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Rose Bengal Photodynamic Antimicrobial Therapy: A Novel Treatment for Resistant *Fusarium* Keratitis

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Abstract

Background—Fungal keratitis is a commonly occurring cause of corneal blindness which does not always respond to standard antifungal treatment. Rose bengal photodynamic antimicrobial therapy represents an alternative treatment that has worked *in vitro* and that was successfully implemented in the clinic to treat an aggressive infectious keratitis case.

Purpose—To evaluate the efficacy of rose bengal photodynamic antimicrobial therapy (PDAT) for the management of a patient with multidrug-resistant *Fusarium keratoplasticum* keratitis unresponsive to standard clinical treatment.

Results—*In vitro* results demonstrated the efficacy of rose bengal PDAT against a multidrug-resistant *Fusarium keratoplasticum* species. There was complete fungal inhibition in our irradiation zone on the agar plates. In the clinical case, the patient was successfully treated with two sessions of rose bengal PDAT, and at the eight month follow-up, there was no recurrence of infection nor adverse effects to report.

Conclusions and Relevance—Rose bengal PDAT is a novel treatment that may be considered in cases of aggressive infectious keratitis. Further studies are needed to understand the mechanisms of PDAT *in-vivo*.

PURPOSE

To evaluate the *in-vitro* and *in-vivo* efficacy of rose bengal photodynamic antimicrobial therapy (PDAT) for the management of multidrug-resistant *Fusarium* keratitis.

INTRODUCTION

Infectious keratitis secondary to fungal species is a common cause of corneal blindness. There are cases of fungal keratitis that can be successfully treated with standard antifungal agents. Not uncommonly, standard therapy is ineffective and a significant number of patients require a therapeutic graft. Surgical therapy may be curative in many patients; however, chances of graft failure are high and severe complications such as corneal neovascularization, corneal graft rejection, secondary glaucoma, and recurrence of the infection can occur.¹

One alternative treatment for infectious keratitis is collagen cross-linking (CXL), a procedure traditionally used to halt progressive corneal ectasia using riboflavin and UV-A light. One specific category of CXL is photoactivated chromophore cross-linking (PACK-CXL) yet *in-vitro* and *in-vivo* results have demonstrated limited efficacy against fungal species.^{2, 3}

Given the limitations of PACK-CXL to treat fungal keratitis, we investigated rose bengal as an alternative photosensitizer. Rose bengal is a commonly available ocular surface stain activated by green light. *In-vitro* investigations of rose bengal PDAT have demonstrated inhibition of numerous fungal species.⁴ The purpose of this report is to present both *in-vitro* and *in-vivo* efficacy of rose bengal PDAT in multidrug-resistant *Fusarium* keratitis.

MATERIALS AND METHODS

We performed *in-vitro* susceptibility testing of a patient's fungal isolate to standard antifungals, riboflavin CXL, and rose bengal PDAT. We also report the treatment outcomes (*in-vivo*) of the patient with *Fusarium* keratitis who received two sessions of rose bengal PDAT at Bascom Palmer Eye Institute (BPEI) after obtaining informed consent.

In-vitro

Corneal scrapings were cultured as previously described by Ansari et al. The corneal tissue was scraped with a surgical blade, smeared onto agar plates (chocolate, blood, Sabouraud Dextrose, and non-nutrient), and prepared for Gram and Giemsa stains.⁵ Only moderate growth of *Fusarium* species was recovered within 24-hours on multiple media. Molecular sequencing and antifungal susceptibility testing were performed at the Fungus Testing Laboratory, San Antonio, Texas. To evaluate susceptibility to rose bengal PDAT and CXL, experiments were performed as previously described by Arboleda et al and Halili F Jr et al.^{4, 6} In short, the isolate was prepared in suspension in tryptic soy broth and adjusted to concentration of 5.0×10^5 colony forming units/mL using a Neubauer chamber. Then, 0.1mL of isolate suspension was mixed with 0.9mL of either 0.1% photosensitizer solutions. Next, 1mL was aliquoted onto Sabouraud-Dextrose plates in triplicate, and plates were exposed to the irradiation source according to experimental group. Irradiation was performed using either a UV-A ($\lambda=375\text{nm}$) or a green ($\lambda=518\text{nm}$) light source developed at BPEI. Test groups were as follows: (1) control, (2) 0.1% riboflavin: (a) dark, (b) 30-minute UV-A irradiation, (c) 15-minute UV-A irradiation, (d) 10-minute UV-A irradiation, (3) 0.1% rose bengal: (a) dark, (b) 30-minute green irradiation, (c) 15-minute green irradiation, (d) 10-

minute green irradiation. After irradiation, plates were stored in an incubator for 72 hours and then photographed.

In-vivo

To perform the rose bengal PDAT procedure on the patient, first, a speculum was placed and topical anesthesia (4% lidocaine) was applied to the cornea. The corneal epithelium was debrided in the area of the ulcer. A custom-made shield and well was placed on the cornea, and 0.1% concentration of rose bengal in balanced saline solution was applied in a dropwise manner for 30 minutes. After application of rose bengal, green light was applied for 15-minutes using a custom-made green light source. After rose bengal PDAT, the eye was flushed with balanced saline solution.

RESULTS

In-vitro

Molecular sequencing identified the isolate as *Fusarium keratoplasticum*. Antifungal susceptibility profiles revealed intermediate resistance to natamycin (Minimum Inhibitory Concentration (MIC) 8 µg/ml), and high-level resistance to amphotericin-B (MIC 2 µg/ml), voriconazole (MIC 16 µg/ml) and fluconazole (MIC 64 µg/ml). Riboflavin CXL demonstrated no inhibition of fungal isolate growth, whereas rose bengal PDAT inhibited fungal isolate growth within the irradiation zone (Figure 1).

Clinical case

A 56-year-old female rigid gas-permeable contact lens wearer, with a history of keratoconus and secondary penetrating keratoplasty 15 years prior was referred to BPEI for treatment of possible graft infection. The patient reported good contact lens hygiene, and denied sleeping or swimming with contact lenses. At presentation, the patient had a 3x3.5mm corneal defect located in the paracentral-temporal area with associated multiple multifocal infiltrates (<1mm). Best corrected visual acuity (BCVA) was 20/400, and her treatment consisted of hourly topical corticosteroids as well as polymyxin B sulfate/trimethoprim and fluoroquinolone drops each every four hours. Corneal scraping revealed fungal hyphal elements and *Fusarium* was identified. Treatment with hourly natamycin 5% drops was initiated, and topical corticosteroid was tapered off. Despite compliance for four weeks, the corneal infiltrate was no smaller.

At this point, an intrastromal amphotericin-B injection (2.25µg/0.05mL) was performed and oral fluconazole 400mg daily was initiated. However, despite the intrastromal injection and three weeks of oral fluconazole and continued good compliance with hourly natamycin drops, clinical appearance continued to worsen. The patient had progressive corneal thinning (50% depth), +3 graft edema, and the infiltrate was now 5x6mm and threatened the graft host junction of the temporal area of the cornea, all associated with constant moderate-to-severe pain and severe photophobia.

Given continued worsening and intermediate resistance to multiple antifungal medications, the option of therapeutic penetrating keratoplasty versus experimental rose bengal PDAT

were discussed with the patient, and she elected rose bengal PDAT. At Day 44, a rose bengal PDAT was performed for a total energy of 0.9 J/cm², as described above in materials and methods section (Figure 2). Within 36 hours, there was resolution of pain, and over the next two weeks, the corneal infiltrate shrank. Four days after treatment, the infiltrate was approximately 1mm smaller in all directions. At Day 57, the infiltrate was almost resolved; at this time, a second session of rose bengal PDAT (1.8 J/cm²) was performed, and all antifungals were discontinued.

On Day 86, the corneal epithelial defect and infiltrate resolved. Four weeks later (Day 108), the patient began topical corticosteroids. Four months after the first rose bengal PDAT procedure (Day 166), corneal scarring and neovascularization decreased, and there was no evidence of any other adverse effects. At latest follow up (Day 290), patient demonstrated a healthy and quiet ocular surface of the left eye and a clear cornea, which suggests that, in this case, the endothelial function of the graft was unaffected and there is no limbal stem cell deficiency. (Figure 3)

DISCUSSION

To our knowledge, this is the first patient with fungal keratitis treated with rose bengal PDAT. Initial results are promising; at six month follow-up, no recurrence of infection or adverse events had occurred and a therapeutic graft was avoided. *In-vitro* tests showed that the patient's isolate was completely inhibited by rose bengal PDAT. Previous studies showed similar results of antimicrobial efficacy of rose bengal PDAT against a variety of fungal species.^{4, 6} The literature reports that riboflavin CXL is less effective against fungal species;^{2, 3} this was also noted in the *in vitro* experiments performed in this study.

Current standard therapy for fungal keratitis unresponsive to antifungal agents is therapeutic keratoplasty. However, Kirkness et al. demonstrated that in 116 therapeutic grafts, 43 failed due to rejection or recurrence of infection with a 5-year survival of 51%.¹ This is in stark contrast to the prognosis of a cornea transplant in eyes with inactive infection which was comparable to grafts in non-infectious indications, with 5-year graft survival of 90%. Partial thickness keratoplasty in this patient would be difficult due to the degree of inflammation and secondary tissue necrosis, with a high chance of leaving infected tissue behind and risk of causing an iatrogenic perforation. In addition, this would have been this patient's second penetrating keratoplasty, which has reduced graft survival because of the eye having significant neovascularization and inflammation.⁷

Rose bengal PDAT has the potential to be a promising treatment for patients with fungal keratitis. However, some factors remain that need further investigation. Laboratory testing has demonstrated a different response to rose bengal PDAT depending on the fungal species. Hence, photosensitizer concentration, irradiation energy, and treatment duration need to be thoughtfully studied and implemented in order to maximize the benefits of rose bengal PDAT.

Encouraging *in-vitro* results reported by Fadlallah et al showed that rose bengal PDAT has the capability of arresting the cornea melting process, in addition to *our in-vitro* results

showing inhibition of growth of microorganisms.⁸ Another determining factor of success is penetration of rose bengal into the cornea. Rose bengal, like other photosensitizers, has limited diffusion into the cornea (~100–150µm).⁹ However, in cases of corneal ulceration, the infection itself might allow rose bengal to reach the depth of the microorganisms. A final point of discussion is that the patient may have benefited from administering rose bengal PDAT earlier in the disease course, which could have possibly reduced healing time and infection-related complications.

This case demonstrates proof-of-principle of the potential of PDAT in the management of resistant infectious keratitis. This patient received aggressive medical treatment, and despite good compliance, her infection continued to progress and worsen. It was not until the rose bengal PDAT treatment that the infection process was halted. This patient was the first to be offered this treatment and received a low energy treatment; however since treating this patient, we have found that 5.4J/cm² over 15-minute exposure has provided the most improvement with no complications. Further investigations need to be performed to understand the precise role of rose bengal PDAT in treating fungal infections.

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References

1. Kirkness CM, Ficker LA, Steele ADM, et al. The role of penetrating keratoplasty in the management of microbial keratitis. *Eye*. 1991; 5:425–431. [PubMed: 1743358]
2. Said DG, Elalfy MS, Gatziofias 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. [PubMed: 24576886]
3. Ehlers N, Fau HJ, Fau NK, et al. Riboflavin-UVA treatment in the management of edema and nonhealing ulcers of the cornea. *J Refract Surg*. 2009; 25:S803–806. [PubMed: 19772255]
4. Arboleda A, Miller D, Cabot F, et al. Assessment of Rose Bengal Versus Riboflavin Photodynamic Therapy for Inhibition of Fungal Keratitis Isolates. *Am J Ophthalmol*. 2014; 158:64–70. e62. [PubMed: 24792103]
5. Ansari Z, Miller D, Galor A. Current Thoughts in Fungal Keratitis: Diagnosis and Treatment. *Curr Fungal Infect Rep*. 2013; 7:209–218. [PubMed: 24040467]
6. Halili F, Arboleda A, Durkee H, et al. Rose Bengal– and Riboflavin-Mediated Photodynamic Therapy to Inhibit Methicillin-Resistant *Staphylococcus aureus* Keratitis Isolates. *Am J Ophthalmol*. 2016; 166:194–202. [PubMed: 27016125]
7. Thompson RW Jr, Price MO, Bowers PJ, et al. Long-term graft survival after penetrating keratoplasty. *Ophthalmology*. 2003; 110:1396–1402. [PubMed: 12867398]

8. Fadlallah A, Zhu H, Arafat S, et al. Corneal Resistance to Keratolysis After Collagen Crosslinking With Rose Bengal and Green Light. *Invest Ophthalmol Vis Sci*. 2016; 57:6610–6614. [PubMed: 27926752]
9. Cherfan D, Verter EE, Melki S, et al. Collagen cross-linking using rose bengal and green light to increase corneal stiffness. *Invest Ophthalmol Vis Sci*. 2013; 54:3426–3433. [PubMed: 23599326]

Figure 1.

In vitro results of rose bengal PDAT and riboflavin CXL against *Fusarium keratoplasticum*.

A standard inoculum (10^6 cfu/ml) was used to evaluate the in vitro efficacy of rose bengal PDAT and riboflavin CXL against *Fusarium keratoplasticum*. Zones of growth inhibition within the irradiated areas were documented for rose bengal PDAT at 72 hours. No zones of inhibition were observed for riboflavin CXL during the same time period.

Figure 2.

Clinical rose bengal PDAT system for green light irradiation using light emitting diodes with central wavelength of 518nm. System is computer-controlled with a centrally located camera for continuous view of the treated ocular surface during rose bengal PDAT.

Figure 3.

Clinical photograph sequence from presentation to 4-month follow-up; Day 1: active corneal ulceration at presentation; Day 44: before rose bengal PDAT; Day 45: 1 day after rose bengal PDAT; Day 57: before second rose bengal PDAT; Day 58: 1 day after second rose bengal PDAT; Day 86: resolved epithelial defect with presumed sterile infiltrate; Day 108: 2 days after start of corticosteroids; Day 118: follow-up showing quiet ocular surface, intact corneal epithelium, no stromal infiltrate and diffuse temporal donor cornea scarring; and Day 290: latest follow-up.