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Photodynamic Therapy for Infectious Keratitis

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Abstract

Infectious keratitis is a sight-threatening microbial infection. The prevalence of antimicrobial resistance in cases of infectious keratitis has increased the demand for fortified compounded antimicrobial drops. Even with proper medical management, severe cases of infectious keratitis can further evolve into corneal perforation, requiring surgical intervention in the form of keratoplasty to control the infectious process. Due to the invasive nature of the procedure and the shortage of available donor tissue around the world, alternative treatments are needed for the management of progressive infectious keratitis. In ophthalmology, photodynamic therapy (PDT) has been used for numerous applications. PDT with Rose Bengal as a photosensitizer combined with green light optical irradiation (RB-PDAT) is a novel treatment with dual purpose: to arrest the infection from progressing and strengthen the collagen of the cornea. RB-PDAT may be considered as an adjunct therapy in severe cases of infectious keratitis to minimize the need for a therapeutic keratoplasty.

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Conflict of Interest

Jean Marie Parel and Guillermo Amescua are authors of a University of Miami invention disclosure on the instrument described herein.

Diego Altamirano, Jaime Martinez, and Katherine D Leviste each declare no potential conflicts of interest.

Human and Animal Rights and Informed Consent

All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

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Keywords

rose bengal photodynamic antimicrobial therapy; cornea; ocular surface; infectious keratitis; epithelial defect

Introduction

Prevalence and Current Standard of Care

The incidence of infectious keratitis (IK) is on the rise. In a rural region in North America, Nevitt et al. demonstrated a notable increase from 2.5 cases per 100,000 people in the 1950s to 11 cases per 100,000 people in 1980.(1) In California, Jeng et al. observed an even greater incidence of IK at a rate of 27.6 cases per 100,000 people annually. IK occurred at a higher prevalence among contact lens wearers, where *Staphylococcus aureus* was identified as the most frequently recovered organism.(2) The predominant type of microorganism may vary based on the geographic area of clinical practice. In India, the most common etiology of IK is fungal keratitis.(3)

Recently, the increased prevalence of antimicrobial resistance requires the use of fortified antimicrobial drops sourced from compounding pharmacy services. However, this method of treatment subjects the ocular surface to significant toxicity.(4) Even with proper medical therapy, some cases of IK will evolve into corneal perforation and require a therapeutic penetrating keratoplasty (TPK). Corneal transplantation on an infected or inflamed ocular surface is associated with many complications and has a guarded prognosis. (5) Therefore, alternative treatments are needed for the management of advanced and severe cases of infectious keratitis.

Collagen Cross-Linking

A recent addition to the armamentarium against IK is the use corneal collagen cross-linking (CXL), a procedure based on combining a photosensitizer chemical (riboflavin) and an ultraviolet light source with a specific wavelength to excite the chemical. Collagen cross-linking results in the formation of stronger chemical bonds between adjacent fibrils, with the added benefit of the cornea becoming more resistant to enzymatic digestion by collagenases. (6) In the 1960s, Tsugita et al. reported that the application of riboflavin and ultraviolet A (UV-A) light led to inactivation of the tobacco mosaic virus.(7) Since then, this knowledge has been used to eradicate microorganisms in many applications, including the sterilization of water, surfaces, and blood products. The mechanism of action is described as the release of reactive oxygen species upon riboflavin activation by UVA light, which compromises the pathogen DNA and RNA.

CXL with riboflavin and ultraviolet (UV) light was introduced as a first-line treatment to prevent the progression of keratoconus and/or corneal ectasias as early as 2003.(8,9) The first publication describing the use of CXL in the context of human corneal infections was by Iseli et al in 2008. Their group reported CXL treatment in five patients with IK that did not respond to standard medical therapy. Post-CXL, they documented that the progression of corneal melting was halted and infiltrate size was effectively reduced in four out of five

patients who received the therapy.(10) Since then, several case reports, case series, and prospective non-randomized studies on the use of CXL for corneal infections and the term Photo-Activated Chromophore for Keratitis Corneal Cross-Linking (PACK-CXL) have been used in many recent publications. (11-16)

The first randomized controlled clinical studies comparing PACK-CXL as an adjunct procedure to conventional medical protocol by Said et al and Kasetsuwan et al, did not show a significant difference in corneal healing and final visual outcome compared to standard antibiotic eye drop therapy in patients with severe IK.(17, 18) Nevertheless, PACK-CXL reduced late-onset complications of IK, such as corneal perforation. Bamdad et al demonstrated a faster recovery of epithelial defects and infiltrates when PACK-CXL was added to the treatment regimen.(19) Summaries of clinical trials are shown in Table 1.

CXL for the Treatment of Fungal Ulcers

A randomized clinical trial reported by Prajna et al showed no evidence in favor of using adjunct PACK-CXL in the primary treatment of filamentous fungal ulcers (Table 1). The use of PACK-CXL in fungal keratitis remains a topic of debate. Li et al reported a successful use of PACK-CXL for eight cases of fungal keratitis.(20) However, Uddaraju et al in a prospective randomized clinical trial, found that PACK-CXL was not helpful for cases of deep stromal fungal keratitis, with five eyes in the PACK-CXL group and three eyes in the non-CXL group having experienced treatment failure by 6 weeks.(21) Interestingly, modifying parameters of the Dresden protocol such as increasing the UV fluence may lead to better outcomes. Richoz et al reported an accelerated protocol using 18mW/cm² for 5 minutes and 36 mW/cm² for 2.5 minutes maintaining high bacterial killing for *Staphylococcus aureus* and *Pseudomonas aeruginosa* in-vitro.(22) Recently Knyazer et al described the use of accelerated PACK-CXL (30mW/cm² for 3 minutes, total dose of 5.4 J/cm²) as additional treatment for resistant IK, with no associated complications.(22, 23) Also, another interesting finding by Hafezi et al, is that the application of fluorescein staining prior to the procedure competes with riboflavin for the absorption of UV-A during PACK-CXL, which reduces the antimicrobial effect of the procedure.(24). Our group reported a case series of three patients with infectious keratitis unresponsive to standard medical treatment (*Pseudomonas aeruginosa*, *Mycobacterium chelonae*, and *Curvularia spp*) treated with PACK-CXL as an adjunct treatment with good long term follow-up and results.(25)

Management of Infectious Keratitis at Bascom Palmer

In our institution, an estimated number of 900 cases of presumed infectious keratitis are treated annually. With the support of our microbiology and pathology laboratory, about 55% of our keratitis cases are confirmed culture-positive for microbiology. Our eye hospital is the largest university eye hospital in the region, with a dedicated ophthalmic microbiology laboratory for clinical and research work. We are able to identify many cases of infectious keratitis given these resources and our geographic area of practice in a tropical climate with easy access to the ocean, swimming pools, and agricultural activities with secondary organic trauma, bring a significant number of patients with presumed infectious keratitis to our emergency room and our clinics. Even with access to standard medical care and the best diagnostic tools available, our institution performs around 70 therapeutic corneal transplants

a year. TPK may be curative in many patients, however, the long-term visual prognosis is guarded in most of these eyes. Severe complications such as corneal neovascularization, corneal graft rejection, secondary glaucoma, and recurrence of infection can occur. (26) In an effort to improve clinical outcomes in patients with progressive infectious keratitis, our group decided to incorporate the use of PACK-CXL in our armamentarium against IK. Our preliminary work in the laboratory showed that the use of PACK-CXL was not effective in-vitro for the 3 most common fungal organism that we see associate with IK. As a result, we decided to investigate the possibility of using a different photosensitizing agent activated by a specific light source.

Rose Bengal Background

Rose Bengal (RB) is a dye routinely used in ophthalmology clinics to stain for corneal or conjunctival epithelial defects and degeneration.²⁷ The main constituents of RB are iodine derivatives of di- and tetrachloro fluorescein. It is commonly used in solution to stain damaged corneal and conjunctival cells. RB neither penetrates nor dyes mucins, and therefore presents better adherence to epithelial cells whose external surface is not covered by an intact coat of mucins.(28) In consideration of the utility of PACK-CXL and as an alternative therapeutic tool, research by our group have focused on the *in vitro* and *in vivo* antimicrobial efficacy of photodynamic antimicrobial therapy (PDAT). By using Rose Bengal as a photosensitizer activated by green light, the RB-PDAT method has proven to kill organisms in the cornea and strengthen the collagen fibers.

Mechanism of Photodynamic Therapy

Photodynamic therapy involves the activation of a photosensitizing agent by light ranging from ultraviolet-A (UV-A) to about 750nm. The photosensitizer reaches an excited state that undergoes a reaction with ambient oxygen to create singlet oxygen (SO) and reactive oxygen species (ROS), singlet oxygen being the most efficacious element produced by rose bengal while riboflavin only produces ROS. These SO and ROS then react with intracellular components and produces cell inactivation and death.²⁹ In the field of ophthalmology, Photodynamic therapy has been used for numerous applications including the treatment of corneal neovascularization, choroidal neovascularization in age-related macular degeneration and experimentally, for tumor treatment and *Acanthamoeba* keratitis.^{30,31} A custom-built LED source was designed and fabricated by the University of Miami Ophthalmic Biophysics laboratory. The irradiation head was assembled using an array of twenty- four light emitting diodes (LEDs). A single green LED (L1-0-G5TH45-1, LED supply, Randolph, VT, USA) has a 518 nm peak irradiance ($I_{40\%}$: 500-541 nm) and produces 2.2mW/cm² over a surface of 28.3 cm². As recently as 2020, Peterson et al established development of a-singlet oxygen dosimeter to optimize RB-PDAT (32). Using our RB-PDT system we compared the *in vitro* efficacy of rose bengal versus riboflavin as photosensitizing agents for photodynamic therapy (PDT) on different microorganisms. Our team demonstrated *in vitro* antimicrobial effects on a variety of microbes, including multiple species of bacteria and fungi.(27,33,34) Our laboratory reported an *in vitro* study using MRSA isolates from patients with keratitis, which resulted in a complete growth inhibition using Rose Bengal PDAT compared to minimal inhibition when riboflavin PDAT was used. Others have demonstrated a possible role for RB-PDAT in treating *Acanthamoeba*

infections. Atalay et al demonstrated *in vivo* and *in vitro* that RB-PDAT plays a fundamental role and is effective in decreasing the parasitic load and clinical severity of *Acanthamoeba castellanii* keratitis(35,36)

Safety and Toxicity Studies

Many studies have validated the safety and efficacy of RB-PDAT *in vivo*. (37). Moreover, our team demonstrated that performing RB-PDAT does not cause toxicity to the corneal endothelium and did not affect the viability of the limbal stem cell *in vivo* (38). Given the potential for RB-PDAT for the use of treatment for progressive infectious keratitis, we were able to establish the safety of this procedure. Resistance to treatment has not been observed with RB-PDAT.(39) The promising *in vitro* and *in vivo* results from our institution were used for clinical guidance toward the use for RB-PDAT to treat patients with progressive IK that have been unresponsive to standard medical therapy.

Bascom Palmer RB-PDAT Protocol

As it is a novel application, our group established the following standard protocol: A lid speculum is placed under topical anesthesia (sterile lidocaine 1% and proparacaine 0.5%), followed by an injection of 2 mL of 2% lidocaine with epinephrine 1:10,000 into the bulbar subconjunctival space at 12 o'clock and 6 o'clock with a 30 gauge needle. If the ulcer has a small epithelial defect, the area surrounding it is debrided to obtain an 8-mm de-epithelialized area to increase Rose Bengal absorption. An 8 mm corneal sponge (Beaver Visitec International, Waltham, Massachusetts, USA) soaked in RB is placed over the cornea. Followed by 3 to 5 drops every 3 minutes over the following 30 minutes to maintain corneal saturation. The sponge is then removed and a custom-made disposable shield measuring 10 mm x 15 mm with a central 9-mm opening was placed to protect the corneoscleral limbus from the green light irradiation. The anterior corneal surface is irradiated with a custom-made 6 mW/cm² green LED light source for 15 minutes for a total energy density exposure of 5.4 J/cm². The anterior corneal surface is irrigated with balanced saline solution (Alcon Laboratories, Fort Worth, Texas, USA) every 3 minutes throughout the light exposure to prevent corneal dehydration. Finally, a bandage contact lens (Airoptix AQUA; Alcon, Fort Worth, Texas, USA) is placed to protect the ocular surface.

The first successful reported case of RB-PDAT was in a patient with *Fusarium keratoplasticum* keratitis. In this case a therapeutic graft was avoided, and we were able to quiet the inflammation and later perform an optic graft with excellent clinical and visual outcomes. This case demonstrated proof-of-principle of the potential of PDAT in the management of resistant infectious keratitis. (40, 41). Our group published a retrospective pilot study using RB-PDAT as an adjunct treatment for severe IK, with a total of 18 patients. Seventeen had positive microbial cultures results, with cases divided between *Acanthamoeba* (n=10), *Fusarium spp.* (n=4), *Pseudomonas aeruginosa* (n=2), and *Curvularia spp.* (n=1). In addition, one patient's cultures showed the presence of multiple microorganisms. All patients had resolution of the infection within 38 days of the first RB-PDAT treatment. The current standard therapy for infectious keratitis unresponsive to treatment is a therapeutic penetrating keratoplasty, which requires surgical intervention and the placement of a donor graft. Kirkness et al. demonstrated that in 116 therapeutic grafts, 43 failed (a rate of 51%)

due to rejection or recurrence of infection within 5-years post-operatively.²⁶ In contrast, the prognosis of a corneal transplant in eyes with inactive infection was much-improved, with 90% of grafts surviving for 5-years. Our pilot study defined RB-PDAT treatment as successful if therapeutic penetrating keratoplasty was avoided. This was achieved in 72% of the patients treated with RB- PDAT.(42) Interestingly, in a second analysis, we have noted that patients with resistant and severe forms of IK that required therapeutic keratoplasty who had previous RB-PDAT treatment demonstrated successful therapeutic graft adherence, these grafts remaining clear for a long time without reinfections, rejection, or failure. (25) We believe that RB-PDAT not only has an antimicrobial effect, also may have a beneficial effect on corneal grafts by regressing blood and lymphatics vessels by induction of apoptosis in vascular endothelial cells. (41). Moreover, the benefit of using CXL in donor corneas to increase graft survival has been published. CXL with riboflavin may prevent the recurrence of infection in cases of infectious keratitis after TPK, increasing the survival rate of corneal grafts.(43-45). Titiyal et al published a prospective interventional study which evaluated the outcomes of therapeutic keratoplasty using riboflavin CXL-treated and non-treated donor corneas in fungal keratitis. Eighty percent of patients in the CXL-treated donor group showed clear corneal grafts at 6 months compared to 22% of patients in the non-treated group. Interestingly, none of the patients in the CXL-treated donor group had recurrence of infections compared to 22% in the non-treated CXL group.(43) This is preliminary data and an exciting area of research that may bring new tools to prolong longevity of high-risk corneal grafts. On the other hand, another benefit of using RB-PDAT is an increased resistance to collagenase digestion (46,47). If antimicrobial eradication is not achieved with RB-PDAT, its protective effect in preventing or delaying corneal melt, can then allow for the use of conventional medical antimicrobial therapy to eradicate the infection.

Conclusion

Although our laboratory and clinical results of using RB-PDT as a potential tool in the management of IK are encouraging, the treatment still has limitations. We understand how deep RB can reach in normal stromal tissue, however the data on RB penetration of corneas with active infection and inflammation remains to be seen. Our clinical work has focused mostly on patients with advanced disease. The use of a light activated photosensitizer agent for Infectious Keratitis continues to be a challenge, but an area of opportunity for developing therapeutics. Novel potential treatments such as PACK-CXL and RB-PDAT have shown success but have yet to be evaluated in prospective clinical trials. New improvements in technology as well as better understanding of the mechanism of action will help improve our patient outcomes. Currently, RB-PDAT may thus be considered as an adjunct therapy in severe cases of infectious keratitis in an attempt to avoid TPK and optimize future visual potential. Currently, additional *in vitro*, *in vivo*, and human clinical trials are underway at different institutions worldwide. Our group hopes that in the future Rose Bengal photodynamic therapy can be considered as a primary treatment for infections and to help mitigate the prevention of blindness from corneal infections.

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Figure 1. A case of a 68-year-old female patient, with a history of Ocular Cicatricial Pemphigoid and a confirmed diagnosis of bilateral *Curvularia* keratitis. (A, B) Clinical appearance at presentation with bilateral deep corneal infiltrates, paracentral melting, thinning and hypopyon. (C, D) RB-PDAT was performed due to lack of response to standard medical treatment. (E, F) Appearance of the ocular response to RB-PDAT procedure where corneal melt and thinning have stopped, a decreased in conjunctival inflammation with less infiltrate, and grossly significant improvement. The corneal epithelium has healed and steroid drops were started. (G, H) Patient with a bilateral clinical resolution of the infection.

Table 1.

Summary of all prospective or randomized clinical trials for cross-linking in IK over the last 10 years

Author year	Methods	Participants Setting	Groups Intervention	Outcomes	Time Fu
Price 2012	Pilot study Prospective Non-randomized	40 patients USA	Organisms Bacterial 24 eyes (<i>Staph spp.</i> , <i>Moraxella</i> , <i>Pseudomonas</i> , <i>Serratia</i> , <i>Enterococcus</i>) Fungal 7 eyes (<i>Penicillium</i> , <i>Fusarium</i> , <i>Aspergillus</i>), Acanthamoeba 2 eyes, Herpes 1 eye. All had Riboflavin CXL treatment (4 patients 30 minutes) 36 patients randomized to different UVA light treatment time from 15 to 45 minutes) + standard medical care treatment Infiltrate diameter range: 1 to 12 mm ²	The success rate was higher for bacterial infections than fungal infections The two cases of Acanthamoeba did not appear to be significantly influenced	
Makdoui 2012	Pilot study Non-randomized	16 patients Sweden	Organisms Bacterial (<i>Staph spp.</i> , <i>Corynebacterium</i> , <i>Micrococcus</i> , <i>Propionibacterium</i>) All received Riboflavin CXL Dresden protocol <u>alone</u> Only 2 patients received additional antibiotic. Corneal ulcer size 0.1 to 2.5 mm	All eyes responded to the photochemical treatment with improvement in symptoms and signs of reduced inflammation. Epithelial healing was achieved in all cases. Antibiotic administration was necessary in two cases	
Said 2014	Randomized Clinical trial	21 eyes Egypt	Organisms Bacterial, Fungi or Acanthamoeba Group 1 PACK-CXL Dresden protocol + Standard medical treatment Group 2 Standard medical treatment Infiltrate diameter range in both groups : 2 to 10 mm	Three patients in the control group had corneal perforation, whereas patients treated with PACK-CXL did not experience this complication.	-
Uddaraju 2015	Randomized controlled trial	13 eyes India	Organisms Aspergillus, Fusarium, Unidentified fungal corneal ulcers. Group 1 Standard medical treatment Group 2 Standard medical treatment + Riboflavin CXL Dresden protocol Infiltrate diameter range in both groups : 5 to 7.48 mm	CXL used as adjuvant therapy for recalcitrant deep stromal fungal keratitis did not improve outcomes. The trial was stopped before full enrollment because of a marked difference in the rate of perforation between the 2 groups.	6 weeks
Bamdad 2015	Randomized clinical trial	22 patients Iran	Organisms Bacterial not species not specified Group 1 Standard medical treatment Group 2 Standard medical treatment + Riboflavin CXL Dresden protocol Infiltrate diameter 19.25 mm ²	Beneficial effect of CXL in patients with moderate bacterial keratitis. In addition to accelerating epithelialization, this method shortens the course of treatment and may minimize or remove the need for surgery or other serious sequelae, such as corneal perforation.	-
Venkatesh Prajna 2020	Randomized clinical trial	403 patients India	Organisms: Filaments Group 1: natamycin alone, Group 2: natamycin plus CXL, Group 3: amphotericin alone, and Group 4: amphotericin plus CXL. Riboflavin CXL Dresden protocol Moderate size corneal ulcers	Unable to find a difference in 24- hour culture positivity between those randomized to amphotericin and those randomized to natamycin when evaluated as a group regardless of whether or not they received CXL (coefficient 1.10; 95% CI, 0.47e2.54; P 1/4 0.84). No difference in infiltrate or scar size, percentage of epithelialized or adverse events when comparing CXL with no CXL or the 2 topical medications.	3 months

PACK-CXL: Photo Activated Chromophore for Keratitis-Corneal Cross-linking; *Spp.* species; CXL: cross-linking; BCVA: Best-corrected visual acuity