Photodiagnosis and Photodynamic Therapy 16 (2016) 66–71

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Effect of photodynamic therapy with two photosensitizers on Streptococcus mutants: In vitro study

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ARTICLE INFO

Article history:
Received 17 July 2016
Received in revised form 2 August 2016
Accepted 7 August 2016
Available online 10 August 2016

Keywords:
Photodynamic therapy
Indocyanine green
Methylene blue
Streptococcus mutans
Laser
Photosensitizing agents

ABSTRACT

Background and objectives: Streptococcus mutans (S. mutans) colonizes the oral cavity and causes dental caries and periodontal diseases. Considering the importance of the treatments that decrease pathogenic microorganisms, the aim of the present research was the assessment of the antimicrobial effect of Photodynamic Therapy (PDT) with Methylene Blue (MB) and Indocyanine Green (IG) photosensitizers on S. mutans.

Materials and methods: In this In vitro experimental study, Sixty four caries-free first premolars were contaminated with 0.5 McFarland S.mutans suspension and were randomly assigned to 4 groups. The teeth in the first group were impregnated with 2% MB while the teeth in the second group were impregnated with 0.2% IG. The teeth in the first group were irradiated with continuous-wave 660 nm dod laser with 40 mw output power, energy density of 2.4 J/cm^2 and 100% duty cycle for 60 s, while the teeth in the second group were irradiated with continuous -wave 810 nm diode laser with 100 mw out power, density energy of 6 J/cm^2 and 100% duty cycle for 60 s in contact mode. In the third group, the teeth were suspended in 0.2% Chlorhexidine for 30 s. The fourth group was considered as the control. The teeth were sampled before and after the interventions and the samples were incubated in Blood Agar for 24 h. Afterwards, the number of S. mutans colonies were counted. Data were statistically analyzed by Kruskal-Wallis, Dunn's and Friedman tests.

Results: In the groups treated with a combination of MB and IG and laser irradiation and also in the Chlorhexidine group, the final number of S. mutans colonies equaled zero. In "MB and IG groups without laser irradiation", although the amount of microorganisms decreased, but the number of colonies did not reach zero. Pair comparisons by Dunn's test showed that there was a significant difference between "MB and IG groups without laser irradiation" and the other experimental groups $p = 0.03$).

Conclusion: PDT with MB and IG photosensitizers and also Chlorhexidine mouthwash, have the ability to completely eradicate S. mutans bacterial colonies.

1. Introduction

Streptococcus mutans (S.mutans) colonizes the oral cavity and comprises 70% of the bacteria in dental plaque [1]. This bacterium causes common oral diseases such as dental caries and periodontal diseases [1]. Therefore, it is necessary to apply specific procedures to decrease the population of this bacterium and/or inhibit its accumulation in dental plaque to prevent these common oral diseases. S. mutans similar to candida albicans, develops dental caries and periodontal diseases in the conditions that enhance its proliferation [2].

Mouthwashes are supplementary tools in dental plaque control to decrease the number of oral microorganisms, however their application in conjunction with the main mechanical plaque control methods can have different effects on oral tissues [3,4]. Mouthwashes with various compositions, have exclusive effects

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http://dx.doi.org/10.1016/j.pdpdt.2016.08.002
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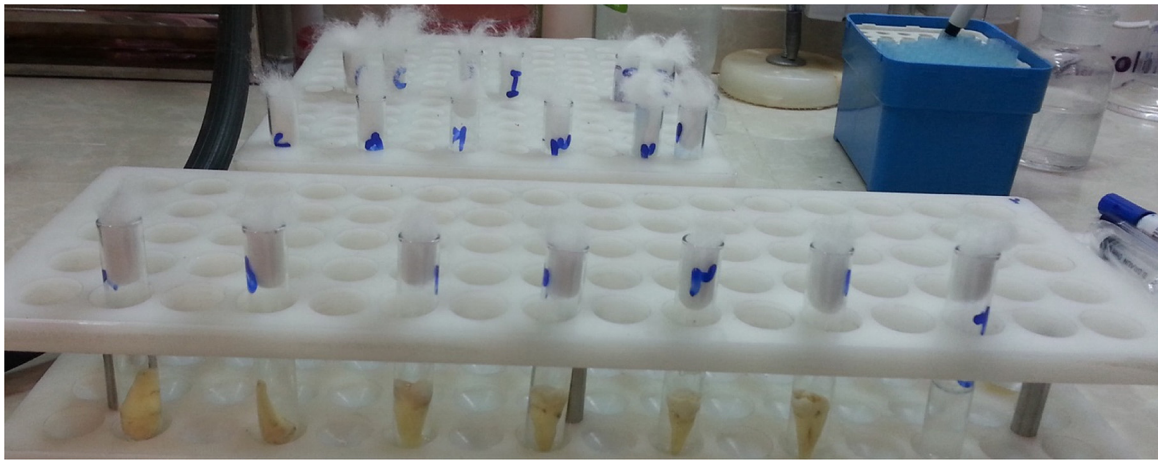

Fig. 1. Impregnation of teeth with S.mutans.

and associated side effects, which limit their benefits. Chlorhexidine is a well-known mouthwash, which despite having some advantages, such as extensive antimicrobial effect, it also has certain side effects including dysgeusia and tooth and restoration discoloration [5].

Currently, the novel photochemical technique for elimination of microorganisms which is called "Photodynamic Therapy" (PDT) has attracted a lot of attention [6]. PDT was first implemented in 1904 by Von Tappeiner and Jodlbauer in cancer therapy [7]. This technique is a combination of the application of nontoxic chemical elements (photosensitizers) and low level light energy, which produce free radicals that have cytotoxic effect on the target cells [8]. In this technique, only the cells that are impregnated with the photosensitizer and irradiated, will be eradicated and it is considered an invasive method against microorganisms [9,10]. PDT and low level lasers are effective in the treatment of a wide range of diseases, including Lichen planus, Temporomandibular Joint (TMJ) disorders and reduction of the number of pathogenic microorganisms such as Candida albicans [11,12].

Numerous studies have been performed on the techniques of reducing the number of oral microorganisms and preventing dental caries and periodontal diseases, but few studies have evaluated the effect of PDT on the reduction of S. mutans colonies in contaminated dental specimens. Therefore, the aim of the present research, was the assessment of the effect of PDT with two types of photosensitizers: Methylene Blue (MB) and Indocyanine Green (IG) on S. mutans.

2. Materials and methods

Sixty four caries-free extracted first premolars were selected for this in vitro experimental study and were sterilized in an autoclave. The teeth were contaminated with 0.5 McFarland S.mutans ATCC 1683 suspension (procured from the health authority of the Ministry of Health and Medical Education, Iran) in an incubator at 37 °C for 120 min (Fig. 1). Afterwards, the teeth were washed with normal saline for 2 min. Before the treatment, the teeth were sampled with use of a sterile swab, and the samples were incubated in Blood Agar culture medium at 37 °C for 24 h. At that point, the number of S.mutans colonies were counted. (Fig. 2) The teeth were randomly assigned to 4 groups. The first group consisted of 20 teeth impregnated with 2% MB (Merck KGaA, Darmstadt, Germany) for one minute (Fig. 3), and the second group consisted of 20 teeth impregnated with 0.2% IG (Merck KGaA, Darmstadt, Germany) for two minutes. (Fig. 4) Then, the teeth in both groups were washed with normal saline for 30 s. The teeth were sampled by swabbing

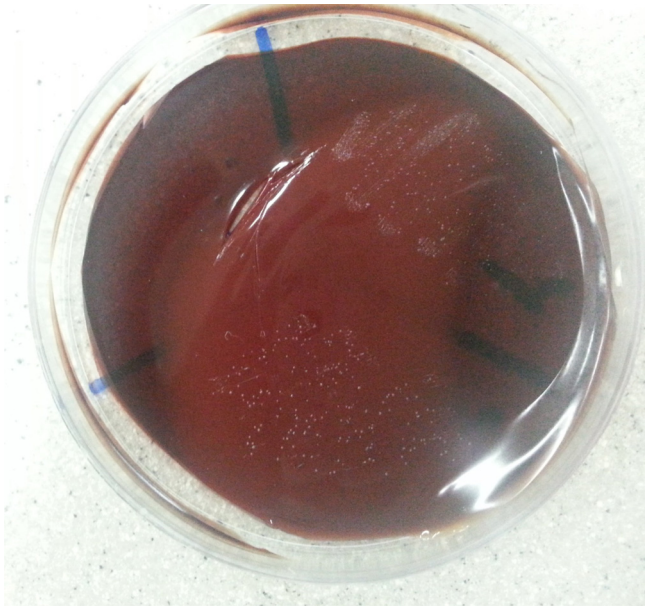

Fig. 2. Proliferated S.mutans colonies in the culture medium before the interventions.

before laser irradiation and the samples were incubated in Blood Agar culture medium at 37 °C for 24 h and afterwards, the number of S. mutans colonies were counted and recorded [7]. The teeth in the first group were irradiated with continuous-wave 660 nm diode laser with 40 mW output power, density energy of 2.4 J/cm2 and 100% duty cycle for 60 s, while the teeth in the second group were irradiated with continuous-wave 810 nm diode laser with 100 mW output power, density energy of 6 J/cm2 and 100% duty cycle for 60 s in contact mode [10,13]. Diode laser (A.R.C laser GmbH, Nurenberg, Germany) at 660 and 810 nm wavelenghts was calibrated and irradiated. The teeth were sampled with use of a swab and the samples were incubated in Blood Agar culture medium at 37 °C for 24 h and next, the number of S. mutans colonies were counted and the data were recorded. (Fig. 5) Twenty teeth of the third group were suspended in 0.2% Chlorhexidine mouthwash (Behsa Pharmaceutical Co., Iran) for 30 s. These teeth were also sampled by a sterile swab and the samples were incubated and the number of S. mutans colonies were counted. (Fig. 6) In the fourth group, two teeth were considered as positive controls (impregnated with S. mutans suspension without adding a dye or Chlorhexidine or laser irradiation) and two teeth were considered as negative controls