Antimicrobial Efficacy of Riboflavin/UVA Combination (365 nm) In Vitro for Bacterial and Fungal Isolates: A Potential New Treatment for Infectious Keratitis

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PURPOSE. To demonstrate the antimicrobial properties of riboflavin/UVA (365 nm) against common pathogens.

METHODS. One group of bacteria (*Pseudomonas aeruginosa* [PA], *Staphylococcus aureus* [SA], and *Staphylococcus epidermidis* [SE]) was tested by using Kirby-Bauer discs with (1) empty disc (Control – C); (2) riboflavin 0.1% (B2); (3) riboflavin 0.1% previously activated by UVA (B2′); (4) UVA alone (UVA); (5) group 2+additional UVA exposure (UVA+B2); and (6) group 3+additional UVA exposure (UVA+B2′). In addition, another group of microbes was tested with the same approach: methicillin-resistant *S. aureus* (MRSA), multidrug-resistant *P. aeruginosa* (MDRPA), drug-resistant *Streptococcus pneumoniae* (DRSP), and *Candida albicans* (CA). The mean growth inhibition zone (GIZ) in square millimeters was measured around the discs. The mean standard deviation (MSD) was calculated to be 3.65 when $\alpha = 0.01$. A mean deviation (MD) > MSD indicates a significant difference.

RESULTS. In the first group, the GIZ was significantly greater after UVA (MD = 14.30), UVA+B2 (MD = 39.61), and UVA+B2′ (MD = 40.45) when compared with C, B2, and B2′. UVA alone was less effective than UVA+B2 (MD = 25.31) and UVA+B2′ (MD = 26.15). The second group demonstrated increased GIZ in UVA (MD = 6.98), UVA+B2 (MD = 17.80), and UVA+B2′ (MD = 21.15) when compared with C, B2, and B2′. UVA alone was less effective against the second group of bacteria than was UVA+B2 (MD = 10.82) and UVA+B2′ (MD = 14.17). CA did not show any GIZ after treatment.

CONCLUSIONS. Riboflavin/UVA was effective against SA, SE, PA, MRSA, MDRPA, and DRSP, but was ineffective on CA and has the potential for use in treatment of microbial keratitis in the future. (*Invest Ophthalmol Vis Sci.* 2008;49:3402–3408) DOI: 10.1167/iovs.07-1592

Corneal ocular infections may have a profound and devastating impact on visual function. Ulcerative keratitis, often microbial in origin and presenting as central or peripheral corneal ulceration or infiltration, is a sight-threatening condition. It remains an important cause of blindness that requires skilled management and effective chemotherapy to preserve vision.[1,2] If diagnosis and initiation of appropriate antimicrobial treatment are delayed, it has been estimated that only 50% of eyes will heal with good visual outcome.[3]

Although the treatment of corneal ulcers with topical antimicrobial agents has been notably successful, with an expanding array of both focused and broad-spectrum antibiotics, there has been an alarming resistance to antimicrobial agents.[4-8] Microbes cleverly develop resistance to antibiotics as a result of chromosomal mutation, inductive expression of a latent chromosomal genes, or exchange of genetic material via transformation, bacteriophage transduction, or plasmid conjugation.[1,4]

Use of the fluoroquinolones in the management of external infections is the most recent example of how a new class of antibiotics has been instrumental in changing management strategies for the treatment of corneal infections. Nonetheless, emerging patterns of resistance even to these new classes of antimicrobial agents (Drake JD, et al. *IOVS* 1997;38:ARVO Abstract S871),[9-22] have stimulated the continuing quest for an agent that provides rapid and complete microbicidal activity with minimal toxic effects and susceptibility to mechanisms of microbial resistance.

Riboflavin, or vitamin B2, is a naturally occurring compound and an essential human nutrient. Japanese scientists demonstrated in the 1960s that riboflavin, when exposed to visible or UV light, could be used to inactivate the RNA containing tobacco mosaic virus.[23] Research has been developing since 2000 to use riboflavin as a photosensitizer to inactivate pathogens in plasma, platelet, and red cell products.[24-26] This development leads us to propose that it could act as a photosensitizer useful for the inactivation of pathogens found in corneal infections, because of its nucleic acid specificity and its limited tendency toward indiscriminate oxidation.

On the other hand, the antimicrobial activity of ultraviolet (UV) irradiation includes sporicidal and virucidal effects.[27,28] Traditional applications of UV light are disinfection of drinking water and air/surface disinfection.[27,28] Limitations of the application of UV are mainly the lack of penetration and a strong dependence on the distance from the UV source, which may result in nonhomogeneous microbial inactivation.

The recent clinical use of a riboflavin/UVA combination for corneal collagen cross-linking and the observations in the laboratory of keratocyte depletion after its application,[29-32] stimulated us to evaluate its application for corneal infection. This project involved a series of in vitro experiments performed in our laboratory to evaluate the bactericidal effects of this therapy, in an effort ultimately to expand the armamentarium of antimicrobial agents for the management of severe microbial keratitis.

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FIGURE 1. One large (100 μL) drop of riboflavin (*arrow*) in the process of photoactivation (previously activated B2) using UVA light at 365 nm wavelength, 3 mW/cm^2 (✶) for 1 hour, before instillation in the culture plates, adjacent to the filter discs.

MATERIALS AND METHODS

Selection of Riboflavin Concentration

In previous published studies in which riboflavin/UVA treatment for the cornea was tested, the purpose was to induce collagen cross-linking to prevent the progression of corneal ectasia.[30-40] As our main objective is to treat corneal infections, we attempted to find an appropriate concentration primarily on the basis of its demonstrated in vitro spectrum of activity. Based on safety studies in corneal collagen cross-linking trials, the riboflavin's concentration established for this study was 0.1%.

Preparation of 0.1% Riboflavin Solution

Riboflavin-5-phosphate 25 mg (Sigma-Aldrich, St. Louis, MO) was diluted with phosphate buffered saline (Invitrogen, Grand Island, NY) to achieve a 0.1% riboflavin solution. The solution was protected from light and used within 24 hours.

Previously Photosensitized 0.1% Riboflavin

Riboflavin solution (0.1%) was exposed to a UVA light source (365 nm wavelength; Conversion Energy Enterprises, Spring Valley, NY) at a power density of 3 mW/cm^2 on multiwell culture plates (Sigma-Aldrich) for 1 hour before use (Fig. 1). Forty microliters of the photosensitized solution were placed in the bacterial isolate plates, adjacent to the 6 mm diameter blank paper discs (BD Biosciences, Sparks, MD).

Selection of a Panel of Pathogens

Some of the microbes used in the study were selected from a panel of human clinical ocular isolates from severe cases of bacterial keratitis treated at The Wilmer Ophthalmological Institute and maintained by the Microbiology Laboratory, Johns Hopkins University School of Medicine. The test panel of human clinical isolates maintained by our microbiology laboratory includes oxacillin-resistant *Staphylococcus epidermidis* (ORSE), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and pan-resistant *Pseudomonas aeruginosa* (PRPA). The other isolates used were individual cultures of freeze-dried microorganisms used to assist in the quality control of microbiologic media (LyfoCults; PML Microbiologicals, Wilsonville, OR), which includes ORSE strain (SE), methicillin-resistant *Staphylococcus aureus* (MRSA), oxacillin-susceptible *Staphylococcus aureus* (SA), and susceptible *Pseudomonas aeruginosa* (PA). *Candida albicans* (CA) strains were selected from human clinical isolates in the Virology Sector and maintained by
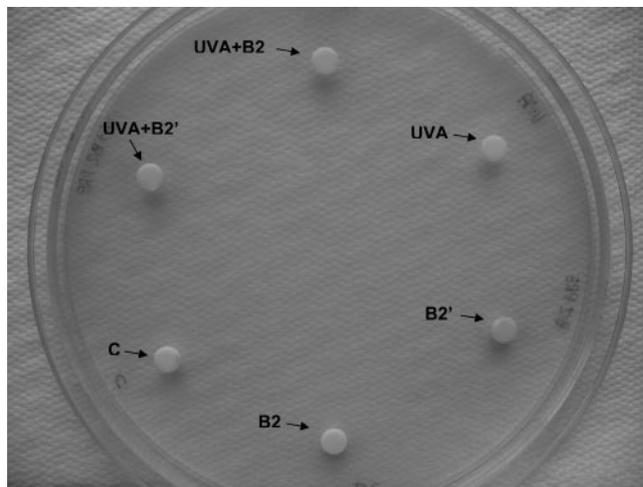

FIGURE 2. Six filter paper discs placed in a Mueller-Hinton agar plate: (C) Control; (B2) riboflavin 0.1%; (B2′) riboflavin 0.1% previously activated by UVA; (UVA) UVA light exposure; (UVA+B2) riboflavin 0.1%/UVA; and (UVA+B2′) riboflavin 0.1% previously activated by UVA+UVA light exposure.

the Microbiology Laboratory (Johns Hopkins University School of Medicine).

In Vitro Experiments: Preparation of Bacteria in Log Phase of Growth

Four or five isolated colonies of similar colony morphology grown overnight on nonselective medium (BD Bioscience) were added to Mueller-Hinton broth aliquot.

Isolates from frozen and lyophilized conditions required two subcultures before testing. These were incubated for 2 to 8 hours until growth equal to or greater than a 0.5 McFarland turbidity standard was reached. The turbidity was visually adjusted, or a turbidometer (Thermo Fisher Scientific Inc., Hampton, NH) was used, adjusted to 0.5 to 0.12 by using the broth.[41]

Inoculation of Agar Plate

Within 15 minutes, the inoculum was adjusted, and a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess inoculum. The entire surface was swabbed of agar plate three times, rotating the plate approximately 60° between streaking to ensure even distribution. Discs were applied to the agar surface by using sterile forceps.[42,43]
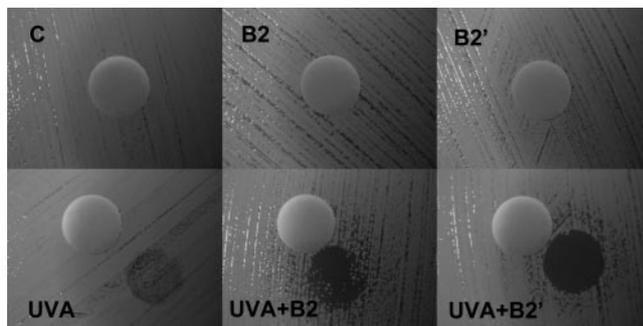

FIGURE 3. Colonies of MRSA were added to a Müller-Hinton agar plate and six filter paper discs were placed in the plate as labeled and as described in Figure 2. The subgroups C, B2, and B2′ did not show any GIZ, whereas in the subgroups UVA, UVA+B2, and UVA+B2′, we observed three different GIZs around the discs.

TABLE 1. Mean Differences in GIZ Area (mm^2) 24 Hours after Riboflavin/UVA Treatment: Group 1—Nonresistant Bacteria

	C	B2	B2 PRE	UVA	UVA+B2	UVA+B2 PRE
C	—	—	—	—	—	—
B2	0.00	0.00	—	—	—	—
B2 PRE	0.00	0.00	0.00	—	—	—
UVA	14.30*	14.30*	14.30*	—	—	—
UVA+B2	39.61*	39.61*	39.61*	25.31*	—	—
UVA+B2 PRE	40.45*	40.45*	40.45*	26.15*	0.84	—

To compare the different treatment subgroups in the first setting of microorganisms (group 1), the MSD was calculated to be 3.65 when α = 0.01. MDs in area of the GIZ are expressed in square millimeters.

* MD > MSD, indicates significant difference.

Experimental Design

We tested the riboflavin/UVA combined treatment against two settings of organisms based on their antibiotic susceptibility. Group 1 comprised nonresistant organisms: SA, PA, and SE. Group 2 was formed by antibiotic-resistant organisms: MRSA, PRPA, PRSP. CA was also tested in the same settings. The assay used was a disc diffusion susceptibility test based on the principle that a standardized inoculum of the organism is swabbed onto the surface of a Mueller-Hinton agar plate, and filter paper discs (Kirby-Bauer discs) impregnated with antimicrobial agents are placed on the agar. In our study, the discs were placed in the culture plates as reference points, to locate the areas were the riboflavin was previously instilled. The discs were also taken as a guide measurement for planimetric assays. Riboflavin drops (40 μL) were placed directly adjacent to the discs, to have an area where the light exposure would be performed. After 20 minutes of diffusion of the drops in the agar media, a beam of UVA light was directed to the selected location for 1 hour, in an attempt to photoactivate the vitamin solution or simply irradiate the area of bacterial growth with UVA alone.

These two settings of isolates were divided in six subgroups, which were tested using these Kirby-Bauer discs with: (1) empty disc (control-C); (2) riboflavin 0.1% (B2); (3) previously exposed riboflavin 0.1% (B2′); (4) UVA light exposure alone (UVA); (5) riboflavin 0.1%/UVA (UVA+B2); and (6) previously exposed riboflavin 0.1%/UVA (UVA+B2′; Fig. 2). The UVA was exposed for 1 hour, and the experiments were performed three times for each microorganism. After UVA irradiation, the agar plates were inverted and incubated for 24 hours at 34°C to 35°C in an ambient-air incubator.[43]

Analyzing Plates

After incubation, each disc and its surrounding agar area was photographed with a digital camera (Coolpix 990; Nikon Inc., Melville, NY) with a 17× macro lens attached. Digital images of each disc and the surrounding agar area were captured (AxioVision software; Axiovert 200M; Carl Zeiss Meditec Inc., Thornwood, NY), to measure the area of inhibition zone to the nearest whole millimeter.[44] The mean growth inhibition zone (GIZ) in square millimeters is inversely proportional to the minimum inhibitory concentration (MIC) of the organisms.[42]

Statistical Analysis

Descriptive statistics are expressed as mean and SD. A two-way ANOVA test was used for the analysis, assuming a theoretical normal population. Multiple comparisons were made post hoc between the different groups to find significant differences. $P < 0.01$ was determined to be significant. To minimize the type I error introduced with multiple comparisons, the Turkey honestly significant difference (HSD) test was used. This conservative test protects well against type I errors and requires the calculation of a minimum significant difference (MSD), which is the critical value. MSD is a function of the studentized range statistic, q, and requires a confidence level (α), which was set at 0.01. A mean difference (MD) between group means is compared to the MSD. When the MD is greater than the calculated MSD, the difference between the two groups compared is significant ($P < 0.01$).[14]

RESULTS

As seen in Figure 3, subgroups C, B2, and B2′ did not show any area of bacterial growth inhibition, but subgroups UVA, UVA+B2, and UVA+B2′ showed a GIZ in all microorganisms tested 24 hours after the treatment was applied.

To compare the different treatment subgroups in the first setting of microorganisms (group 1), we calculated the MSD to be 3.65 when α = 0.01. MDs in the area of the GIZ are presented in Table 1.

The mean area of the GIZ was 11.37 ± 0.92 mm^2 (SD) in the UVA/SA subgroup, 29.45 ± 0.46 mm^2 in the UVA+B2/SA subgroup, and 31.99 ± 0.72 mm^2 (SD) in the UVA+B2′/SA subgroup; for PA-susceptible microorganisms, the GIZ was 5.27 ± 1.54 mm^2 (SD) in the UVA subgroup, 7.62 ± 1.19 mm^2 in the UVA+B2 subgroup, and 7.88 ± 2.36 mm^2 in the UVA+B2′ subgroup. In the SE group, the area of the GIZ was 8.44 ± 2.39 mm^2 in the UVA subgroup, 32.37 ± 1.33 mm^2 in the UVA+B2 subgroup, and 31.05 ± 3.05 mm^2 in the UVA+B2′ subgroup. No GIZ was found in the subgroups C, B2, and B2′.

TABLE 2. MDs in GIZ Area 24 Hours after Riboflavin/UVA Treatment: Group 2—Resistant Bacteria

	C	B2	B2′	UVA	UVA+B2	UVA+B2′
C	—	—	—	—	—	—
B2	0.00	0.00	—	—	—	—
B2′	0.00	0.00	0.00	—	—	—
UVA	6.98*	6.98*	6.98*	—	—	—
UVA+B2	17.80*	17.80*	17.80*	10.82*	—	—
UVA+B2′	21.15*	21.15*	21.15*	14.17*	3.35	—

To compare the different treatment subgroups in the second setting of microorganisms (group 2), the MSD (critical value) was calculated to be 3.58 when α = 0.01. MDs in area of GIZ are expressed in square millimeters.

* MD > MSD, indicates significant difference.

TABLE 3. MDs in GIZ Area 24 Hours after Riboflavin/UVA Treatment, Comparing the Bacteria: Group 1—Nonresistant Bacteria

	PA	SE	SA
PA	—	—	—
SE	20.61*	—	—
SA	20.99*	0.38	—

To compare the bacteria responses in group 1, the MSD (critical value) was calculated to be 3.11 when $\alpha = 0.01$. MDs in area of GIZ are expressed in square millimeters.

* MD > MSD, indicates significant difference.

To compare the different treatment subgroups in the second setting of microorganisms (group 2), we calculated the MSD (critical value) to be 3.58 when $\alpha = 0.01$. MDs in the area of the GIZ are presented in Table 2.

For this setting of microorganisms being tested (the resistant bacteria), the GIZ areas for MRSA microorganisms were 9.41 ± 2.19 mm^2 in the UVA subgroup, 25.51 ± 3.67 mm^2 in the UVA+B2 subgroup, and 29.18 ± 0.14 mm^2 in the UVA+B2′ subgroup. In the PRPA group, the area of the GIZ was 0.36 ± 0.15 mm^2 in the UVA subgroup, 3.26 ± 1.20 mm^2 in the UVA+B2 subgroup, and 5.05 ± 2.08 mm^2 in the UVA+B2′ subgroup. In the ORSE group, the GIZ was 5.59 ± 2.92 mm^2 in the UVA subgroup, 21.84 ± 8.22 mm^2 in the UVA+B2 subgroup, and 29.52 ± 5.20 mm^2 in the UVA+B2′ subgroup. For the PRSP group, the area of GIZ was 12.89 ± 2.85 mm^2 in the UVA subgroup, 21.46 ± 2.64 mm^2 in the UVA+B2 subgroup, and 21.87 ± 2.27 mm^2 in the UVA+B2′ subgroup.

The area of the GIZ was significantly increased when each of the riboflavin/UVA-treated groups was compared with the control group, with both settings of bacteria. There was no GIZ in the CA culture plates, at two B2 concentrations tested for this particular microorganism (0.1% and 0.5%).

To compare the bacterial responses in group 1, we calculated the MSD (critical value) to be 3.11 when $\alpha = 0.01$. MDs in the area of the GIZ are presented in Table 3. The greatest GIZ was achieved by the SE and SA groups. The GIZ was significantly greater for SE and SA when compared with that of the PA group (SE MD = 20.61 and SA MD = 20.99). There was no significant difference between the SE and SA groups (Fig. 4).

In the second setting of microorganisms, the MSD (critical value) was calculated to be 3.29 when $\alpha = 0.01$, to make comparisons between the resistant bacteria. MDs in the area of the GIZ are presented in Table 4. The greatest GIZ was achieved by MRSA. The GIZ was significantly greater in the PRSP-, ORSE-, and MRSA-treated groups when compared with the PRPA-treated group (PRSP MD = 11.18, ORSE MD = 9.74, and MRSA MD = 9.59). However, we found no significant difference when comparing the PRSP, ORSE, and MRSA groups (Fig. 5).

DISCUSSION

The work outlined was directed toward the development of riboflavin and UVA as a novel method for the treatment of microbial keratitis. Bacterial keratitis is a cause of significant morbidity worldwide and can cause rapid and devastating visual loss.[45] Approximately 90% of cases of bacterial keratitis in the United States are caused by one of four groups of organisms (1): PA (2) SA and *Micrococcaceae*, (3) SP, and (4) *Enterobacteriaceae*.[46] *Pseudomonas* keratitis is one of the most serious corneal infections and represents one of the most threatening bacterial infections of the eye. Because of its aggressive behavior and the frequency and context in which it occurs, PA was chosen as a pathogen in this study. SA was also used because of its frequency of occurrence as a clinical pathogen. SE is an uncommon clinical corneal pathogen; however, its common presence at the ocular surface and its occasional conversion to an opportunist led to its selection as a comparison test organism. SP is commonly associated with keratitis in the United States (3%–15% of cases).[47]

The antimicrobials currently in use are sometimes problematic because of their toxic effects on the ocular surface (e.g., punctate keratitis, delayed re-epithelialization, hyperemia, chemosis) and, more important, the emerging and increasing patterns of resistance.[48,49] The treatment based on UVA light and the photosensitizer riboflavin was introduced recently by Wollensak et al.,[29,30,38] to induce collagen cross-linking in corneal ectasias. Pits et al.[50] found corneal damage at the surface UVA dose (365 mV) of 42.5 J/cm^2, and Wollensak et al.[51] described that riboflavin/UVA treatment leads to dose-dependent keratocyte damage in human corneas. Coincidentally, this approach has also been researched for pathogen inactivation via the byproducts of riboflavin after UVA exposure. This mechanism affects a large list of pathogens, including parasites and viruses.[23-26] The chemistry, toxicity, and ability of riboflavin to

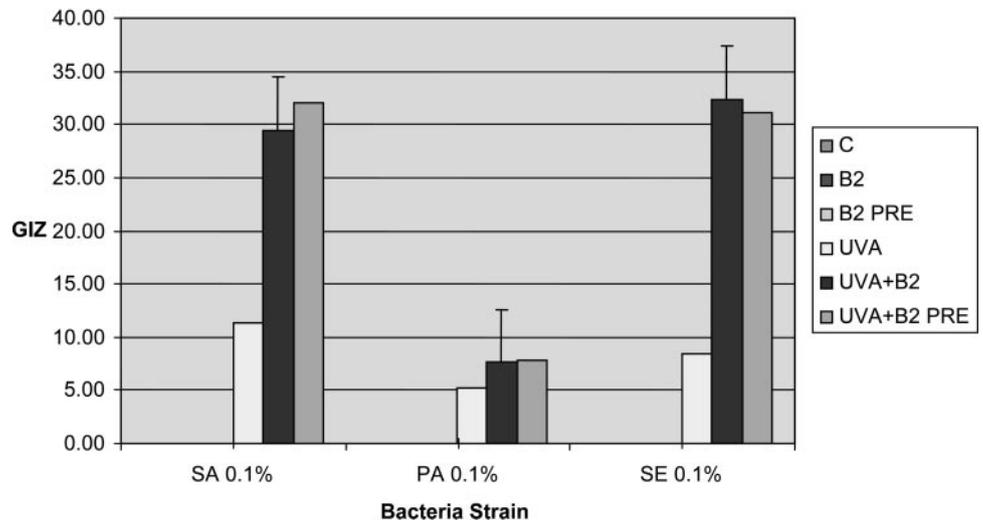## Correlation of GIZ by Susceptible Bacteria Strain

FIGURE 4. The greatest GIZ was achieved by SE and SA. The GIZ was significantly greater for SE and SA when compared with that in the PA group (SE MD = 20.61 and SA MD = 20.99).

TABLE 4. MDs in GIZ Area 24 Hours after Riboflavin/UVA Treatment, Comparing the Bacteria: Group 2—Resistant Bacteria

	PRPA	PRSP	ORSE	MRSA
PRPA	—	—	—	—
PRSP	9.59*	—	—	—
ORSE	9.74*	0.15	—	—
MRSA	11.18*	1.59	1.44	—

In the second setting of microorganisms, the MSD (critical value) was calculated to be 3.29 when $\alpha = 0.01$ to make comparisons between the resistant bacteria. MDs in the area of the GIZ are expressed in square millimeters.

*MD > MSD, indicates significant difference.

interact with nucleic acids after UVA photograph activation have been extensively studied.[52] Riboflavin and UVA (280-370 nm) may damage nucleic acids by direct electron transfer, production of singlet oxygen, and generation of hydrogen peroxide with formation of hydroxyl radicals. Pathogen DNA/RNA may be affected in the absence of oxygen. This process has proven effective against a wide range of pathogens, including bacteria, intracellular HIV-1, West Nile virus (WNV), and porcine parvovirus in preclinical studies of platelets and plasma, but this is the first report of a test of this process in culture plates against common corneal pathogens. The process also damages leukocyte DNA in a manner that makes repair by normal pathways unlikely. Riboflavin products, including lumichrome, are present and consumed in a wide range of foods and natural products in common use. UVA/riboflavin therefore may offer high efficacy with low protein damage and little toxicity. As part of an extensive evaluation of this potential new treatment, we have undertaken an experimental study to assess our assumption that the combined riboflavin/UVA could have an effect in killing common corneal pathogens.

The results in the present study showed significant in vitro inhibition growth of test isolates using combined UVA alone (UVA subgroup) and combined riboflavin/UVA treatment (UVA+B2 and UVA+B2′ subgroups) compared with the other types of treatment used in the study (B2 alone and B2 previously activated by UVA), for both setting of microorganisms, and our results showed that previously activated riboflavin and the combination riboflavin/UVA may be effective methods for the inhibition of bacterial growth in culture plates (Tables 1,

2). Seemingly, the results also demonstrate that UVA treatment alone is less effective in killing test isolates when compared with riboflavin 0.1%/UVA combined treatment and riboflavin 0.1% previously activated by UVA/UVA treatment, in the groups of bacteria tested.

Of interest, a very localized response to the area of irradiation was observed, with well-defined margins of bactericidal activity, which may be particularly useful for corneal application (Fig. 3). Riboflavin alone did not seem to have any effect as an antibacterial agent, but UVA alone may be effective against all test isolates in this study but resistant PA. The combined riboflavin/UVA treatment did not seem to have any effect on CA at the riboflavin concentrations tested (0.1% and 0.5%).

In the nonresistant group, the efficacy of riboflavin/UVA treatment was greater against SA and SE, when compared with the treatment applied against PA. In the resistant group, we found the same effect, with the treatment being more effective against the Gram-positive microorganisms than against PA. Despite those findings, we cannot exclude PA as a potential microorganism to be treated with riboflavin/UVA treatment, as it showed some GIZ in both groups (Figs. 4, 5). The levels of bacterial inactivation in platelet concentrates according to Lin et al.,[53] expressed as log reductions, was 4.5 ± 0.1 for PA, which was the lowest log reduction between the aerobic bacteria in photochemical treatments. Viable bacteria were still detected after the treatment, but they were not considered resistant to inactivation. These results are similar to ours, in that we found a smaller GIZ for PA, but we could see that the UVA/riboflavin combined treatment was still effective.

It is well known that UV light can damage the eye in several ways. Photokeratitis[54] has been shown to occur in the cornea at wavelengths of 270 to 315 nm (UVB), at power densities ranging from 0.12 to 0.56 J/cm^2. For the development of cataract, various power values have been reported in the literature at wavelengths between 290 and 365 nm.[50,55,56] The retina is damaged by thermal or visible-light–induced photochemical damage in the wavelength range of 400–1400 nm.[57] Recently, a safety study was performed by Spoerl at al.[58] to evaluate potential damage to ocular tissues during corneal collagen cross-linking by means of the riboflavin/UVA (370 nm) approach. They concluded that damage to the corneal endothelium, the lens, or the retina is not expected when the

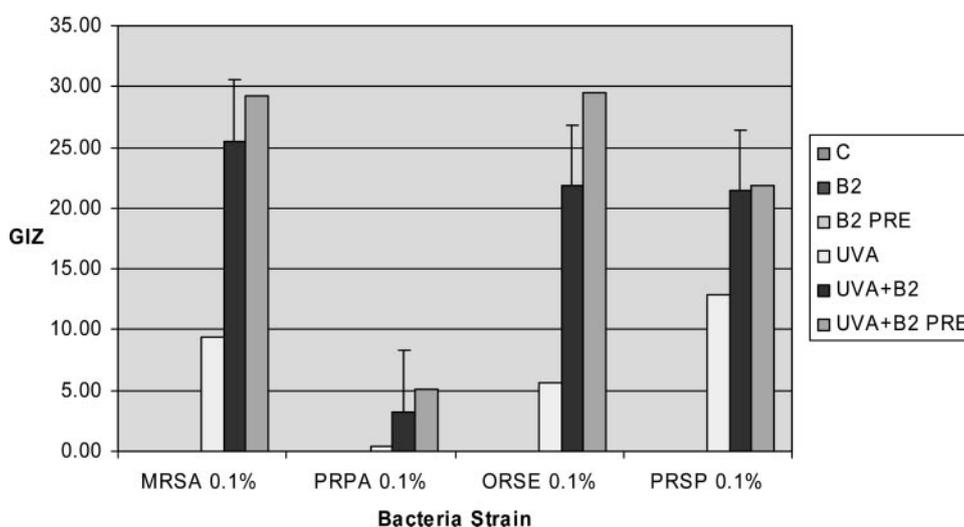## Correlation of GIZ by Resistant Bacteria Strain

FIGURE 5. The greatest GIZ was achieved by MRSA in all resistant bacteria. The GIZ was significantly greater for PRSP-, ORSE-, and MRSA-treated groups when compared with PRPA (PRSP MD = 11.18, ORSE MD = 9.74 ORSE, and MRSA MD = 9.59). We found no significant difference when comparing the PRSP, ORSE, and MRSA groups.

established criteria for the UVA/riboflavin treatment are fulfilled.[58]

There is a possibility that the riboflavin already present in the cornea serves as a natural antimicrobial mechanism. However, riboflavin concentration in the cornea is not enough to produce antimicrobial effects in overt keratitis. Since the riboflavin is photosensitive, it is more likely that the small content of corneal riboflavin will be depleted when exposed to sunlight.[59]

The data selected in these series reflect a recent effort to demonstrate a potential new treatment for corneal infection. The results demonstrated that in an in vitro environment, riboflavin/UVA treatment was effective against the clinical bacterial isolates we tested. Against PA, susceptible and resistant strains, the results were less impressive, and against CA, the treatment in the test conditions was not effective. In summary, of the experimental results, we can conclude the following:

1. UVA, UVA+B2, and UVA+B2′ treatments were successful in destroying the bacterial isolates, in a 0.1% riboflavin concentration.
2. The UVA+B2 and UVA+B2′ subgroups were the most effective approaches against all bacteria, and demonstrated augmented activity against the Gram-positive organisms.
3. Riboflavin/UVA treatment did not show any effect against CA at either 0.1% or 0.5% riboflavin concentration.

The fact that we were able to demonstrate in vitro activity of UVA/riboflavin against these bacteria—especially SA, SE, and SP—suggests that there may be ways of treating corneal infections using this approach. Since the results obtained in vitro do not always correlate with in vivo efficacy, further tissue culture models and animal studies are under way to test the efficacy of this treatment for infectious keratitis.

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