Rose Bengal Photodynamic Antimicrobial Therapy for Patients With Progressive Infectious Keratitis: A Pilot Clinical Study

ANDREA NARANJO, ALEJANDRO ARBOLEDA, JAIME D. MARTINEZ, HEATHER DURKEE, MARIELA C. AGUILAR, NIDHI RELHAN, NEDA NIKPOR, ANAT GALOR, SANDER R. DUBOY, ROGER LEBLANC, HARRY W. FLYNN JR, DARLENE MILLER, JEAN-MARIE PAREL, AND GUILLERMO AMESCUA

OBJECTIVE: To report clinical outcomes of rose bengal photodynamic antimicrobial therapy (RB-PDAT) as an adjunct treatment for severe, progressive infectious keratitis.

METHODS: Consecutive interventional case series.

RESULTS: The current study included 18 patients (7 male and 11 female) ranging from 17 to 83 years old. Acanthamoeba was the most frequent microbe (10/17; 59%), followed by Fusarium spp. (4/17; 24%), Pseudomonas aeruginosa (2/17; 12%), and Curvularia spp. (1/17; 6%); 1 patient had no confirmed microbiologic diagnosis. Main clinical risk factor for keratitis included contact lens wear (79%). The average area of epithelial defect prior to first RB-PDAT was 32 ± 27 mm² and average stromal depth hyperreflectivity area of epithelial defect prior to first RB-PDAT was 32 (10/17; 59%), followed by Fusarium spp. (4/17; 24%), Pseudomonas aeruginosa (2/17; 12%), and Curvularia spp. (1/17; 6%); 1 patient had no confirmed microbiologic diagnosis. Main clinical risk factor for keratitis included contact lens wear (79%). The average area of epithelial defect prior to first RB-PDAT was 32 ± 27 mm² and average stromal depth hyperreflectivity measured with anterior segment optical coherence tomography was 269 ± 75 μm. Successful RB-PDAT (avoidance of therapeutic keratoplasty) was achieved in 72% of the cases, with an average time to clinical resolution (decreased pain and inflammation with re-epithelialization and infiltrate resolution) of 46.9 ± 26.4 days after RB-PDAT. Time of follow-up after RB-PDAT was 13.3 ± 5.7 months.

CONCLUSION: RB-PDAT can be considered as an adjunct therapy for cases of severe, progressive infectious keratitis before performing a therapeutic keratoplasty. (Am J Ophthalmol 2019;208:387–396. © 2019 Elsevier Inc. All rights reserved.)

Infectious keratitis is an ophthalmic emergency owing to its potential rapid progression and devastating consequences if not managed promptly and effectively. A common sequel of infectious keratitis is corneal stromal scarring, which constitutes the fourth-leading cause of blindness globally. More severe complications can include iris synechiae with secondary ocular hypertension and optic nerve damage, corneal perforation, endophthalmitis, and hemorrhagic choroidal detachment with secondary permanent vision loss.

The standard of care for infectious keratitis consists of frequent application of topical antimicrobials and in some cases systemic and/or periocular administration of antimicrobial agents; however, there has been a recent increase in antibiotic-resistant organisms. Moreover, access to compounded medications is limited in rural areas of the United States and the rest of the world. The increased resistance to standard medical treatment and limited access to treatment has led to a rise in the clinical complications previously mentioned. In order to prevent corneal perforations, or further dissemination of the disease to the scleral tissue or inside the eye, a therapeutic penetrating keratoplasty (TPK) or therapeutic lamellar keratoplasty may become necessary. Unfortunately, the probability of graft failure is high after performing a corneal graft in the setting of active corneal inflammation. Owing to potentially visually threatening consequences and limited treatment strategies, alternative therapies are considered in severe, progressive corneal infections.

During the last decade, corneal collagen crosslinking (CXL) procedures have been proposed as a novel treatment strategy for the management of resistant microbes and cases with progressive presumed infectious keratitis. The initial treatment was known as photo-activated chromophore corneal collagen crosslinking (PACK-CXL), which consisted of riboflavin irradiated with ultraviolet A (UV-A) light. Later, rose bengal photodynamic antimicrobial
therapy (RB-PDAT) was shown in vitro to be more efficient as a treatment for fungal and methicillin-resistant Staphylococcus aureus (MRSA) keratitis.\textsuperscript{10,11} This therapy involved a photochemical process using rose bengal (RB), a frequently used diagnostic dye in ophthalmology,\textsuperscript{12} excited with green light (wavelength: 500-550 nm) to generate reactive oxygen species (ROS).\textsuperscript{13} Two types of ROS mechanisms have been described. Type I is associated with electron transfer reactions that form free radicals, superoxide anion, hydrogen peroxide, and hydroxyl peroxide. In type II, energy is transferred from the triplet state of the photosensitizer to the ground state of triplet molecular oxygen, which leads to the formation of toxic singlet oxygen. Singlet oxygen has higher reactivity to lipids, proteins, and nucleic acids and is considered to have the greatest antimicrobial effect of all the ROS.\textsuperscript{14} In RB-PDAT, RB is activated by the green light to stimulate the transfer of energy to nearby molecular triplet oxygen ($^3$O$_2$). This energy transfer results in the formation of reactive singlet oxygen ($^1$O$_2$), which interacts with surrounding organic compounds in cells and tissues to produce a variety of effects, including eradication of a wide array of bacteria, viruses, fungi, and protozoa.\textsuperscript{15}

Several publications have demonstrated the in vitro efficacy of RB-PDAT in inhibiting the growth of different organisms including MRSA, Fusarium solani, Aspergillus fumigatus, and Candida albicans.\textsuperscript{10,11,15,16} In vivo and ex vivo experiments have evaluated the safety profile of green light–activated RB and have shown no significant adverse effects on keratocytes and no evidence of harm to deeper tissues such as the iris and retina.\textsuperscript{17} However, there is a paucity of information on the clinical effects and efficiency of this therapy for the treatment of infectious keratitis. The current article reports the clinical outcomes of patients diagnosed with infectious or presumed infectious keratitis unresponsive to standard medical treatment that underwent RB-PDAT as a last resort before considering a therapeutic corneal transplant.

**METHODS**

**PATIENT SELECTION:** Infections were considered resistant to treatment if they had received appropriate standard medical therapy without any clinical improvement for at least 2 weeks. RB-PDAT was considered in those patients with evidence of progressive disease in spite of maximal medical treatment and 2 faculty members of the cornea service at Bascom Palmer Eye Institute agreed the next step in treatment was surgical intervention. Exclusion criteria were age less than 12, pregnancy, and inability to remain supine for 45 minutes. There were no exclusion criteria related to morphometric ulcer characteristics (eg, residual corneal thickness, depth and size of infiltrate).

**OPHTHALMIC EXAMINATION:** All of the patients underwent a thorough ophthalmic examination prior to RB-PDAT. Risk factors for infection were evaluated (eg, contact lens use, trauma, diabetes). The ophthalmic evaluation consisted of measurement of best-corrected visual acuity (BCVA), slit-lamp examination with and without fluorescein, slit-lamp photography, corneal pachymetry, and anterior segment optical coherence tomography (AS-OCT). During the slit-lamp examination, characteristics of infection such as location, size of the epithelial defect, and area of stromal infiltration were recorded. The slit-lamp examination also included scraping the cornea for microbiological staining, culture, and antibiotic susceptibility testing as per the Bascom Palmer Microbiology laboratory protocol.\textsuperscript{18} Infection depth was determined using the digital caliper of an optical coherence tomographer (iVue; Optovue, Fremont, California, USA) by selecting 2 different images of the cornea and averaging hyperreflectivity depth at 3 different points of the cornea on each of these 2 images.

**SOLUTION PREPARATION OF ROSE BENGAL:** Four or 8 strips of RB (GloStrips 1.3 mg; Amcon, St. Louis, Missouri, USA) were diluted for 1 minute in 5 mL of balanced salt solution (Alcon Laboratories, Fort Worth, Texas, USA), while shaking the container to achieve an RB solution concentration of 0.1% or 0.2%, respectively. Concentration of RB solution was selected by the clinician based on the severity, clinical response to prior treatments, and/or in vitro data regarding the susceptibility of the microorganisms to RB-PDAT.

**ROSE BENGAL PHOTODYNAMIC ANTIMICROBIAL THERAPY PROCEDURE:** A sterile lid speculum was 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 ulcers had a small epithelial defect, the area surrounding it was debrided to obtain an 8-mm de-epithelialized area to increase RB absorption. An 8 mm corneal sponge (Beaver Visitec International, Waltham, Massachusetts, USA) was placed over the cornea. Three drops of 0.1% or 0.2% of RB solution was applied to the sponge surface, followed by 1 drop every 3 minutes over the following 30 minutes to maintain saturation. The sponge was then removed and a
custom-made disposable shield measuring 10 mm × 15 mm with a central 9-mm opening was placed to protect the corneoscleral limbus from the green light irradiation. Afterwards, the anterior corneal surface was 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 was irrigated with 1 drop of 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) was placed to protect the ocular surface at the conclusion for pain management after the procedure and the ocular surface was examined at the slit lamp. Patients were evaluated after 1, 3, and 7 days of treatment and reevaluated after 2 weeks of treatment to determine if they would benefit from another treatment. If no significant improvement was seen, but physician and doctors agreed that there was some benefit, an additional treatment was given. Patients were afterwards followed biweekly, weekly, or monthly depending on their clinical progression. Patients continued their standard medical therapy, specific to the type of microorganism, after RB-PDAT until the infection clinically resolved.

• **STATISTICAL ANALYSIS:** The main outcome measure was frequency of RB-PDAT success, defined as avoidance of TPK. Secondary outcome measures included number of RB-PDAT treatments, time from first RB-PDAT treatment to clinical resolution (clinical resolution defined as re-epithelialization of the epithelial defect with decreased pain and inflammation and resolution of infiltrate), time from first RB-PDAT to TPK or optical penetrating keratoplasty (OKP), and BCVA 6 months and 1 year after first RB-PDAT. Visual acuity progression was reported by using a value of 1/400 Snellen (logMAR = 2.6) to represent vision of counting fingers and further extrapolated hand movement, light perception, and no light perception as 2.7, 2.8, and 2.9 logMAR, respectively. For statistical purposes, we summarized the descriptives based on the 18 individuals treated that had available follow-up. We included information from the most severely affected eye in the patient with bilateral disease. Logistic regression analysis was performed to evaluate which factors (patient demographics, ulcer characteristics, length of standard medical treatment before presentation, BCVA at first RB-PDAT session) affected success. A paired, 2-tailed t test was additionally performed to compare BCVA before and after treatment. P value of <.05 was considered statistically significant.

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**RESULTS**

• **STUDY POPULATION:** Between January 2016 and March 2018, 19 patients (20 eyes) underwent RB-PDAT for the indications previously outlined. Of those, follow-up was available for 18 patients (19 eyes). Of these 18 patients, 7 (39%) were male and 11 (61%) were female. One patient presented with bilateral keratitis and underwent RB-PDAT in both eyes and 17 individuals had unilateral keratitis and received treatment in 1 eye only. Demographics and clinical characteristics of each patient are reported in Table 1. Average age at the time of RB-PDAT was 45.4 years (range 17-83 years). A microbiologic diagnosis was confirmed through culture and/or biopsy in 17 patients; no microbiologic diagnosis was determined in 1 patient. Of the cases identified, *Acanthamoeba* spp. was the most frequent microbe identified (59%, 10/17), followed by *Fusarium* spp. (24%, 4/17), *Pseudomonas aeruginosa* (12%, 2/17), and *Curvularia* spp. (6%, 1/17). The patient who had a negative culture (Patient 6) had a clinical presentation strongly suggestive of *Acanthamoeba* spp.; thus, even though microbiologic diagnosis was negative, she was classified and treated as an *Acanthamoeba* spp. infection. Patient 15 had multiple co-infections with *Acanthamoeba* spp., *Staphylococcus* spp., *Candida* spp., and *Streptococcus* spp., in the setting of a chemical burn.

The majority of individuals reported a history of contact lens wear (79%) and 11% reported an occupation that required frequent contact with soil. Other comorbidities in our cohort included active and progressive ocular cicatricial pemphigoid (n = 1), iridocorneal endothelial syndrome (n = 1), and history of chemical burn (n = 1). The average area of epithelial defect prior to first treatment with RB-PDAT was 32 ± 27.3 (range: 0.81 mm²) with an average stromal opacification area of 29.2 ± 27.5 mm² (range: 2.81 mm²). The ulcer was central in 5 patients, paracentral in 5, peripheral in 3, and diffuse (limbus to limbus) in 5. By AS-OCT, average stromal depth hyper-reflectivity was 269 ± 75 µm (range: 116-387 µm).

The length of standard medical treatment prior to the first RB-PDAT ranged from 2 to 64 weeks depending on the organisms (*Acanthamoeba* spp. = 18.5 weeks, *Fusarium* spp. = 3 weeks, *Curvularia* spp. = 4 weeks, and *Pseudomonas aeruginosa* = 3.5 weeks) with a mean of 12.5 weeks. Nine patients received 1 treatment with RB-PDAT, 8 patients received 2 treatments, and 1 patient underwent 3 treatments. Patients who received multiple treatments presented with very severe cases of infectious keratitis. Clinicians decided whether a repeat treatment was indicated if they observed minimal clinical improvement at follow-up.

• **ROSE BENGAL PHOTODYNAMIC ANTIMICROBIAL THERAPY OUTCOMES:** RB-PDAT was considered successful in 13 individuals, defined as control of infection without the need for a TPK. Clinical endpoints were defined as no infection with no treatment for a minimum of 3 months for bacterial and fungal keratitis and 6 months for *Acanthamoeba* keratitis. In these 13 individuals, time to clinical resolution after the first RB-PDAT session was
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BCVA = best-corrected visual acuity; CE+IOL = cataract extraction + intraocular lens placement; CK = chemical keratitis; CL = contact lens; HM = hand motion; LP = light perception; MRSA = methicillin-resistant Staphylococcus aureus; OCP = ocular cicatricial pemphigoid; PKP = penetrating keratoplasty; SMT = standard medical treatment; TPK = therapeutic penetrating keratoplasty.
FIGURE 1. Slit-lamp findings and clinical course of patients that underwent optical corneal transplantation after successful rose bengal photodynamic antimicrobial therapy (RB-PDAT). FU = follow-up; OPK = optical penetrating keratoplasty.
46.9 ± 26.4 days. Individuals without evidence of recurrence or inflammation were given the option of undergoing optical keratoplasty at least 6 months after RB-PDAT. Seven optical penetrating keratoplasties and 2 deep anterior lamellar keratoplasties were thus successfully performed with no recurrent infection or graft failure on mean follow-up of 6.47 ± 3.22 months (range 0.25-10 months). No organisms were identified on microbiology or pathology from the host tissue removed at the time of surgery. Figure 1 depicts the graft outcomes of these patients. Three of the remaining patients have not yet undergone any additional procedure and 1 was lost to follow-up. The average BCVA prior to PDAT treatment was 2.48 logMAR and improved to 1.87 logMAR after infectious resolution (P = .046). BCVA at 6 months and 1 year after PDT was 1.83 logMAR and 0.54 logMAR in those who underwent optical corneal transplantation and 0.73 logMAR and 0.57 logMAR for those who have not yet undergone any additional intervention. Therefore, in those considered successful, BCVA improved 1.92 ± 0.8 logMAR (P < .01) from presentation to 1-year follow-up. Clinical evolution of the patients is seen in Figure 2.

RB-PDAT was a failure when corneal perforation occurred after treatment with RB-PDAT. This occurred in 5 patients and after a mean of 15.8 ± 8 days after the first RB-PDAT, a TPK had to be performed. Three of the 5 individuals were found to have microbes identified in the perforated tissue: 1 with Acanthamoeba spp. cysts noted in the pathology specimen, 1 with Acanthamoeba spp. cyst on pathology and culture, and 1 with fungal elements on pathology. Two individuals had no microbes identified in the tissue, suggesting microbial elimination. Outcomes after TPK were not as good as after optical corneal transplantation. While 1 graft remained clear after TPK, 3 grafts had corneal edema (n = 2) and neovascularization (n = 1) on mean follow-up 10.3 ± 4.86 months after TPK (range: 3-13 months). One patient underwent enucleation 2 days after TPK, owing to intractable pain and intraocular involvement. The average BCVA prior to PDAT treatment in these patients was 2.73 logMAR and with slight improvement in vision to 1.95 and 1.78 logMAR 6 months and 1 year, respectively, after the procedure. BCVA improved only 0.96 ± 1.4 logMAR (P = .19) from presentation to 1-year follow-up in patients who failed RB-PDAT treatment (Patient outcomes are illustrated in Figure 3.)

### Risk Factors for Rose Bengal Photodynamic Antimicrobial Therapy Failure

Demographics (age, sex, ethnicity, race) did not predict RB-PDAT outcome.
Non–contact lens wearers had a higher risk of RB-PDAT failure as compared to contact lens wearers (100% vs 14%, \( P = .01 \)). Clinical characteristics of infection (eg, depth, epithelial defect size, and area of infiltrate) and length of medical treatment before RB-PDAT also did not predict outcomes. Additionally, there was no difference in outcomes between those patients treated with 1% RB solution vs 2% RB solution. Comparison of characteristics of patients and infection are summarized in Table 2.

**DISCUSSION**

INFECTIOUS KERATITIS CAN LEAD TO SIGNIFICANT OCULAR morbidity, greatly impacting patients’ quality of life. This sight-threatening condition can be caused by a wide range of bacteria, fungi, protozoa, and/or viruses. Antimicrobial-resistant strains are increasing and are associated with worse clinical presentation and visual impairment. Consequently, great efforts are being made to develop novel therapies to control these infections. We found that 1-3 sessions of RB-PDAT, in conjunction with medical therapy, eliminated infection and prevented the need for TPK in 72% of individuals with severe corneal ulcers. Even in the 5 individuals who failed RB-PDAT and underwent TPK, no residual infection was noted in 2.

The results of the current study are supported by basic science data showing that RB-PDAT is more effective against microbes than CXL with riboflavin and UV-A light. In vitro studies of CXL with riboflavin and UV-A have demonstrated efficacy against certain bacteria, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa*; however, this treatment has been shown to be ineffective against fungi including *Candida* spp., *Fusarium* spp., and *Aspergillus* spp. On the contrary, RB-PDAT has been shown to fully inhibit growth of both fungal (*Candida* spp., *Fusarium* spp., and *Aspergillus* spp.) and bacterial (MRSA) isolates. Clinically, PACK-CXL has also

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<th>N</th>
<th>Pre RB-PDAT</th>
<th>Time to perforation after first PDAT</th>
<th>Perforation</th>
<th>Time until last FU</th>
<th>Last Slit-lamp appearance available</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td></td>
<td>7 days</td>
<td></td>
<td>13 months</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>10 days</td>
<td></td>
<td>13 months</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>20 days</td>
<td></td>
<td>12 months</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>27 days</td>
<td></td>
<td>N/A</td>
<td>Enucleated</td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>15 days</td>
<td></td>
<td>3 months</td>
<td>Picture not available</td>
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</table>

**FIGURE 3.** Slit-lamp findings and clinical course of patients that had an unsuccessful rose bengal photodynamic antimicrobial therapy (RB-PDAT) and required therapeutic keratoplasty or enucleation. FU = follow-up.
been used for the treatment of infectious keratitis associated with melt. A meta-analysis of PACK-CXL reported this treatment stabilized corneal melt in gram-negative bacteria (13/14; 92%), gram-positive bacteria (37/44; 84%), Acanthamoeba (5/7; 71%), and fungus (8/13; 61%). However, thamoeba involved and is higher for bacteria (394 DECEMBER 2019AMERICAN JOURNAL OF OPHTHALMOLOGY)

A randomized controlled trial found that the risk of perforation was higher with adjunct PACK-CXL and medical therapy as compared to medical therapy alone in cases of deep fungal keratitis. Current study outcomes are favorable compared to the current standard of care of TPK for resistant infections. The risk of graft failure is dramatically higher in TPK compared to OPK. Survival rates for therapeutic grafts have been reported to be 78.4%, 58.3%, and 37.3% at 1, 3, and 5 years. However, success depends on the organism involved and is higher for bacteria (76.6%) compared to fungi (51%–84%) at 1 year. Furthermore, eradication of the infection is not ensured after TPK and ranges from 90% to 100% in bacterial keratitis, 69% to 90% in fungal keratitis, and 45% to 81% in Acanthamoeba. In our cohort, infection was clinically eradicated in 72% (13/18) and microbiologically eradicated in 83% (15/18) after RB-PDAT and the 1-year graft survival in those who later underwent OPK was 100%.

The safety of 0.1% RB-PDAT has been evaluated in vivo and ex vivo in animal models and no significant adverse effects on keratocytes, iris, or retina were observed. These studies, along with our data demonstrating no difference between patients treated with 0.1% and 0.2% RB solution, led us to select a 0.1% RB solution as the standard for our RB-PDAT protocol. RB is thought to interact strongly with collagen, thus limiting its penetration into healthy stroma to approximately 100 μm. However, considering our average depth of infection of 269 ± 75 μm and our good clinical outcomes, we hypothesize that diffusion of the dye and its effects are enhanced by the stromal melting and inflammation present in keratitis. Beyond a direct antimicrobial effect, another potential consequence of photochemical crosslinking using RB and green light is an increased resistance to collagenase digestion. Evidence to support the process of photochemical crosslinking was observed in some patients with the presence of a demarcation line. We therefore consider that even if antimicrobial eradication was not achieved with RB-PDAT, corneal melting and extension of the infection were halted, allowing the conventional antimicrobials administered as standard medical therapy to then eradicate infection. In the long term, RB-PDAT may also have a beneficial effect on subsequent OPK. Studies in murine models have demonstrated that CXL regresses pathologic vessels and lymphatics in high-risk corneal grafts, and can thus increase survival after OPK.

As with all studies, our findings must be considered in light of the study limitations, which include its retrospective nature and limited number of subjects. Additionally, owing to regulatory issues, this treatment was offered only to advanced, resistant cases and these results do not apply to less severe cases or early treatment. Thus, owing to the severity of the infections, no minimum corneal thickness could be determined as an inclusion criterion as corneal thickness measurements were limited by dense infiltrates, inflammation, and edema. We believe RB-PDAT outcomes were unfavorable in some patients owing to inconsistent attendance of follow-up appointments and earlier treatment could have resulted in a better outcome. A controlled randomized clinical trial with a larger number of patients would be needed to compare RB-PDAT with medical treatment to medical treatment alone and determine which subtypes of infection are best treated with combined therapy. Despite these limitations, the current pilot clinical study results indicate that RB, a photosensitizing agent available to most ophthalmologists, when combined with a green LED light, resolved infection and prevented the need for TPK in a majority of individuals with sight-threatening infections. This therapy 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.
REFERENCES