ORIGINAL ARTICLE

Combination of Curcumin Photosensitizer With Laser Diode to Reduce Antibiotic Resistant Bacterial Biofilms

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ABSTRACT

Introduction: : Photodynamic Inactivation is a method for inactivating bacteria using a combination of light and photosensitizer (PS) with the appropriate spectrum. This study aims to determine the energy density to reduce Methicillin Resistent Staphylococcus aureus (MRSA) bacteria with the addition of turmeric extract as a PS. **Methods:** The samples were divided into 3 groups, namely T0 untreated control group, T1 laser diode treatment group, T2 laser diode with curcumin extract treatment group. Treatment uses a 403 nm diode laser with a biofilm life of 48 hours. Samples was treated with variation of irradiation time of laser 30 s, 60 s, 90 s, 120 s and 150 s. **Results:** The results of the absorbance test showed that the uptake of turmeric extract on the 403 nm diode laser was 95%. The results of the treatments showed that the 403 nm diode laser irradiation with an energy density of 13.56 J/cm^2 with the addition of turmeric extract to the MRSA biofilm resulted in a reduction percentage of 83.93% of the biofilm, greater than the 403 nm diode laser treatment with an energy density of 10.17 J/cm^2 without turmeric extract of 81.67%. So the addition of PS extract of turmeric increases the effectiveness of photoinactivation of biofilm bacteria that are resistant to antibiotics.

Keywords: Photoinactivation, MRSA, Curcumin, Laser diode

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INTRODUCTION

Methicillin resistant Staphylococcus aureus (MRSA) is a Staphylococcus aureus bacteria that has been resistant to methicillin antibiotics (1). MRSA is able to form self-protection against antibiotics by forming a biofilm layer, that structured communities of bacterial cells and are attached to each other to form colonies capable of producing a hydrated polymer matrix from exopolymeric substances, polysaccharides, nucleic acids and proteins on a biotic or abiotic surface (2). Bacteria that have been resistant to antibiotics will be difficult to treat.

Photodynamic Inactivation (PDI) is a method used for microbial inactivation by irradiating photons of light (3,4). There are three main factors that play a role in the success of PDI, light source, photosensitizer (PS), and free radicals that are reactive to biological systems such as cells (5,6). Various light sources have been used in PDI, including the use of diode lasers at wavelengths in the visible light range. The successful use of laser diode (7,8) and light emitting diode (LED) (9,10) for bacterial photoinactivation has been reported.

PS is a non-toxic chemical that has a certain absorbance and will activate Radical Oxigen Singlet (ROS) through photooxidation when exposed to a light source with a wavelength that matches its absorption (11). Exogenous PS helps the formation of excess ROS thereby increasing the probability of damage to bacterial cell membranes that will cause bacteria to die, one example is curcumin (12). Curcumin has an absorption spectrum at 350 nm to 500 nm. Various studies have reported the success of PDI with curcumin PS in bacterial pathogens (12, 13). Continuing previous research with PDI (14), the aim of this study was to determine the effectiveness of curcumin PS with diode laser activator to reduce MRSA bacterial biofilm.

MATERIALS AND METHODS

Biofilm Development Assay

The pure culture of MRSA approximated 108 CFU / mL or 1.0 McFarland Standard was used for this study (14). 100µL bacteria culture was placed in 96-well microplate and was added 20 µL 20% sucrose solutions. Samples were treated with a crystal violet assay, rinsed by posphat buffer saline (PBS) with pH 7.4 three times, added 100 µL 1% crystal violet solutions and rinsed by saline water, added 50µL 33% Glacial Acetic Acid (GAA) solution and measured using a micro plate reader 595 nm (14). Then 0.3% curcumin extract (38.4 mg) was dissolved with DMSO 10% 1 ml, added with distilled water until the total volume reached 10 ml.

Sample Treatments

The samples were divided into 3 groups, namely T0 untreated control group, T1 laser diode treatment group, T2 laser diode with curcumin extract treatment group. Treatment uses a 403 nm diode laser with a biofilm life of 48 hours, with variation of irradiation time of laser 30; 60; 90; 120 and 150 s (3.39; 6.78; 10.17; 13.56 and 16.95)J/cm^2. The light source used is laser diode from Sony with an output wavelength of 403 nm, power output 22.6 mW and spot area 0.2 cm^2. The percentage of biofilm reduction was calculated from: $\textit{Percentage biofilm reduction}\ (\%) = \frac{\textit{Control - Treatment}}{\textit{Control}} \times 100\%$. The data would be tested statistically using ANOVA one-way test. The significant value p = 0.05 was used as a determinant of statistical conclusion results.

RESULTS

The results showed that curcumin had an absorption spectrum at 350 nm to 500 nm. Based on the results of the UV-Visible spectrophotometric test, it showed that the extract absorption of curcumin at the 403 nm diode laser light source used was (15): Transmitance = exp (-absorbance) = 0.0497; Thus, % absorbance = (1-0.0497) x 100% = 95%. Table I show the result of diode laser and curcumin treatment for biofilm reduction. Figure 1 and 2 show the results of the percentage reduction of MRSA biofilm with a diode laser and diode laser with adding PS curcumin. The results of the treatments showed that the 403 nm diode laser irradiation with an energy density of 13.56 J/cm^2 with and without the addition of turmeric extract to the bacteria resulted in a biofilm reduction percentage of 83.93% and 81.67%.

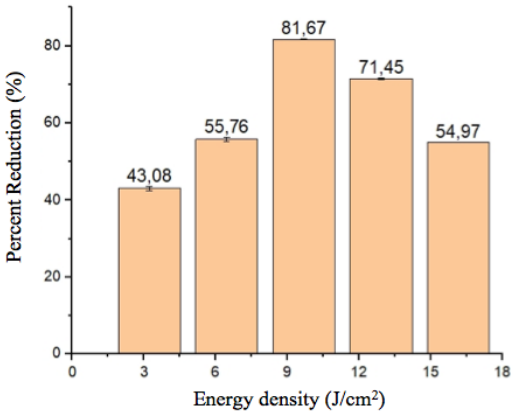

Figure 1 : The results of the percentage reduction of MRSA biofilm with a diode.

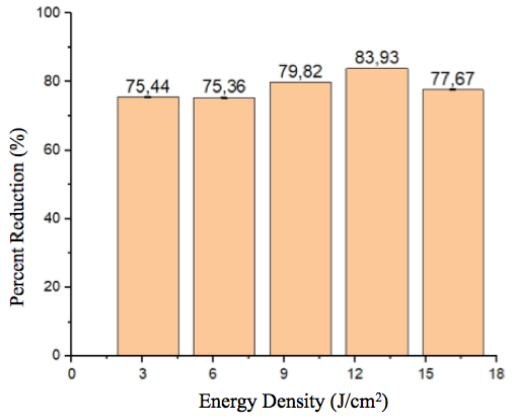

Figure 2 : The results of percentage reduction in MRSA biofilm with a combination of diode laser and curcumin treatment.

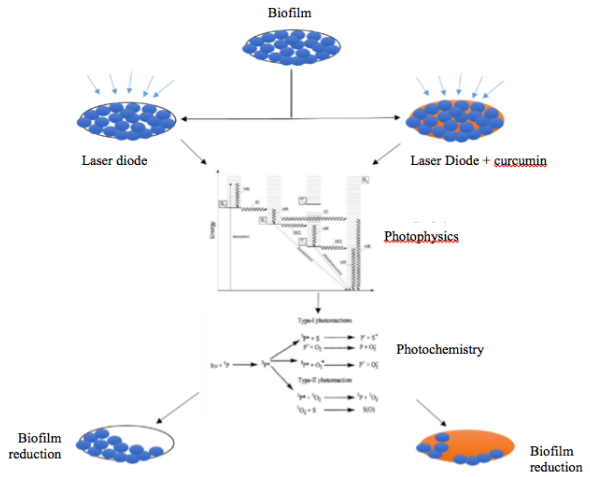

Figure 3 : The photodynamic Mechanism of inactivation on an MRSA biofilm.

DISCUSSION

MRSA bacteria are bacteria from the S. aureus strain that are resistant to methicillin antibiotics (16). The mechanism of resistance in MRSA bacteria is due to the structure of PBP2 (Protein Binding Penicillin) in bacteria mutating (17). The resistance of Staphylococcus aureus bacteria is caused by the presence of PBP2a in bacteria although in relatively small numbers. Figure 3 show the photodynamic Mechanism of inactivation on an MRSA biofilm.

PDI is a modality of antimicrobial therapy utilizing light and a PS. A photophysical process occurs in the form of absorption of photons on electrons which can cause the PS molecule to become active. When the photon energy is absorbed by the curcumin PS molecule, there will be an excitation process to a higher energy level (1P *) and a spin reversal occurs through the intersystem crossing to the triplet excitation level (3P *) (18). Then it triggers a photochemical reaction. In type 1, optically excited PS molecules react directly with substrates such as linoleic acid, cell membranes or molecules, and transfer a proton or electron to form radical anions or cations. These radicals will react with oxygen and produce reactive oxygen (ROS). In type 2, the triplet PS transfers its energy directly to the oxygen molecule to produce singlet oxygen. Singlet oxygen is very reactive, if it sticks to the membrane it causes peroxidation of lipids and membrane proteins resulting in cell leakage and lysis (19).

In biofilms, the mechanism of bacterial death occurs due to disruption of the peptidoglycan constituent components of the bacterial biofilm, damaging the bacterial membrane layer, so that ion exchange is uncontrolled and causes the bacterial cell death. In addition, curcuminoids are phenolic compounds. This phenol derivative will interact with the bacterial cell wall, then it will be absorbed and penetrated into the bacterial cell, causing protein precipitations and denaturation, consequently lysing the bacterial cell membrane.

CONCLUSION

The absorbance test results showed that the uptake of turmeric extract on the 403 nm diode laser was 95%. The results of the treatments showed that the 403 nm diode laser irradiation with an energy density of 13.56 J/cm^2 with the addition of turmeric extract to the MRSA biofilm resulted in a reduction percentage of 83.93% of the biofilm, greater than the 403 nm diode laser treatment with an energy density of 10.17 J/cm^2 without turmeric extract of 81.67%. So the addition of PS extract of turmeric increases the effectiveness of photoinactivation of biofilm bacteria that are resistant to antibiotics.

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