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Research Report

The duration effectivity of diode laser 405 nm with erythrosine photosensitizer in killing *Streptococcus mutans*

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ABSTRACT

Background: *Dental caries is a multifactorial disease caused by the interaction of pathogenic bacteria, especially Streptococcus mutans. Various caries prevention chemically and mechanically can help reduce the number of bacteria. However, this approach does not cover the entire bacterial population with a photodynamic therapy approach with the addition of photosensitizers and low-intensity light radiation with the right wavelength able to eliminate the number of S. mutans bacteria.* ***Purpose:*** *To determine the duration of effective irradiation time on a 405 nm diode laser with erythrosine photosensitizer in killing S. mutans bacteria.* ***Methods:*** *This research was an experimental laboratory type with 25 S. mutans samples divided into 5 groups treated with erythrosine photosensitizer 0.1 mg / ml and 405 nm diode laser irradiation with 45 seconds, 60 seconds, 75 seconds duration and no erythrosine photosensitizers and without irradiation. The growth of S. mutans bacteria were calculated then the data were analyzed statistically.* ***Results:*** *the research found that S. mutans bacteria decreased gradually in each group; erythrosine without photosensitizer and without irradiation had the highest average growth rate of 71.6 CFU / ml; without photosensitizer with irradiation of 40.6 CFU / ml; irradiation with photosensitizer 45 seconds at 20.6 CFU / ml; irradiation with 60 seconds photosensitizer at 11 CFU / ml; and irradiation with 75 seconds photosensitizer at 0 CFU / ml. In statistical tests, the data are normally distributed and homogeneous. There are significant differences between groups.* ***Conclusion:*** *The effective duration in this study was found at 75 seconds.*

Keywords: *Exposure duration; laser diode; erythrosine photosensitizer; Streptococcus mutans.photodynamic therapy*

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INTRODUCTION

Dental caries is a multifactorial infectious disease as the result of pathogen bacteria interaction, especially *Streptococcus mutans* in oral colonization. Caries prevention could be done by controlling dental plaque accumulation by mechanical means. Treatment of caries lesions include infected dentine removal by restoration of the affected teeth with various restorative materials options such as amalgam, composite resin and glass ionomer cement. Antibacterial chemical agents such as chlorhexidine also help reduce the number of pathogenic bacteria. However, this preventive approach cannot reach the entire population. Thus, the tooth cavities are still formed[1]. An approach is needed which can provide the possibility of reducing oral bacteria number efficiently with minimum damage to dental health (preventive approach) and secondary caries formation prevention which reduces the possibility of material substitution and pulp inflammation (curative approach).

Photodynamic therapy provides new modalities to reduce pathogenic bacteria and prevent new dental caries lesions. To achieve low bacterial cavity in order to achieve successful treatment, cavity disinfection is carried out. Cavity disinfection is a process to eliminate microbes contained in the cavity, before it is restored [2]. As a fast, non-toxic, and non-invasive antimicrobial approach, photodynamic therapy has emerged as a suitable process to reduce bacterial contamination and increase treatment success. Another advantage of photodynamic therapy compared to antibiotics is that bacteria do not form resistance to oxygen species because there is no target specificity.

The effectivity of photodynamic therapy based on few aspects: photosensitizer ability to interact with bacteria membrane, its ability to penetrate and action inside cells, and the formation of ROS (Reactive Oxygen Species) around bacterial cells by photosensitizer illumination[1]. Photodynamic therapy for caries bacteria is carried out as a process, where microorganisms are added to the

photosensitizer drug and then irradiated with low intensity lamps and appropriate wavelength [3]. The effectivity of this therapy depends on its strength level, duration, light absorption from tissue, photosensitiser concentration and distance of the tip of the photodynamic device to the target cell.

A research by (Ara´ujo Neto *et al.,* 2017)[4] reported that the usage of photodynamic therapy with methylene blue photosensitizer for 90 seconds, significantly could decrease the number of *Streptococcus mutans*. Based on a research results by (Citrasari, 2018) [5] reported that the 75-second irradiation time by using a 650 nm diode laser and methylene blue photosensitizer was the most effective irradiation time in killing *Streptococcus mutans* bacteria. Research by Astuti, et al in 2016 reported that photodynamic therapy with chlorophyll k-link photosensitizers using a 405 nm diode laser, with an irradiation distance of 1.5 cm, decreased the number of *Streptococcus mutans* by 74% with a long exposure time of 75 seconds[6].

Based on the existence of various opinions about the advantage of photodynamic therapy using erythrosine photosensitizer material. and based on the research obtained by the effective laser irradiation time of the diode with chlorophyll and methylene blue photosensitisers at 75 seconds. So, we conducted the research on the most effective irradiation time on the Streptococcus mutans bacteria using photosensitiser erythrocin, with 75 seconds irradiation time. It is hoped that this research can provide new innovations in clinical practice in the treatment of carious lesions in increasing antimicrobial effects using 405 nm diode laser beam with the most effective irradiation time using erythrosine photosensitizers optimal and prevent the risk of re-infection.

MATERIALS DAN METHODS

This is a laboratory experiment research with post-test control group design, where sample will be treated in the form of laser diode 405 nm with power 50mWin various durations then after treatment, the number of *S. mutans* bacteria colonies was observed. The sample used was *Streptococcus mutans*, divided into 25 samples in 5 groups. Diode lasers are semiconductor devices that emit diode rays. The wavelength used in this study was 405 nm which was exposed to *Streptococcus mutans* bacteria with a power of 50mW and a beam distance of 20 mm. Photosensitizer is a non-toxic photoactive coloring agent which is erythrosine B which has been diluted with deionized water to a concentration of 0.1 mg / ml or equivalent to 0.11 mM [7].

The independent variable was the exposure duration of 405 nm laser diode. the duration of exposure to photodynamic therapy was the length of time the device is irradiated as photoactivation on *Streptococcus mutans* which has been given erythrosine photosensitizer, which is used as an antibacterial with a long exposure time of 45, 60, and 75 seconds. The dependent variable in this research was the number of *Streptococcus mutans* bacteria colonies that lived after the application of erythrosine photosensitizer material, and laser diode irradiation on Agar Nutrient Media (MHA). The number of *Streptococcus mutans* bacteria were measured in units of Colony Forming Units per millimeter (CFU / ml).

Streptococcus mutans bacteria obtained from the stock Research Center of the Faculty of Dental Medicine, Universitas Airlangga, Surabaya, were cultured in the Brain Heart Infusion Broth (BHIB) medium and incubated at 37°C for 48 hours anaerobic atmosphere. Bacterial cultures were standardized with a Mc Farland scale of 0.5 (1.5 x 108 CFU / ml). The cultures were transferred to 25-wells consisting of 90 µl BHIB and 20 µl bacterial cultures. Samples were grouped and treated differently for each well: Group I are samples without photosensitizer, without irradiation; Group II are samples with 90µl erythrosine photosensitizer without irradiation; Group III are samples with 90µl erythrosine photosensitizer and 45 seconds irradiation of diode laser; Group IV are samples with 90µl erythrosine photosensitizer with 60 seconds irradiation of diode laser; Group V are samples with 90µl erythrosine photosensitizer with 75 seconds irradiation of diode laser.

A total of 50µl of the treated sample was taken and planted on a nutrient agar and flattened using a spreader. Incubated for 48 hours at 37 ° C in an anaerobic atmosphere. The number of *Streptococcus mutans* growing colonies was counted manually by the standard plate count method and averaged in each group. Data were analyzed using the SPSS Program consisting of normality tests: Shapiro-Wilk, homogeneity tests: Levene test, Kruskal-Wallis test, and *follow-up* tests with Mann-Whitney, with a p value of 0.05

RESULTS

Data from overall the total sample were 25 samples that have been collected, divided into 5 groups, groups I, II, III, IV, and V. In each group there were 5 samples of *Streptococcus mutans* colonies that are given different treatment in each group Figure 1.

In the normality test using Shapiro-Wilk test in each group, if it is found that the value of $p > \alpha = 0.05$ can indicate

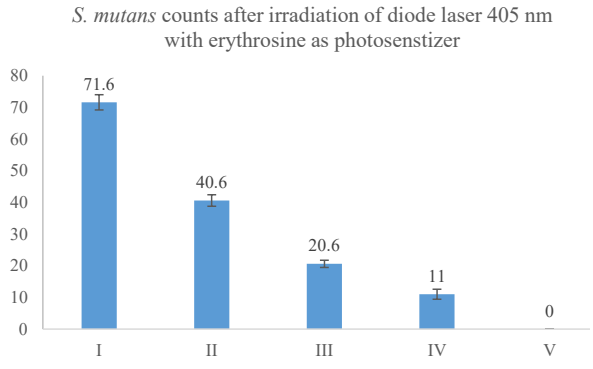

Figure 1. Diagram mean and standard deviation.

Table 1. The Mann Whitney test results in groups I, II, III, IV and V

Group	I	II	III	IV	V
I		0.009	0.009	0.009	0.005
II			0.009	0.009	0.005
III				0.009	0.005
IV					0.005
V					

normal data distribution. In Table 2, the results obtained in group I p = 0.787, group II with p = 0.254, group III with p = 0.814, group IV with p = 0.967 and in group V the data is constant and cannot be calculated for p value, thus it can be drawn conclusions for all data groups are normally distributed.

In the homogeneity test using Levene's test, the result p = 0.006, which means the sample is not homogeneous due to the value of p <0.05, then proceed with the Kruskal Wallis test to determine the comparison of the average number of cells between groups and if there are significant differences. Obtained significance of 0,000. It can be concluded that there are significant differences between groups, indicated by the value of p <0.05.

The statistical analysis was continued by calculating the Mann Whitney Test to determine differences between groups. From Table 3. the whole group showed a significant difference which indicates that there are significant mean differences between groups (P <0.05).

DISCUSSION

Photodynamic therapy consists of photosensitive molecules that absorb light with adequate wavelengths. This light-excited molecule, the photosensitizer, can induce two reactions that occur simultaneously (Type I and II reactions). In Type I reactions, excited photosensitizer triplets react with biomolecules found in *Streptococcus mutans*, such as nucleic acids, lipids, and proteins by transferring electrical charges that produce radicals and radical ions. These radicals react with molecular oxygen to form reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals, and superoxide anions. In a Type II reaction, photosensitizers in the excited triplet state, transferring energy to oxygen in the base triplet state (a process called triplet destruction), forming singlet oxygen[8]. Type 1 reactions and type 2 reactions can damage cells and bacterial metabolic processes, causing bacterial death. The longer the irradiation time of the laser diode, the more light absorbed by photosensitizers.

This research showed that erythrosine has the potential of photosensitiser for PDI (photodynamic inactivation) against Gram (+) bacteria. The maximum light absorbance is at 530 nm in aqueous solution and undergoes photodegradation. Erythrosine maintains a positively charged surface. Therefore, it can directly lead to Gram-positive and Gram-negative bacteria. The positively charged photosensitizer shows high affinity towards the negatively charged outer bacterial membrane. This mainly induces local damage, which helps penetration and erythrosine's ability to initiate photochemical degradation reactions is well explained [9].

Erythrosine has some antimicrobial activity against Gram-positive and Gram-negative bacteria. In addition, erythrosine also included in a cyclic class compounds called xanthenes, which absorb light in the visible region, and the erythrosine ability to initiate photochemistry. The ability of erythrosine in non-oral microbial sensitization to kill with light supposed to be effective when compared with other types of photosensitisers. Erythrosine also has advantages over other photosensitizers in the form of erythrosine itself already has a receptor that is able to target dental plaque and has been declared oral safe [10].

Based on this research, it is known that increasing the exposure of erythrosine photosensitizers can increase effectiveness in killing *Streptococcus mutan*s. Overall *Streptococcus mutans* has been successfully killed by photodynamic therapy in the 75th second. This showed that to achieve maximum therapeutic levels in killing *Streptococcus mutans* takes time gradually. The increase of irradiation duration causes an increase in good disinfection results, because with short irradiation time causes the ROS concentration to be formed low [11]. The more light absorbed, causing erythrosine in the ground phase (singlet) state (S0), the more that will react. This results in energy transfer and causes erythrosine in the ground (singlet) state which turns into the excited singlet state (S1) more. The more erythrosine in the excited singlet state (S1) phase, the more erythrosine in the triplet state (T1) phase will also increase, causing more ROS and singlet oxygen produced. This causes more damage to the bacterial cell wall and bacterial cytoplasmic membrane, due to lipid peroxidation, thus causing more and more bacterial death.

However, an experimental and clinical research conducted by Shi, 2017, stated that there are various anti-inflammatory effects and immunological activities on the use of PDT (Photodynamic Therapy) for too long [7]. There is an irritant reaction to the skin and mucosa after photodynamic therapy. This showed that the use of photodynamic therapy with any kind of photosensitizers needs to time limited to prevent side effects[8].

CONCLUSION

The most effective duration in killing *Streptococcus mutans* in illumination of laser diode 405 nm with erythrosine photosensitizer is 75 seconds compare with 45 and 60 seconds.

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