Collagen Cross-linking with Photoactivated Riboflavin (PACK-CXL) for the Treatment of Advanced Infectious Keratitis with Corneal Melting

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Purpose: To investigate the efficacy and safety of corneal collagen cross-linking with photoactivated riboflavin (PACK-CXL) in the management of infectious keratitis with corneal melting.

Design: Prospective clinical trial.

Participants: Forty eyes from 40 patients with advanced infectious keratitis and co-existing corneal melting.

Methods: Twenty-one patients (21 eyes) underwent PACK-CXL treatment in addition to antimicrobial therapy. The control group consisted of 19 patients (19 eyes) who only received antimicrobial therapy.

Main Outcome Measures: The slit-lamp characteristics of the corneal ulceration, corrected distance visual acuity (CDVA), duration until healing and complications were documented in each group. Mann-Whitney Rank Sum Test was used for statistical analysis. p values less than 0.05 were considered statistically significant.

Results: The average time until healing was 39.76±18.22 days in the PACK-CXL group and 46.05±27.44 days in the non-PACK-CXL group (p=0.68). CDVA after treatment and healing was 1.64±0.62 in the CXL group and 1.67±0.48 in the non-PACK-CXL group (p=0.68). Corneal ulceration’s width and length was significantly bigger in the PACK-CXL group (p=0.004 and 0.007). Three patients of the non-PACK-CXL group developed corneal perforation; one had their infection recur. No serious complications occurred in the PACK-CXL group.

Conclusions: PACK-CXL did not shorten the time to corneal healing; however, the complication rate was 21% in the non-PACK-CXL group, whereas there was no incidence of corneal perforation or recurrence of the infection in the PACK-CXL group. These results indicate that PACK-CXL may be an effective adjuvant therapy in the management of severe infectious keratitis associated with corneal melting.
Infectious keratitis is a potentially sight-threatening condition of the cornea often presenting as an ophthalmic emergency. Delayed treatment of infectious keratitis can lead to significant visual loss in as many as 50% of cases. A wide range of fungi, bacteria, protozoa, and viruses has been identified as infectious agents in microbial keratitis. The majority of community-acquired cases of microbial keratitis resolve with empiric treatment using broad-spectrum topical antimicrobials. However, the emergence and spread of antimicrobial-resistant organisms remain a serious clinical and public health concern. In infectious keratitis, antimicrobial-resistant strains are associated with a worse clinical presentation and marked visual impairment. A considerable amount of research is directed towards developing newer antibiotics or defining alternative modalities of treatment. Corneal degradation and melting occurs when specific proteinases are upregulated following corneal damage. These matrix metalloproteinases are synthesized either in the keratocytes (MMP-2) or by corneal epithelial cells (MMP-9) and are also responsible for delayed epithelial wound healing.

Corneal collagen cross-linking (CXL) is a novel technique, which was developed and introduced in 1999 for the treatment of keratoconus (KC) and postoperative ectasia. During the standard CXL procedure, the corneal epithelium is removed and the corneal tissue is irradiated with ultraviolet-A (UV-A) light after instillation of the photo-sensitizer, riboflavin. The induced photochemical reaction facilitates the strengthening of the collagen lamellae in corneal stroma, thereby stabilizing the cornea biomechanically.

It has been shown that CXL demonstrates excellent antimicrobial efficacy against a variety of common pathogens in vitro. The evidence that CXL can
effectively treat clinical microbial keratitis and arrest the progression of corneal melting, however, is limited.\textsuperscript{15-17} To better distinguish the use of CXL for the treatment of infectious keratitis from CXL for progressive keratoconus, the term PACK-CXL (\textbf{Ph}oto\textbf{A}ctivated \textbf{C}hromophore for infectious \textbf{K}eratitis-CXL) was created at the ninth cross-linking congress in Dublin, Ireland in 2013.

Within this context, the aim of our study was to investigate the clinical efficiency and safety of PACK-CXL in the management of infectious keratitis associated with corneal melting.

\textbf{Methods}

This prospective clinical trial was conducted at the Cornea Unit of the Research Institute of Ophthalmology (RIO), Cairo, Egypt in collaboration with the Department of Ophthalmology, Beni-Suef University Hospitals, Egypt, the Rowad Correction Centre, Cairo, Egypt, the Department of Ophthalmology, University of Nottingham, UK, and the Department of Ophthalmology, University of Geneva, Switzerland, during the period between January 2010 and April 2013. The RIO Institutional Review Board approved the study protocol, which adhered to the tenets of the declaration of Helsinki, and written informed consent was obtained by all participants before inclusion.

\textit{Participants}

The study included adult patients (>18 years old) presenting to the cornea clinic of RIO with infective corneal ulcer with a possible bacterial, fungal, acanthamoeba or mixed origin with evident corneal melting. Exclusion criteria were age under 18 years, corneal ulceration in proximity (1mm) to the corneal limbus, underlying
autoimmune disease, history of herpetic eye disease, corneal thickness less than 400 micrometer with epithelium and pregnant or nursing women. Patients who agreed to be enrolled in the study and provided informed consent were randomized according to the order of presentation alternately into either of the 2 groups: a “PACK-CXL-treated group” that received PACK-CXL and medical treatment, and a “non-PACK-CXL group” (control group) that received medical treatment alone.

**Ophthalmological Examination and Medical Treatment**

After admission, the initial examination included the patient’s medical history, specifically the history of contact lens (CL) wear, the duration and type of treatment prior to first visit, the presence of systemic diseases, measurement of corrected distance visual acuity (CDVA), slit-lamp examination, slit-lamp photography, and ultrasound pachymetry of corneal thickness. The parameters evaluated during slit-lamp examination included the localization and extent (longest diameter and at right angles to it) of corneal ulcer, the site and extent (diameter) of infiltrate, the localization of corneal vascularization and the presence of hypopyon.

On presentation, all pre-existing treatment was interrupted for 24 hours and corneal scrapes for direct smears and cultures were done. Initial antimicrobial therapy for both groups consisted of fortified vancomycin eye drops 50 mg/ml, fortified ceftazidime eye drops 50 mg/ml hourly, and the antifungal agent itraconazole 100 mg orally twice per day. This regimen was subject to change according to response or culture results.

**PACK-CXL Treatment**

Patients allocated to the PACK-CXL group were assessed and treated by PACK-
CXL within 48 hours. Topical anesthesia was achieved using 0.4% benoxinate hydrochloride drops. Epithelium was removed up to 9 mm diameter. Corneal thickness of the area to be treated was measured (without epithelium) aiming for a starting thickness of no less than 350 and no more than 500 microns. Corneas thicker than 500 microns were deswelled using 70% Glycerol drops (prepared at our local pharmacy) applied topically at intervals of 2-3 seconds for five minutes.

Iso-osmolar riboflavin drops (Medio-Cross® 0.1% riboflavin/dextran solution, Peschke Meditrade, Huenenberg, Switzerland) were instilled topically on the cornea for a period of 30 minutes at an interval of 2 minutes and the thickness re-measured every 5 minutes to ensure that the thickness remained below 500 microns during the course of instillation. In one case, the cornea deswelled to 260 micron and hypo-osmolar riboflavin was instilled until the corneal thickness reached 350 microns.

The cornea was illuminated using a UVX lamp (Peschke Meditrade GmbH, Huenenberg, Switzerland), UVA 365 nm with an irradiance of 3 mW/cm² for 30 minutes and a total dose of 5.4 J/cm² during which riboflavin was instilled every 2 minutes and corneal pachymetry performed every 5 minutes. CXL was performed in a 9mm-diameter zone. After PACK-CXL treatment, antimicrobial treatment was continued as before and daily follow-up examination was performed until healing was complete. Complete healing was defined as re-epithelialization of the corneal epithelial defect with disappearance of hypopyon with no anterior chamber activity and clearing of stromal infiltrate. All complications including perforation of the corneal stroma were recorded.

Statistical analysis
Data were analyzed with the Statistical Package for Social Sciences (SPSS®, version 17.0). All data are expressed as the mean ± standard deviation (SD). Normal distribution of data was evaluated by the Shapiro-Wilk test. Groups were compared using the Mann-Whitney Rank Sum Test and \( p \) values less than 0.05 were considered statistically significant.

**Results**

The PACK-CXL-treated group included 21 patients (8 males and 13 females) with a mean age of 37.3 years. The non-PACK-CXL group included 19 patients (10 males and 9 females) with a mean age of 49.8 years. The baseline CDVA at presentation was 2.16±0.35 logMAR (logarithm of the minimum angle of resolution) in the PACK-CXL group and 2.01±0.44 logMAR in the non-PACK-CXL group (\( p=0.11 \)). The isolated causative microorganisms for each group are shown in Table 1 for the PACK-CXL group and Table 2 for the non-PACK-CXL group. Staphylococcus and Aspergillus were the most common bacterial and fungal genera isolated in culture, respectively. Slit-lamp findings of the corneal ulcer in both groups are depicted in Table 1 and 2. The mean size of the ulcer was larger in the PACK-CXL group (5.62±1.88 x 6.22±1.98 mm) than in the non-PACK-CXL group (3.97±2.5 x 4.22±2.18 mm; \( p_1=0.004; \ p_2=0.007 \)). The mean duration to complete healing was 39.76±18.22 days in the PACK-CXL group and 46.05±27.44 in the non-PACK-CXL group (\( p=0.68 \)). The average CDVA after complete healing was 1.64±0.62 logMAR in the PACK-CXL group and 1.67±0.48 logMAR in the non-PACK-CXL group (\( p=0.68 \)). Figure 1 and Figure 2 show colored fundus photography and fluorescein staining of patients number 19 and 20, respectively, before and after PACK-CXL.

Three patients in the non-PACK-CXL group had corneal perforation, while
PACK-CXL-treated patients did not suffer this complication. Infection did recur in one patient from the non-PACK-CXL group, making the total complication rate 21%. In contrast, no severe complications occurred in the PACK-CXL group. Corneas that exceeded 500 microns in thickness were treated with glycerol, which caused conjunctival chemosis in some patients. In one eye, glycerol application caused excessive de-swelling that was treated with hypo-osmolar riboflavin prior to PACK-CXL. All PACK-CXL-treated patients exhibited limbitis after treatment, which resolved in 5-7 days in all but one case. In one patient (acanthamoeba keratitis), limbitis persisted for 3 weeks after the PACK-CXL treatment despite a reduction in the size of the ulcer.

**Discussion**

Infectious keratitis is a severe ocular infection and one of the leading causes of monocular blindness worldwide. The incidence of microbial keratitis ranges from 6.3 to 710 cases per 100,000 population per year, and is even more common in contact lens wearers. Various microorganisms, including bacteria, viruses, fungi, and parasites, may cause infectious keratitis. This infection and inflammatory reaction may lead to ulceration, corneal melting and perforation if not treated adequately. The increasing resistance to antimicrobial agents has contributed to a dramatic increase of keratitis-related complications with devastating consequences. Therefore, current research focuses on innovative treatment options beyond antimicrobials for the management of infectious keratitis, particularly for the therapy of resistant forms.

There is a body of clinical evidence, which supports the antimicrobial efficacy of
PACK-CXL.\textsuperscript{15-17} Iseli et al. demonstrated immediate regression of the corneal melting process and significant decrease in infiltrate size after PACK-CXL in five patients with therapy-resistant bacterial or fungal ulcerative keratitis.\textsuperscript{15} Makdoumi et al. used PACK-CXL as primary therapy in patients (seven eyes, six patients) with bacterial keratitis and reported symptomatic relief and arrest of progression of melting in all cases.\textsuperscript{16} Panda et al. treated patients with antimicrobial-refractory, keratitis-associated corneal melting by PACK-CXL. The melting was halted and avoided emergency keratoplasty in all seven eyes.\textsuperscript{17} Similarly, Kozobolis et al. presented excellent clinical outcomes after PACK-CXL in two patients with combined bullous keratopathy and ulcerative keratitis.\textsuperscript{20} Skaat et al. reported good results of PACK-CXL in the management of refractory infectious keratitis in 6 patients,\textsuperscript{21} while PACK-CXL has also been applied successfully in the treatment of fungal keratitis and post-LASIK keratitis associated with corneal melt.\textsuperscript{22, 23} Finally, PACK-CXL healed corneal ulceration and eliminated edema and painful symptoms in a patient with \textit{Escherichia coli} keratitis.\textsuperscript{24} Our results concur with published reports; however, to our knowledge we are the first to report a series of more than 7 eyes and that includes a control comparator. This control group afforded us the opportunity to estimate the rate of serious complications. Patients receiving only antimicrobial therapy demonstrated a significant complication rate compared to those who additionally underwent PACK-CXL (21\% vs. 0\%). No significant differences regarding the time to healing and the final visual outcome were observed between the two groups. CXL was associated with transient limbitis, a non-serious complication not seen in the control group.

We have also noticed a transient increase in the size of hypopyon in the first 24 hours after PACK-CXL in some patients. Thereafter, the hypopyon decreases gradually in size until it disappeared. (Figure 1 A,B). This might be due to a reaction to
the massive and simultaneous death of microorganism and release of endotoxins, similar to a Jarisch-Herxheimer reaction.²⁵

PACK-CXL halts corneal melting and improves infectious keratitis by at least two mechanisms, and these probably operate in synergy. First, it is well established that pathogens implicated in corneal melting may act by enzymatic digestion.²⁶, ²⁷ Since PACK-CXL increases tissue resistance to enzymatic digestion, the cross-linking procedure may help the corneal stroma resist proteolysis by enzymes from polymorphonuclear leukocytes participating in the inflammatory process.²⁸ A fortified stroma may also block the penetration or effect of toxins from the pathogenic organism. Secondly, the phenomenon of apoptosis induced by PACK-CXL likely does not only kill keratocytes, but also microbes, which decelerates the infectious process.²⁹ Indeed, in vitro studies support this latter antimicrobial mechanism. PACK-CXL has documented bactericidal activity against some common pathogens in vitro, such as Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, methicillin-resistant S. aureus (MRSA), multidrug-resistant P. aeruginosa and drug-resistant Streptococcus pneumoniae.³⁰ PACK-CXL has also exhibited antimicrobial effects against fungal pathogens in vitro, such as Candida albicans, Fusarium sp., and Aspergillus fumigatus.³⁰

Our results demonstrate the beneficial effect of PACK-CXL in cases of infectious keratitis with corneal melting. PACK-CXL could serve as valuable adjuvant therapy in the management of infectious keratitis with corneal melting. This treatment may minimize or avoid severe complications such as corneal perforation and/or recurrence of the infection.
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References


