Evaluation of the efficacy of AmPDT of oral microorganisms with Photogem® associated to red LED (λ640ηm±5ηm): In vitro

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ABSTRACT

The aim of this study is to evaluate the Antimicrobial Photodynamic Therapy (AmPDT) of microorganisms mediated by red LED (λ640 ± 5 ηm, 120 mW, spot of 0.785 cm^2) associated with Photogem®. Microorganisms of the posterior dorsal region of the tongue and oral mucosa were collected and inoculated in 8 mL of TSB medium overnight followed by inverse homogenization. Culture plates with 24 wells were used for the irradiated and non-irradiated species. Each well received 400 µL of the suspension containing the microorganisms. In eight wells no Photogem® was used and they were the irradiated and non-irradiated controls. The remain of the wells had Photogem® applied with a pre-irradiation time of 5 min in concentrations of 40, 20, 10, 5 e 2.5 µg/mL. LED was applied for 5 min and 45 sec (50 J/cm^2). After agitation, 200 µL were withdrawn from each well and colorimetric measurements were immediately taken. Other 200 µL were withdrawn from the wells after 1 hour in bacteriological incubator for a second measurement. The best results found were for 40 µg/mL of Photogem® associated with LED irradiation and immediate (36.7%) and for 5 µg/mL with LED irradiation with measurements one hour after incubation (42.8%). This study suggests that antimicrobial photodynamic therapy with Photogem® associated to red LED can be a potential mechanism of control of oral microorganisms.

Keywords: LED, infections, photosensitizing agents, mouth.

1. INTRODUCTION

The oral cavity is colonized by a highly diverse community of microorganisms which are mostly harmless. However, some have been identified as the etiological agents of a variety of diseases both within and outside of the oral cavity, ranging from those with a very high prevalence but life-threatening such as caries and periodontal diseases, to those with a lower prevalence but a high mortality including bacterial endocarditis, pneumonia and cardiovascular diseases[1].

Antimicrobial Photodynamic Therapy (AmPDT) or lethal photosensitization has been used recently in dentistry to control of periodontal pathogens, root canal disinfection and for the treatment of some oral infections, representing an alternative to antibacterial, antifungal and antiviral treatments, especially against resistant organisms to conventional treatment. AmPDT is a therapeutic technique that involves the use of a photosensitizer active by visible light with adequate wavelength in the presence of oxygen. Photosensitizer exposed to light results in the formation of oxygen reactive species which may cause cell damage or death[2-3].

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The development of resistance against AmPDT seems unlikely because the singlet oxygen and all other generated free radicals interact with diverse microbial cell structures by different mechanisms inactivating a wide array of pathogenic microorganisms[3-6]. Infection control using different types of photosensitizers (methylene blue, rose of bengal, chlorophyll, phthalocyanines and others) have been effective on both Gram negative and Gram positive microorganisms[4]. Red Light Emitting Diode (LED) increased the inhibitory AmPDT effect of methylene blue in prokaryotic and eukaryotic cells[5].

However photosensitizer concentration and light parameters yet have to be established in order to provide satisfactory results in AmPDT treatment for oral cavity microorganisms. Photogem® is a hematohematoporphyrin-derived compound that has been recently tested against some oral species[3]. Non-coherent light sources, represented by LEDs, have a low cost and simple technology compared to lasers resulting in more accessible treatment[3,7].

It would be of interest in dentistry to have an alternative way of reducing the number of microorganisms immediately before an oral interventions, specially previously to surgeries, minimizing the risk of infections during and after the procedure. Therefore, the objective of this study was to evaluate the effect of lethal photosensitization of microorganisms of the oral cavity with Photogem® associated with red LED (λ640ηm±5ηm).

2. MATERIAL AND METHODS

Microorganisms from the oral mucosa and posterior dorsal region of the tongue of three patients were collected with sterilized swabs and inoculated in 8 mL of TSB medium overnight, followed by inverse homogenization. Two swab samples were collected from each patient. Culture plates with 24 wells were used for the irradiated and non-irradiated species. Each well received 400 µL of the suspension containing the microorganisms. In eight wells no Photogem® was used and they were the irradiated and non-irradiated control. The remaining wells had Photogem® applied with a pre-irradiation time of 5 min in concentrations of 40, 20, 10, 5 e 2.5 µg/mL. A prototype red LED (São Carlos, SP) was used and the LED irradiation time in these wells was of 5 min and 45 s. The LED parameters used are presented in Table 1. After agitation, 200 µL were withdrawn from each well and colorimetric measurements were immediately taken. Other 200 µL were withdrawn from the wells after 1 hour in bacteriological incubator for a second measurement. Those measurements were carried out with the spectrophotometer SpectraMax (Medical Device).

Table 1: LED Parameters.

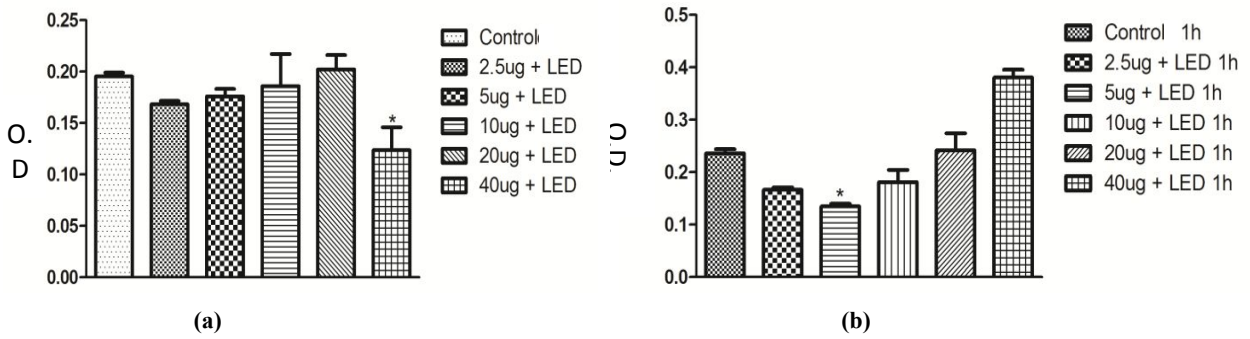
Parameters	
Wavelenght	640ηm±5ηm
Output	120mW
Irradiation time	5´45´´
Tip	Straight
Energy Density	50

3. RESULTS

The best results in the reduction of microorganisms was found in the group treated with 40 µg/mL Photogem® associated with LED irradiation and immediate measurement (36.7%) and in the 5 µg/mL group with LED irradiation with measurements one hour after incubation (42.8%), both with statistical significant difference ($p<0.05$) in ANOVA with Dunnett and Tukey multiple comparison tests (Table 2 and Figs1,2). No statistical difference was found between the Photogem® groups without LED irradiation and the control.

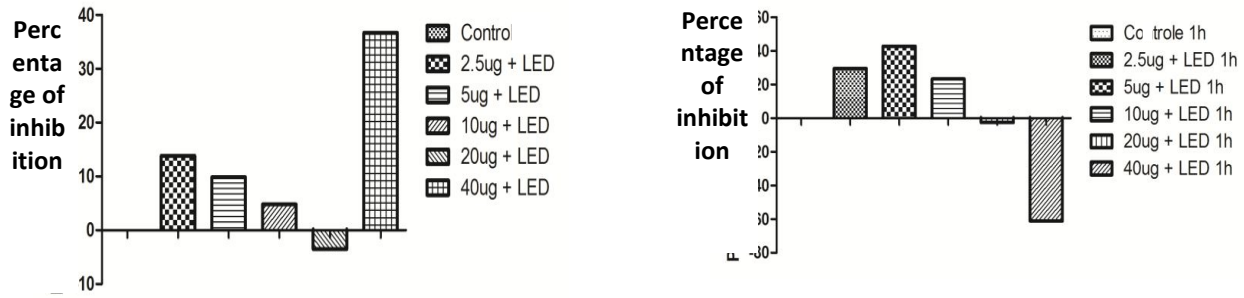
Table 2: different experimental groups

	Imediate						1 Hour					
GROUPS	Control	2.5ug	5ug	10ug	20ug	40ug	Control	2.5ug	5ug	10ug	20ug	40ug
	0.1877	0.1615	0.1902	0.1952	0.2218	0.1622	0.2231	0.1637	0.1436	0.202	0.2937	0.3539
	0.1979	0.1712	0.1696	0.1276	0.2097	0.0851	0.2507	0.1599	0.1263	0.2068	0.2503	0.407
	0.2003	0.172	0.1683	0.2348	0.175	0.1236	0.2343	0.1756	0.1349	0.134	0.1821	0.3804



(a) (b)

Figure 1: Results of the Optical density for Photogem® + LED with immediate (a) and 1 hour after the readings (b).



(a) (b)

Figure 2: Inhibition Potential for Photogem® + LED with immediate (a) and 1 hour after the readings (b).

4. DISCUSSION

AmPDT for the treatment of microbial infection is of interest due to the growing resistance of microorganisms to conventional antimicrobial agents[2]. Antibiotic resistance of pathogenic bacteria species is considered a serious problem for public health[8].

In this study, the group treated with 40 μg/mL Photogem® associated with LED irradiation and immediate measurement presented one of the highest reductions of microorganisms of all groups tested. Nevertheless, after one hour of incubation, this concentration presented microorganism growth instead of inhibition. We could speculate that a concentration of 40 μg/mL might eliminate some microorganisms which justifies the percentage of microorganisms reduction, but within time allows their competitors to grow, similarly to the possible effects as to when using antibiotics. Other researches using multiple bacterial species found from 47 % up to 99 % of killing rate in different patients using toluidine blue and a Diode Laser. However, the authors also suggested that variance between patient could be related to the variety of microorganisms in the sample with a possible complex competence and dependence relationship among different species, significantly decreasing some bacteria species while promoting an increase in other species[9].

A recent study has demonstrated that AmPDT with Photogem® and blue LED can result in a substantial reduction, up to 90 %, in the viable microorganism counts from dentures infected with *Candida* spp., *Staphylococcus* spp. and *Streptococcus mutans*[2]. The use of AmPDT, *in vitro,* to induce *Staphylococcus aureus* reduction with a Photogem® using concentration of 75 μg/ml has been demonstrated to be effective in microbial inhibition of around 98%[4]. Nevertheless, the need of further studies to evaluate whether a mixture of several bacterial species is less sensitive to photodynamic action than single-type bacteria *in vitro* has also been pointed out in the literature[9]. In this study, with a lower dosage of 5 μg/mL of Photogem® associated with LED irradiation presented the best result in microorganism inhibition when measured one hour after incubation. Following biological principles, lower dosages of any type of drug are always recommended and preferable to use.

AmPDT can be used against various microorganisms including viruses, bacteria, and fungi with multiantibiotic-resistant strains killed as easily as naïve strains and side effects minimized by the use of photosensitizers targeted towards microorganisms rather than host cells. The development of resistance to AmPDT has not been shown to occur and is thought to be very unlikely[6].

This study demonstrates that AmPDT, with Photogem® associated to red LED, could be an alternative method to oral microorganism's reduction. The method could be used before oral surgeries decreasing possible post-operative infections, as well as in hospital inpatients avoiding secondary infections such as pneumonias and reducing hospital expenses. This methodology can be used against microbial biofilms, however the protocol of application need to be carefully design. The parameters most important to determinate successful of AmPDT protocols are energy density, photosensitizer concentration, pre-irradiation time and cell target[10].

5. CONCLUSION

This study suggests that AmPDT with Photogem® associated to red LED (λ640 ηm ± 5ηm) can be a potential mechanism of control of oral microorganisms.

REFERENCES

[1] Wilson, M., "Lethal photosensitisation of oral bacteria and its potentialapplication in the photodynamic therapy of oral infections," Photochem. Photobiol. Sci. 3, 412-418 (2004).

[2] Ribeiro, D. G., Pavarina, A. C., Dovigo, L. N., et al, "Photodynamic inactivation of microorganisms present on complete dentures. A clinical investigation," Lasers Med. Sci. 27,161-168 (2012).

[3] Trindade, F. Z., Pavarina, A. C., Ribeiro, A. P. D., Bagnato, V. S., Vergani, C. E., Costa, C. A. S., "Toxicity of photodynamic therapy with LED associated to Photogem®: An in vivo study," Lasers Med. Sci. 27, 403-11 (2012).

[4] Ferraz, R. C. M. C., Fontana, C. R., Ribeiro, A. P., et al., "Chemiluminescence as a PDT light source for microbial control," J. Photochem. Photobiol. B: Biology 103, 87–92 (2011).

[5] Peloi, L. S., Soares, R. R. S., Biondo, C. E. G., Souza, V. R., Hioka, N., Kimura, E. "Photodynamic effect of light-emitting diode light on cell growth inhibition induced by methylene blue," J. Biosci. 33, 231–237 (2008).

[6] Demidova, T. N., Hamblin, M. R., "Photodynamic therapy targeted to pathogens," Int. J. Immunopathol. Pharmacol. 17, 245–254 (2004).

[7] Giusti, J. S. M., Fontana, C .R., Guimarães, O. C. C., Pinchemel, L. C. P., Bagnato, V. S., "Single equipment combines simultaneous application of mechanical ultrasound and photodynamic action for microbial control," Photodiagn Photodyn. Ther. 7, 137-138 (2010).

[8] Kurek, A., Grudniak, A. M., Kraczkiewicz-Dowjat, A., Wolska, K. I., "New Antibacterial Therapeutics and Strategies," Polish J. Microbiol. 60, 3-12 (2011).

[9] Qin, Y., Luan, X., Bi, L., et al., "Toluidine blue-mediated photoinactivation of periodontal pathogens from supragingival plaques," Lasers Med. Sci. 23, 49-54 (2008).

[10] Panhóca, V. H., Florez, F. L. E., Faria Júnior, N. B., Rastelli, A. N. S., Tanomaru, J. M. G., Kurachi, C., Bagnato, V. S., "Evaluation of Antimicrobial Photodynamic Therapy against Streptococcus mutans Biofilm in situ," J. Contemp. Dent. Pract. 17, 184-191 (2016).