ORIGINAL RESEARCH PAPER

Internal Medicine

EFFECTIVENESS OF BLUE-LIGHT THERAPY AGAINST STAPHYLOCOCCUS CLINICAL ISOLATES

KEY WORDS:

Kim Chung-Jong, Cho Ran*

MD PhD, Department of Internal Medicine, Ewha Womans University School of Medicine 260, Gonghangdae-ro, Gangseo-gu, Seoul, Korea *Corresponding Author

ABSTRACT

We tried to evaluate whether blue light in a specific area has an antibacterial effect on *S. aureus* and *S. epidermidis* strains, which are mainly skin flora, or contamination of the environment or equipment. A light source was constructed using LEDs with wavelengths of 410 nm, 450 nm, and 470 nm with a fixed irradiation distance of 15 mm. The irradiation time was 15, 30, 45, 60, and 120 minutes. Under 410nm light source, in all three bacterial species, the PDT-treated samples showed a significant decrease ($p<0.05$) in viability. In both bacterial species, the bacterial killing effect was more pronounced when the irradiation time was increased.

INTRODUCTION

The use of antibiotics has changed the history of not only the treatment of infectious diseases, but also the treatment of diseases. This is because the use of appropriate antibiotics, such as the use of prophylactic antibiotics before surgery, has greatly expanded the scope of other treatments.

However, as the use of antibiotics increases, various problems with the use of antibiotics follow . The most typical problem is the increase in resistant bacteria due to the use of antibiotics. In addition, problems such as side effects and C. difficile infection may occur due to the use of systemic antibiotics.

Recently, various methods of treating non-antibiotic infectious diseases have been in the spotlight[1-3]. Light therapy is a method to obtain an antibacterial effect using light of a specific wavelength. Light therapy with or without photosensitive substances is being tried a lot. Antibacterial treatment using light therapy has the advantage that it is not systemic therapy by antibiotics. On the other hand, the wavelength range used for light therapy is short, making it difficult to penetrate deep into the skin, making it difficult to treat deep or systemic infections[3,4].

In this study, we tried to evaluate whether blue light in a specific area has an antibacterial effect on S. aureus and S. epidermidis strains, which are mainly skin flora, or contamination of the environment or equipment.

METHODS

Clinical isolates of S. aureus and S. epidermidis were used among clinical isolates obtained at the hospital where the study was conducted. The bacterial isolates were collected at the participating hospital and stored at 70°C using Microbank (Pro-Lab Diagnostics, Austin, TX, USA). Frozen bacterial isolates were thawed and resuspended in Trypticase soy broth (TSB), and grown overnight. After overnight growth, 1 mL of bacterial suspension was added to 9 mL of fresh TSB and grown to mid-log phase. Bacteria were then collected by centrifugation, and resuspended in fresh TSB medium in each experiment.

After incubation, 200 μL of bacterial suspension (5×10^7 CFU/mL) was added to each well and incubated for 60 min at 37°C.

Light source

In this study, a light source was constructed using LEDs with wavelengths of 410 nm, 450 nm, and 470 nm.

Plate preparation and experimental process

The bacterial suspension prepared above was diluted and adjusted to $5X10^7$ CFU/ml. And 100 ul of this bacterial suspension was dispensed in a 96 well plate. In this case, the suspension contained in the well is pooled to a height of 3 mm. Prepare the light source prepared above to illuminate one LED per well vertically so that light can enter each well evenly. The light source was designed to fit a 96 well plate with a fixed irradiation distance of 15 mm. The irradiation time was 15, 30, 45, 60, and 120 minutes. The entire experiment was performed under minimal daylight conditions and the surface temperature of the sample was continuously recorded to avoid a significant increase in temperature. After each irradiation period, serial dilutions were performed. Survival counts for each sample were performed by triplicate.

Confirm the sterilization effect

20ul of bacterial suspension was extracted from the wells after light treatment for a certain period of time, serial dilution was performed, inoculated on a blood agar plate, incubated overnight for 24 hours, and the number of strains was measured.

Statistical analysis method

Results obtained were expressed as mean ± standard deviation and statistically analyzed using one way ANOVA tests. Statistical differences were considered significant at $p<0.05$. All experiments were performed in triplicate and repeated three times. Statistical analysis was performed using SPSS software.

Results

Baseline characteristics of bacterial isolates

The strains used in the study were clinical isolates. MSSA, MRSA, and MRSE were each collected by 10 strains. MSSA, MRSA, and MRSE were all collected from blood culture bacteria. As the strain, strains grown by different people were used. The MSSA strain was in 6 cases of infectious endocarditis and 4 cases of skin soft tissue infection. In the MRSA strain, there were 8 cases of central venous tube-related infection and 2 cases of unclear primary site. All MRSE strains were central venous duct-associated infections.

Light source evaluation

The emission spectrum of the LED array showed peak spectra at 410, 450, and 470.84 nm.

Time Kill assay

Under 410nm light source, in all three bacterial species, the PDT-treated samples showed a significant decrease ($p<0.05$) in viability. In both bacterial species, the bacterial killing effect was more pronounced when the irradiation time was increased. There was no difference in irradiation time to achieve the same mortality rate between MRSA, MSSA, and MRSE. (figure).

In other frequency, the killing effect of light emission was decreased.

DISCUSSION

Photodynamic therapy using blue light has been widely studied as an promising antimicrobial therapy. It shows positive results in in vitro trials and shows benefits as a treatment when compared to photodynamic therapy and UV light[1,5-7].

Nevertheless, it is difficult to identify ideal luminescence parameters to adopt with confidence because the experimental protocols using blue light for bacterial inhibition are very diverse. Some investigations using blue light have reported 100% inhibition of S. aureus proliferation in in vitro culture[1,3,8,9].

Previous studies have used various wavelengths and various strains and have used various methods such as adding a photosensitizer such as riboflavin.

The present study successfully demonstrated the efficacy of blue LEDs in in vitro inactivation of Staphylococcus aureus and MRSE. Blue light of different wavelengths has been successfully used to inactivate a wide range of bacterial pathogens in vivo and in vitro. The 405 nm blue led array alone has been successfully used for in vitro inactivation of a wide range of bacterial pathogens[2,5,10]. Even a 470 nm blue LED alone has been used to kill methicillin-resistant Staphylococcus aureus in vitro. Similarly, PDT using a 470 ± 20 nm blue led was found to be successful in in vivo inactivation of P. aeruginosa[3,7,8].

A longer irradiation time was required to kill P. aeruginosa than Staphylococcus aureus. This has been shown to be due to the complex cell wall structure of the gram-negative species, which allows less photosensitizer and less light to penetrate the cell wall structure of the gram-negative species. Blue light with a killing soft tissue penetration depth of 2-3 mm can be used to treat superficial wound infections.

As the high patterns of antibiotic resistance of these bacterial species effectively defeat the use of antibiotics, these new therapeutic modalities could prove effective in treating wound infections. Further insight into PDT from in vivo experiments is needed to ensure the effectiveness of antimicrobial PDT.

Previous studies have used various wavelengths and various strains and have used various methods such as adding a photosensitizer such as riboflavin. However, as can be seen in the literature, many experiments show only the relative inhibition of in vitro culture when exposed to different patterns of blue light emission[5-7,11].

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