ORIGINAL RESEARCH

Photoactivated Chromophore for Keratitis-Corneal Collagen Cross-Linking (PACK-CXL) Improves Outcomes of Treatment-Resistant Infectious Keratitis

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Purpose: To investigate the efficacy of photoactivated chromophore corneal collagen cross-linking (PACK)-CXL in the management of treatment-resistant infectious keratitis.

Design: Observational cohort study.

Participants: Forty-two eyes from 41 patients with treatment-resistant infectious keratitis.

Methods: Eyes underwent PACK-CXL treatment with the Dresden modified protocol in addition to standard antimicrobial therapy. The primary endpoint was the size of the corneal ulcer. Descriptive statistics, Wilcoxon rank test, McNemar test and Spearman correlation coefficient were used for statistical analysis, and *p* values lower than 0.05 were considered statistically significant.

Results: Success rate at third postoperative month was of 90.5%. Statistical analyses showed a significant effect of (PACK)-CXL with standard antimicrobial therapy to reduce corneal ulcer size (p=0.031).

Conclusion: As adjuvant therapy to standard antimicrobial treatment, PACK-CXL improves the outcomes in patients with treatment-resistant corneal ulcers.

Keywords: cross-linking, PACK-CXL, resistant keratitis

Introduction

Infectious keratitis is associated with a risk of permanent and devastating visual loss worldwide, especially in developing countries.[1–5] Treatment comprises topical broad-spectrum antibiotics, but if therapy is delayed, it is estimated that only 50% of the eyes will have a good visual outcome; actually, infectious keratitis can lead to corneal perforation or endophthalmitis.[6]

To improve treatment outcomes and enhance microbial eradication with fewer side effects, new approaches like corneal cross-linking (CXL) have been proposed. This technique was introduced in the late 1990s as a primary treatment for corneal ectasia, for which is widely used. It is a simple and non-invasive technique that combines ultraviolet A radiation and a chromophore (riboflavin) to stiffen corneal tissue through collagen fiber photopolymerization.[7]

CXL is proposed to be effective for treating infectious keratitis based on the antibacterial properties of photoactivated chromophore and UVA light. Its mechanism of action includes inhibition of microbial replication, DNA and RNA damage,[8,9]

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intercalation of the chromophore with microbial nucleic acids,[10] direct damage to the pathogen cell walls and oxidation of nucleic acid residues by reactive oxygen species,[11,12] as well as increased resistance of the stiffened cornea to enzymatic damage from the microorganisms.[13] Other potential advantages of UVA and riboflavin application over antibiotics include eliminating ocular surface toxicity and avoiding adherence issues associated with the need for frequent eyedrop administration, among others.[14]

In 2000, Schnitzler et al reported the use of CXL for stabilization of non-infectious corneal melting in four patients.[15] The melting process stopped in three of four patients, delaying surgical treatment. This early trial showed the efficacy of CXL in biomechanical stabilization of structurally altered corneas without inducing ectasia. Other case studies about the effect of CXL on melting corneas with advanced and treatment-resistant keratitis showed similar results and proved to be effective not only in stabilizing melting but also in eliminating several pathogens.[16–19]

To encourage the exploration of different applications of CXL and modifications to the Dresden protocol,[20] the ninth CXL congress in 2013[8,21] established separate designations to distinguish between CXL for ectasia and CXL for infectious keratitis. The latter is also known as photo-activated chromophore for infectious keratitis (PACK)-CXL.[22]

We present an observational cohort study of antibiotic-resistant infectious keratitis treated with PACK-CXL plus standard antibiotic treatment in a tertiary eye care center in Mexico City.

Materials and Methods

The participants were enrolled from the Cornea Department of the "Asociación para Evitar La Ceguera en Mexico I.A. P., Hospital Dr. Luis Sánchez Bulnes", a tertiary eye care center located in Mexico City, from November 2014 to January 2015. The ethics committee of the "Asociación para Evitar La Ceguera en Mexico I.A.P., Hospital Dr. Luis Sánchez Bulnes", approved this observational cohort study and all research followed the tenets of the Declaration of Helsinki. A written informed consent was obtained from all participants.

Patients eligible for the study were male and female adult patients (18 years or older) with treatment-resistant infectious keratitis. A patient was considered treatment resistant when after one week with treatment, there was a worsening of clinical presentation (increased epithelial defect). Treatment consisted in one of the following

therapies: 1) If a bacterial infection was suspected or documented, a fluoroquinolone and an aminoglycoside were prescribed 2) If a fungal infection was suspected or documented, natamycin was prescribed 3) For suspected polymicrobial etiology, both strategies were used. Exclusion criteria were diagnosis of rheumatologic disease, perforation or a descemetocele with high risk of corneal perforation, endophthalmitis, and pregnancy.

All patients underwent a comprehensive ophthalmic examination that included uncorrected visual acuity (UCVA), slit lamp biomicroscopy, fundoscopy and intraocular pressure. The severity of keratitis was graded by slit lamp examination and the longest diameter of the ulcer in the first exam (initial size) was registered. Patients were classified according to the size of the ulcer in 3 categories: ulcers with size <3 mm, 3–6 mm and >6 mm. Ulcer size was defined as the corneal epithelial defect, regardless of infiltrating size. A minimum 400-micron corneal stromal thickness was considered safe to perform CXL, obtaining pachymetry by Pentacam® rotating Scheimpflug camera topography.

After obtaining corneal scrapings, they were sent for standard microbiology culture and antibiotic susceptibility testing, as following: after tetracaine instillation, a sample of the desired area of the cornea was taken with a spatula or cotton swab, proceeding to spread C-shaped streaks on chocolate agar, blood agar (5% ram blood), staphylococcus 110, Sabouraud agar, Sabouraud-Emmons medium for fungal growth, and Biggy agar for Candida.

For microscopic observation, the sample is placed in an area marked with a circle with a diamond pencil to prepare the following stains: Gram for positive and negative bacteria, Ziehl-Neelsen for acid-alcohol-fast bacilli, Periodic acid-Schiff (PAS) and calcofluor white (CFW) for Acanthamoeba and fungi under fluorescent light.

(PACK)–CXL with ultraviolet A and riboflavin was applied under topical anesthesia on the day of diagnosis (day 0). According to the Dresden modified protocol, riboflavin 0.1% solution was administered to the cornea every minute for 15 minutes, followed by exposure to 370-nm UVA light (with a fluence of 3 mW/cm^2) from a distance of 1 cm for 30 minutes.

After PACK-CXL treatment, the eye was rinsed with saline followed by bandage contact lens placement; post-operative fluorometholone acetate 0.1% drops were given 4 times a day for 2 days and then 3 times a day for one week. Contact lens was removed one day after placement. Patients received topical antibiotic eyedrops based on the antibiogram

results and artificial tear eyedrops until epithelial healing was observed, and as part of the postoperative care, patients were advised to wear UV protection glasses. The primary endpoint was the size of the ulcer. Treatment success was defined as the complete closure of the epithelial defect, with no corneal stromal infiltrates and lack of symptoms.

Standard antimicrobial treatment was initiated as monotherapy with fourth-generation fluoroquinolones and subsequently modified according to clinical response, gram stains culture and antibiotic susceptibility results. Antimicrobials given to the patients were moxifloxacin 0.5% ophthalmic solution (Vigamoxi®, 17 eyes, 40.47%); ofloxacin 0.3% ophthalmic solution (Ocuflox®, 8 eyes, 19.04%), gatifloxacin 0.5% ophthalmic solution (Zymar®, 15 eyes, 35.71%), natamycin 0.5% ophthalmic suspension (Pimaricin®, 7 eyes, 16.66%), netilmicin 0.3% ophthalmic solution (Netira®, 26 eyes, 61.9%), tobramycin 0.3% ophthalmic solution (Trazil®, 3 eyes, 7.14%), ceftazidime 5% ophthalmic solution (500 mg/ 2mL solution; 2 mL of this solution are added to 8 mL of hypromellose; 2 eyes, 4.76%), sulfacetamide 10% ophthalmic solution (Blef®-10, 1 eye, 2.38%), chloramphenicol 0.5% ophthalmic (Cloran®, 2 eyes, 4.76%) solution and/or voriconazole 1% ophthalmic solution (Vozole®, 8 eyes, 19.04%).

Statistical analysis was performed using descriptive statistics with the software IBM SPSS Statistics for Windows, Version 23.0 Armonk, NY: IBM Corp. Since the data followed a normal distribution (verified by Kolmogorov– Smirnov test), for quantitative variables we used measures of central tendency and dispersion. Qualitative variables were expressed as absolute and relative frequencies, using tables to present the results. For inferential statistics, the Wilcoxon rank test was used to compare changes in corneal ulcer size, considering that the size was categorized on an ordinal scale. McNemar test was used to compare treatment response; both tests were used to evaluate initial and each follow-up time-point after treatment, always comparing with the previous immediate visit. Finally, Spearman´s correlation coefficient was used to correlate treatment stages with the percentage of healed eyes. For all tests, an α level of significance was assumed if the p-value was <0.05.

Results

We analyzed 42 eyes corresponding to 41 patients; the average age of the patients was 48.24 years; 52.38% were male and 47.62% female. All patients were Mexican Mestizos (a mixture of indigenous and European ancestry). Of the eyes analyzed, 26 were OD and 16 were OS. Corneal scraping results showed Gram-positive organisms as the

more frequent isolated strains followed by Gram-negative and fungal. There were also nine negative culture results. *Staphylococcus epidermidis* was the most frequent isolated strain (14 eyes, 33.33%) followed by *Klebsiella* (4 eyes, 9.52%), *Fusarium dimerum* (3 eyes, 7.14%), *Serratia marcescens* (2 eyes, 4.76%), *Enterobacter cloacae* (2 eyes, 4.76%) and *Bacillus* (2 eyes, 4.76%). The initial size (Day 0) of the corneal ulcer was of <3 mm in 21.43%, 3 to 6 mm in 47.62% and >6 mm in 30.95% (Table 1).

Corneal ulcer size was evaluated at different time-points after treatment, always comparing with the immediately preceding time-point. The results showed significant differences in the size of the corneal ulcer on the first postoperative day (Day 1) compared to the initial size (p-value = 0.008), where 16.67% of the eyes showed an increased size of the ulcer, while 83.33% maintained the initial size. At this point, four eyes needed surgery due to imminent perforation, so two underwent tectonic corneal

Table 1 Clinical Characteristics of Patients with Infectious Keratitis

Characteristics	Values
Age (average (SD)) in years	48.24 (13.34)
Sex (n (%))	
Female	20 (47.62%)
Male	21 (52.38%)
Evaluated eye (n (%))	
Right	26 (61.90%)
Left	16 (38.10%)
Culture (n (%))	
Negative	9 (21.43%)
Gram-negative	10 (23.81%)
Gram-positive	20 (47.62%)
Fungal	3 (7.14%)
Microorganism (n (%))*	
Staphylococcus epidermidis	14 (33.33%)
Klebsiella	4 (9.52%)
Fusarium dimerum	3 (7.14%)
Serratia marcescens	2 (4.76%)
Enterobacter cloacae	2 (4.76%)
Bacillus	2 (4.76%)
Initial corneal ulcer size (Day 0, n (%))	
<3 mm	9 (21.43%)
3 to 6 mm	20 (47.62%)
<6 mm	13 (30.95%)

Note: *There were six instances of different individual isolations and 9 negative results.
Abbreviation: SD, standard deviation.

transplant, one underwent conjunctivoplasty (conjunctival flap) and one was eviscerated. It is worth mentioning that these four cases were referred to our center and had been previously treated with steroids, which may explain the negative outcomes. Three of these eyes (75%) showed a mycotic isolate by culture and the other was negative. These eyes were maintained as failures (corneal ulcer or increased size of the ulcer) for the rest of analyses. For the remaining eyes, between Day 1 and Week 1, there were also significant size differences with a p-value = 0.000. In this case, 76.19% of the eyes showed a decrease in the size of the ulcer and 14.28% maintained the size. When comparing Week 1 with Month 1, we observed significant differences in the size of the ulcer (p-value = 0.000), where 85.71% of the eyes showed a decrease in the size of the ulcer or healing. At Month 3 compared to Month 1, significant differences (p-value = 0.020) were detected, with 14.28% showing a decrease of the ulcer size or healing, and the remaining 84.21% unchanged at Month 3, corresponded to patients that at the Month 1 time-point had already healed (Table 2).

Treatment response (healed or presence of corneal ulcer) was compared between the different time-points after treatment, always making comparisons with the previous time-point. The results showed that between Day 1 and Week 1 after treatment, no significant differences were observed, with treatment response in a few percentage of the cases. Between Week 1 and Month 1, significant differences were observed with a high percentage of the eyes healing and a few percentage with the corneal ulcer remaining. Finally, between Month 1 and Month 3, significant differences were

also observed, achieving healing of all treated eyes except the four cases that needed emergency surgery (Table 3).

When correlating each time-point with the percentage of healed eyes, a direct linear relationship was observed (p-value = 0.005, Spearman´s correlation coefficient 0.975), indicating that treatment effect increased with time. Regarding this result, it is important to highlight that the percentage of healing eyes increases dramatically between one week and one month of treatment (from 4.77% to 76.20%).

Discussion

Some authors have reported the use of CXL as a treatment for advanced corneal melting in treatment-resistant cases of infectious keratitis[23–25] alone or in combination with surgery.[26] A small cohort of five patients,[23] unresponsive to standard topical and systemic antimicrobial therapy, prompted the use of CXL as a rescue measure, observing that melting stopped in four of five patients. This confirmed previous results from Schnitzler and colleagues[15] and proposed that CXL may be effective for the treatment of infectious corneal ulcers. Several case series, two randomized controlled trials,[22,27] a systematic review and meta-analysis[28] and one meta-analysis[29] showed similar findings.

The PACK-CXL protocol, widely described in the literature, uses conventional 365 nm UVA light at 3 mW/cm^2 applied for 15, 30 or 45 minutes followed by 9 mW/cm^2 accelerated UVA light for 10 minutes. In our institution, we follow the Dresden protocol modified by Wolensack[20] which is the standard in most institutions, with 30 minutes of UVA light exposition.

Table 2 Comparison of Ulcer Size by Time-Points

Change in the Size of the Corneal Ulcer	Day 1/Initial (Day 0)		Week 1 vs Day 1		Month 1 vs Week 1		Month 3 vs Month 1	
	n (%)	p-value	n (%)	p-value	n (%)	p-value	n (%)	p-value
Reduced	0 (0.00%)	0.008*	32 (76.19%)	0.000*	36 (85.71%)	0.000*	6 (14.28%)	0.020*
Without changes	35 (83.33%)		6 (14.28%)		2 (4.76%)		32 (76.19%)	
Increased	7 (16.67%)		4 (9.53%)		4 (9.53%)		4 (9.53%)	

Notes: *Significant changes in ulcer size, significance with a p-value <0.05; based on Wilcoxon signed-rank test.

Table 3 Comparison of Treatment Response by Time-Points

Treatment Response	Day 1/Initial (Day 0)		Week 1 vs Day 1		Month 1 vs Week 1		Month 3 vs Month 1	
	n (%)	p-value	n (%)	p-value	n (%)	p-value	n (%)	p-value
Healed	0 (0.00%)	-	2 (4.77%)	0.500	32 (76.20%)	0.000*	38 (90.50%)	0.031*
Corneal ulcer	42 (100.00%)		40 (95.23%)		10 (23.80%)		4 (9.50%)	

Notes: *Significant changes in treatment response, significance with a p-value <0.05; based on McNemar test.

Causative microorganisms of infectious keratitis found in our study were similar to those reported in the literature, with bacteria being prominent. We did not identify Acanthamoeba trophozoites or cysts on Gram, Giemsa–Wright or hematoxylin and eosin stains. Although other authors have described viral etiologies (eg, herpesvirus), the clinical evolution of our patients suggested a bacterial or fungal etiology, and additionally we do not have the laboratory resources to perform specific tests like immunofluorescence assays (IFA) or polymerase chain reactions (PCR), so we cannot exclude that negative cultures may have been of viral origin. We found a low incidence of fungal infections, consistent with a 30-year survey exploring causative organisms in infectious corneal ulcers that reported that only 1% of the cases were fungal.[30]

Papaioannou et al[29] reported a healing rate of 87.2% (159 of 175 eyes) in a systematic review and meta-analysis of 2 randomized controlled clinical trials, 13 case series, and 10 case reports, and we found a similar rate in our study (90.50%). They also showed a low success rate in fungal infections consistent with our findings in which from the four eyes that needed surgery due to imminent perforation, three (75%) had a fungal isolate by culture. This finding is consistent with other studies[31–34] where bacterial keratitis cases show improvement after PACK-CXL treatment, whereas fungal keratitis show less or no improvement at all,[35] like in our study, where the 3 cases with a fungal isolate had a torpid evolution and needed urgent surgery to avoid organ loss, which unfortunately could not be prevented in one case. Nevertheless, there are studies that report a high success rate for fungal ulcers.[36] The late application of PACK-CXL in our cases may explain the huge reduction in effectiveness and the low healing rate leading to treatment failure. Also, Zhang´s[37] observations regarding lack of effect in deep fungal keratitis should be taken into consideration; perhaps our cases corresponded to deep infections and this would explain the bad outcomes. Finally, the efficacy of PACK-CXL correlates with the causative agent, a factor that should be considered when deciding to use this treatment in an individualized manner.

It is important to highlight that between Day 1 and Week 1 after treatment, the ulcer size does not decrease or even increases slightly compared to baseline and then decreases after Week 1. This should not be a reason for considering that the treatment is not successful, since this response is due to the mechanical de-epithelialization of the ulcer margins for CXL application and not a sign of deterioration. The slow healing rate in the first week (consistent with re-epithelialization) contrasts with the strong and sustained response to treatment showed by the decreased ulcer size and the healing response after this time-point, reaching a maximum at Month 3.

An important contribution of this work is the results achieved with the early use of fluorometholone in all patients. It is recommended to start fluorometholone only after re-epithelialization is complete; nevertheless, we have observed that post-CXL inflammation may induce corneal lysis and hence complications, but in our experience, the early use of fluorometholone helps to avoid negative lysis-related outcomes in bacterial cases.

This study was conducted in patients with resistant forms of corneal infections in whom antimicrobial therapy was administered for a long time without response; usually this cases progress and cause serious damage to the cornea and as a consequence, devastating visual or organ loss. The healing rates obtained with PACK-CXL are unprecedented positive results for this patient population, hence the importance of sharing our results to prompt a wider use of this procedure in cases unresponsive to standard therapy.

Also, as microbial resistance to antibiotics increases, new lines of treatment are needed. PACK-CXL may be a promising new alternative and its use is recommended due to the potential benefit obtained by controlling infection regardless of drug resistance, stopping the melting process, avoiding emergency keratoplasty and decreasing the possibility of performing lamellar grafts for visual rehabilitation.

Disclosure

Rosario Gulias-Cañizo reports she is an employee of Alcon Laboratories, outside the submitted work. Everardo Hernández-Quintela reports consultant fees from Allergan, Sifi and Thea Laboratories, outside the submitted work. The authors declare that there is no other conflicts of interest.

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