Accelerated Corneal Cross-Linking With Photoactivated Chromophore for Moderate Therapy-Resistant Infectious Keratitis

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Purpose: To evaluate the effect of accelerated corneal cross-linking with photoactivated chromophore (PACK-CXL) as additional treatment for therapy-resistant infectious keratitis.

Methods: In this interventional cohort study, 20 patients (11 men and 9 women), aged 65.5 (interquartile range = 21.5–78.5) years, who were hospitalized for moderate-sized therapy-resistant bacterial corneal ulcers (11/20 microbiologically confirmed) were treated with hypoosmolar 0.1% riboflavin solution and Ultraviolet A (UVA) irradiation for 3 minutes at 30 mW/cm^2 (5.4 J/cm^2) as additional therapy to standard antimicrobial treatment.

Results: We did not observe any adverse effects of accelerated PACK-CXL on the corneal stroma or limbus. The median ulcer size was 3.00 (2.63–4.50) mm, the median time to reepithelialization was 6.50 (5.00–18.0) days, and the mean hospitalization period was 8.5 ± 4.5 days. Tectonic keratoplasty became necessary in 1 patient (5%).

Conclusions: Our results suggest that accelerated PACK-CXL may provide an antimicrobial effect similar to the 1 low-intensity, slow setting (30 minutes at 3 mW/cm^2) and may be used as additional treatment in moderate-sized therapy-resistant infectious keratitis.

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Infectious keratitis is a potentially sight-threatening condition. It can be of various origins—bacterial, fungal, viral, protozoal, and parasitical.[1] Several risk factors are known including ocular trauma, contact lens wear, ocular surgery, and ocular surface disease.[2,3] The World Health Organization classifies infectious keratitis as a "silent epidemic," with an estimated 800,000 new cases every year in India alone.[4]

The standard bacterial keratitis treatment according to the guidelines of the American Academy of Ophthalmology includes the use of broad-spectrum topical antibiotics. However, antimicrobial resistance (AMR) is rising at an alarming speed, and the World Health Organization has published an urgent call to identify alternatives to antibiotics in its global report on AMR.[5]

Corneal cross-linking (CXL) with riboflavin and UVA might represent such a method. Originally introduced for the treatment of keratoconus by improving the biomechanical features of the cornea,[6,7] CXL was used in 2008 in a pilot study in therapy-resistant infectious keratitis by Iseli et al.[8] They successfully treated 5 eyes using the standard cross-linking settings of 3 mW/cm^2 for 30 minutes (Dresden protocol).[8] In the same year, Martins et al[9] used the same technical settings in vitro to demonstrate the effect of UVA and riboflavin against a variety of pathogens involved in infectious keratitis.

From 2008 until today, published studies cover a total of 190 eyes treated with CXL as an adjunct to antimicrobial treatment, of whom 189 were treated with the Dresden protocol settings.[10,11] Only in 1 case of infectious keratitis, CXL was performed using 9 mW/cm^2 for 10 minutes.[12,13]

In this study, we tested whether CXL for infectious keratitis can be accelerated according to the Bunsen–Roscoe law of reciprocity to 3 minutes at 30 mW/cm^2 and whether it can maintain its efficacy in killing bacteria.

MATERIALS AND METHODS

We retrospectively analyzed a cohort of 20 cases with suspected therapy-resistant bacterial keratitis who were admitted to the Department of Ophthalmology at Soroka

University Medical Center, Beer-Sheva, Israel, between April 2015 and May 2016. Inclusion criteria were clinical signs of corneal ulcers of suspected bacterial origin up to a diameter of 7 mm and a depth of a maximum of 300 μm as determined by pachymetry (PachyPen Handheld Pachymeter; Accutome, Inc., Malvern, PA) and by slit-lamp examination so that the ulcer did not extend beyond two-thirds of the cornea.

Exclusion criteria were suspicion of noninfectious keratitis, viral or *Acanthamoeba* keratitis or sterile infiltrate, corneal perforation, descemetocele, pregnancy or breastfeeding, active corneal herpetic disease, systemic treatment involving steroids, immunosuppressed/immunocompromised patients, and diagnosed eczema (or atopic dermatitis). A total of 5 patients were excluded from this study because of the exclusion criteria. A total of 20 eyes from 20 patients (11 men and 9 women) with a median age of 65.5 (interquartile range = 21.5–78.5) years have been included in the study. The study protocol was approved by the Institutional Review Board of the Ben-Gurion University of the Negev and adhered to the tenets of the Declaration of Helsinki.

Initial antimicrobial therapy consisted of fortified vancomycin eye drops (50 mg/mL), fortified ceftazidime eye drops (50 mg/mL) hourly, artificial tears, and cycloplegia (when culture was positive for fungi, antifungal drops were added). In 11 patients (55%), the underlying pathogen that caused bacterial keratitis was identified. In 9 patients (45%), corneal scrapes remained inconclusive. Patients who did not show clinical improvement after 72 hours of antibiotic therapy underwent corneal cross-linking with photoactivated chromophore (PACK-CXL) treatment once written informed consent had been obtained.

Surgical Technique

The PACK-CXL procedure was performed under sterile conditions in an operating room setting. Topical anesthesia was achieved using 0.4% benoxinate hydrochloride drops. First, the epithelium was removed 1 mm around the borders of the ulcer. Second, hypoosmolar 0.1% riboflavin solution (Medio-Cross 0.1%; Peschke Meditrade GmbH, Huenenberg, Switzerland) was instilled topically on the entire cornea every 2 minutes for 20 minutes. The cornea was irradiated at 365 nm with an intensity of 30 mW/cm^2 for 3 minutes (total dose of 5.4 J/cm^2) using a commercially available device (LightLink-CXL; LightMed, San Clemente, CA). After PACK-CXL, antimicrobial treatment was continued. Complete healing was defined as re-epithelialization of the corneal epithelial defect with resolution of (eventual) hypopyon.

Statistical Analysis

Descriptive statistical analysis was performed with SPSS Statistics software (Version 23; IBM).

RESULTS

The Shapiro–Wilk test did indicate a non-normal distribution of the ulcer diameter ($P = 0.02$), time to reepithelialization ($P = 0.016$), and age ($P = 0.009$). Therefore, results are presented as median (interquartile range). The initial median ulcer diameter was 3.00 (2.6–4.5) mm. Five patients (25%) presented with hypopyon on admission to our department. The median duration to complete reepithelialization was 6.5 (5.0–18.0) days, and the

TABLE 1. Demographic and Clinical Characteristics of Patients

Case	Age	Sex	Etiology	Pathogen Identified (y/n)	Type of Pathogen	Corneal Ulcer Size, mm	Hypopyon (y/n)	Time to Healing, d
1	19	M	CL	Yes	PA	2.0	No	4
2	67	M	BK	Yes	PA	5.0	No	22
3	90	F	IS	Yes	*Candida parapsilosis*	4.5	No	16
4	44	M	FB	No		3.0	No	4
5	79	M	BK	No		5.0	No	20
6	26	F	IS	No		2.0	No	14
7	20	M	IS	Yes	*Staphylococcus aureus*	3.0	No	5
8	64	M	BK	Yes	*Proteus mirabilis*, *C. parapsilosis*	3.0	Yes	Keratoplasty
9	67	M	CA	Yes	*Staphylococcus epidermidis*	3.0	No	6
10	15	F	CL	Yes	PA	4.0	No	7
11	74	F	IS	No		2.0	No	4
12	88	F	CA	Yes	*S. aureus*	3.5	No	30
13	19	F	CL	Yes	PA, KP, *Serratia*, and *Morganella*	4.5	Yes	21
14	73	F	IS	No		2.5	No	18
15	77	M	CA	No		7.0	No	14
16	86	M	CA	No		3.0	No	5
17	85	F	IS	Yes	KP	3.0	Yes	4
18	19	M	CL	No		2.0	Yes	6
19	27	M	CL	Yes	PA	4.5	No	5
20	52	M	IS	No		3.0	Yes	8

BK, bullous keratopathy; CA, corneal abrasion (posttraumatic); CL, contact lens; F, female; IS, infected sutures (postkeratoplasty); KP, *Klebsiella pneumoniae*; M, male; PA, *Pseudomonas aeruginosa*.

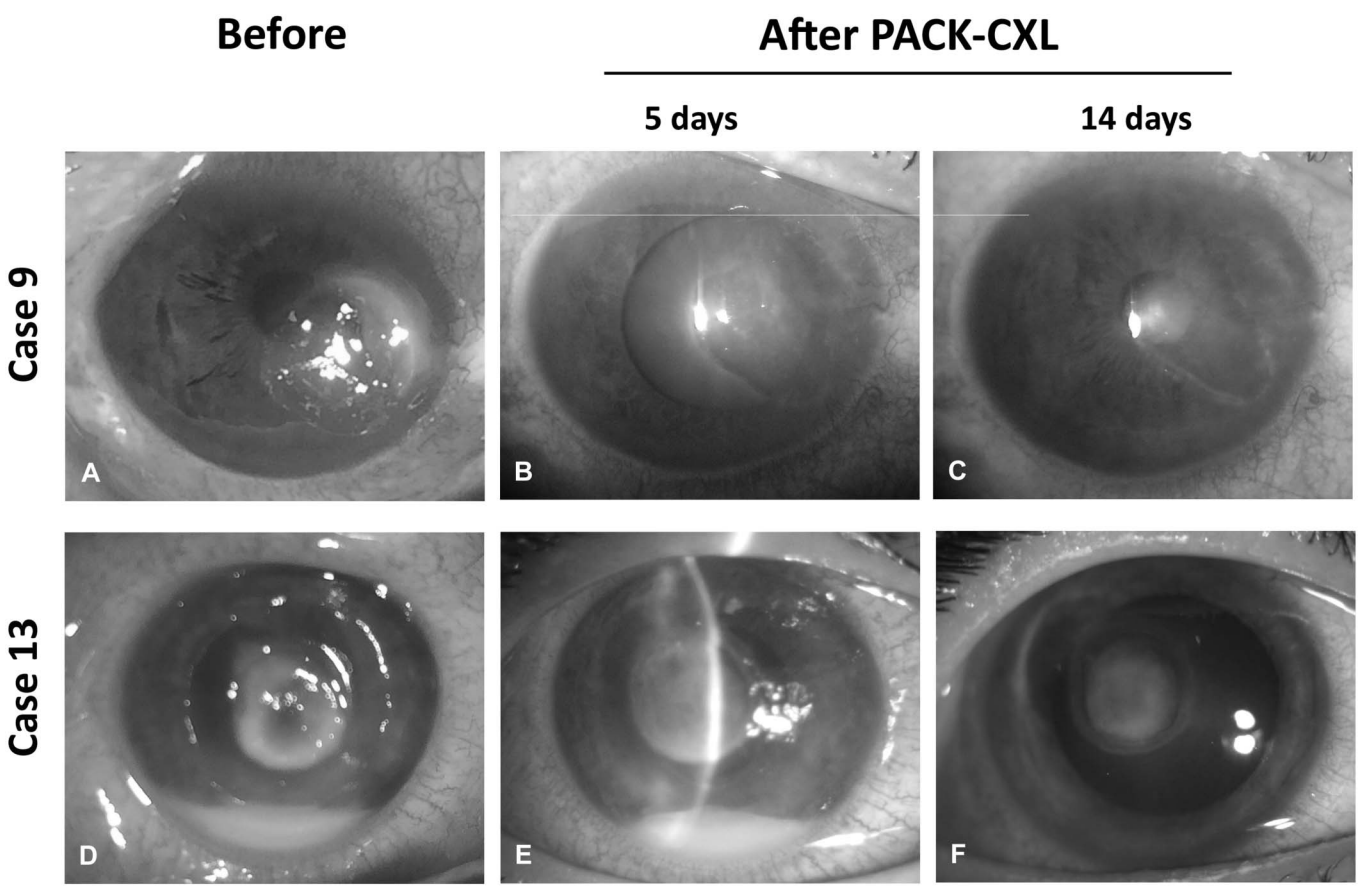

FIGURE 1. Slit-lamp images from patient 9 (A–C) with *S. epidermidis* infectious keratitis and from patient 13 (D–F) with a mixed infection (*Klebsiella pneumoniae, Pseudomonas aeruginosa, and Serratia* and *Morganella*), at presentation (A and D), and at 5 (B and E) and 14 (C and F) days after PACK-CXL treatment.

mean hospitalization duration was 8.5 ± 4.5 days. One patient (5%) showed a *Proteus mirabilis* and *Candida parapsilosis* corneal ulcer (case 8 in Table 1). After PACK-CXL, we observed stabilization of the melting process, but persistence of deep intrastromal infection and hypopyon. On day 14 after PACK-CXL, we performed tectonic corneal transplantation. PACK-CXL may have been able to postpone corneal transplantation and be further away from the acute infection phase.

The demographic, pre- and post-PACK-CXL data are presented in Table 1. Figure 1 shows slit-lamp images of patients 9 and 13 before and after accelerated PACK-CXL.

DISCUSSION

To better distinguish the use of CXL for the treatment of infectious keratitis from CXL used for ectasia, the term "photoactivated chromophore for infectious keratitis" (PACK)-CXL was proposed and adopted at the ninth cross-linking congress in Dublin, Ireland, in 2013.[14] The new PACK-CXL terminology helps unite the literature and simplify communication on the topic.

Recent studies demonstrated, both clinically and experimentally, that accelerating CXL for ectasia must be handled with care: irradiation times below 10 minutes significantly reduced the efficacy of the method clinically,[15–17] probably because of insufficient oxygen levels.[18–20] PACK-CXL for infectious keratitis, however, seems to maintain efficacy under laboratory conditions, even when accelerated to 3 minutes.[21] This is likely because photodynamic inactivation of microorganisms arises from the generation of free radicals and therefore depends largely on the totally administered energy and less on oxygen availability. Free radicals rapidly oxidize biomolecules and induce damage in major cell components, mainly in the cell wall and DNA, which finally lead to killing of microorganisms, but also of corneal keratocytes. Given that keratocytes do not survive the treatment, potentially induced mutations are not a problem. Clinical data on PACK-CXL are not yet available. In this preliminary report, we describe the outcome of 20 cases that were treated in our hospital for therapy-resistant infectious keratitis and underwent accelerated PACK-CXL (3 minutes irradiation at 30 mW/cm^2) as an adjuvant to topical antibiotic treatment.

CXL is based on the use of riboflavin as a photosensitizer, which generates reactive oxygen species when activated by UVA light at 365 nm. Riboflavin associates with nucleic acids by intercalation. Upon photoactivation, it sensitizes reactive oxygen species, including hydroxyl radicals with the potential of causing base damage and strand breaks, and

singlet oxygen with the potential of causing guanine lesions[22,23] and extracellular matrix components oxidation. Using higher ultraviolet irradiances for photoactivation shifts the production to more hydroxyl radicals and less singlet oxygen, which reduces extracellular matrix oxidation and hence corneal stiffening. In the context of this study, this implies that no mechanical stiffening can be expected for the accelerated PACK-CXL protocol.

Infectious keratitis is a well-known sight-threatening condition and leads to legal blindness in many cases, even when maximal conventional systemic and topical antibiotic therapies are available.[1] The disease may occur in all age groups, and treatment of bacterial keratitis is a costly and socioeconomic issue. In the light of increasing AMR in ophthalmology, new treatment modalities are needed for the treatment of this commonly encountered disease.[5,24,25]

Our study suggests that the PACK-CXL protocol may safely be accelerated from 30 minutes at 3 mW/cm^2 (Dresden protocol) to 3 minutes at 30 mW/cm^2. PACK-CXL should be considered in cases of severe unresponsive infectious keratitis before undertaking emergency keratoplasty. Further randomized prospective studies are needed to further investigate this accelerated mode of treatment.

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