

Corneal Collagen Cross-linking for Treatment of Non-healing Corneal Ulcers

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Abstract

Purpose: To evaluate the efficacy of corneal collagen cross-linking (CXL) for treatment of corneal ulcers not responding to antimicrobial therapy.

Methods: Eight patients with corneal ulcers associated with corneal melting, not responding to conventional antibiotic therapy, were treated with CXL. The procedure was performed according to the standardized protocol for keratoconus. Preoperative medications were continued after CXL in all cases. Microbiological exams revealed *Pseudomonas aeruginosa* in 3 cases. Follow up continued from 1 to 10 months.

Results: In 6 of 8 eyes, progression of corneal melting was halted and complete epithelialization occurred. In one eye emergency keratoplasty was needed due to corneal perforation. A conjunctival flap was performed to treat severe localized corneal thinning in one of the patients a few days after CXL. Significant clinical improvement occurred in all cases of *Pseudomonas aeruginosa* keratitis.

Conclusion: CXL can be considered as a promising new treatment in the management of refractory non-healing corneal ulcers, including *Pseudomonas aeruginosa* keratitis.

Keywords: Corneal Collagen Cross-linking; Corneal Ulcer; Riboflavin; Ultraviolet-A

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INTRODUCTION

Infectious keratitis, often microbial in origin, is a devastating ocular disorder which can lead to blindness if not treated promptly. This urgent ophthalmic condition needs to be treated with appropriate antimicrobial agents.^[1-3] However, some ulcers do not respond to conventional antimicrobial treatments.^[4] Resistance of microbes to antibiotics is one of the main causes of treatment failure.^[4-6] Furthermore, severe and refractory corneal ulcers are usually associated with corneal melting. As the enzymes that are released from inflammatory cells (neutrophils), some bacteria and fungi dissolve stromal collagen fibers and lead to corneal melting.^[4,7,8]

Cross linking is a method of tissue stabilization.^[9] Corneal collagen cross linking (CXL) was first introduced for corneal stiffening and stabilization of progressive keratoconus.^[10] The procedure is performed using

ultraviolet-A (UVA) irradiation and a photosensitizer (riboflavin, vitamin B2).^[10,11]

There is also a known disinfecting effect for UVA light. Traditionally, it has been used for disinfection of drinking water and air/surface disinfection. This procedure has been used to inactivate viruses, bacteria and parasites from the time (1960s) Japanese scientists showed riboflavin exposed to visible or UV light could be used to inactivate the RNA containing tobacco mosaic virus.^[12] Several *in vitro* and *in vivo* studies have shown that the use of riboflavin and UVA is effective against different pathogens.^[13-20] In addition to this capability, cross-linking, using riboflavin and UVA, could stop collagenolysis and corneal melting in severe corneal ulceration.^[21]

Herein, we report our experience with CXL for treatment of infectious keratitis resistant to conventional therapy in 8 patients.

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METHODS

Eight patients with severe corneal ulcers were treated at the Department of Ophthalmology, Ahvaz Jundishapur University of Medical Sciences. Slit lamp biomicroscopy was performed to evaluate the ulcer details, anterior chamber condition and to measure ulcer size. Ultrasonography was performed to find any posterior segment pathology in cases with severe corneal haze. Samples were taken from the ulcer margins, conjunctiva, lid margins, contact lens and its cases in order to perform appropriate laboratory tests. Symptom duration before CXL was between 7-35 days. Three patients had history of previous ocular surgery. In one patient a therapeutic contact lens had been fitted because of an epithelial defect following DSAEK surgery. Patients' details are presented in Table 1. Despite intensive topical and systemic antimicrobial therapy, corneal infiltration and melting progressed in all cases. Microbiological evaluation reports were negative in 2 of 8 eyes but positive for *Pseudomonas aeruginosa* in 3 eyes, and Enterobacter, Streptococcus and filamentary fungi each in one eye.

The conventional CXL protocol for keratoconus, as described by Wollensak et al, was used in all patients after obtaining informed consent. In one eye with severe localized thinning, conjunctival flap was performed shortly after CXL. Preoperative medications were continued after surgery in all cases. During the postoperative period, fortified antibiotics were tapered gradually and discontinued one week after complete re-epithelialization. Cycloplegics, topical tear substitute drops, and systemic doxycycline were used in conjunction with antimicrobial drugs. All eyes were photographed prior to intervention and during each postoperative visit. Follow-up period ranged from 1 to 10 months.

CXL Procedure

CXL was performed under sterile conditions in the operating room. Tetracaine drops (0.1%) were used for topical anesthesia. After removal of the loose epithelium

around the ulcer, riboflavin drops (Medio-Cross® riboflavin 0.1%/dextran solution 20%, Switzerland) were instilled every 3 min over the surface of cornea for 30 min. Thereafter, the cornea was irradiated for 30 min using a UVX lamp (Peschke Meditrade GmbH, Huenenberg, Switzerland), with 365 nm wavelength, irradiance of 3 mW/cm² and distance of 5 cm. During the period of UVA irradiation, riboflavin was administered every 3-4 min.

Slit lamp microscopy, determination of visual acuity and slit lamp photography were performed at each postoperative follow up visit.

RESULTS

Eight patients with corneal ulcers associated with corneal melting not responding to intensive topical and systemic antimicrobial therapy were treated with CXL. The age of the patients ranged from 27 to 83 years.

All patients experienced improvement in symptoms, such as epiphora, photophobia and pain within 24-48 h after CXL. Hypopyon was present in 6 eyes but regressed completely within 2-4 days after treatment in all eyes except one. The progression of corneal melting stopped rapidly after CXL in six cases. Re-epithelialization of the cornea started within a few days and complete epithelialization was achieved with residual stromal scarring [Figures 1-3]. Wound healing was completed by 2-5 weeks. Best-corrected visual acuity ranged from light perception to counting fingers before CXL, which improved to hand motions to 20/100 during the postoperative period.

One eye developed corneal perforation 6 days after CXL. Therefore, urgent penetrating keratoplasty was performed. Table 2 presents more details on the cases.

DISCUSSION

This series showed promising results from CXL for treating refractory infectious corneal ulcers associated with melting. Except one eye, all patients showed clinical improvement in ulcer characteristics and reported a decrease in pain and other symptoms. These results are

Table 1. Demographic characteristics of patients with corneal ulcer treated with corneal collagen cross-linking

Patient	Age (years)	Sex	Eye	Symptom duration (days)	Ocular history in the eye with keratitis
1	28	Female	Left	14	None
2	80	Female	Right	12	Phacoemulsification
3	76	Male	Right	7	Contact lens user, trabeculectomy, DSAEK
4	76	Female	Left	11	Dry eye
5	45	Female	Left	30	Dry eye
6	27	Female	Right	15	None
7	55	Male	Left	35	Nonpenetrating trauma
8	83	Female	Left	30	Phacoemulsification Dry eye Trachomatous keratopathy

DSAEK, descemet stripping automated endothelial keratoplasty

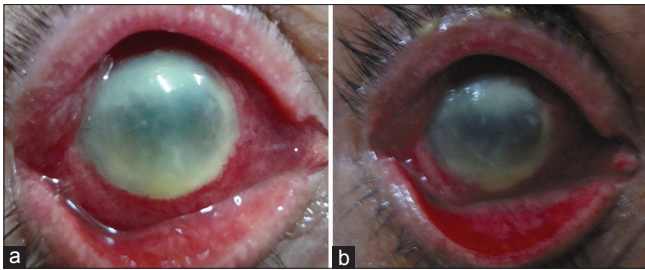


Figure 1. (a) Right eye of patient number 3, with a large *Pseudomonas aeruginosa* corneal ulcer at presentation, after 1-week medical treatment. (b) The same eye, 2 days after treatment with corneal collagen cross-linking (CXL) showing improvement with a significant decrease in infiltration density.

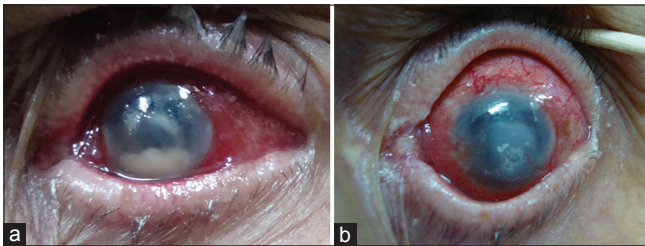


Figure 2. (a) Left eye of patient number 4, 11 days after medical treatment for *Pseudomonas aeruginosa* corneal ulcer. (b) The same eye shows significant healing with scar formation and corneal vascularization 4 weeks after CXL.

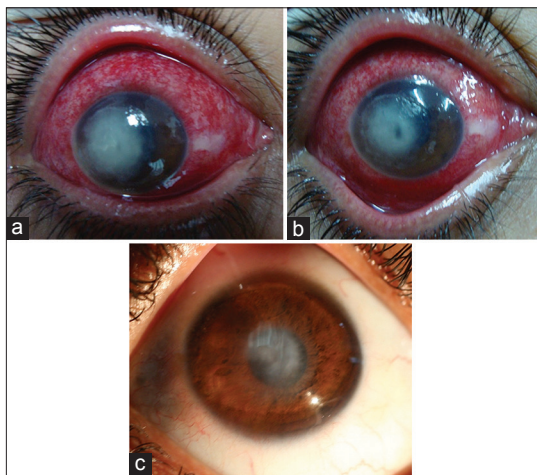


Figure 3. (a) Right eye of case number 6 with large corneal infiltration 15 days after topical antibiotic therapy. (b) The same eye 2 days after CXL showing a decrease in peripheral and central corneal infiltration. (c) The same eye 4 months after CXL showing a small corneal scar.

compatible with other reports on the beneficial effect of CXL for refractory infectious keratitis.^[13-19,22]

Collagen cross-linking is a new horizon in the treatment of corneal diseases. The corneal stroma is the thickest part of the cornea and is mainly composed of collagen fibrils, charged with stromal maintenance and wound healing.^[23,24] Corneal collagen cross-linking by UVA and riboflavin creates more covalent bands

between collagen fibers. It has a stiffening effect that stabilizes the corneal stroma and increases its resistance to enzymatic degradation.^[24-26] Although, this procedure was originally introduced as a treatment for corneal ectasia,^[10] there are increasing reports of its effectiveness in the treatment of a variety of other corneal disorders such as symptomatic Fuch's corneal dystrophy,^[27] pseudophakic bullous keratopathy,^[28] and more recently, infectious keratitis.^[13-19]

Resistance to antibiotics, which happens because of chromosomal changes or the exchange of genetic material via plasmids or transposons, is a major cause of refractory non-healing corneal ulcers.^[6] CXL seems to be a valuable adjunctive treatment in such cases because its mechanisms of action are different from antibiotics.

The therapeutic effect of CXL in corneal ulcers could be related to its toxic action against the pathogens and the increase in collagen resistance against enzymatic degradation. UV irradiation has antimicrobial activity and has been traditionally used for disinfection of blood transfusion products, drinking water and air or surfaces.

Hiraku et al demonstrated that UVA radiation (365 nm) in the presence of a photosensitizer such as riboflavin and pterin derivatives induces DNA damage.^[29] After UVA absorption, the riboflavin molecules cross-over to a triplet state and transfer energy to generate singlet oxygen. Singlet oxygen and superoxide anions then react with available groups nearby.^[29,30]

Martins et al studied the *in vitro* antimicrobial properties of riboflavin/UVA (365) against common pathogens including, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *epidermidis*, methicillin-resistant *S. aureus* (MRSA), multidrug-resistant *P. aeruginosa* (MDRPA), drug-resistant *Streptococcus pneumoniae* (DRSP), and *Candida albicans* (CA). They demonstrated that UVA/riboflavin was effective against all of them except *Candida albicans*.^[20]

Among our eight patients, three cases had keratitis due to *P. aeruginosa* with large progressive corneal ulcers unresponsive to antibiotic therapy. All of these cases were controlled and treated successfully with CXL. Clinically, *P. aeruginosa* keratitis ulcers usually show severe corneal ulcerations with a rapidly progressive course and stromal necrosis. Treatment of *P. aeruginosa* keratitis is generally thought to be more difficult, with poorer visual outcomes than other bacterial corneal ulcers.^[4,31] There are several reports that have described cases of infectious keratitis due to *mycobacteria*, *S. aureus*, *S. epidermidis*, *Enterobacter*, *fungi*, and *Acanthamoeba* corneal infection successfully treated with CXL.^[13-19,32] However, there are not enough reports on treating *P. aeruginosa* keratitis using CXL. In our small series, three eyes had refractory keratitis secondary to *P. aeruginosa*. According to this study, CXL may be an effective adjunctive therapy in controlling advanced refractory *P. aeruginosa* keratitis.

In addition to its antibacterial effect, several studies suggested that CXL could be effective to treat sterile

Table 2. Ocular characteristics and outcomes of treatment with corneal collagen cross-linking in corneal ulcers

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8
Ulcer size (mm)	7×7	5×5	8×8	5×5.5	2×2	7×5	Near total	5.5×6
Ulcer site	Central	Central	Central	Central	Paracentral		Near total	Central
Hypopyon	Yes	Yes	Yes	Yes	No	No	Yes	Yes
Detected microbe	FF	PA	PA	PA	E	N	NS	N
Antibiotic prior to CXL	Nat + Cip	Ami + Cef + Cip	Ami + Cef + Cip	Ami + Cef + Cip	Ami + Cef + Cip	Van + Cef	Van + Cef + Cip	Van + Cef + Cip
BCVA at presentation	3	3	3	3	3	1.8	3	3
BCVA at follow-up (logMAR)	1.8	3	3	1.8	1.5	0.70	3	3
Complete healing	4	5	5	3	2	4	-	4
Additional surgical treatment	No	No	Conjunctival flap	No	No	No	PK	No

mm, millimeter; FF, filamentous fungi; PA, *Pseudomonas aeruginosa*; E, Enterobacter; NS, nonhemolytic Streptococcus; N, negative; Nat, natamycin; Cip, ciprofloxacin; Ami, amikacin; Cef, ceftazidime; Van, vancomycin; CXL, corneal collagen cross-linking; BCVA, best-corrected visual acuity; logMAR, logarithm of the minimum angle of resolution; PK, penetrating keratoplasty

keratitis.^[21,33] Gao et al studied the effect of CXL on prevention of melting in rabbit corneas after alkali burn and found that it could prevent and delay corneal melting. They showed that CXL reduced the destruction of corneal collagen fibers and infiltration of inflammatory cells in the cornea.^[21]

Ehlers et al performed CXL for treating corneal decompensation and non-healing corneal ulcers including sterile keratitis. They reported that CXL could be effective in some cases of sterile keratitis. One of the probable hypotheses explaining such an effect is that free oxygen radicals produced during CXL may directly inactivate proteolytic enzymes or damage leukocytes thus reducing the production of such enzymes.^[34] Spoerl et al showed that CXL markedly increased corneal collagen resistance against enzymatic digestion by trypsin and pepsin.^[26]

In another study by Wollensak et al, the authors found that CXL led to an increase in mechanical rigidity of the cornea, which was more prominent in human corneas as compared to porcine corneas.^[24] Considering our study, melting was a prominent feature in all ulcers which stopped in 75% of cases after CXL. These findings are compatible with similar studies proposing a positive effect from CXL in treating stromal melting.^[13-19]

The potency of CXL to halt progressive ulceration and melting in infectious keratitis could prevent the need to perform emergency penetrating keratoplasty. In the presence of active corneal infection and anterior chamber inflammation, penetrating keratoplasty is associated with higher rates of rejection and more post-keratoplasty complications such as anterior and posterior synechiae, glaucoma, cataracts, need for resuturing, stromal haze and graft infection.^[34,35] In our study, only one case required penetrating keratoplasty because of corneal perforation. It seems that CXL could prevent such complications in severe corneal ulcerations.

In conclusion, CXL could be considered as a useful adjunct for treating progressive and non-healing

corneal infections including *P. aeruginosa* keratitis. This intervention may decrease the need to perform emergency penetrating keratoplasty. However, more research on a larger number of eyes and more randomized clinical trials comparing the safety of CXL application to routine antibiotics are recommended.

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