Original Article

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Corneal Collagen Cross-linking for Treatment of Bacterial and Herpetic Keratitis

Ophthalmology Section

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ABSTRACT

Introduction: Optimal management of infective keratitis is a formidable challenge and subject of ongoing studies. Recently, Collagen Cross-Linking (CXL) of the cornea has been considered to be a new effective therapeutic approach for resistant infectious keratitis.

Aim: Aim of the study was to evaluate the effectiveness of CXL with Ultraviolet-A (UV-A) and riboflavin for treatment of the refractory bacterial and Herpes Simplex Virus (HSV) keratitis.

Materials and Methods: In this prospective interventional study, eight patients with diagnosis of infectious keratitis who were referred to Khalili Hospital eye emergency room, between 2014 and 2015 were included in the study. There were six patients with bacterial keratitis and two patients with HSV keratitis; they were resistant to conventional treatment and underwent CXL. Response to the treatment was considered as good if rapid epithelialization and rapid decrease in stromal infiltration occurred.

Results: Microbial culture in the bacterial keratitis group showed coagulase negative *Staphylococcus* in two patients, *Staphylococcus aureus* in one patient, mixed infection in one patient and *Pseudomonas aeruginosa* in two patients. Good response and rapid epithelialization and resolution of stromal infiltration were seen in the four out of six eyes. Two patients showed no response and underwent penetrating keratoplasty for eradication of infection. Furthermore, one patient showed a good response to CXL in the HSV keratitis group and another patient had a relative response but recurrence occurred.

Conclusion: Although, CXL seems promising in the treatment of patients with refractory bacterial keratitis, but in some cases, it is ineffective. CXL may be an alternative treatment for refractory cases of HSV keratitis but recurrence is possible.

Keywords: Herpes simplex virus, Infectious keratitis, Keratoplasty

INTRODUCTION

Microbial keratitis, also known as infectious corneal ulcer occurs because of the proliferation of bacteria, viruses, fungi or parasites within the corneal tissue and is associated with inflammation and tissue destruction. It is a leading cause of blindness especially in the developing world [1,2]. Corneal destruction may be caused by infectious agents or by the associated inflammatory response [2]. Optimal management of infective keratitis is a formidable challenge and a subject of ongoing research. Several therapies such as topical broad-spectrum antibiotics [3], topical steroids [4] and Amniotic Membrane Transplantation (AMT) [5] have been used to eradicate the infection or to decrease the inflammatory response of the cornea.

In severe and unresponsive cases, it may be necessary to perform emergency keratoplasty to eradicate the infection and to preserve the eye, a procedure that may be ineffective or it may be associated with several complications, especially in the acute inflammatory phase of the infection. In addition, recurrence of infection remains a significant problem [6,7]. Corneal CXL with UV-A and riboflavin has been used as a new modality in the management of keratoconus to halt the progression of the disease, to prevent visual loss and to postpone the need for surgical intervention. Because CXL has been an effective and a relatively safe modality in the management of keratoconus, it has been used empirically in several other disorders of the cornea such as corneal edema [8], Fuchs dystrophy [9], nonhealing corneal ulcers and infectious melting keratitis [10,11].

This prospective interventional study was conducted to evaluate the effects of therapeutic corneal CXL with UV-A and riboflavin for the treatment of patients with refractory bacterial and HSV keratitis.

MATERIALS AND METHODS

In this prospective interventional study, eight patients with a diagnosis of refractory bacterial or HSV keratitis who were referred to Khalili Hospital eye emergency room, affiliated to Shiraz University of Medical Sciences, Fars, Iran, between 2014 and 2015 were included in the study. There were six patients with bacterial keratitis and two patients with HSV keratitis and were resistant to conventional treatment and underwent therapeutic CXL. Patients with perforated corneal ulcer, those with a corneal ulcer that had produced a descematocele and patients with collagen vascular disease and immunocompromised patients were excluded from the study.

This study was performed in accordance with the declaration of Helsinki and approved by our Institution's Ethics Committee. Written informed consent was obtained from all subjects before inclusion.

All patients underwent complete ophthalmologic examination including corrected visual acuities and slit lamp biomicroscopy. Corneal ulcers were graded according to their location, size, depth and severity of anterior segment infiltration. Grade 1 ulcers were nonaxial, <2 mm in size, involved superficial one-third of the cornea with mild anterior segment infiltration. Ulcers were graded as 2 if their area was between 2 mm and 6 mm, involved superficial two-third of the cornea and had moderate to severe fibrinous exudates in the anterior chamber. Grade 3 ulcers were more than 6 mm in size, extending to the inner one-third of the cornea with severe hypopyon.

After treatment with CXL, the patients were examined daily to evaluate their response to treatment and time to re-epithelialization and time to resolution of stromal infiltrate were recorded. Response to the treatment was considered as good if rapid epithelialization and rapid decrease in stromal infiltration (significant change in the course of the disease) occurred after CXL. If there was no change in the course of healing, if ulcer deteriorated after treatment and if surgical treatment was necessary for eradication of infection despite enough time after CXL, response of the patient was defined as poor (no response). The response was considered as

relative if the treatment was only partially effective (partial resolution of stromal infiltration and incomplete epithelialization) or if there was recurrence of infection in the early postoperative course.

Stages of Treatment

First, the cornea was anesthetized with topical tetracaine 0.1% drops every three minutes for at least 15 minutes in the patient with supine position. After inserting a lid speculum, corneal epithelium within a 8 mm diameter zone that included all microbial infiltrates was removed using a thin blunt metal (Hockey knife) and then 0.1% riboflavin in dextran 500 20% drops (MEDIOCROSS™ Medio-Haus Medizinprodukte GmbH, Behrensbrook 6, D-24214 neudorf) were administered every three minutes for 30 minutes. Thereafter, the cornea was exposed to UV-A rays (365 nm) in an optical zone of 8 mm for 30 minutes with an irradiance of 3 mW/cm2 (UV-X™; Peschke Meditrade, Cham, Switzerland). Light distance from the corneal apex was about 50 mm. During the procedure, the cornea was moistened every five minutes with 0.1% riboflavin and tetracaine drops. After the treatment, the eye was addressed with a therapeutic soft contact lens (T-lens) and artificial tear and previous topical medications.

RESULTS

Bacterial Keratitis Group

In the group, six patients were treated for resistant bacterial keratitis. Their age ranged from 19 to 96 years. Predisposing factors were trauma with vegetative matter in two cases and previous corneal graft in another two cases. Two patients had no predisposing factor. Visual acuities ranged from 20/200 to light perception. Corneal ulcer was Grade 3 in all patients. Microbial analysis showed coagulase negative Staphylococcus in two patients, Staphylococcus aureus in one patient, mixed infection (Streptococcus and coagulase-negative Staphylococcus) in one patient and Pseudomonas aeruginosa in two patients. CXL was performed from 6 to 45 days after presentation of corneal ulcers (mean±standard deviation was 15.0±15.2 days). Four of six eyes showed good response after treatment and rapid epithelialization and resolution of stromal infiltration occurred. Postoperatively, signs of response such as decrease in stromal infiltration occurred within the first 72 hours in all responsive patients. Two patients showed no response and corneal graft (penetrating keratoplasty) was necessary for eradication of infection. Details of three patients are described below (one responsive patient and two unresponsive patients).

Case 1 (Responsive Patient)

A 49-year-old woman with a past ocular history of corneal graft in her right eye one year ago because of corneal ulcer was referred because of bacterial keratitis in her right eye. The patient complained of red eye but only minimal pain. She was pseudophakic and also had a history of uncontrolled advanced glaucoma that had undergone transscleral cyclophotocoagulation two months ago. Visual acuities were hand motion in the right eye and 20/40 in the left eye. Slit lamp examination showed a central corneal ulcer that was 2 mm × 8 mm in size. The ulcer had a branching (arborizing) pattern that was located deeply in the corneal stroma (80% depth) suggestive of infectious crystalline keratopathy. Corneal scraping specimen was sent to microbiology laboratory and Gram-stain showed Gram-positive cocci. Culture showed mixed infection (*Streptococcus* and coagulase-negative *Staphylococcus*).

She was diagnosed to have bacterial keratitis and fortified vancomycin and ceftazidime eye drops were started. There was no response to the treatment and keratitis was deteriorated with increasing stromal suppuration and corneal thinning. Four days later, it was decided to treat the eye by CXL. After obtaining informed consent, the patient underwent an uncomplicated CXL procedure. Two days later, stromal suppurative infiltration decreased and seven days later, branching (arborizing) pattern disappeared. Three weeks after CXL, keratitis completely resolved with scarring and cornea was epithelialized. At the eight months follow-up, no recurrence was observed and visual acuity was hand motion in the right eye.

Case 2 (Unresponsive Patient)

This 74-year-old woman was referred to eye emergency room because of a central corneal ulcer since two weeks that had been unresponsive to fortified antibacterial eye drops. The patient had a history of previous corneal ulcer six months ago for which conjunctival flap had been performed because of corneal thinning. Visual acuities were 20/80 in the right eye and hand motion in the left eye. Slit lamp examination showed a central corneal ulcer that was 2 mm × 4 mm in size with corneal thinning and 1 mm hypopyon.

Corneal scraping specimen was sent to microbiology laboratory. Gram-stain showed Gram-positive cocci and culture showed coagulase negative Staphylococcus. Despite systemic cefazolin and gentamicin and topical fortified antibiotics (vancomycin and ceftazidime fortified eye drops every hour), the corneal ulcer progressed over the next four days with increasing stromal infiltration and corneal thinning.

As an alternative to immediate corneal transplantation, the patient was offered corneal CXL. After obtaining the informed consent, CXL was performed with standard protocol within the 8 mm treatment zone. After CXL, the corneal ulcer deteriorated with stromal suppuration and descematocele formation. Penetrating keratoplasty was performed to eradicate the infection five days later. At five month follow-up, no recurrence was occurred and visual acuity was 4 m Counting Fingers (CF) in the same eye.

Case 3 (Unresponsive Patient)

The patient was a 69-year-old man referred to our hospital with impression of bacterial keratitis in his left eye. The patient had a history of trauma to the eye 10 days before referral and complained of ocular pain and red eye and had received fortified cefazolin and gentamicin for five days before referral to our hospital. Visual acuity was 1 m CF in the left eye. Slit lamp examination showed a peripheral corneal ulcer that was 3.5 mm × 4.5 mm in size. The ulcer was located deeply in the corneal stroma (90% depth) and was associated with a 3.5 mm × 2.5 mm corneal epithelial defect and hypopyon (1 mm).

Corneal scraping specimen was sent to microbiology laboratory and Gram-stain showed Gram-negative cocci. KOH smear did not show fungi. He was diagnosed as bacterial keratitis and fortified cefazolin and gentamicin were continued. There was no response to the treatment. Culture showed P. aeruginosa two days later. Despite treatment, keratitis deteriorated with increasing stromal infiltration and hypopyon. Three days later, because of the proximity of the ulcer to the limbus and progression of the ulcer, we decided to treat it by corneal CXL.

After obtaining informed consent and instillation of one drop of topical tetracaine and one drop of povidone iodine 5%, the patient underwent CXL procedure. After CXL, no response was observed and the corneal ulcer deteriorated with progression toward the limbus. Penetrating keratoplasty was performed to eradicate the infection four days later. At six months follow-up, no recurrence was observed.

HSV Keratitis Group

This group comprised two patients treated for HSV keratitis that was resistant to conventional treatment. One patient had a history of previous corneal graft because of HSV keratitis and showed a good response to CXL. Another patient had a relative response,

but recurrence was obeserved. The ulcers were Grade three in these patients. Details of two patients were described below.

Case 1

This 81-year-old man had an ocular history of corneal graft in his right eye 16 months before referral because of HSV stromal keratitis. He was referred because of keratitis in his right eye after suture removal one month ago. Before admission, the patient had received oral acyclovir (400 mg PO every five hours) and topical fortified antimicrobial agents for two weeks. Visual acuities were 1 m CF in his right eye and 20/200 in the left eye. Slit lamp examination showed a corneal ulcer that was 5 mm × 3.5 mm in size. The ulcer was associated with 50% corneal thinning and was located near the graft-host junction.

Corneal scraping specimen was sent to microbiology laboratory. HSV-DNA was detected by Polymerase Chain Reaction (PCR) technique. He was diagnosed to have HSV necrotizing stromal keratitis. Oral acyclovir continued, prednisolone eye drop (every six hours) and moxifloxacin (Vigamox™) eye drop were started. There was no response to the treatment and corneal thinning was progressed. Two weeks after admission, it was decided to treat the eye by CXL.

After obtaining the informed consent, the patient underwent an uncomplicated CXL procedure. Five days later, stromal infiltration decreased and 15 days later, it disappeared. After 25 days of CXL, the cornea was epithelialized and keratitis completely resolved with scarring. At seven months follow-up, no recurrence was observed.

Case 2

This 60-year-old woman was referred to our in-patient service because of a central corneal ulcer in the left eye. Visual acuities were 20/40 in the right eye and hand motion in the left eye. Slit lamp examination showed a central corneal ulcer that was 1.5 mm × 3 mm in size. Corneal scraping specimen was sent to microbiology laboratory. Gram-stain, bacterial and fungal cultures were negative, but PCR test was positive for HSV. During the following three weeks, the patient received topical prednisolone acetate eye drop every six hours and systemic acyclovir (400 mg every five hours). Despite this maximal treatment, corneal ulcer increased in both diameter and depth. Hence, the patient was referred for CXL.

After obtaining informed consent, CXL was performed. After CXL, over the next four days, the stromal infiltration gradually decreased. At day 11, infiltration size was 2 mm × 0.5 mm and the cornea was epithelialized. After 28 days of CXL, AMT was performed because of corneal thinning. Thereafter, acyclovir decreased to 400 mg two times/day (prophylactic dose) and topical steroid gradually decreased. Two weeks after AMT, stromal infiltration recurred and corneal epithelial defect was developed. Hence, dose of oral acyclovir increased to the therapeutic dose and topical steroid frequency also increased. During the following two weeks, stromal infiltration decreased and the cornea was epithelialized. At four month follow-up, no recurrence was observed and visual acuity was 2 m CF in the left eye.

DISCUSSION

As CXL has been an effective and a relatively safe modality in the management of keratoconus, investigators have tried it for other different disorders of the cornea such as corneal edema [12,13], bullous keratopathies [8], Fuchs dystrophy [9], non-healing corneal ulcers [14], corneal erosive disorders and infectious melting keratitis [9,10,13].

This study was conducted to assess the effectiveness of Riboflavin/UV-A Corneal CXL in the treatment of refractory cases of bacterial and HSV keratitis. According to our results, four out of six patients treated for refractory bacterial keratitis showed a good response. Two patients showed no response and penetrating keratoplasty was necessary to eradicate the infection in these two patients. Of the two patients treated for refractory HSV stromal keratitis, one had a history of previous corneal graft because of HSV keratitis and had a good response to CXL. Another patient had a relative response, but recurrence of infection occurred after treatment.

The exact mechanisms underlying therapeutic effects of CXL in the treatment of infectious keratitis are unclear. Several mechanisms may underlie these therapeutic effects. The first underlying mechanism is the direct antimicrobial effect of CXL on the infectious agents. It has been demonstrated that combined application of Riboflavin and UV light could inactivate viruses and bacteria. These effects are the consequence of damage of DNA at the molecular level such as damage to the guanine bases in DNA induced by Riboflavin/UV light [15]. It has also been shown that riboflavin/UV-light induces a photochemical inactivation of viruses and bacteria in platelet concentrates, fresh frozen plasma and in red blood cells [15-17]. Antimicrobial efficacy of riboflavin/UV-A combination for bacterial isolates has been tested in vitro [18]. Martins SA et al., evaluated the antimicrobial efficacy of riboflavin/UV-A for the most common offending pathogens of microbial keratitis including S. aureus, P. aeruginosa, Staphylococcus epidermidis, methicillin-resistant S. aureus (MRSA), multidrug-resistant P. aeruginosa and drug-resistant Streptococcus pneumoniae. They showed that combination riboflavin/UV-A is an effective method for the inhibition of bacterial growth in culture plates. Their results also demonstrated that UV-A treatment alone is less effective in killing test isolates when compared to riboflavin/UV-A combined treatment and riboflavin 0.1% previously activated by UV-A treatment [18]. In another in vitro study performed by Schrier A et al., the antibacterial action of riboflavin alone, UV light alone and the combination of riboflavin and UV light has been assessed on S. aureus, MRSA and P. aeruginosa. According to their results, no bacterial death was seen in plates of riboflavin alone and all S. aureus and MRSA plates exposed to UV light alone; however, significant bacterial death was observed in all plates exposed to the combination of riboflavin and UV light [19].

Direct microbicidal effects of CXL on the infectious agents are very advantageous. Eradication of infection without application of topical antibiotics is of paramount importance especially in the presence of antibiotic-resistant organisms such as antibiotic-resistant *S. aureus* and *Pseudomonas*. This effect may also be useful in the initial stages of microbial keratitis when the offending pathogen has not yet been identified or in slow-growing microorganisms such as *Mycobacteria*. In addition, intensive topical broad-spectrum antibiotics are toxic to the corneal epithelium and may contribute to a prolonged corneal epithelial defect.

An increase in biomechanical and thermal stability of the cornea induced by cross-linking process is another mechanism that may underlie beneficial effects of CXL in the treatment of infectious keratitis [20,21]. A strengthened cornea is potentially less susceptible to the microbial and inflammatory destructive processes. It has been shown that photochemical cross-linking of the cornea using riboflavin and UV-A results in a markedly increased resistance against enzymatic digestion [20]. Therapeutic effects of CXL in melting ulcers of the cornea of various origins including sterile keratitis that has been demonstrated in several studies also provide evidence that CXL may reduce a load of proteolytic enzymes in the diseased cornea [13,22].

In a clinical study performed by Ehlers N et al., 14 eyes were treated for chronic, non-healing corneal ulceration. According to their results, six out of the 14 eyes healed after the treatment, three

patients had some healing and in fives eyes, there was no clear effect from CXL [13]. In another study by Iseli HP et al., five patients with infectious keratitis associated with corneal melting were treated with CXL. In all cases, it was indicated that CXL treatment could halt the progression of corneal melting and emergency keratoplasty was avoided [23].

CXL by riboflavin/UV-A has been used for the treatment of corneal edema, bullous keratopathies and in Fuchs dystrophy. Bullous keratopathies are among the predisposing factors for the development of microbial keratitis. Considering its ability in decreasing corneal edema and also its antibacterial effects, combined riboflavin/UV-A is especially useful for such cases. Such a therapeutic effect of corneal cross-linking has been reported in two cases of combined bullous keratopathy and infectious keratitis that have been resistant to traditional topical therapy [11].

Treatment of refractory keratitis with riboflavin/UV-A may allow us to avoid an emergency keratoplasty, a procedure that is usually needed to eradicate the infection and to preserve the eye, in severe and unresponsive cases. Emergency keratoplasty has several disadvantages such as the high rates of reinfection and rejection and avoiding it by another safe therapeutic option such as CXL may provide the opportunity to perform elective keratoplasty with lower risk of complications [23,24].

Although, our results showed that CXL is effective in the treatment of refractory bacterial and HSV keratitis and four of six eyes with bacterial keratitis had good response to this treatment; from two patients with HSV keratitis, one patient had good response and another patient had a relative response; in some cases, it was ineffective and the disease aggravated after treatment. Aggravation of keratitis after CXL may be a coincidental occurrence and related to the natural course of the disease but it may also be attributed to the procedure complications. It could be caused by treatment out of the security limit; corneal thickness was not measured in our study because of difficulties in the measurement of corneal thickness in the setting of corneal ulcer and its possible complications such as corneal perforation in a thinned cornea. In addition, the effect of UV-A light and or riboflavin on fortified antibiotics is actually unknown and they may have toxic effects on an already diseased cornea.

Melting of the cornea after corneal cross-linking has been reported in literature [25]. Several cases of bacterial keratitis such as Pseudomonas keratitis [26], Escherichia coli keratitis [27] and polymicrobial keratitis [28] have also been reported after UV-A/riboflavin corneal cross-linking. Herpetic keratitis with iritis after corneal cross-linking has also been reported [29]. Although, keratitis following CXL have been attributed to several factors such as the postoperative use of a soft bandage contact lens and topical corticosteroids and the presence of an epithelial defect, as trials of corneal CXL in treatment of corneal disorders are increasing, it seems necessary to conduct studies to evaluate the possible adverse effects of the UV light on the immune mechanisms of the cornea and the corneal wound healing processes.

CONCLUSION

Overall, it could be said that CXL with UV-A and riboflavin is effective in some cases of refractory bacterial and HSV keratitis and it can be added to the armamentarium of antimicrobial agents for the management of severe and refractory cases of infectious keratitis. Because both the experimental and clinical results are still preliminary and CXL may potentially aggravate the course of keratitis, we recommend its use only in refractory cases of microbial keratitis to avoid emergency keratoplasty. There is a demand for further studies to evaluate the efficacy and safety of this modality as the initial therapy for patients with infectious keratitis.

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