Accelerated Photoactivated Chromophore for Keratitis–Corneal Collagen Cross-linking as a First-line and Sole Treatment in Early Fungal Keratitis

David Tabibian, MD; Olivier Richoz, MD; Arnaud Riat, MS; Jacques Schrenzel, MD; Farhad Hafezi, MD, PhD

ABSTRACT

PURPOSE: To report the use of accelerated photoactivated chromophore for keratitis–corneal collagen cross-linking (PACK-CXL) as a first-line treatment in a patient with an atypical fungal keratitis.

METHODS: Case report and literature review.

RESULTS: A patient who presented with a painful peripheral corneal infiltrate underwent PACK-CXL with a local limited abrasion and accelerated ultraviolet-A irradiation at 365 μm and 9 mW/cm^2 for 10 minutes. Cultures grew *Aureobasidium pullulans*. The corneal epithelium closed completely within 3 days and the infiltrate was completely eradicated without administration of antibiotics.

CONCLUSIONS: Accelerated PACK-CXL was successfully used as a first-line and sole treatment in a case of early fungal keratitis caused by *Aureobasidium pullulans*. Further characterization of the antifungal effect of PACK-CXL is needed in prospective studies.

[*J Refract Surg*. 2014;30(12):855-857.]

Besides the established indication of corneal ectatic disorders, corneal collagen cross-linking (CXL) has more recently been used as photoactivated chromophore for keratitis–CXL (PACK-CXL) to treat infectious keratitis.[1-5]

From the Laboratory for Ocular Cell Biology, University of Geneva, Geneva, Switzerland (DT, OR, FH); Genomic Research Lab, Division of Infectious Diseases, Geneva University Hospitals, Geneva, Switzerland (AR, JS); and the Department of Ophthalmology, Keck School of Medicine, University of Southern California, Los Angeles, California (FH).

Submitted: October 3, 2014; Accepted: October 29, 2014; Posted online: December 5, 2014

Supported by grants from the Gelbert Foundation, Geneva, Switzerland, and the Swiss National Science Foundation, Bern, Switzerland.

Drs. Richoz and Hafezi are co-inventors of the PCT/CH 2012/000090 application. The remaining authors have no financial or proprietary interest in the materials presented herein.

Correspondence: Farhad Hafezi, MD, PhD, University of Geneva, Rue Michel-Servet 1, 1211 Geneva 4, Switzerland. E-mail: farhad@hafezi.ch

doi:10.3928/1081597X-20141113-06

Iseli et al. published the first report on the use of PACK-CXL as a treatment in microbial ulcers resistant to conventional therapy in 2008. Two of the five cases presented were of fungal origin.[1] In 2012, Makdoumi et al. observed 16 eyes of 16 patients with bacterial keratitis that received PACK-CXL as first-line treatment. Postoperatively, only 2 of the 16 eyes required additional topical antibiotic treatment and epithelial healing occurred within a mean of 7.1 days in 15 of the 16 cases.[3] These promising results encouraged others to explore PACK-CXL as a treatment for fungal keratitis, both clinically[4,5] and experimentally in animal models.[6]

We report successful PACK-CXL as a first-line and sole treatment in a patient with an atypical keratitis caused by *Aureobasidium pullulans*, a fungus with rare occurrence in corneal infections.

CASE REPORT

A 27-year-old woman presented to our department in June 2014 complaining about a red, painful, and tearing right eye since the morning. The patient had worn daily soft contact lenses since 7 years of age and had no other relevant medical and ocular history. Corrected distance visual acuity (CDVA) was 0.1 logMAR (6/7.5 Snellen) in the right eye (with -3.50 -0.25 × 9) and 0.0 logMAR (6/6.0 Snellen) in the left eye (with -3.50 -0.75 × 174). Slit-lamp examination showed a marked conjunctival hyperemia and a round corneal stromal infiltrate with a diameter of 1 mm at the 2-o'clock position in the peripheral cornea, surrounded by a mild localized corneal edema (**Figure 1A**). Minimum corneal thickness was 519 µm, as measured optically by Scheimpflug imaging (Pentacam; Oculus, Wetzlar, Germany), and the infiltrate extended to a stromal depth of 170 µm (**Figure 1D**, arrow), as assessed by anterior segment optical coherence tomography (Spectralis; Heidelberg Engineering, Inc., Heidelberg, Germany). A slight anterior chamber reaction with few cells was present.

Corneal and conjunctival swabs were taken and sent to the bacteriology laboratory. The patient's contact lens was not available. With the consent of the local Institutional Review Board, PACK-CXL was offered as a first-line and sole treatment to the patient. The patient's consent was obtained and surgery was planned for the same day. No topical or systemic antibiotics were administered prior to surgery.

The patient was treated as described previously[5] with two modifications: an accelerated protocol was used and the epithelium was only removed partially. Briefly, prior to the irradiation, the epithelium was removed locally 1 mm around the infiltrate with a

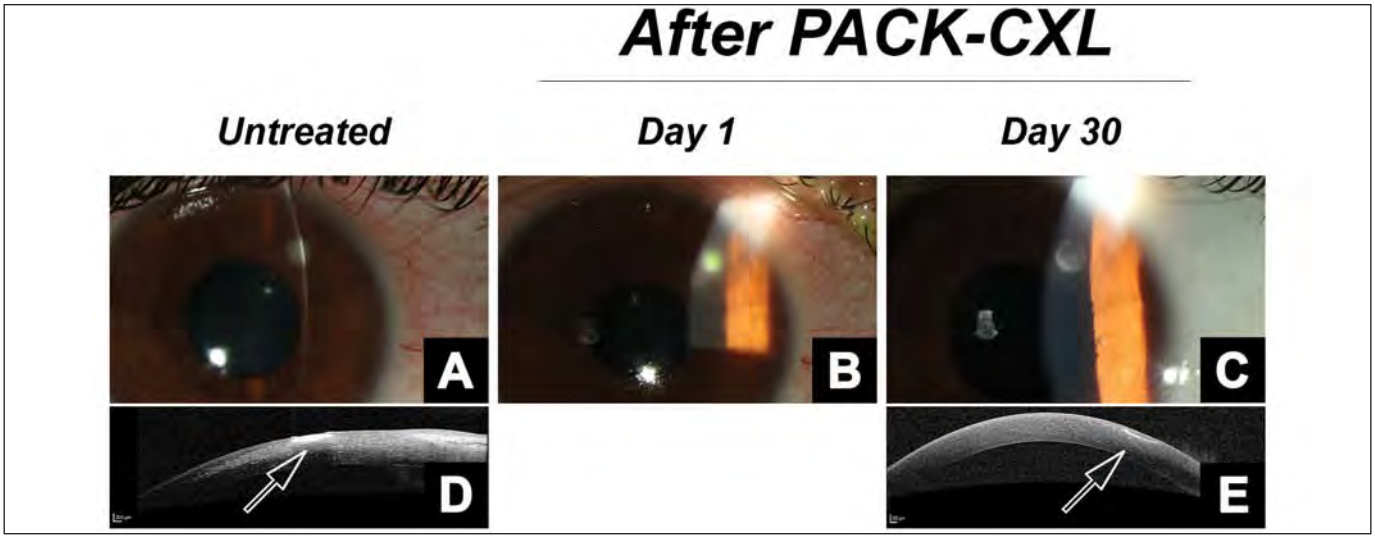

Figure 1. Slit-lamp photographs of the corneal infiltrate in the right eye (A) prior to photoactivated chromophore for keratitis–corneal collagen cross-linking (PACK-CXL) and at (B) 1 and (C) 30 days after PACK-CXL. Anterior segment optical coherence tomography showing the infiltrate (depth: 170 μm) (D) prior to and (E) 30 days after PACK-CXL.

hockey knife, creating a round abrasion of 3 mm diameter total. Proparacaine hydrochloride 0.5% eye drops containing 0.01 benzalkonium chloride were administered three times within 10 minutes, followed by instillation of 0.1% hypo-osmolaric riboflavin solution (MedioCross TE; Peschke Meditrade, Zug, Switzerland) on the entire cornea every 3 minutes for 30 minutes. Irradiation was performed at 365 µm using 9 mW/cm^2 for 10 minutes (CCL365; Peschke Meditrade). During the procedure, riboflavin solution was added once after 5 minutes. After the procedure, the riboflavin was rinsed off with saline solution, vitamin A ointment was applied, and the eye was patched. Acetaminophen was administered for analgesia. No topical or systemic antibiotics were prescribed.

The following day, CDVA in the right eye was 0.1 logMAR (6/7.5 Snellen) and slit-lamp examination showed a deepithelialized zone of 2.5-mm diameter. Slight conjunctival hyperemia was observed and the minimal anterior chamber reaction was still present (**Figure 1B**). The patient reported discomfort, but no pain. Because the evolution of the infiltrate was favorable at 24 hours after treatment, no additional medication besides vitamin A ointment was used after the time of surgery. Reepithelialization occurred gradually and the epithelium was completely closed and inflammatory signs on the conjunctiva and anterior chamber were resolved at 72 hours after PACK-CXL. At 1 week after the procedure, CDVA was 0.00 logMAR (6/6.0 Snellen) in both eyes. At 1 month after PACK-CXL, slit-lamp examination and anterior segment optical coherence tomography (**Figures 1C-1E**, arrow) showed a reduction in depth and density of the residual stromal scar associated with a regular healing process. CDVA was 0.0 logMAR (6/6.0 Snellen) in the right eye (with -3.50 -0.75 × 163) and 0.0 logMAR (6/6.0 Snellen) in the left eye (with -3.50 -1.00 × 1).

Microbial cultures revealed that a black yeast-like fungus caused the infection. The analysis by both MALDI ToF MS and ITS sequencing identified the strain as *Aureobasidium pullulans*, a type of fungus rarely associated with ocular infection in the literature. To determine the exact phenotype, the strain was cultured for 7 additional days at room temperature in the dark on potato dextrose agar, yielding the specific color of *Aureobasidium pullulans var pullulans*.

DISCUSSION

We report accelerated PACK-CXL as a first-line and sole treatment in a contact lens wearer presenting with a beginning fungal keratitis caused by *Aureobasidium pullulans*, a yeast-like species that shows a filamentous growth. *A. pullulans* is ubiquitous and can be responsible for various infections (mainly in immune-compromised patients, such as pneumonia, catheter-related bacteremia, peritonitis, and abscesses of the spleen, and also infectious keratitis).[7-9]

Microbial keratitis represents an indication for PACK-CXL that is rapidly developing. For bacterial keratitis, PACK-CXL has been successfully applied in clinical case series and studies and showed promising results in both advanced and early ulcers caused by various strains of bacteria.[1-5] This case report suggests that this technology might be useful as a first-line treatment not only in early bacterial, but also in early fungal keratitis. Furthermore, we applied recently published

findings indicating that accelerated irradiation protocols (9 mW/cm^2 for 10 minutes) might be as effective as conventional irradiation with 3 mW/cm^2 for 30 minutes.[10] The ultrastructure of fungi and especially their cell walls are distinctly different from bacteria, and further experimental studies are needed to explore the efficacy of PACK-CXL against various types of fungi.

AUTHOR CONTRIBUTIONS

Study concept and design (FH, DT); data collection (DT); analysis and interpretation of data (FH, AR, OR, JS, DT); drafting of the manuscript (FH, DT); critical revision of the manuscript (FH, AR, OR, JS, DT); administrative, technical, or material support (FH); supervision (FH)

REFERENCES

1. Iseli HP, Thiel MA, Hafezi F, Kampmeier J, Seiler T. Ultraviolet A/riboflavin corneal cross-linking for infectious keratitis associated with corneal melts. *Cornea*. 2008;27:590-594.
2. Makdoumi K, Mortensen J, Crafoord S. Infectious keratitis treated with corneal crosslinking. *Cornea*. 2010;29:1353-1358.
3. Makdoumi K, Mortensen J, Sorkhabi O, Malmvall BE, Crafoord S. UVA-riboflavin photochemical therapy of bacterial keratitis: a pilot study. *Graefes Arch Clin Exp Ophthalmol*. 2012;250:95-102.
4. Price MO, Tenkman LR, Schrier A, Fairchild KM, Trokel SL, Price FW Jr. Photoactivated riboflavin treatment of infectious keratitis using collagen cross-linking technology. *J Refract Surg*. 2012;28:706-713.
5. Said DG, Elalfy MS, Gatzioufas Z, et al. Collagen cross-linking with photoactivated riboflavin (PACK-CXL) for the treatment of advanced infectious keratitis with corneal melting. *Ophthalmology*. 2014;121:1377-1382.
6. Galperin G, Berra M, Tau J, Boscaro G, Zarate J, Berra A. Treatment of fungal keratitis from *Fusarium* infection by corneal cross-linking. *Cornea*. 2012;31:176-180.
7. Maverick KJ, Conners MS. *Aureobasidium pullulans* fungal keratitis following LASEK. *J Refract Surg*. 2007;23:727-729.
8. Huang YT, Liaw SJ, Liao CH, et al. Catheter-related septicemia due to *Aureobasidium pullulans*. *Int J Infect Dis*. 2008;12:e137-e139.
9. Salkin IF, Martinez JA, Kemna ME. Opportunistic infection of the spleen caused by *Aureobasidium pullulans*. *J Clin Microbiol*. 1986;23:828-831.
10. Richoz O, Kling S, Hoogewoud F, et al. Antibacterial efficacy of accelerated photoactivated chromophore for keratitis-corneal cross-linking. *J Refract Surg*. 2014;30:850-854.