Original Article

Newly Developed Head-Mounted Ultraviolet-Activated Riboflavin Device for Corneal Cross-Linking Therapy

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Edy Wibowo[1], Datu Respatika[2], Indra Tri Mahayana[3]
Suhardjo Pawironanu[4], Muhammad Bayu Sasongko[5]
[1-5]Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada – Dr.Sardjito General Hospital, Yogyakarta, Indonesia

ABSTRACT

Purpose: To compare the effectiveness of a newly developed head-mounted device (UV-GAMA) with traditional UV-CXL in terms of bacterial and fungal eradication.

Study Design: Pre-clinical experimental study.

Place of Study: Department of Microbiology Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada, Yogyakarta, Indonesia.

Methods: Bacterial and fungal isolates were cultured, and suspensions were created to make a solution of 10^8/ml. Each test series had a non-illuminated control vessel without bacterial or fungal inoculation as a negative control. Every bacterial and fungal isolate was treated with 30 minutes of UV radiation (UV-GAMA head mounted device vs Corneal Collagen Cross-Linking/CXL device). The bacterial and fungal suspension was cultured for 24 hours and 96 hours, respectively. The number of CFU was counted for each solution, as well as the corresponding control solution, and the concentration of bacteria was calculated. One-way ANOVA and post-hoc pair wise comparison using independent t-test were used for statistical analysis. The results of each bacterial/fungal count were examined separately.

Results: Treatment with riboflavin + UV-GAMA and riboflavin + UV-CXL showed significantly reduced bacterial and fungal colonies compared to the positive control. Thus, riboflavin + UV-GAMA and riboflavin + CXL showed a similar outcome in terms of reducing bacterial colonies.

Conclusion: The newly developed UV-GAMA head-mounted device shows a comparable result to the established CXL device. This finding emphasizes that the newly developed UV-GAMA can be used as an alternative to the existing CXL device.

Key words: Corneal Cross-linking, Ultraviolet A, Riboflavin, Bacterial Ulcer, Fungal ulcers.

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Correspondence: Muhammad Bayu Sasongko
Faculty of Medicine, Public Health and Nursing,
Universitas Gadjah Mada – Dr. Sardjito General Hospital
Email: mb.sasongko@ugm.ac.id

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INTRODUCTION

The second most common cause of blindness worldwide after cataract is corneal infection-related blindness.[1] Bacterial and fungal infections of cornea are common worldwide.[2,3] The prevalence rate, however, varies not only between nations but also within a single nation and between populations.[4-5] The management of corneal ulcers has remained largely inadequate within the health systems of developing countries.[6] Treatment is started based on clinical judgment and smear results while the treatment plan can be modified depending on culture results and clinical response.[7]

Several in vitro and in vivo studies have shown that UV-activated riboflavin in Corneal Crosslinking

(CXL) can effectively eradicate bacteria and fungi that cause corneal ulcers. Martins et al, demonstrated that the riboflavin/UVA combination was effective against a range of bacterial and fungal isolates.[8] Similarly, other studies reported that corneal crosslinking effectively treated infectious keratitis, which was unresponsive to conventional therapy.[9] They also found that UVA-riboflavin therapy was effective in treating bacterial keratitis in a pilot study.[10] A successful outcome has also been reported with the use of collagen crosslinking, employing UV-activated riboflavin, in the management of advanced, non-resolving microbial keratitis.[11] These studies prove that UV-activated riboflavin in CXL can be a safe and effective alternative treatment for bacterial and fungal corneal ulcers, particularly those that are difficult to treat with conventional therapy.

The current CXL therapy requires an expensive device that might not be available in certain places, especially in developing countries. Another concern that needed to be addressed is the current protocols for CXL, which require a patient to lie down during the entire CXL procedure.[12] This might be difficult for patients who have special conditions such as chronic obstructive pulmonary disease, Cor pulmonale, heart failure, and obesity. To address these issues, we developed a portable Ultraviolet activated riboflavin CXL head-mount that is affordable and allows patients to undergo CXL therapy in a supine and prone position. The aim of this present study was to compare the objective level of bacterial and fungal population density(CF/m3) of UV-GAMA (Ultraviolet Gadjah Mada head mounted device) vs UV-CXL.

METHODS

The UV-GAMA head-mount is a portable UV device capable of performing UV-activated riboflavin for CXL procedures. The main UV device of UV-GAMA head-mount consists of 5 main subsystems, namely a rechargeable battery type 18650 with a capacity of 3000mAh (Block 1 – Fig. 1); this type of battery will last for 60 minutes to turn on the 3 watts UVA LED (Block 3 – Fig. 1). Rechargeable batteries require a power regulator so that the current released can flow constantly and can be recharged. This battery management module, called a battery management system (BMS) (Block 2 – Fig. 1), is equipped with a timer to adjust the UVA LED exposure. For recharging a battery, the BMS requires a power supply (Block 5 – Fig. 1) with a power capacity of 15 watts and an output voltage of 5V DC. The power supply has been engineered using a manual switch to replace the battery's function. Therefore, the UVA LED will not turn on during the battery recharging process. Nevertheless, once we turn the switch to power supply mode, the UVA LED will be turned on, and the charging process will be turned off. The UVA LED light beam will be passed through a flat-convex lens (Block 4 – Fig. 1) so that the UVA light beam can focus with a fixed diameter of 5mm.

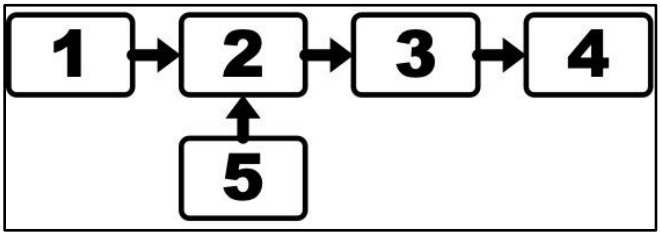

Figure 1: UV-GAMA head-mounted diagram block.

The UV-GAMA head-mounted device is also equipped with an adjustable head-mount that can fit a diverse head circumference (Fig. 2). This feature allows CXL procedures to be performed in numerous positions, such as standing, sitting, Fowler, supine, prone, lateral recumbent, and even Trendelenburg. To make the UV-GAMA head-mounted device portable and comfortable, its case is built from robust, lightweight polycarbonate material and weighs just under 240 grams.

Figure 2: UV-GAMA head-mounted device.

Bacterial and fungal isolates were cultured in the Department of Microbiology, Faculty of Medicine,

Public Health, and Nursing Universitas Gadjah Mada. Bacterial isolates consisting of Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus pneumoniae, and Pseudomonas aeruginosa were chosen for these experiments. While fungal isolates consisted of Aspergillus fumigatus, Candida albicans, and Fusarium sp. The bacteria were cultured on the plates of Columbia agar with 5% sheep blood for 24 hours, and the strains were dispersed in 0.9% NaCl to a concentration of approximately 10^8/ml. The fungi were cultured on the plates of Dextrose Sabouraud agar for 96 hours, and the strains were dispersed in PBS (GIBCO no: 14190, Invitrogen) to a concentration of approximately 10^8/ml. The 100µl suspension that contained approximately 10^8/ml bacterial and fungal isolates were placed in 96-well microtiter plate.

The Ultraviolet Gadjah Mada (UV-GAMA) head-mounted device was originally built in the Production House Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. The head-mounted-device was made from polyvinyl chloride and rubber with a variable adjustment to accommodate various head circumferences. It was powered by 3000mAh rechargeable lithium polymer battery packs to activate the 3-watt UVA LED. The UVA source in UV-GAMA head-mounted device and the UVA source in CCL-Vario Crosslinking (UV-CXL, Peschke Meditrade GmbH, Zurich, Switzerland) were measured with spectroradiometer HR4000CG UV-NIR that was certified by The National Standardization Agency of Indonesia. The UV-GAMA wavelength was measured at 373 nm, and the UV-CXL wavelength was measured at 370 nm. Thus, both UV-GAMA and UV-CXL have a comparable wavelength.

For every preparation of riboflavin/bacteria or riboflavin/fungal solution, a non-illuminated control vessel was prepared as a positive result. Each test series had a non-illuminated control vessel without bacterial or fungal inoculation as a negative control. First, we cultured the bacterial/fungal isolates in 3 different microtiter plates (plate number 1 for positive control/no treatment, plate number 2 for riboflavin + UV-GAMA treatment, plate number 3 for riboflavin + UV-CXL treatment). Plate number 4 was prepared for the negative control. Therefore, Columbia agar with 5% sheep blood or Dextrose Sabouraud agar was plated without bacterial or fungal inoculation. We added 33µl of 0.1% Riboflavin into plate number 1 and 2. Then we added 3 mW/cm^{2}of UV light (UV-GAMA for plate number 2 and UV-CXL for plate number 3) for 30 minutes with a working distance of 5 cm between the UV light and plates. Lastly, after 30 minutes of UV radiation, the bacterial suspension was moved into Mueller Hinton agar at 35˚C for 24 hours. The fungal suspension was also moved into Mueller Hinton agar at 25˚C for 96 hours. After the last incubation, the viable bacterial and fungal suspensions were counted. The number of CFU was counted for each solution, and the corresponding control solution and the concentration of bacteria was calculated. Each experiment was performed nine times.

The statistical analysis used were one-way ANOVA for each bacterial and fungi (S. Aureus, S. Epidermidis, S. Pneumoniae, P. Aeruginosa, S. Fumigatus, C. Albicans and Fusarium Sp.), followed by post-hoc pairwise comparison using independent t-test (Positive control vs UV-GAMA, Positive control vs UV-CXL). The results of each bacterial/fungal count were examined separately. The statistical significance of comparing solutions with and without riboflavin was determined. The level of significance was fixed at 5%. Analysis was performed using SPSS® 12.0 (SPSS Inc. Chicago, IL, USA).

RESULTS

In our experiments, the treatment with riboflavin + UV-GAMA and riboflavin + UV-CXL significantly reduced the bacterial colony in S. aureus, S. epidermidis, S. pneumoniae, and P. aeruginosa groups when compared to the positive control ($p<0.05$ for post-hoc pairwise comparison using independent t-test for positive control vs UV-GAMA, positive control vs UV-CXL) (Fig. 3). Thus, riboflavin + UV-GAMA and riboflavin + CXL showed a similar outcome in terms of bacterial colony reduction.

Concordantly, the treatment with riboflavin + UV-GAMA and riboflavin + CXL also showed a significant reduction of fungal colony in A. fumigatus, C. albicans, and Fusarium sp. group when compared to positive control ($p<0.05$ for post-hoc pairwise comparison using independent t-test for positive control vs UV-GAMA, positive control vs UV-CXL) (Fig. 4). Thus, riboflavin + UV-GAMA and riboflavin + CXL showed a similar outcome in terms of reduction of fungal colony.

DISCUSSION

A CXL procedure has been shown to be effective in arresting the progression of keratoconus and corneal ulcer treatment.[13-16] However, the high cost of CXL device (approximately $47,000 – $55,000), may limit the applicability of CXL procedures in some countries, especially in developing countries. To fix this problem, we developed an affordable CXL device that is applicable to remote areas.

To prove the functionality of UV-GAMA head-mounted device, we showed that both UV-GAMA-activated riboflavin and UV-CXL-activated riboflavin were equally effective in bacterial and fungal eradication. Several studies have shown that UVA-activated riboflavin is effective for bacterial and fungal eradication in corneal ulcers.[17-19] The mechanism of PDT with riboflavin that leads to the eradication of pathogens involves the generation of reactive oxygen Both UV-GAMA and UV-CXL PDT with riboflavin were very effective in eradicating S. aureus, S. epidermidis, and S. pneumoniae. Although there was a significant reduction of P. aeruginosa after UV-

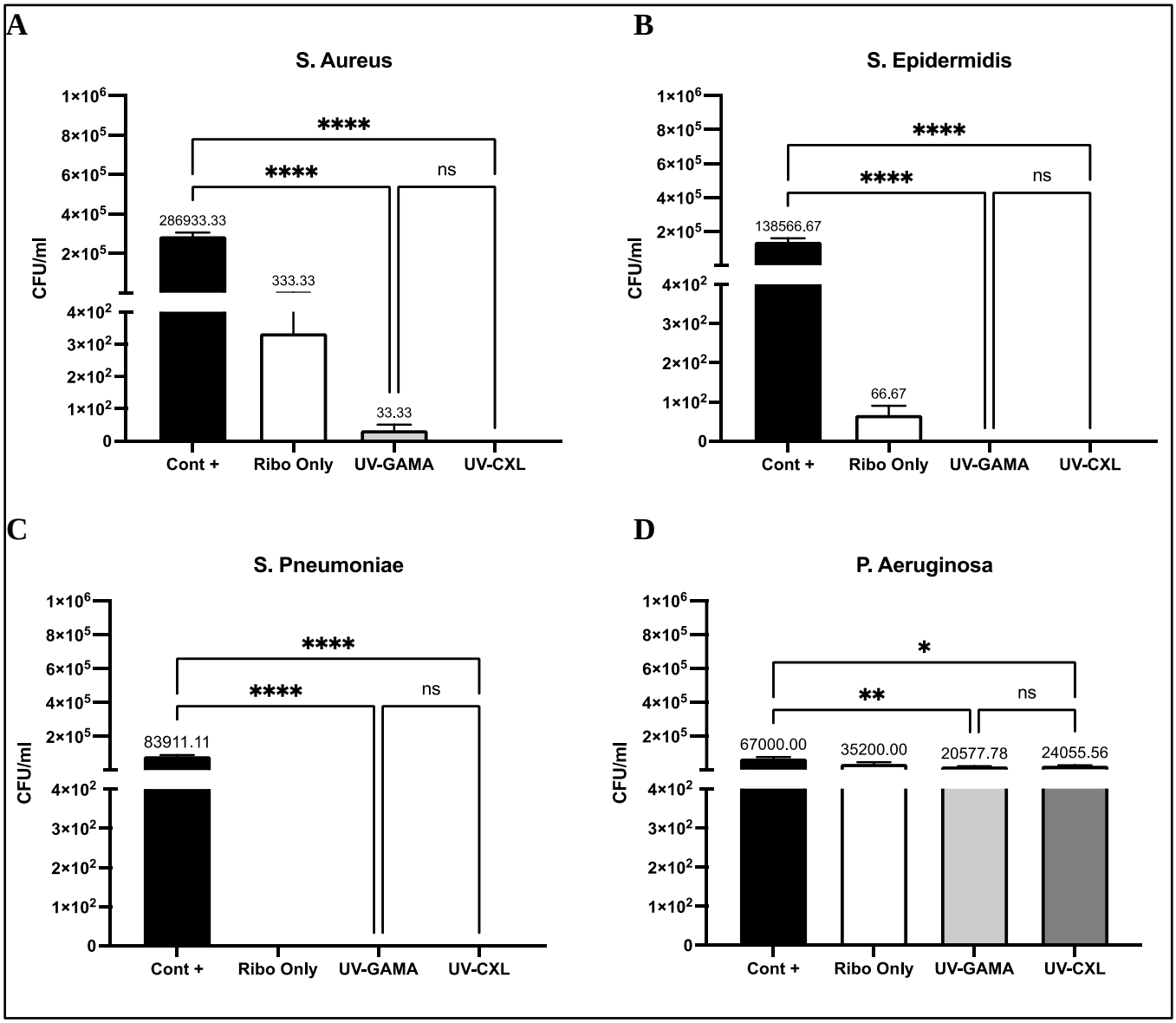

Figure 3: Experimental results of bacteria group: S. aureus (A), S. epidermidis (B), S. pneumoniae (C), and P. aeruginosa (D) cultured and treated with riboflavin only (Ribo Only), riboflavin + UV-GAMA (UV-GAMA), or riboflavin + UV-CXL (UV-CXL). Positive control (cont. +) was obtained by inoculating the bacteria without any treatment[$p<0.05$ for one-way ANOVA for each bacterium (S. Aureus, S. Epidermidis, S. Pneumoniae, and P. Aeruginosa)].

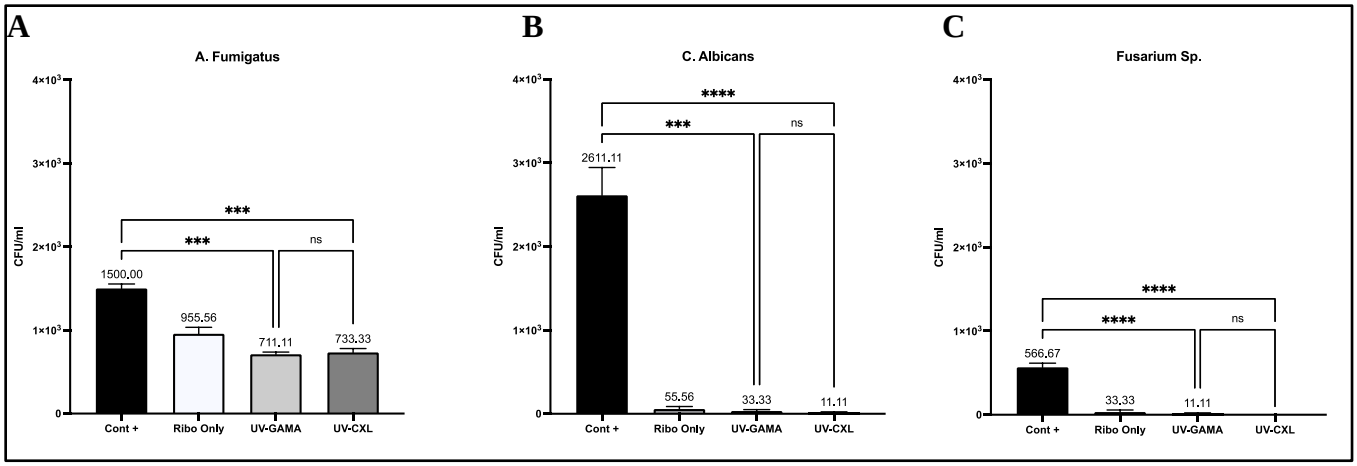

Figure 4: Experimental results of fungi group: A. fumigatus (A), C. albicans (B), and Fusarium sp. (C) cultured and treated with riboflavin only (Ribo Only), riboflavin + UV-GAMA (UV-GAMA), or riboflavin + UV-CXL (UV-CXL). Positive control (cont. +) was obtained by inoculating the bacteria without any treatment[$p<0.05$ for one-way ANOVA for each fungus(S. Fumigatus, C. Albicans and Fusarium Sp.)].

GAMA and UV-CXL PDT with riboflavin, the result did not seem as effective as other bacterial groups.

A previous study mentioned that P. aeruginosa was more resistant to PDT with riboflavin when compared to S. aureus.[22] Concordantly, another study also mentioned that PDT in rabbit eyes infected with P. aeruginosa had higher Hobden clinical scores compared to rabbit eyes infected with S. aureus after PDT with riboflavin.[23] Another study also mentioned that the bacterial killing ratios of accelerated photoactivated chromophores for keratitis cross-linking were better in S. aureus when compared to P. aurigenosa.[24] It is postulated that the complex cell wall structure may result in less photosensitivity, less penetration of light, and ROS penetrating in gram-negative organisms treated with UV activated riboflavin.[22,25]

We applied UV radiation for one session and one riboflavin dose. As a result, we were unable to draw any conclusions about how the effect of UV-GAMA PDT might differ from UV-CXL PDT at various therapy duration and doses. Further study is required for this subject.

CONCLUSION

The newly developed UV-GAMA head-mounted device shows a comparable result to the established PDT with CXL device. This finding emphasizes that the newly developed UV-GAMA can be used for PDT as an alternative to the existing CXL device. Further study is required to investigate UV-GAMA head-mounted side effects, efficacy, and patient experiences for treating bacterial and fungal corneal ulcers.

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Patient's Consent: Researchers followed the guidelines set forth in the Declaration of Helsinki.

Conflict of Interest: Authors declared no conflict of interest.

Ethical Approval: The study was approved by the Institutional review board/Ethical review board **(KE/FK/1276/EC/2021)**.

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Authors Designation and Contribution

Edy Wibowo; *Consultant Ophthalmologist: Concepts, Design, Literature search, Data acquisition, Manuscript preparation, Manuscript review.*

Datu Respatika; *Assistant Professor: Literature search, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review.*

Indra Tri Mahayana; *Assistant Professor: Literature search, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review.*

Suhardjo Pawironanu; *Professor: Concepts, Design, Manuscript review.*

Muhammad Bayu Sasongko; *Professor: Concepts, Design, Literature search, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review.*