=0.070).

## DISCUSSION

The incidence of corneal edema has increased in the past decades, especially because of the increased number of intraocular surgeries being performed, mainly cataract surgeries. Postsurgical persistent corneal edema has been one of the main indications for penetrating keratoplasty<sup>[1,11,12]</sup>. Corneal transplantation remains the definitive treatment for endothelial dysfunction. A successful procedure provides both visual rehabilitation and relief of symptoms. However, immediate keratoplasty is not a reality in many countries, and the time spent waiting for a donated cornea could range from weeks to years<sup>[1,13]</sup>. Among the options for the management of painful bullous keratopathy, several treatment modalities have been proposed. Topical hypertonic solutions can reduce epithelial edema, although it has little effect on stromal edema<sup>[1,14]</sup>. Therapeutic soft contact lenses are useful to relieve discomfort but carry the risk of infection<sup>[1,15]</sup>. Surgical procedures include anterior stromal cauterization, manual anterior stromal puncture or anterior stromal puncture aided with yttrium-aluminum-garnet laser<sup>[16,17]</sup>, excimer laser phototherapeutic keratectomy<sup>[18]</sup>, conjunctival flaps, and amniotic membrane transplantation<sup>[19]</sup>. Some of these approaches could only bring short-term relief of symptoms or could interfere on the visual recovery after penetrating keratoplasty. In recent years CXL with riboflavin has been

proposed as a promising treatment modality. Theoretically, riboflavin causes new bonds to form across adjacent collagen strands in the stromal layer of the cornea, which increases the cornea's biomechanical strength. In patients with bullous keratopathy, the corneal extracellular spaces between collagen fibers accumulate fluids. After CXL, the fibers would theoretically be more compact, thus reducing corneal edema<sup>[6,8]</sup>. In a study of the hydration behavior of porcine corneas cross linked with riboflavin and UVA, Wollensak *et al*<sup>[3]</sup> found beneficial effects of CXL in corneal edema. The behavior of previously cross-linked corneas was better compared to those not previously cross-linked, showing decreased CCT and improved corneal transparency. These findings are theoretically a consequence of stromal compaction after CXL<sup>[8]</sup>.

One advantage of corneal cross-linking is the absence of scars on the corneal surface, allowing the performance of techniques such as the deep lamellar endothelial keratoplasty or the descemet stripping endothelial keratoplasty for the visual recovery of patients. Other methods such as anterior stromal micropuncture or cauterization lead to scarring of the corneal surface, leaving the penetrating keratoplasty as the only therapeutic option for these cases<sup>[1]</sup>. All patients in our study had an important reduction on corneal thickness after treatment, and this effect was probably a consequence of the cross-linking procedure itself, which included corneal deepithelialization and the hyperosmolar effect of the riboflavin-dextran solution. However, at the 1-mo follow-up visit, corneas remained thinner than before the procedure. In an attempt to overcome the limitations imposed by increased corneal thickness in patients with bullous keratopathy, modifications in the surgical technique have been proposed to maintain reproducible and efficient UVA light riboflavin penetration in the stroma. Krueger *et al*<sup>[6,8]</sup> propose intrastromal delivery of riboflavin with 2 pockets created by a femtosecond laser. It is important to note that Wollensak *et al*<sup>[7]</sup> used 40% glucose for 1d before cross-linking in his case series that allowed a better control of the corneal thickness before the procedure. In addition, whereas the Wollensak *et al*<sup>[9,20]</sup> standard protocol recommends a 5-min application period of riboflavin/dextran before the irradiation, we chose a 30-min application period as proposed by Spoerl *et al*<sup>[10]</sup> also leading to a reduction of corneal thickness before cross-linking because of the dextran. Some participants had recurrence of symptoms and epithelial bullae formation. Ten individuals developed bullous edema between 1 and 5mo after the procedure, and 4 developed bullous edema after 6mo. As mentioned previously the cross-linking procedure does not cause scarring of anterior corneal surface, and it might reduce anterior stromal edema, resulting in less epithelial edema and a smoother surface. However, in advanced cases of endothelial failure, the effect of crosslinking might not be strong enough to avoid recurrence of corneal edema and bullous keratopathy. From this case series, we observed that those with more advanced corneal edema had little short-term benefits. These patients usually have a significantly impaired

endothelial function and thicker corneas, which may interfere with riboflavin diffusion in the stroma resulting in less benefit<sup>[1]</sup>. Other previous studies<sup>[6,8]</sup> report an improvement in corneal transparency. In our study, we also observed an initial improvement in corneal transparency, although it did not last more than 6mo. In corneal edema, the interfiber collagen spacing is increased because of fluid accumulation. The compaction of fibers in the stroma, combined with an enhanced resistance to osmotic and hydrostatic fluid accumulation after the crosslinking procedure, indicates that this technique could be useful in patients with corneal edema<sup>[3,21]</sup>. We observed an increase of CCT measured by both instruments after the first month of follow-up. Former studies also demonstrated similar results but with fewer cases. The increase of CCT over time might be explained by the fact that fluid could have infiltrated through newly formed bonds. In addition, this effect could have been influenced by the residual endothelial function<sup>[1,3]</sup>. Besides means<sup>[8,21]</sup> urements done by the UP, Pentacam was used. Studies have shown high correlation between measurements of corneal thickness using ultrasound pachymetry, Pentacam and anterior segment OCT. However, the OCT has underestimated pachymetric measurements in some studies and overestimated in others compared with the UP. The reasons for these differences were not clear<sup>[22,23]</sup>.

Consistent with Krueger *et al*<sup>[6]</sup> study, in our study the clinical outcomes were improved; VA increased, bullous changes of epithelium improved, FBS decreased and corneal thickness reduced, but their procedure was different and they used two consecutive corneal pockets (350 and 150 $\mu$ m deep) that were created using a femtosecond laser and sequential intrastromal injection of 0.1% riboflavin. Constantin *et al*<sup>[24]</sup> showed that one month after CXL, a gradual reduction of corneal edema was observed with subsequent improvement of the corneal clarity and corneal epithelium integrity and an improvement of the symptoms without any side effects. Constantine *et al*<sup>[24]</sup> procedure and results was similar with ours, but their sample size (1 eye) and follow-up time (1mo) was not enough to evaluate the effects of CXL on PBK parameters. Bikbov G study<sup>[28]</sup> with similar to ours procedure and one year follow-up time in 9 eyes of BK, showed that, the VA raised in all patients, the pain subsided and corneal edema (thickness) reduced by 99.05 $\mu$ m in 11.8mo. In our study the corneal thickness reduced 74 $\mu$ m in 6mo, postoperatively. Gadelha *et al*<sup>[26]</sup> treated 12 eyes with similar procedure and 2mo, follow-up time. They reported, significant pain reduction ( $P < 0.001$ ) but corneal thickness and VA measurements presented with no significant difference. Outcomes of Gharaee *et al*<sup>[27]</sup> study with standard procedure after 6 months follow-up, indicated CXL is not an effective treatment for bullous keratopathy with respect to VA and CCT, although it can improve irritation and discomfort. In contrast with Gharaee *et al*<sup>[27]</sup> and Gadelha *et al*<sup>[26]</sup> studies, our study after 6 month leads to improving of VA in 70% and reducing of corneal thickness by 80% of eyes. Similar to their studies, pain and FBS improved in 70% of eyes, in our

study. Cordeiro Barbosa *et al*<sup>[1]</sup> interventional case series included 25 eyes with corneal edema demonstrated that, mean CCT, measured by ultrasound and OCT, decreased significantly after 1 and 3mo. 14 (56%) patients developed new epithelial bullae and 11 (44%) patients, remained asymptomatic until their last visit. In our intervention 3 patients (30%) developed new epithelial bullae, but only one of them increased corneal thickness. In other one patient, corneal thickness increased by 177µm but no developed new epithelial bullae.

Limitations of our study was the lack of a control group and short follow up time. Our study suggests that corneal collagen cross-linking reduced corneal edema temporarily. This effect was demonstrated through the reduction of corneal thickness of the patients submitted to this treatment. The procedure was demonstrated to be safe with few complications. Although the effect seemed to be moderate in most cases, it still provided relief of symptoms to many patients in this study, indicating that this procedure might be helpful for patients with bullous keratopathy awaiting

penetrating keratoplasty. Future studies will help determine the profile of individuals with corneal edema who will get the most benefit from this procedure<sup>[1]</sup>. In conclusion and according previous studies mentioned above, our study, confirmed that; using of CXL for treating of PBK leads to compaction of corneal stroma, decreases corneal thickness, pain and FBS, increases corneal clarity and VA, in short time after cross-linking, thus supplies the patient's comfort, extends the time of interval for corneal transplantation and increases visualization during DSAEK procedure. But, it seems that, due to sustained endothelial dysfunction, the symptoms will be reversible, and further clinical studies with longer follow-up period are needed to accurate evaluation of these early changes.

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