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BRIEF REPORT

Effect of photodynamic therapy with malachite green on non-surgical periodontal treatment in HIV patients: a pilot split-mouth study

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Introduction

A number of oral lesions are among the early features of HIV infection [1, 2]. It has been described that HIV patients are at risk for severe periodontal diseases [3, 4]. In addition, there is a higher prevalence of periodontal pathogens such as Actinobacillus actinomycetemcomitans (Aa), Porphyromonas gingivalis (Pg), and Tannerella forsythensis (Tf) in HIV patients compared with non-HIV patients [5, 6].

Polymerase chain reaction (PCR) is a diagnostic method that enables assessing microorganisms with very high levels of accuracy. For this reason, PCR is helpful to detect periodontal pathogens at levels below the ones detectable by cell culture or other diagnostic methods [7].

In most cases, the sole use of repeated scaling and root planing (SRP) as periodontal therapy leads to satisfactory clinical outcomes. However, the aforementioned approach may not be enough to achieve periodontal health in more challenging cases, such as residual deep pockets and furcation areas [8]. In addition, SRP often needs to be followed by adjunctive therapy such as local delivery and systemic antimicrobials and host modulation [9, 10].

Summary Adjunctive periodontal treatment with PDT using malachite green leads to significant reduction of microbiological levels of periodontal pathogens in HIV patients.

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One of the most recent methods used in combination with SRP in the periodontal treatment is the photodynamic therapy (PDT). In PDT, a photosensitizing agent is used to apply light therapy selectively to target specific cells. It is useful for sensitizing bacterial cells, leading to effective antimicrobial activity due to the production of cytotoxic oxygen free radicals (singlet oxygen) [11]. However, the efficacy of PDT is dependent on various factors, such as the laser wavelength and its interaction with the photosensitizer. One of the photosensitizers that have been used for the aforementioned purpose is the malachite green (MG) [12], which leads to dissipation of the cell membrane potential in both gram-positive and -negative bacterial species. MG is defined as a cationic dye of the triarylmethane family that shows satisfactory absorption at the red end of the visible spectrum [13].

However, little is known on the effect of PDT with MG as an adjunctive periodontal treatment in combination with SRP.

Thus, the aim of this pilot split-mouth study was to assess clinical and microbiological effects of PDT with MG on non-surgical periodontal treatment in HIV patients.

Materials and methods

Subjects

This pilot study was conducted with subjects attending in the Center for Study and Care of Special Patients (CEAPE-UNIP). Subjects were consecutively selected from June 2010 to May 2011. All subjects willing to participate in this study signed an informed consent form. Ethical approval was obtained from the Ethics Committee of the Paulista University (protocol number 408/11 CEP/ICS/UNIP). The

guidelines of the Helsinki Declaration were followed in this investigation.

Inclusion and exclusion criteria

HIV-positive subjects (ELISA/Western blot) who had undergone HAART for at least 3 years and presenting mild periodontitis—according to an updated concept previously described in the literature [14]—and at least two contralateral periodontal pocket sites with depth greater than 4 mm were included in this study. In addition, only sites initially presenting bleeding on probe were considered in the analyses.

Patients with recent mandibular tooth extractions (less than 6 months of follow-up) were excluded to avoid the socket-remodeling period. Patients with metabolic disorders, such as diabetes and vitamin D deficiency, were excluded. The presence of other oral pathologies and alveolar bone disorders was also considered as exclusion criteria.

Test and control group definition

Right and left sides of each subject were randomly allocated to either a test or a control group by using a computer-generated list. Sides receiving non-surgical periodontal treatment based solely on SRP were classified in the control group, whereas sides receiving PDT with MG, in combination with SRP, were classified in the test group.

Treatment timetable

Prior to periodontal treatment, all subjects had been required to undergo any calculus removal, exodontia, provisional restorations, and supragingival plaque control. Microbiological sample collection, bleeding on probing (BOP) assessment, and periodontal probing depth (PPD) measurement were performed prior to the beginning of treatment (baseline). Gingival bleeding index (GBI) and plaque index (PI) were also recorded at the baseline. SRP was performed in all compromised periodontal pockets under local anesthesia in a single appointment using Gracey periodontal curettes (Hu-Friedy, Chicago, IL) and an ultrasonic scaler (Cavitron, Dentsply, Tulsa, OK).

In the test group, SRP was always followed by the application of PDT with MG at 0.01 %. The laser system was composed by a hand-held battery-operated diode laser (Thera Lase, DMC, Sao Paulo, SP, Brazil) at 4 J/point with a wavelength of 660 nm, a power output of 30 mW for 133 s, resulting in an energy dose of approximately 47.57 J/cm^2 (considering an energy density loss of around 15 % due to the non-collimated laser beam). MG at 0.01 % was applied to the deepest part of the pocket for 5 min and then exposed to the laser light with a laser spot area of 0.07 cm^2 (tip diameter = 3 mm). Laser light was applied on three equidistant points (mesial, central, and distal) in each buccal and lingual aspects of the periodontal pocket, with a 90° angle in relation to the tooth long axis and a distance to the tissue of approximately 1 mm, following a previously described methodology [15]. The pocket was then rinsed with a saline solution to remove the photosensitizer.

After the first appointment, all subjects received oral hygiene instructions. Post-treatment microbiological sample collection, BOP assessment, and PPD measurements were carried out after one week. In addition, GBI and PI were recorded after 1-week, 1-month, and 3-month follow-up periods.

Microbiological analyses

After removal of the supragingival biofilm, the areas corresponding to residual pockets were rinsed with saline solution and dried. All microbiological samples were collected by a single trained examiner (DMRAS). A sterile paper point was inserted into the periodontal pocket for 30 s. The paper points were placed into sterile tubes containing 300 mL of 0.1 mM Tris-EDTA and immediately stored at −20 °C. Microbiological assays, primers, and reaction templates were performed following a previously described methodology [16] to determine positivity for Aa, Tf, and Pg at a level of detection of 10^3 bacteria per plaque sample.

Statistical analysis

Relative risk analyses were carried out to understand the association between the two groups and outcomes from the different non-continuous variables. Because of our split-mouth study design, relative risks were not adjusted to confusion variables such as age and gender. In addition, Friedman's test was used to assess differences in GBI and PI between the different follow-up periods. A p value under 0.05 was considered to indicate a statistically significant difference.

All statistical analyses were performed using the same software SPSS Statistics 17 software (SPSS®, Inc., Chicago, IL).

Results

Ten subjects (6 females and 4 males, mean age of 48.6 ± 5.8 years) totaling 93 periodontal compromised sites (48 in the test group and 45 in the control group) were included in the study. The presence of BOP and microbiological positivity for Aa, Pg, and Tf were confirmed for all sites analyzed at the baseline. Mean PPD at the baseline was 4.3 ± 0.4 for the test group and 4.5 ± 0.5 for the control group. One week after periodontal treatment, mean PPD was 3.9 ± 0.7 for the control group and 3.8 ± 0.6 for the test group.

Table 1 Relative risk of periodontal alterations treated with SRP and PDT

Variables	Treatment type		RR (95 % CI)	*p* value*
	SRP (control group)	SRP + PDT (test group)		
Bleeding on probing				
No	8	5	1	
Yes	37	43	1.08 (0.92–1.29)	.313
PCR for Aa				
Negative	12	31	1	
Positive	33	17	0.48 (0.32–0.73)	.001
PCR for Tf				
Negative	13	7	1	
Positive	32	41	1.20 (0.96–1.50)	.102
PCR for Pg				
Negative	3	15	1	
Positive	42	33	0.73 (0.60–0.91)	.003
PPD				
<4 mm	32	29	1	
≥4 mm	13	19	1.37 (0.76–2.43)	.284

SRP scaling and root planning, *PDT* photodynamic therapy, *RR* relative risk, *CI* confidence interval, *PCR* polymerase chain reaction, *Aa* Actinobacillus actinomycetemcomitans, *Tf* Tannerella forsythia, *Pg* Porphyromonas gingivalis

*Statistically significant when $p < 0.05$

Risk relative analyses revealed that Aa (RR = 0.48, 95 % CI = 0.32–0.73, $p = 0.001$) and Pg (RR = 0.73, 95 % CI = 0.60-0.91, $p = 0.001$) microbiological levels were significantly reduced in the test group (Table 1). No clinical parameters presented significant differences between groups ($p > 0.05$). In addition, Friedman's test showed no significant differences in PI ($p > 0.05$) and a significant reduction in GBI (Fig. 1) after a 3-month follow-up ($p < 0.05$).

Discussion

The association between HIV infection and oral lesions such as periodontal disease is not surprising, considering the altered immune system of the subject [2, 3]. One of the main alterations is the decreased number of CD4-T cells that leads to neutrophil hyperactivity and consequent damage to the periodontal tissues [4]. As a result, significant microbiological changes have been described by studies on this type of patients [15, 17], emphasizing the clinical relevance of the present study.

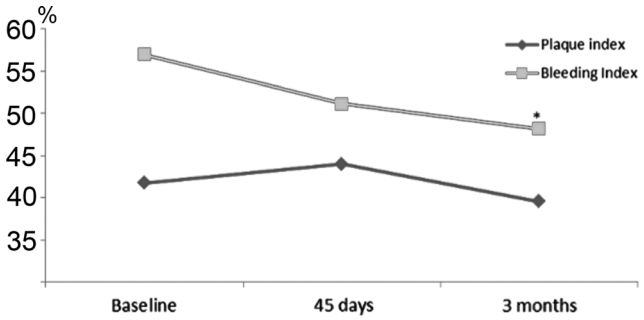

Fig. 1 GBI and PI baseline and follow-up results. Statistically significant (*$p < 0.05$) according to Friedman test

The advent of PDT with methylene blue as an adjunct periodontal treatment modality has been documented in the literature [15, 18]. However, antimicrobial activity against periodontal pathogens with methylene blue in systemic compromised patients seems to be low [15]. In the present study, the use of MG—a few researched photosensitizer—led to a significant reduction of periodontal bacterial levels. To our knowledge, this is the first clinical split-mouth study assessing the influence of PDT by using MG as photosensitizer. According to our risk relative analysis, PDT with MG was useful to reduce Aa and Pg bacterial levels. Our results support findings from an in vitro study indicating the efficacy of this method against Aa [19]. Furthermore, PDT with MG has also been described as useful against other oral pathogens such as *Streptococcus mutans* [20]. On the other hand, our methodology did not allow for reduction of Tf bacterial levels. This finding is in agreement with that of a previous study on PDT in cases of periodontitis [21].

Despite of the satisfactory microbiological benefits of PDT with MG presented herein, there were no significant

differences in clinical features between test and control groups, as also observed by another study on PDT [21]. This may be due to the immunologic conditions of HIV patients, which may worsen clinical prognosis [3, 4]. Different results have been reported by studies on adjunctive periodontal treatment with PDT using methylene blue as photosensitizer in non-HIV patients. Two studies [22, 23] found a significant reduction of PPD but not of number of sites with BOP after SRP and PDT with methylene blue, as compared with SRP alone. Other investigators [24, 25], in turn, found significant improvements in both PPD and BOP.

The present findings also contrast with those of a previous similar study on HIV patients receiving adjunctive periodontal treatment with PDT associated with methylene blue [15]. The authors of the aforementioned study reported that PDT led to a significant improvement in clinical parameters such as PPD and BOP, whereas no significant reduction of bacterial levels could be observed, as compared with the control group. This may be explained by the difference in maximum wavelength absorption between MG and methylene blue. Each photosensitizer absorbs light with an appropriate wavelength to induce singlet oxygen and free radicals, which are toxic to certain bacterial species [4, 26, 27]. Further comparative studies with larger sample sizes would be required to address details on the benefits of using MG, methylene blue, and other photosensitizers in the adjunctive periodontal treatment with PDT for HIV patients.

In contrast with the abovementioned studies, the present methodology did not include mean comparisons to assess clinical parameters. Instead, we conducted a series of relative risk analyses, which is an appropriate method to assess differences in efficacy of two different treatment modalities [28]. One of the limitations of the present study design, however, is that only short-term comparative results are provided by our relative risk analyses. In accordance, it has been suggested that the literature still lacks information on the long-term benefits of PDT as an adjunctive periodontal treatment [29]. Further long-term randomized clinical trials and comparative studies using different photosensitizers would be recommended to address the long-term benefits of PDT as a periodontal treatment modality. On the other hand, our follow-up results for GBI indicate that potential long-term clinical results with satisfactory outcomes could be expected for the use of PDT with MG in combination with non-surgical periodontal treatment.

In conclusion, within the limitations of this study, the present results suggest that the use of MG as a photosensitizer for PDT adjunct to SRP led to significant reduction of microbiological levels of periodontal pathogens with no significant improvement of clinical parameters, as compared with SRP alone.

Compliance with ethical standards

Ethical approval Ethical approval was obtained from the ethics committee of the Paulista University (protocol number 408/11 CEP/ICS/UNIP).

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent All subjects willing to participate in this study signed an informed consent form.

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References

1. Reznik DA (2005) Oral manifestations of HIV disease. Top HIV Med 13:143–8
2. Coogan MM, Greenspan J, Challacombe SJ (2005) Oral lesions in infection with human immunodeficiency virus. Bull World Health Organ 83:700–6
3. Alpagot T, Duzgunes N, Wolff LF, Lee A (2004) Risk factors for periodontitis in HIV patients. J Periodontal Res 39:149–57
4. Yeung SC, Stewart GJ, Cooper DA, Sindhusake D (1993) Progression of periodontal disease in HIV seropositive patients. J Periodontol 64:651–7
5. Patel M, Coogan M, Galpin JS (2003) Periodontal pathogens in subgingival plaque of HIV-positive subjects with chronic periodontitis. Oral Microbiol Immunol 18:199–201
6. Rams TE, Andriolo M Jr, Feik D, Abel SN, McGivern TM, Slots J (1991) Microbiological study of HIV-related periodontitis. J Periodontol 62:74–81
7. Sanz M, Lau L, Herrera D, Morillo JM, Silva A (2004) Methods of detection of Actinobacillus actinomycetemcomitans, Porphyromonas gingivalis and Tannerella forsythensis in periodontal microbiology, with special emphasis on advanced molecular techniques: a review. J Clin Periodontol 31:1034–47
8. Anderson GB, Palmer JA, Bye FL, Smith BA, Caffesse RG (1996) Effectiveness of subgingival scaling and root planing: single versus multiple episodes of instrumentation. J Periodontol 67:367–73
9. Kornman KS (1993) Controlled-release local delivery antimicrobials in periodontics: prospects for the future. J Periodontol 64: 782–91
10. Garcia Canas P, Khouly I, Sanz J, Loomer PM (2015) Effectiveness of systemic antimicrobial therapy in combination with scaling and root planing in the treatment of periodontitis: a systematic review. J Am Dent Assoc 146:150–63
11. Demidova TN, Hamblin MR (2005) Effect of cell-photosensitizer binding and cell density on microbial photoinactivation. Antimicrob Agents Chemother 49:2329–35
12. Kowaltowski AJ, Turin J, Indig GL, Vercesi AE (1999) Mitochondrial effects of triarylmethane dyes. J Bioenerg Biomembr 31:581–90
13. Yamaoka K, Sasai R (2000) Pulsed electric linear dichroism of triphenylmethane dyes adsorbed on montmorillonite K10 in aqueous media. J Colloid Interface Sci 225:82–93

14. Eke PI, Page RC, Wei L, Thornton-Evans G, Genco RJ (2012) Update of the case definitions for population-based surveillance of periodontitis. J Periodontol 83:1449–54
15. Noro Filho GA, Casarin RC, Casati MZ, Giovani EM (2012) PDT in non-surgical treatment of periodontitis in HIV patients: a split-mouth, randomized clinical trial. Lasers Surg Med 44:296–302
16. Casarin RC, Ribeiro Edel P, Mariano FS, Nociti FH Jr, Casati MZ, Goncalves RB (2010) Levels of Aggregatibacter actinomycetem comitans, Porphyromonas gingivalis, inflammatory cytokines and species-specific immunoglobulin G in generalized aggressive and chronic periodontitis. J Periodontal Res 45:635–42
17. Goncalves LS, Soares Ferreira SM, Souza CO, Souto R, Colombo AP (2007) Clinical and microbiological profiles of human immunodeficiency virus (HIV)-seropositive Brazilians undergoing highly active antiretroviral therapy and HIV-seronegative Brazilians with chronic periodontitis. J Periodontol 78:87–96
18. Correa MG, Oliveira DH, Saraceni CH, Ribeiro FV, Pimentel SP, Cirano FR et al (2015) Short-term microbiological effects of photodynamic therapy in non-surgical periodontal treatment of residual pockets: a split-mouth RCT. Lasers Surg Med
19. Prates RA, Yamada AM Jr, Suzuki LC, Eiko Hashimoto MC, Cai S, Gouw-Soares S et al (2007) Bactericidal effect of malachite green and red laser on Actinobacillus actinomycetemcomitans. J Photochem Photobiol B 86:70–6
20. Rolim JP, de-Melo MA, Guedes SF, Albuquerque-Filho FB, de Souza JR, Nogueira NA et al (2012) The antimicrobial activity of photodynamic therapy against Streptococcus mutans using different photosensitizers. J Photochem Photobiol B 106:40–6
21. Polansky R, Haas M, Heschl A, Wimmer G (2009) Clinical effectiveness of photodynamic therapy in the treatment of periodontitis. J Clin Periodontol 36:575–80
22. Al-Zahrani MS, Austah ON (2011) Photodynamic therapy as an adjunctive to scaling and root planing in treatment of chronic periodontitis in smokers. Saudi Med J 32:1183–8
23. Andersen R, Loebel N, Hammond D, Wilson M (2007) Treatment of periodontal disease by photodisinfection compared to scaling and root planing. J Clin Dent 18:34–8
24. Braun A, Dehn C, Krause F, Jepsen S (2008) Short-term clinical effects of adjunctive antimicrobial photodynamic therapy in periodontal treatment: a randomized clinical trial. J Clin Periodontol 35: 877–84
25. Campos GN, Pimentel SP, Ribeiro FV, Casarin RC, Cirano FR, Saraceni CH et al (2013) The adjunctive effect of photodynamic therapy for residual pockets in single-rooted teeth: a randomized controlled clinical trial. Lasers Med Sci 28:317–24
26. Atieh MA (2010) Photodynamic therapy as an adjunctive treatment for chronic periodontitis: a meta-analysis. Lasers Med Sci 25:605–13
27. Silva EJ, Coutinho-Filho WP, Andrade AO, Herrera DR, Coutinho-Filho TS, Krebs RL (2014) Evaluation of photodynamic therapy using a diode laser and different photosensitizers against enterococcus faecalis. Acta Odontol Latinoam 27:63–5
28. Thiese MS (2014) Observational and interventional study design types; an overview. Biochem Med (Zagreb) 24:199–210
29. Sgolastra F, Petrucci A, Severino M, Graziani F, Gatto R, Monaco A (2013) Adjunctive photodynamic therapy to non-surgical treatment of chronic periodontitis: a systematic review and meta-analysis. J Clin Periodontol 40:514–26