EVALUATION OF PHOTODYNAMIC THERAPY USING A DIODE LASER AND DIFFERENT PHOTOSENSITIZERS AGAINST *ENTEROCOCCUS FAECALIS*

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ABSTRACT
Photodynamic therapy (PDT) has been proven to be effective in disinfecting root canals. The aim of this present study was to evaluate the effects of PDT on the viability of Enterococcus faecalis using methylene blue (MB) and malachite green (MG) as photosensitizers. Solutions containing E. faecalis (ATCC 29212) were prepared and harvested by centrifugation to obtain cell suspensions, which were mixed with MB and MG. Samples were individually irradiated by the diode laser at a distance of 1mm for 30, 60, or 120 seconds. Colony-forming units (CFU) were determined for each treatment. PDT for 60 and 120 seconds with MG reduced E. faecalis viability significantly. Similar results were obtained when MB was used as photosensitizer. PDT using MB and MG have antibacterial effect against E. faecalis, showing potential to be used as an adjunctive antimicrobial procedure in endodontic therapy.

Key words: *Endodontics; Photodynamic Therapy; Enterococcus faecalis*

AVALIAÇÃO DA TERAPIA FOTODINÂMICA UTILIZANDO LASER DIODO E DIFERENTES FOTOSENSIBILIZANTES FRENTE O *ENTEROCOCCUS FAECALIS*

RESUMO
A terapia fotodinâmica (PDT) tem demonstrado ser eficaz na desinfecção de canais radiculares. O objetivo do presente estudo foi avaliar os efeitos da PDT sobre a viabilidade de Enterococcus faecalis, utilizando azul de metileno (MB) e verde malaquita (MG) como fotosensibilizantes. Soluções contendo E. faecalis (ATCC 29212) foram preparadas e colhidas por centrifugação, para se obter suspensões de células que foram misturadas com o MB e MG. As amostras foram irradiadas individualmente pelo diodo laser a uma distância de 1 milímetro por 30, 60 ou 120 segundos. Unidades formadoras de colônias (UFC) foram determinados para cada tratamento. PDT por 60 e 120 segundos com MG reduz significativamente a viabilidade de E. faecalis. Resultados semelhantes foram observados quando MB foi utilizado como fotosensibilizante. PDT utilizando MB ou MG tem efeito antibacteriano contra E. faecalis, mostrando o potencial de ser usada como um adjuvante do processo antimicrobiana em endodontia.

Palavras-Chaves: *Endodontia; Terapia Fotodinâmica: Enterococcus faecalis*

INTRODUCTION

Endodontic treatment is the clinical management of a microbiological problem, and the main targets of the treatment are the microorganisms residing within the root canal system[1]. *Enterococcus faecalis* is a facultative anaerobic bacterium that has been associated with secondary endodontic infections, refractory infected lesions, and periapical biofilms[2]. Techniques, instruments, and equipment have been developed to improve clinical work and achieve a high disinfection rate. In this context, photodynamic therapy (PDT) has been used to target microorganisms in root canals, suggesting its usefulness as an adjunct to current endodontic disinfection techniques[3-5]. In PDT, the photosensitizer is activated by exposure to light of a specific wavelength in the presence of oxygen. The transfer of energy from the activated photosensitizer to available oxygen results in the formation of toxic oxygen species, such as singlet oxygen and free radicals. These reactive chemical species destroy proteins, lipids, nucleic acids, and other cell components[5]. Many studies

have demonstrated the efficacy of methylene blue (MB) and toluidine blue in PDT for the reduction of bacteria[3,5,6]. Another substance reported as a good photosensitizer is malachite green (MG)[4]. Thus, the objective of this study was to evaluate the effects of PDT for 30, 60 and 120 s on the viability of *E. faecalis*, using MB and MG as photosensitizers.

MATERIAL AND METHODS

The irradiation source was a diode laser (Twin Laser; MM Optics, São Paulo, Brazil). Its wavelength and output power were 660 nm and 40mW, while the energy density and the diameter of quartz optical fiber were 120J/cm² and 400 μm respectively. One percent solutions of MB and MG were used to photosensitize the microorganisms studied. These solutions were prepared by dissolving the powder (Synth, São Paulo, Brazil) in physiological saline solution (0.85% NaCl) and filtering it through a sterile membrane (0.22-mm pore diameter; MFS, Dublin, CA).

E. faecalis was cultured in brain-heart infusion (BHI) broth (Difco, Detroit, MI) at 37° C/18 h in an atmosphere of 5% CO_2. The organisms were harvested by centrifugation at 10,000× g/5 min and suspended in saline solution and adjusted to 3×10^6 cells/mL using a spectrophotometer. Bacterial cell suspensions were mixed with MB and MG solution in sterilized test tubes for a final suspension of 1.5×10^6 cells/mL, then individually irradiated by the diode laser at a distance of 1mm for 30, 60, or 120 seconds. Saline solution and 5.25% sodium hypochlorite were used as positive and negative control respectively. Following irradiation, ten-fold serial dilutions were carried out for each suspension and aliquots were seeded in BHI agar plates, which were incubated at 37°C/18 h in an atmosphere of 5% CO_2. The colony-forming units (CFU/mL) were counted using the spread plate method.

Triplicate experiments were performed throughout this study. All assays were repeated three times to ensure reproducibility. Data from the assays are presented as means ± standard deviation (SD). The results were subjected to the Kolmogorov–Smirnov test to evaluate the normal distribution, and data were analyzed using one-way analysis of variance (ANOVA). Statistical differences amongst the groups were analyzed using Tukey's test at a significance level of 5%. Data were analyzed using the statistical software SPSS (SPSS Inc., Chicago, IL, USA).

RESULTS

Fig. 1 shows the antimicrobial effects of different study protocols against *E. faecalis*. Incubation with saline solution did not have antimicrobial effect, while NaOCl completely eliminated *E. faecalis*. When the diode laser was used with both photosensitizers for 30 seconds, there was a little decrease in bacteria viability, with statistically significant difference from the positive control group and from the negative control group ($P<0.05$). In contrast, a significant decrease in the viability of *E. faecalis* was observed following laser irradiation with both photosensitizers for 60 and 120 seconds when compared to the positive control group ($P<0.01$).

DISCUSSION

E. faecalis is able to survive for long periods without nutrients. It invades dentinal tubules, which protect it against the usual irrigating agents[7].

PDT has been suggested as a good option to maximize root canal disinfection, which confers it with potential to be used to predictably disinfect canals in one visit[3-5]. However, a PDT protocol to be used as an effective antibacterial supplement to chemomechanical therapy remains to be established. There are many variables to be taken into account when developing a PDT protocol, including light

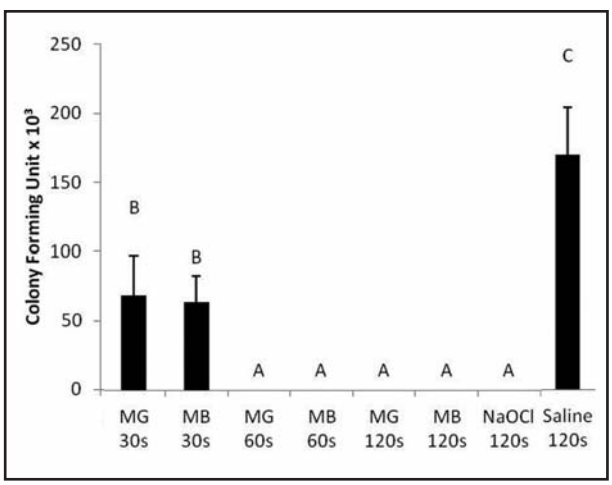

Fig.1: Antimicrobial effects of diode laser on E. faecalis in test tubes. The bacteria were exposed to different disinfection protocols (saline solution; photodynamic therapy with MG- Malachite Green and MB – Methylene Blue for 30, 60 and 120 seconds; and sodium hypochlorite). The number of viable cells after each treatment was counted. Data are expressed as the mean ± standard deviation of triplicate determinations. The experiment was performed three times, with similar results obtained in each. Different letters show statistical difference (p< 0.05).

parameters, photosensitizers, and light delivery techniques. This study evaluated the effect of two different photosensitizers on *E. faecalis* viability. In order for a photosensitizer be effective, the peak of absorption should match the wavelength of the light used for irradiation, promoting the formation of singlet oxygen, a very reactive oxygen species primarily responsible for PDT-mediated bacterial killing. The photosensitizers used in this study, MB and MG, have a maximum wavelength absorption at 660 nm and 674 nm, respectively[8]. Antibacterial effectiveness of PDT may also depend on the photosensitizer concentration[5]. In this study, for the sake of direct comparison between antibacterial efficacies, the same 0.1% concentration was used for both photosensitizers.

These results show that photodynamic therapy with MB and MG was effective in reducing *E. faecalis* strains with PDT for more than 60 seconds. The results for both dyes in PDT were similar to those in several previous studies using different dyes, such as MB, toluidine blue and MG[3,4,6,9].

Our findings suggest that both photosensitizers have the potential to be used as an adjunctive antimicrobial procedure in endodontic treatment. The advantages of this noninvasive technique in the hypothetical case of its *in vivo* application would be rapid application of the drug in the root canal and rapid bacterial killing after a short treatment time. Further studies are needed to investigate the potential clinical use of the proposed photosensitizers, as well as the dentin staining promoted by both dyes.

In conclusion, diode laser and PDT using MB or MG have *in vitro* antibacterial effect against *E. faecalis*. The results of this study suggest that both tested photosensitizers could be used in the clinical application of PDT.

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