# Riboflavin and Ultraviolet Light A Therapy as an Adjuvant Treatment for Medically Refractive *Acanthamoeba* Keratitis

## *Report of 3 Cases*

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***Purpose:*** To present the first 3 cases of *Acanthamoeba* keratitis (AK), unresponsive to medical treatment, that were successfully treated with a novel adjunctive therapy using ultraviolet light A (UVA) and riboflavin (B2).

***Design:*** Interventional case series.

***Participants:*** Two patients with confirmed AK and 1 patient with presumptive AK, which were all refractive to multidrug conventional therapy.

***Intervention:*** Two treatment sessions involving topical application of 0.1% B2 solution to the ocular surface combined with 30 minutes of UVA irradiation focused on the corneal ulcer.

***Main Outcome Measures:*** Clinical examination by slit lamp, confocal microscopy, and histopathology, when available.

***Results:*** All patients in these series showed a rapid reduction in their symptoms and decreased ulcer size after the first treatment session. The progress of the clinical improvement began to slow after 1 to 3 weeks of the first application and was then renewed after the second application. All ancillary signs of inflammation mostly resolved after the second treatment session. The ulcers in all patients continued to decrease and were closed within 3 to 7 weeks of the first application. Two patients developed dense central corneal scars, and penetrating keratoplasty was performed for visual rehabilitation. Histopathologic examination of the excised tissue revealed no *Acanthamoeba* organisms. The remaining patient had no symptoms or signs of infection, both clinically and by confocal microscopy, and was left with a semitransparent eccentric scar that did not affect visual acuity.

***Conclusions:*** The adjunctive use of UVA and B2 therapy seems to be a possible alternative for selected cases of medication-resistant AK.

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Since the initial successful medical strategies for *Acanthamoeba* keratitis (AK) in 1985, there have been many developments in the antimicrobial agents and treatment protocols for the management of this infection.[1] Despite these advances, there is still no ideal medical therapy for AK, and more than 15% of patients who are infected and treated still have severe loss of vision.[2] The current treatment approaches, which involve long and intensive regimens requiring multiple drops to be applied sometimes around the clock, are still marred by resistant infections and adverse effects from the medications.[3–5] The cases that are refractory to medical therapy sometimes require surgical management, most commonly by penetrating keratoplasty (PK). Transplantation does not always offer a complete cure, because approximately 30% of transplanted grafts become reinfected, and most recurrences occur within 2 to 3 weeks.[6,7]

Although still rare, AK remains a major concern not only because of its protracted and grim clinical course but also because its incidence seems to be steadily increasing.[8] Contact lens wear and improper lens hygiene remain the most important risk factors, and as the number of contact lens wearers continues to increase, so do the cases of AK.[9,10] Despite the recent interest in AK because of tainted contact lens solution, contamination more commonly occurs from other seemingly innocuous sources, such as swimming pools, hot tubs, lakes, seawater, and tap water.[11–13]

The application of riboflavin (B2) and concurrent ultraviolet light A (UVA) exposure to the cornea has successfully been used by ophthalmologists to treat keratoconus, post-LASIK keratectasia, and certain corneal melting disorders.[14–16] It has been suggested that the therapy depends on free radicals generated by UVA irradiation of B2 to cause oxidation of corneal collagen, inducing cross-linking and strengthening the collagen matrix.[17] However, the photochemical reaction created from combining UVA and B2 was first exploited by other scientists for pathogen reduction



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in blood products.[18] The free radicals cause oxidative damage to DNA and RNA molecules, which is complemented by structural DNA/RNA damage by activated flavins that associate with and cause conformational changes in those molecules.[19,20] The UVA and B2 pathogen reduction treatment has been proven useful against bacterial, viral, and parasitic contaminates in blood products.[21,22]

With these applications in mind, we have proposed to use this approach to treat infectious keratitis. Our previous work showed that the combination of UVA and B2 can inhibit the growth of common pathogens implicated in bacterial keratitis in vitro[23] and can be used to treat an experimental model of *Staphylococcus aureus* in vivo (Khan YA, Martins SAR, Camacho W, et al. Riboflavin/UVA combined treatment in a rabbit Staphylococcus aureus keratitis model: a new approach for corneal infections. Poster presented at The Association for Research in Vision and Ophthalmology [ARVO] Annual Meeting, May 1, 2008, Fort Lauderdale). This report presents 2 confirmed cases and 1 highly presumptive case of AK refractory to medical therapy, which were all effectively treated with adjuvant UVA/B2 therapy. To the best of our knowledge, this is the first report of confirmed infectious keratitis caused by *Acanthamoeba* that was successfully treated with UVA and B2 as an adjunctive therapy.

## Materials and Methods

The institutional review board of The Johns Hopkins University, Baltimore, Maryland, granted individual emergency therapeutic use approval for the treatment to be conducted on patients 1 and 2 of this series. Similar approval was obtained for patient 3 from the institutional review board of Instituto de Cirugía Ocular in San Jose, Costa Rica. Because this is a non-approved treatment of microbial keratitis in the United States, the US Food and Drug Administration authorized the emergent use of this treatment in patients 1 and 2 under the Investigational New Drug or Device Exemption Process. Informed consent was obtained from each patient in this series, and the guidelines of the Health Insurance Portability and Accountability Act were observed. In all cases, this interventional research adhered to the stipulations presented in the Declaration of Helsinki.

A literature review was conducted to verify that no previous reports of our findings had been presented. The EMBASE database and the PubMed service, including the MEDLINE database, were searched from 1970 to the present, and included the following search terms: *acanthamoeba, amoeba, amoebic, infection, keratitis, treatment, therapy, ultraviolet light, UVA, riboflavin, B2, collagen cross-linking, photochemical,* and *photodynamic.*

### Ultraviolet Light A and Riboflavin Treatment Protocol

The periorbital area of the infected eye was cleaned with povidone iodine solution. A drop of 0.5% proparacaine hydrochloride solution was administered, and an eye speculum was inserted in the involved eye. A small square of filter paper was cut from sterile Schirmer's test paper and soaked in 0.1% B2 solution in 20% Dextran 500. The paper was then placed onto the surface of the corneal ulcer for 5 minutes and then removed. A 365-nm wavelength UVA light source was used to irradiate the ulcer, carefully avoiding the limbal area in the extent of the spot diameter. The spot size of the light was adjusted to 8 mm in diameter to ensure the irradiance at the corneal surface was approximately 3 mW/cm$^2$, as was previously determined in the calibration of the unit. Drops of the B2 solution were administered every 5 minutes for the length of the procedure, and the proparacaine solution drops were administered every 10 minutes and as required. The UVA light exposure and drop instillation were continued for 30 minutes and then ceased.[24]

### Case Reports

**Patient 1.** A healthy 61-year-old African American male soft contact lens wearer was referred to the Cornea Division of The Wilmer Ophthalmological Institute for a presumed bacterial keratitis that was unresponsive to medical treatment. He reported periodic extended wear of his contact lenses 1 week before his symptoms developed. He had a 7-day history of progressive pain, photophobia, and tearing in his right eye before presenting to the referring surgeon. Initial cultures performed at the referring center were positive for *Pseudomonas aeruginosa*, but negative for fungi and protozoa; thus, topical 0.3% ciprofloxacin hydrochloride drops every hour, 1% povidone iodine drops 3 times per day, and 0.5% erythromycin ointment at bedtime were prescribed. After 1 week, there was no improvement in the patient's pain or photophobia and no change in the size of the ulcer. His treatment regimen was modified to include 0.3% gentamycin sulfate drops 3 times per day and 0.5% moxifloxacin hydrochloride drops every hour. The patient reported no improvement in his symptoms, and the size of his ulcer progressed after 5 days of the modified therapy. At this time, an anterior chamber wash and intracameral injection of 0.5% moxifloxacin hydrochloride were performed. The patient's pain and photophobia worsened, and the size of the ulcer increased 1 week later, and he was subsequently referred to our clinic.

Upon arrival to the Wilmer Eye Institute, the patient had continued photophobia and exquisite pain. Examination revealed a 4.5×6.7-mm central corneal ulcer with marked circumcorneal injection, severe chemosis, and bipalpebral edema (Fig 1A). Corneal cultures repeated at our institution were negative for bacteria, fungi, and protozoa. The poor clinical response to antibacterial agents and the negative cultures led to the suspicion of AK. Confocal microscopy (Nidek Confoscan, Aichi, Japan) was performed, and findings were compatible with *Acanthamoeba* cysts (Fig 1B).[11]

The patient was started on a regimen of 0.1% propamidine isethionate, 0.02% polyhexamethylene biguanide (PHMB), and 0.02% chlorhexidine gluconate eye drops every hour. After 1 month of treatment, the patient continued to have severe pain and photophobia, with minor clinical improvement. The chemosis and bipalpebral edema had improved, but the circumcorneal injection and corneal ulcer remained stationary. Because of the medically resistant nature of the AK, the patient was treated with the UVA and B2 therapy, and the topical medications were continued. Guided by clinical progression, a second treatment was applied 1 week after the first session.

**Patient 2.** A 35-year-old male soft contact lens wearer in good health was referred to our clinic for a presumed herpetic ulcer that was unresponsive to medical treatment. He reported 5 days of blurred vision, photophobia, and increasing pain in his right eye before presentation to the referring center. He had a history of swimming in lake water 1 week before onset of his symptoms. The patient was initially treated with topical 1% trifluridine ophthalmic drops 8 times daily. After 2 weeks of treatment, there was no change in the size of the ulcer and no clinical improvement in his pain, photophobia, or blurred vision, at which time he was referred to The Wilmer Eye Institute. At our first encounter, the patient

**Figure 1. A,** Slit-lamp photograph of patient 1 after 30 days of medical treatment, showing a 4.5×6.7-mm central corneal ulcer with surrounding stromal infiltrate and circumcorneal injection. **B,** Confocal microscopy image of patient 1 showing reflective and high-contrast round bodies suggestive of *Acanthamoeba* cysts. **C,** Slit-lamp photograph of the same patient, 1 week after the first UVA and B2 treatment; the ulcer measures 3.3×4.78 mm with decreased stromal infiltrate. **D,** Slit-lamp photograph 6 weeks after the first UVA and B2 therapy in patient 1, a dense corneal scar is present and the corneal ulcer is closed. **E,** Histologic photograph of the excised cornea from patient 1, with no *Acanthamoeba* organisms present. B2 = riboflavin; UVA = ultraviolet light A.

reported intense pain, photophobia, and blurred vision that had become more severe since his prior treatment. The patient was noted to have a 2×3-mm ulcer in the central cornea with surrounding infiltration and edema. Corneal ulcer scrapings mounted on 10% potassium hydroxide revealed *Acanthamoeba* cysts, which coincided with a positive *Acanthamoeba* culture in *Escherichia coli*-enriched media.

The patient was prescribed a regimen including 0.1% propamidine isethionate, 0.02% PHMB, and 0.02% chlorhexidine gluconate, each to be applied hourly for the first 2 weeks. After 45 days of treatment, there was no resolution of his symptoms. The ulcer continued to grow and was observed to be 2.4×3.5 mm (Fig 2A). The patient was then treated with the UVA and B2 therapy, and the antiamoebic agents were continued after the therapy. Two weeks later, the rate of clinical recovery was noted to be decreased and the UVA/B2 therapy was repeated.

**Patient 3.** A 30-year-old male soft contact lens wearer presented to Instituto de Cirugía Ocular with symptoms of burning left eye pain and excessive tearing. He reported that his symptoms began 10 days earlier and were intensifying. He stated he had been swimming in seawater with his contact lenses in place 1 week earlier, and he also had an untreated corneal abrasion before his water exposure. Examination at the clinic revealed an eccentric corneal ulcer measuring 3.5×3.7 mm and severe circumcorneal injection (Fig 3A). Corneal culture results were positive for *Acanthamoeba*, but negative for bacteria and fungi. Confocal microscopy confirmed the presence of corneal ulcer cysts. A treatment protocol including 0.02% PHMB every 30 minutes and 0.02% chlorhexidine gluconate every hour was then initiated.

After 30 days of topical medical therapy, there was no change in the corneal ulcer and the circumcorneal injection was still present. The patient also had continued pain and severe photosensitivity. He was then treated with the UVA and B2 therapy in conjunction with his continued topical therapy. Two weeks after the treatment, the rate of improvement began to plateau and a second UVA and B2 treatment was applied.

## Results

The clinical resolution of AK in these series was determined by improvement of symptoms, decreased ulcer size, reduction in signs of infection, and histopathology. Notably, all patients reported considerable pain within the first 48 hours after the application of the UVA/B2 treatment. However, this was followed by a dramatic decrease in pain and photophobia after 3 days of the UVA and B2 treatment, with no significant exacerbations of symptoms. The ulcers in all patients were visibly decreasing in size after 1 treat-

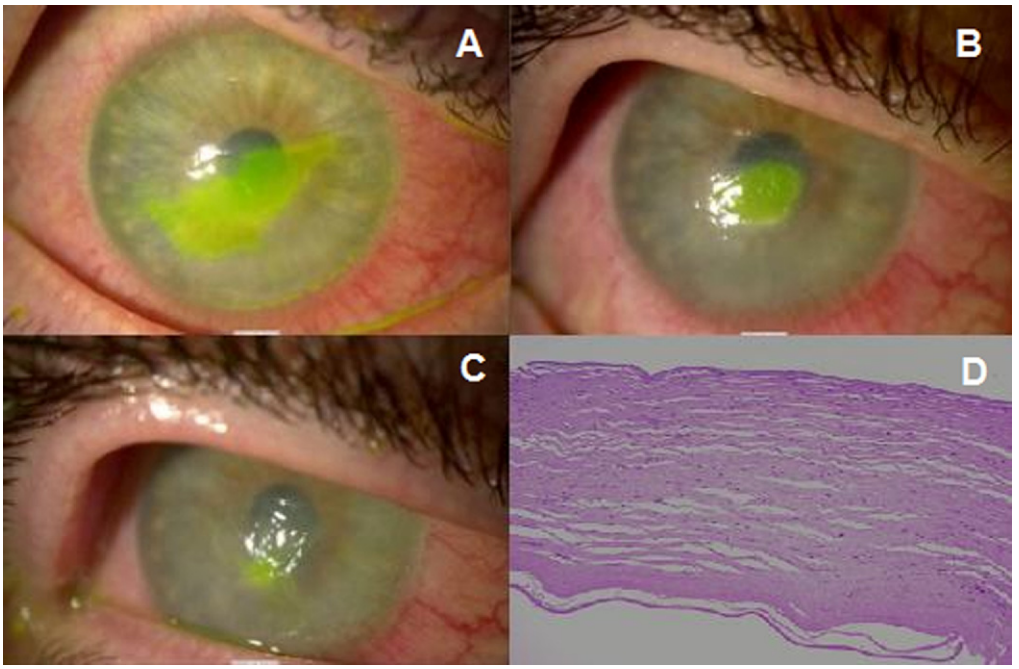


**Figure 2. A,** Slit-lamp photograph of patient 2 after 45 days of medical treatment, showing a 2.4×3.5-mm central corneal ulcer and severe circumcorneal injection. **B,** Slit-lamp photograph of the same patient 1 week after the first UVA and B2 treatment; the corneal ulcer has decreased to 1.5×2.5 mm. **C,** Slit-lamp photograph of patient 2 taken 3 weeks after the initiation of UVA and B2 therapy; the ulcer is closed and a central corneal scar is present. **D,** Tissue histology of the excised corneal tissue. No *Acanthamoeba* protozoa are visible. B2 = riboflavin; UVA = ultraviolet light A.

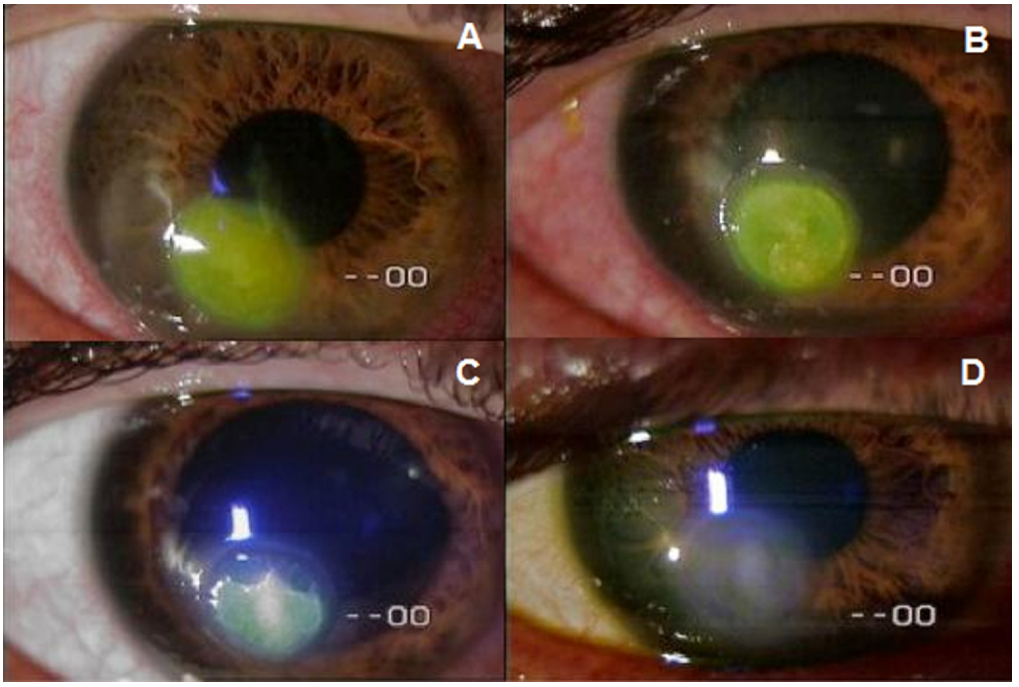


**Figure 3. A,** Slit-lamp photograph of patient 3 after 30 days of medical treatment, showing a 3.5×3.7-mm eccentric corneal ulcer with significant circumcorneal injection. **B,** Slit-lamp photograph of the same patient, 1 week after the first UVA and B2 therapy; the ulcer measures 2.6×3.1 mm, and the circumcorneal injection remains. **C,** Slit-lamp photograph of patient 3 taken 1 week after the second UVA and B2 application; the ulcer measures 1.26×2.6 mm, and the circumcorneal injection is resolved. **D,** Slit-lamp photograph of the same patient 7 weeks after the first treatment; the ulcer is closed and a semitransparent corneal scar is present outside the visual axis. B2 = riboflavin; UVA = ultraviolet light A.

ment, and all epithelial defects were closed after 2 months of the initial treatment. One week after the first session, the corneal ulcer in patient 1 was reduced to 3.3×4.78 mm, 1.5×2.5 mm in patient 2, and 2.6×3.1 mm in patient 3 (Figs 1C, 2B, and 3B).

This rapid rate of improvement began to decline 1 to 3 weeks after the first session, when a second treatment was applied after the ulcer sizes became static. After the second treatment the ulcers continued to close, the circumcorneal injection cleared, and the symptoms resolved in all patients (Fig 3C). Complete ulcer closure occurred 6 weeks, 3 weeks, and 7 weeks after the first UVA and B2 treatment in patients 1, 2, and 3, respectively (Figs 1D, 2C, and 3D).

After complete AK resolution, patients 1 and 2 were left with central corneal scars dense enough to obstruct vision. Penetrating keratoplasty was performed in these patients for visual rehabilitation. Histopathologic examination of the excised corneal tissue revealed no *Acanthamoeba* trophozoites or cysts (Figs 1E and 2D). Patient 3 was left with a nonobstructive, semitransparent scar outside of the visual axis; PK was not required. At confocal microscopy, no cysts of *Acanthamoeba* were identified after the treatment.

Clinical examinations have been performed regularly for 12 and 8 months after PK in patients 1 and 2, respectively. In this time, there has been no evidence of graft infection. Patient 3 has been followed for 4 months after the final UVA and B2 treatment, and there has been no indication of infection recurrence.

## Discussion

The patients presented in this report were of different ages and races, and from different geographic locations. They used different brands of contact lenses and lens care products. The only commonality between them illustrates a salient point about AK: Improper contact lens use and hygiene remain the most important risk factors for developing the infection.[8] Patient 1 reported extended and periodic overnight wear of his contact lenses before his symptom onset.[9] Patients 2 and 3 reported swimming while wearing their lenses before developing their infections in lake and sea water, respectively.[11,12]

There is some indication that monotherapy with biguanides, such as chlorhexidine and PHMB, or a diamidine, such as propamidine, is effective against AK.[25,26] However, in vitro evidence of an additive effect between antiamoebic agents has led to the preference of multidrug regimens; current protocols combine propamidine isethionate with chlorhexidine or PHMB.[2,27,28] All patients in these series received intensive multidrug regimens for at least 30 days before being treated with UVA and B2. Patients 1 and 3 were treated with PHMB, combined with propamidine isethionate and chlorhexidine gluconate, respectively. Patient 2 received therapy with PHMB, propamidine isethionate, and chlorhexidine gluconate.

These long and demanding therapeutic regimens are not always able to cure AK, as was observed in all our patients.[2,4] The *Acanthamoeba* protozoa exist in 2 forms: the active and replicating trophozoite and the dormant cyst. The cysts are impervious to adverse conditions and many antimicrobials, and they present the greatest obstacle to medical therapy.[3,29,30] The success of medical treatment for AK depends on prompt initiation of therapy before encystation, and the best outcome seems to occur when treatment is started within 18 days of the first symptoms.[7,31] Of the patients in these series, only patient 3 was treated for AK within this timeline.

The poor recognition of and difficulty diagnosing AK often lead to the delayed treatment of the disease.[6] From clinical examinations alone, AK is most commonly misdiagnosed as herpetic keratitis, as was the case in patient 2.[11] Corneal cultures for *Acanthamoeba* can help identify the infection, but the test has poor sensitivity and smaller communities may not have the facilities or expertise to perform these specialized tests.[11,32] *Acanthamoeba* can also coinfect the cornea with bacteria or fungi; when a false-negative *Acanthamoeba* culture is obtained, this can contribute to the misdiagnosis of the infection.[7] This was the cause of delayed diagnosis in patient 1, who had 2 negative *Acanthamoeba* cultures.

The inability to treat resistant and advanced cases of AK is not the only shortcoming of current medical therapy. The antiamoebic agents are potent disinfectants that are associated with many adverse effects, and these are amplified because of the prolonged nature of the treatment protocols.[13] Propamidine isethionate alone can cause corneal epithelial keratopathy, and when combined with PHMB can lead to punctate keratopathy.[30,33] The side effect profile of chlorhexidine is even more severe. Corneal necrosis, cataract formation, and iris atrophy have all been associated with prolonged treatment of AK with chlorhexidine.[34,35]

The poor access to these medications can also contribute to treatment delay. Propamidine isethionate is not approved for use in the United States, and ophthalmologists require off-label use of the medication in the United States.[32] Chlorhexidine and PHMB are not readily accessible at most pharmacies and are synthesized extemporaneously from industrial-grade components. The delay from compounding these medications and the tissue toxicity from potential impurities further compromise their utility.[32]

The relationship between contact lens wear and AK has stimulated considerable interest into lens disinfection systems. Hydrogen peroxide is an effective antimicrobial agent that can eradicate many pathogens through oxidative damage.[36] It has been found that contact lens cleaning solutions with hydrogen peroxide are able to neutralize both *Acanthamoeba* trophozoites and cysts.[37] Unfortunately, corneal epithelial injury, stromal swelling, and endothelial toxicity hinder the use of hydrogen peroxide as a topical agent.[38,39]

The first use for the combined UVA and B2 therapy was to neutralize pathogens in blood products, and there are 2 main mechanisms by which this therapy exerts its antimicrobial effect. Light-activated flavins intercalate between base pairs of DNA, causing structural damage to these molecules and preventing genome replication.[20] The combination of UVA and B2 also generates free radicals, which lead to genomic damage through less-specific oxidative damage to DNA molecules.[19] In the field of transfusion medicine, the UVA and B2 therapy has been used effectively against several bacteria, viruses, and parasites.[21,22]

The free radical-induced oxidation reactions that occur from combining UVA and B2 have recently been used to treat corneal ectatic and melting diseases by stimulating

cross-linking between corneal collagen molecules, strengthening the collagen matrix, and making it less susceptible to enzymatic digestion.[14–17] The damage to DNA that makes this therapy effective against pathogens also leads to keratocyte apoptosis. Fortunately, the depth of cell loss is predictable and occurs between 300 and 350 $\mu$m when the UVA irradiance is 3 mW/cm$^2$ and the exposure lasts 30 minutes.[40,41] The keratocyte loss is temporary because of the regenerative ability of these cells, and repopulation is complete within 6 months.[40] By discounting the temporary keratocyte loss, this treatment has few complications. There is some transient stromal edema but no endothelial cell loss or changes to corneal or lens transparency.[14,42]

With the current applications and properties of the UVA and B2 therapy in mind, we have ventured to explore its potential use for treating infectious keratitis. Our previous studies have shown that it is effective against both drug-sensitive and resistant strains of *S. aureus, S. epidermidis,* and *Streptococcus pneumoniae* in vitro.[23] We have also found the UVA and B2 therapy to be effective for treating experimental *S. aureus* keratitis in vivo in an animal model (Khan YA, Martins SAR, Camacho W, et al. Riboflavin/UVA combined treatment in a rabbit Staphylococcus aureus keratitis model: a new approach for corneal infections. Poster presented at the ARVO Annual Meeting, May 1, 2008, Ft. Lauderdale). The knowledge about the free radicals generated by irradiating B2, combined with the reports of successful *Acanthamoeba* cyst neutralization by hydrogen peroxide-based oxidation, has provided the rationale to test the efficacy of combined UVA and B2 against medically refractory AK.

There are 3 ways in which the UVA and B2 treatment can combat AK. Genome damage from activated flavins and free radical insult to DNA can prevent replication of the active trophozoite.[19,20] Oxidative injury from free radicals are also known to be cysticidal.[36,37] Furthermore, *Acanthamoeba* penetrates the cornea by secreting collagenase to dissolve the collagen matrix.[29,43] The UVA and B2 treatment is known to increase the resistance of the corneal collagen to enzymatic digestion; thus, it likely confers some protection against this aspect of *Acanthamoeba* pathogenesis.[17]

The treatment of ectatic disorders involves a single session of UVA and B2 therapy, whereas our patients each underwent 2 sessions.[14] All 3 patients showed a rapid clinical recovery after the first treatment, and the second treatment was applied when the rate of improvement began to decline. The therapy is useful in the management of ectasia because of the lasting biomechanical changes to the collagen matrix, but it is the activated flavins and free radicals that confer the antipathogenic effect.[17,24] Further research will be needed to determine the clearance rates of the flavins and free radicals from the cornea to best exploit this therapy for infection management.

To ensure that standards of clinical care were maintained, all the patients continued their medical treatment concurrently with the UVA and B2 therapy. Thus, no statements can be made about the efficacy of the UVA and B2 as an independent treatment for AK. The UVA and B2 treatment is presumed to be safe for the corneal endothelium and central retina when the appropriate irradiance levels are used in corneas thicker than 400 $\mu$m.[42,44] Thus, it is important to assess corneal thickness, especially in patients with keratitis who may have stromal loss. The optimal protocol for treating infections with UVA and B2 is not yet known, and this experiment replicated the procedure used in the treatment of ectasia.[24] It will be important to conduct more research to determine whether UVA and B2 can be used as a stand-alone treatment for AK and to ascertain the optimal parameters for this use. Our laboratory is currently engaged in in vitro and in vivo experiments to address these queries.

There is some anecdotal evidence that certain cases of AK may be unresponsive to UVA and B2 therapy. The main factor that differentiates these patients from ours was the severity of the keratitis. All the cases presented in this report involved superficial infections with no significant loss of corneal transparency, whereas the patients treated by our colleagues had a more advanced disease, with a deeper penetration and corneal opacification. Neither UVA nor B2 alone is effective against infectious keratitis, and any impediment to the penetration of either of these would limit the antimicrobial effect of this treatment (Khan YA, Martins SAR, Camacho W, et al. Riboflavin/UVA combined treatment in a rabbit Staphylococcus aureus keratitis model: a new approach for corneal infections. Poster presented at the ARVO Annual Meeting, May 1, 2008, Fort Lauderdale). Thus, it stands to reason that corneal opacities dense enough to prevent the transmission of UVA would likewise limit depth of the photochemical reactions that are required to treat corneal infections.

This presents a potential hurdle when treating advanced cases of AK that are associated with both deeply penetrating infections and corneal opacification. Therefore, in addition to evaluating the utility and optimal treatment protocols of the UVA and B2 therapy for AK, it will be important to consider the stage of infection as another variable. It will also be useful to develop a detailed summary of criteria for this therapy, with special attention to clinical features that should exclude cases from treatment.

In conclusion, the combination of UVA and B2 has been used successfully as an adjunctive treatment for the 3 cases of medically refractory AK presented in this report. Although this therapy is primarily used in ophthalmology to treat corneal ectatic disorders, its use for treating corneal infections could be optimized by modifying certain parameters of the current protocols and by establishing the clinical criteria to help identify appropriate candidates. Further research is needed and currently under way to examine how best to use photochemical therapy for the treatment of infectious keratitis.

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