

Ultraviolet A/Riboflavin Corneal Cross-linking for Infectious Keratitis Associated With Corneal Melts

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Purpose: To evaluate the efficacy of ultraviolet-corneal cross-linking (CXL) for treating infectious melting keratitis.

Methods: Five patients with infectious keratitis associated with corneal melting were treated with CXL at the outpatient departments of the Institut für Refraktive und Ophtho-Chirurgie and the eye hospital at the University of Zurich. CXL was performed when the infection did not respond to systemic and topical antibiotic therapy. Follow-up after cross-linking ranged from 1 to 9 months.

Results: In all cases, the progression of corneal melting was halted after CXL treatment. Emergency keratoplasty was not necessary in any of the 5 cases presented.

Conclusions: CXL is a promising option for treating patients with therapy-refractory infectious keratitis to avoid emergency keratoplasty.

Key Words: ultraviolet-corneal cross-linking, corneal infectious keratitis, riboflavin

(*Cornea* 2008;27:590–594)

Biomechanical,¹ thermo-mechanical,^{2,3} and biochemical² properties of the cornea can be modified by using ultraviolet (UV) light in combination with riboflavin to induce cross-links within or between corneal collagen molecules. In 2000, Schnitzler et al¹ reported 4 cases of noninfectious corneal ulcers that were successfully treated with corneal cross-linking (CXL). In *in vitro* experiments, Spoerl et al² found that cross-linked corneas had increased resistance against enzymatic digestion with pepsin and collagenase. Bacteria and fungi can produce enzymes that digest human collagen, which leads to corneal melting.^{4,5} UV light^{6,7} and oxygen radicals⁸ can have an antimicrobial effect because oxygen radicals interfere with

cell membrane integrity. Hence, the combination of UV light and the radicals induced during cross-linking may synergistically protect corneas with microbial keratitis by actively destroying microbes and protecting the collagen against enzymatic degradation.

Here we report our experience with 5 patients with corneal infectious keratitis resistant to conservative treatment who underwent therapeutic CXL.

According to the agreement of the Corneal Cross Linking Congress 2007 (Zurich, Switzerland), we use the new term CXL for corneal cross-linking.

MATERIALS AND METHODS

Patients

Five patients with infectious keratitis-associated corneal melting were treated at the outpatient departments of the Institute for Refractive and Ophthalmic Surgery (n = 4) and the eye hospital at the University of Zurich (n = 1). The age of the patients ranged from 27 to 66 years. Three patients were women. Four patients had a history of laser-assisted *in situ* keratomileusis (LASIK) surgery and an early postoperative infiltrate in the interface. Despite intensive treatment with topical and systemic antibiotics, the corneal infiltrates progressed. One patient suffered from a contact lens-induced fungal keratitis that progressed despite intensive topical and systemic treatment. The follow-up time after cross-linking ranged from 1 to 9 months. We describe 4 cases in detail. The fifth case was similar to case 1 and is not described in detail (Table 1).

Treatment

After topical anesthesia of the cornea (alternating oxybuprocaine 0.4% and tetracaine 0.1% drops every 3 minutes for at least 15 minutes), the corneal epithelium within a 9-mm-diameter zone that included all microbial infiltrates was removed. After inserting a lid speculum, 0.1% riboflavin drops were administered every 3 minutes for 30 minutes. The riboflavin drops were prepared immediately before the treatment by mixing 0.5% aqueous riboflavin solution (Vitamin B₂; Streuli, Uznach, Switzerland) and 20% dextran T-500 solution. Successful penetration of riboflavin through the cornea was assured by visualizing the riboflavin in the anterior chamber by slit-lamp biomicroscopy (by using blue light). Thereafter, the eye was irradiated for 30 minutes with UVA (UVA) with an irradiance of 3 mW/cm² (UV-X; Peschke Meditrade, Cham, Switzerland). During that time, the cornea was moistened every 5 minutes with 0.1% riboflavin drops and

Received for publication July 7, 2007; revision received January 7, 2008; accepted January 10, 2008.

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Supported by a research grant from the University of Zurich.

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TABLE 1. Patient Data

Patient	Age (y)	Sex	Side	History	Initial BSCVA	Symptoms Started (d)	Detected Microbe
1	66	Male	Right	LASIK	CF	14	<i>Mycobacterium chelonae</i>
2	27	Male	Right	LASIK	LP	13	Filamentous fungus acremonium
3	29	Female	Left	LASIK	20/16	5	Nontuberculous <i>Mycobacterium</i>
4	32	Female	Right	CL	20/40	—	<i>Fusarium</i>
5	62	Female	Right	LASIK	CF	5	Nontuberculous <i>Mycobacterium</i>

CL, contact lenses; LP, light perception.

oxybuprocaine drops at the patient's discretion. After the treatment, a bandage lens soaked with 0.3% preservative-free ofloxacin drops (Floxal UD 3 mg; Chauvin Novopharma, Steinhausen, Switzerland) was placed to cover the cornea.

RESULTS

Case 1

Case 1 was a 66-year-old man with a history of uneventful bilateral LASIK for +4 D performed in South America; he was referred to our department because of increasing keratitis in his right eye 2 weeks after surgery. The visual acuity had dropped from 20/25 at the early postoperative stage to counting fingers (CF). On slit-lamp examination, we saw a central white infiltrate in the interface and a small epithelial erosion over this area (Fig. 1A). The stroma adjacent to the opacity appeared infiltrated and edematous. The eye showed mixed injection. The patient complained of photophobia but only minimal pain.

We lifted the flap, took a microbial culture, irrigated the interface with 0.3% gentamycin solution, and repositioned the flap. During this procedure, it became obvious that the flap was centrally perforated, probably because of melting. Immediately thereafter, we performed a standard riboflavin-UVA cross-linking (CXL) procedure. The patient was instructed to apply preservative-free ofloxacin and gentamycin drops alternating every 30 minutes for 3 days. By day 3, the epithelium was healed, and the bandage lens was removed. The patient was instructed to use ofloxacin drops hourly for another 4 days and fluorometholone drops twice per day. One week after CXL, the eye appeared calm, the cornea was without stromal edema, and the white plaques were clearly regressive (Fig. 1B) and gradually disappeared within 4 weeks. Visual acuity had increased to 20/400.

Microbial analysis showed *Mycobacterium chelonae*. Eight months later, the patient received a penetrating keratoplasty abroad.

Case 2

This 27-year-old man received a unilateral LASIK in his right eye for -3 D. The early postoperative period was uneventful; however, 13 days after surgery, his vision deteriorated significantly, and a diffuse lamellar keratitis grade 4 was diagnosed. Consequently, the interface was floated, but after 3 days, plaques developed at the interface. The patient was referred to the in-patient service at the eye hospital of the University of Ulm, Ulm, Germany, and received another floating of the interface, including microbial culture.

The microbial analysis detected filamentous fungus subtype *Acremonium*. During the following weeks, the patient received maximal systemic and topical treatment including topical natamycin 5% and amphotericin 0.1%, alternating

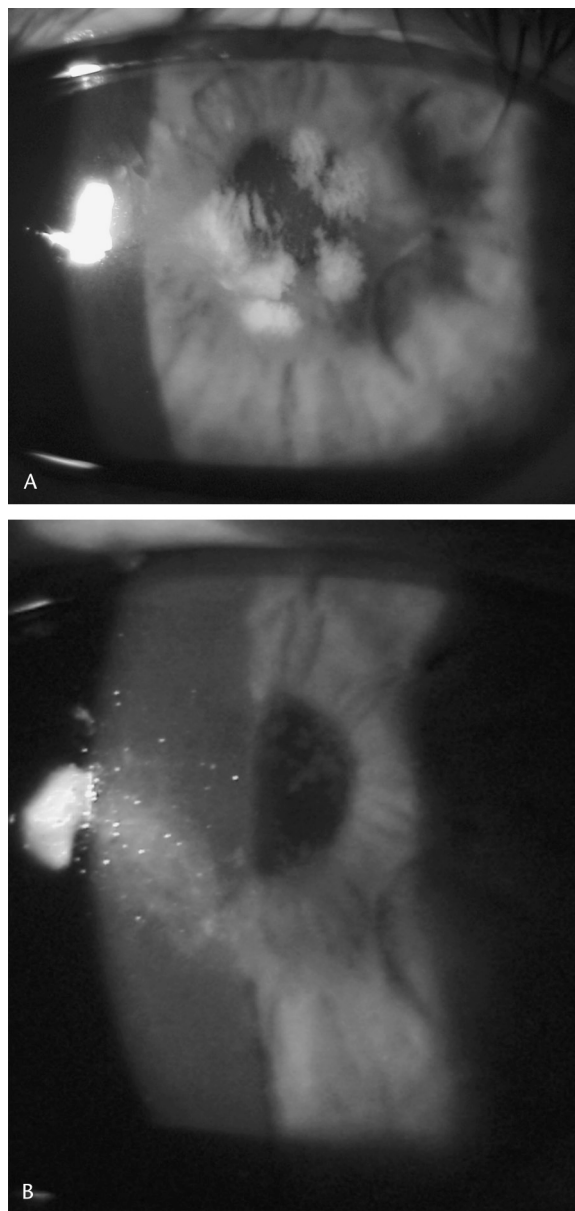


FIGURE 1. Patient 1, corneal ulcer after LASIK. Before CXL (A) and 1 week after treatment (B).

hourly, and systemic ketoconazole. Despite this maximal treatment, a corneal ulcer gradually developed, increasing in both diameter and depth. Ten weeks after the initial LASIK operation, the patient was referred for CXL (Figs. 2A,B). Postoperative treatment included antibiotic (ofloxacin) and antimycotic (natamycin) ointment until epithelial healing was complete, which took 5 days (Fig. 2C). Thereafter, tobramycin-dexamethasone drops (Tobradex; Alcon, Forth Worth, TX) were used 5 times/d, with treatment gradually decreasing over the next 4 weeks. One month after CXL, visual acuity had increased from light perception to CF.

Eight months later, the patient received a deep lamellar keratoplasty with an uneventful follow-up. Histologic examination of the removed tissue showed scarring but no signs of infection. Three months after suture removal, we performed topography-guided LASIK; unaided visual acuity was 20/30 at the 1-month follow up.

Case 3

Case 3 was a 29-year-old woman with a history of uneventful bilateral LASIK for -4.5 D. After 1 month, unaided visual acuity was 20/16 in both eyes. Two months later, she suffered a contusion of her left eye, accompanied by a reduced best spectacle-corrected visual acuity (BSCVA) of 20/100. Macrostriae of the flap were visible at slit-lamp examination, and the surgeon lifted and stretched the flap. At the first postoperative day, unaided visual acuity was 20/30, and the flap showed minimal microstriae. Five days later, the patient returned complaining of pain and a red eye. Slit-lamp examination showed a small, snowflake-like plaque in the interface, surrounded by significant stromal edema in the flap and wound bed. During the following days, plaque diameter and stromal edema increased. Eleven days after flap stretching, we performed a standard CXL procedure including a microbial culture. Postoperative medications were identical to those for case 1, until the microbial cultures identified a nontuberculous *Mycobacterium*. At that time, the cornea was clear except a few small, stationary white opacities, and the eye was white. We applied specific therapy⁹

(topical amikacin, systemic clarithromycin, topical moxifloxacin 0.5%) for 2 months. Because we did not see any additional effect, we reduced this therapy stepwise. At 4 months after cross-linking, all medications were stopped, and the eye remained calm, with a BSCVA of 20/50. Currently, we are considering an automated lamellar keratoplasty to improve visual acuity.

Case 4

This 32-year-old woman was referred to the clinic because of a midperipheral corneal ulcer, probably induced by a contact lens. Repeated microbiologic cultures were negative, but 2 consecutive polymerase chain reaction tests were positive for *Fusarium* (Figs. 3A,B). Despite systemic voriconazole (2×200 mg) and topical antibiotics (natamycin 5% every hour, voriconazole 1% every hour, polyhexamethylene biguanide every hour), the corneal ulcer progressed over the next 3 weeks. As an alternative to immediate corneal transplantation, the patient was offered corneal CXL. The patient consented to the treatment, which followed the standard protocol with a 9-mm treatment zone. After CXL, the patient reported minimal discomfort, and the epithelial wound closed rapidly. The antifungal treatment was continued.

After initial improvement (Figs. 3C,D), the corneal infiltrate progressed 3 weeks after the CXL (Figs. 3E,F), and the patient underwent an uneventful penetrating keratoplasty 1 month after cross-linking. The subsequent clinical outcome was unremarkable. The excised corneal tissue was examined by light and electron microscopy, and parts of the tissue were cultured for bacteria and fungi. Culture results remained negative, and the histologic workup showed an infiltrate with granulocytes and macrophages but no intact fungal cells.

DISCUSSION

The key finding of this small case series is that CXL is a viable option for treating patients with infectious keratitis to postpone keratoplasty (either lamellar or penetrating). In 4 of

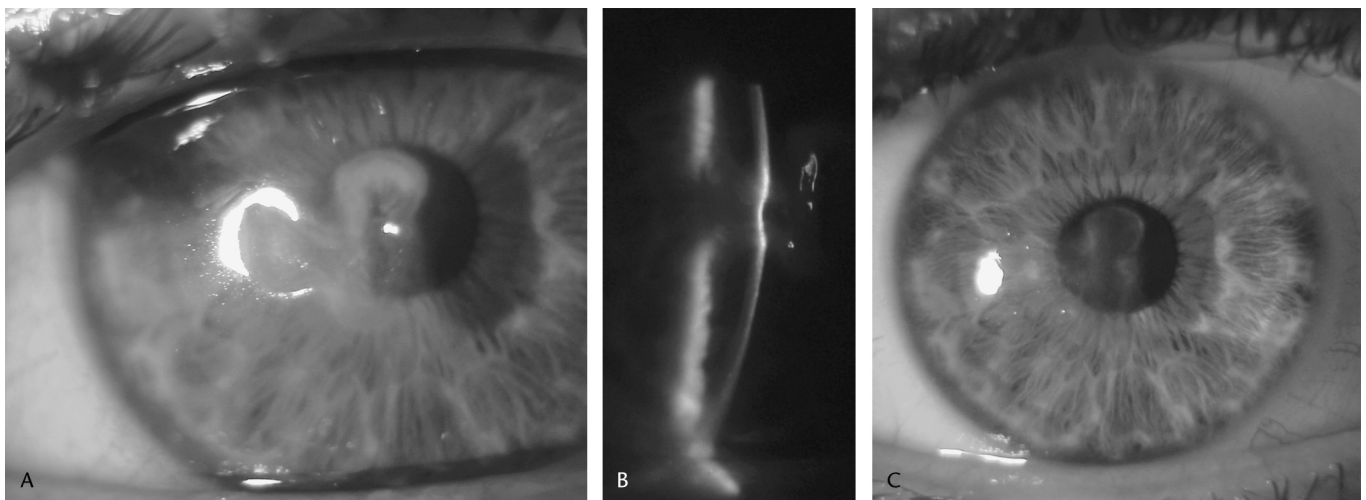


FIGURE 2. Patient 2, corneal ulcer after LASIK. Overview (A) and slit-lamp inspection (B). Same eye 5 days after cross-linking (C).

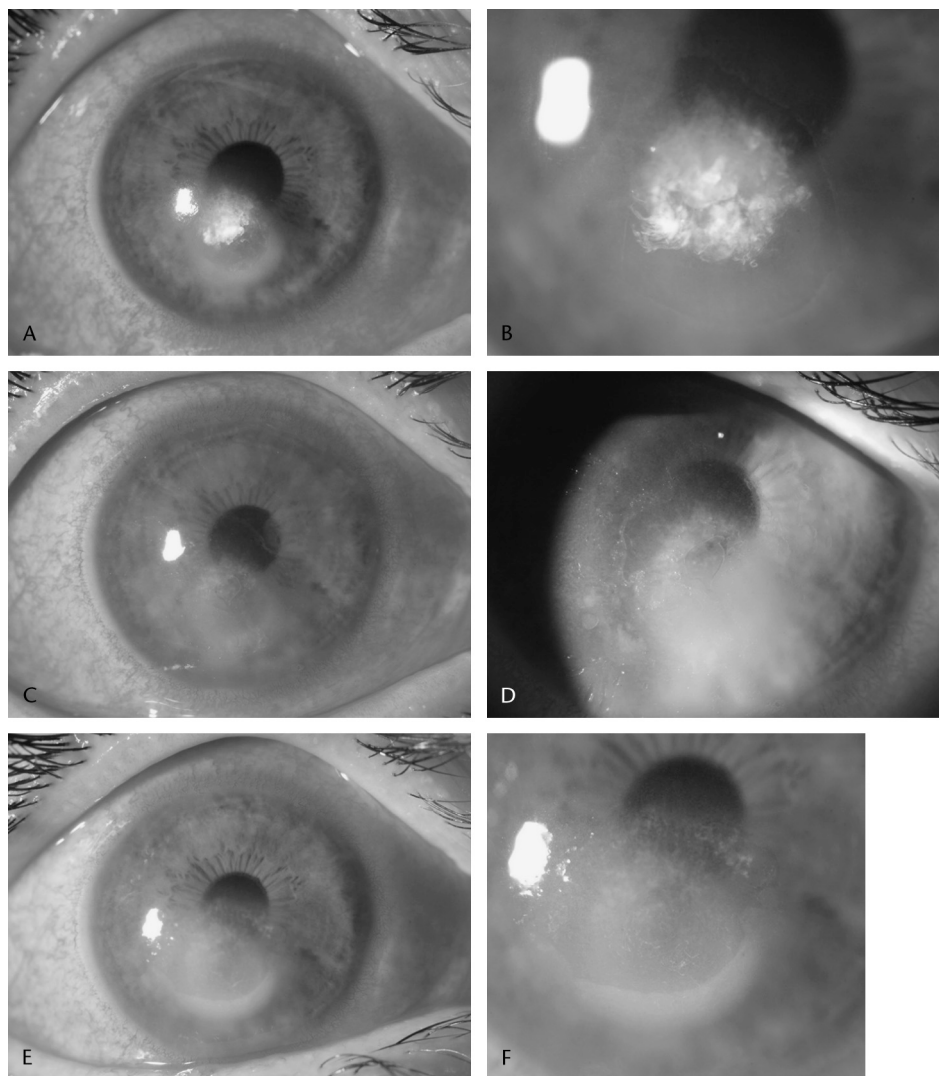


FIGURE 3. Patient 4. Despite systemic and topical voriconazole and topical natamycin, the contact lens-induced corneal ulcer progressed over a period of 3 weeks (A and B). The same patient 2 days after CXL; initial improvement had occurred (C and D). Four weeks after CXL and before penetrating keratoplasty (E and F).

the 5 cases, the infiltrate size and the melting process immediately regressed after CXL and scarring occurred. In the remaining patient (case 4) with recurrent inflammation who underwent corneal transplantation 4 weeks after CXL, histology showed that the progressive keratitis was caused by an immune reaction without persistent active fungal disease. In retrospect, this patient could have been treated with topical corticosteroids alone. Obviously, the CXL procedure can destroy microbes because no cases of recurrent infection occurred after cessation of specific therapy.

Avoiding emergency keratoplasty has several advantages. First, emergency keratoplasty is generally performed by means of a penetrating graft to minimize reinfection, whereas scheduled keratoplasty may provide the opportunity to perform lamellar grafts. Second, even when using a penetrating graft, the reinfection rate can reach 15%.¹⁰ Third, obtaining an antibiogram of slowly growing microbes (eg, mycobacteria) can take as long as 3–4 weeks, during which time specific antibiotic therapy cannot be applied, and graft reinfection is likely. Finally, the rejection rate after emergency keratoplasty is as high as 38%,¹⁰ much higher than standard keratoplasty

($\leq 10\%$).¹¹ From this perspective, it is helpful to have another therapeutic option for treating infectious and melting keratitis that does not respond to broad-spectrum antibiotics.

Spoerl et al² found that cross-linking the cornea by using riboflavin and UVA results in a marked increase in collagen resistance to digesting enzymes. Bacteria and fungi can produce enzymes that digest human connective tissue in the cornea, inducing tissue melting.^{4,5} CXL can slow down or even stop ulcer progression, which occurred in 4 of the 5 cases presented here. The use of cross-linking for treating noninfectious ulcers was reported by Schnitzler et al.¹ They reported stabilizing only 3 of 4 eyes; however, they administered riboflavin for only a few minutes, which might not have been enough to achieve the appropriate riboflavin concentration in the stroma. In contrast, we used topical riboflavin for 30 minutes before UV treatment.

The second component of the assumed effect of CXL is the antimicrobial power of the cross-linking itself. UVA (wavelength, 315–380 nm) alone can inhibit the growth of bacteria and fungi.^{12–14} The free radicals produced during cross-linking interfere with the microbial cell wall.^{15,16} However, it has not yet been fully decided which of the 2

effects, increased resistance against enzymatic digestion or antimicrobial power, is the predominant force in stabilizing the infectious process in the cornea. On the other hand, in 3 of the 5 eyes, there was a remarkable immediate response to the CXL, which led us to believe that the microbes are destroyed during CXL. More in vitro work is necessary to improve our knowledge about the antimicrobial power of CXL.

Corneal infections seen in our institutions are usually caused by soft contact lenses or occur after LASIK and can be difficult to treat because they are often resistant to broad-spectrum topical antibiotic therapy.^{17,18} CXL may become a therapeutic alternative for managing antibiotic-resistant microbial infections. In 4 of our cases, the primary spot of infection was in the interface after LASIK. The interface is a closed space where microorganisms survive and reproduce easily, protected against wash out and shielded from antibiotics and tear film by the flap. Riboflavin can easily diffuse into the stroma once the epithelial barrier is broken, and cells (eg, keratocytes) are killed as deep as 250–300 μm inside the stroma.¹⁹ The same process of cell death can kill infectious microbes located in the interface, which is usually located <200 μm deep.

The safety of CXL is of concern because the UVA could damage intraocular structures. Recently, a detailed analysis of the expected damage compared with accepted damage thresholds was published by Spoerl et al.² During standard CXL of a cornea with a 400- μm thickness, the irradiances of the UV light reaching the iris, lens, and retina are orders of magnitude smaller than the damage thresholds, and the only cell populations at risk are the microbes, the corneal endothelium, and the keratocytes. The alternative procedure used in desperate cases, penetrating emergency keratoplasty, would sacrifice these cells. Moreover, endothelial decompensation was not observed in any of the cases presented.

In conclusion, CXL is a promising option for treating patients with infectious keratitis who do not respond to broad-spectrum antibiotic therapy. Once corneal melting occurs in such cases, CXL should be considered before undertaking emergency penetrating keratoplasty.

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