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Case report

Antimicrobial Photodynamic Therapy mediated by Photodithazine® in the treatment of denture stomatitis: A case report

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ABSTRACT

Antimicrobial Photodynamic Therapy (aPDT) mediated by Photodithazine® (PDZ) has shown efficacy in the inactivation of *Candida* spp. in *in vitro* and in *vivo* studies. This preliminary study reports five clinical cases of patients with denture stomatitis (DS) treated with PDZ-mediated aPDT. Five individuals diagnosed with DS were selected and submitted to aPDT 3 times a week for 15 days (6 sessions). In each session, 200 mg/L of PDZ gel was applied on the upper prostheses and on the palate of the patients for 20 min, then, illuminated by a light emitting diode at 660 nm (50 J/cm^2). Microbiological samples from prostheses and palates were also performed and cultured on Sabouraud Dextrose Agar and Blood Agar. The values of colony forming units per milliliter (CFU/mL) were determined. Standardized photographs of the palates were taken prior the treatment (initial), at the end (final) and until 45 days after the completion of treatments. The results demonstrated that the aPDT treatment reduced *Candida* spp. and the total microbiota viability at the end of the treatment. For most patients, the CFU/mL values obtained in the last microbiological collection (day 45) were lower than those found before the treatment (initial). Three patients presented clinical resolution of DS (no DS signal) after aPDT treatment. One individual demonstrated reduction in palatal inflammation and another one did not show improvement in the oral lesion. Recurrence of DS was observed in all individuals in the follow-up period. PDZ-mediated aPDT may be a promising treatment for DS.

1. Introduction

Candida albicans is a polymorphic yeast, which is able to invade the host tissue and cause cutaneous, mucosal and invasive infections. *C. albicans* is the most prevalent specie isolated from oral candidosis, and other species have also been found, such as *Candida dubliniensis, Candida glabrata, Candida tropicalis, Candida krusei, Candida kefyr* and *Candida parapsilosis* [1]. Denture stomatitis (DS) is an erythematous candidiasis related to the use of denture, considered a chronic atrophic OC, present in up to 65% of denture wearers [2]. Its etiological factors include: continuous denture wearing, poor denture hygiene, decreased salivary flow, medication, immunosuppression, smoking, increased age of denture and mucosal trauma [2]. All of these factors appear to increase the ability of *C. albicans* to colonize both the denture and oral mucosal surfaces [2]. According to Newton [3], DS might be classified as limited to local signs of disease with pinpoint petechial hemorrhage and local inflammation (type I), generalized simple inflammation (type II), or erythema of the central hard palate or with papillary hyperplasia of the oral mucosa in contact with dentures (type III) [3]. Patients with DS may display various symptoms including burning, painful sensation, change of taste and swallowing difficulty, but most often are asymptomatic [2].

The treatment of DS includes the use of topical and systemic antifungal drugs. The prescription of Nystatin, in cream or suspension form, to treat mild DS is recommended by the Infectious Diseases Society of America (IDSA) [2,4]. For immunocompromised patients systemic antifungal therapy, e.g. azoles, have been used. However, the management of DS can be considered difficult and complex due to its multifactorial etiology, common recurrences, as well as lack of antifungal drug efficacy [4]. Moreover, the prolonged or recurrent use of antifungal drugs, such as fluconazole, has resulted in the development of resistant *Candida* species [1]. Besides that, the disinfection of the denture is an important procedure to be performed when treating patients with DS, once the plastic denture is the main source of the *Candida* infection [4].

Therefore, the development of alternative therapies for the

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treatment of DS have been required. Antimicrobial Photodynamic Therapy (aPDT) has been suggested as a promising therapy for DS. aPDT combines a photosensitizing agent (PS), an appropriate wavelength light and the presence of oxygen, which will generate cytotoxic reactive species [5]. The use of aPDT for the management of DS had been evaluated. Mima et al. [6], conducted a clinical trial that compared the efficacy of aPDT mediated by Photogem with Nystatin. Despite being observed that aPDT was as effective as Nystatin, a high concentration of Photogem (500 mg/L) was used and it required a long time of irradiation, equivalent to 20 min [6]. Therefore, the use of a more effective PS was evidenced. Chlorin e6 derivatives have been studied as PS in the inactivation of microorganisms [7–9]. These derivatives demand a lower PS concentration, shorter period of illumination and absorption bands more strongly in the red regions of 650–680 nm, which penetrate deeper into tissues [10]. Photodithazine® (PDZ), a chlorine e6 derivative, has demonstrated photodynamic efficacy against *Candida* spp. *in vitro* [7–9], and *in vivo* [11]. Carmello et al. [11] evaluated the effectiveness of successive applications of PDZ-mediated aPDT on the treatment of OC in mice and they verified that aPDT was effective in reducing the infection and oral lesions [11]. Although, PDZ-mediated aPDT has demonstrated its effectiveness against *Candida* spp. *in vitro* and *in vivo*, there is no clinical study that evaluated the aPDT mediated by PDZ for the management of DS. The present study reports five cases of patients with DS treated with six sessions of aPDT and were followed-up at the time intervals of 15, 30 and 45 days.

2. Case report

Five complete denture wearers clinically diagnosed with DS were selected for the present study. This investigation was approved by the Ethics Committee of the School of Dentistry, Araraquara, São Paulo State University (Permit number – CAAE: 23558614.8.0000.5416) and was conducted according to the Helsinki Declaration. Each individual was informed about the risks and benefits of the treatment and, then, they signed voluntarily the informed consent form. The DS was classified according to Newton's criteria [3].

The chlorin e6 derivative, Photodithazine® (PDZ), produced by VETA-GRAND Co., was used as PS. PDZ is extracted from the *Spirulina platensis* cyanobacteria, and it is a noncovalent complex of N-methyl-D-glucosamine chlorine e6 salt on basis of chlorophyll A derivatives [10]. The PDZ was formulated in Natrosol hydrogel (Santa Paula Pharmacy, Araraquara, SP, Brazil) at the concentration of 200 mg/L. Two light emitting diode (LED) devices in the red region of the spectrum (peak at 660 nm) were fabricated. The device designed to irradiate the dentures (Fig. 1A) was confectioned with 24 LEDs homogeneously positioned all over the apparatus, with an intensity of 50 mW/cm^2, and one air cooler to avoid denture heating. The device designated to irradiate the patient's palate (Fig. 1B), was fabricated in a circular shape with 10 LEDs homogeneously positioned on the platform (240 mW/cm^2 of intensity). This device also had a semiconducting chip called Peltier and an air cooler, to scatter and avoid the heat produced by the apparatus. During the procedure, the LED platform was positioned inside the month of the patient to irradiate the palate.

All patients were oriented to brush their dentures with coconut soap and, then, with toothpaste after every meal and before going to sleep [6,12]. They were instructed to remove their dentures while sleeping and maintain it in filtered water. For the treatment, the denture was incubated with the PDZ gel, then, it was kept in the dark for 20 min. Then, the denture was positioned in the LED apparatus and illuminated for 17 min (50 J/cm^2). Simultaneously, the PDZ gel was applied on the palate for 20 min and, then, the palate was illuminated with the other LED. This apparatus was positioned on the palate and it was irradiated for 4 min (50 J/cm^2). The patients were submitted to aPDT 3 times a week during 15 days (6 sessions).

Microbiological assessment was also performed. For all patients, the microorganisms were recovered by swabbing the palate and the inner surface of the denture for 1 min. Then, the swab was immersed in a Falcon tube containing 5 mL of 0.9% sterile saline solution and vortexed for 1 min to suspend the organisms from the swab. To evaluate *Candida* spp. survival, serial 10-fold dilutions from 10^0 to 10^1 were plated onto Sabouraud Dextrose Agar (SDA; Hi-Media, France), and the plates were incubated at 37 °C for 48 h. Additionally, to assess the total microbiota survival (fungal and bacteria), serial 10-fold dilutions from 10^2 to 10^4 were plated onto Blood Agar (Laborclin, Pinhais, Brazil). The blood Agar plates were incubated at 37 °C for 4 days in a 5% carbon dioxide atmosphere. Colonies from all plates were quantified and the number of colony-forming units per milliliter (CFU/mL) determined. The recovery of microorganisms was performed before treatment (initial), at the end of the treatment (final), and at the follow-up time intervals of 15, 30 and 45 days after the end of treatments. Besides that, Standardized photographs of the palate were taken at the same periods evaluated.

From all patients submitted to aPDT, 3 of them were female and 2 male, the mean age of the patients was 63 years old and the mean age of the denture was 8 years. During aPDT treatment, no suffering, such as discomfort or pain, was reported by the patients. The CFU/mL values obtained from dentures (D) and palates (P) of all patients cultured in SDA and Blood Agar are presented in Table 1. In summary, the aPDT treatment reduced *Candida* spp. and the total microbiota viability at the end of the treatment. For most patients, the CFU/mL values obtained in the last microbiological collection (day 45) were lower than those found before the treatment (initial). The clinical evaluation of the DS

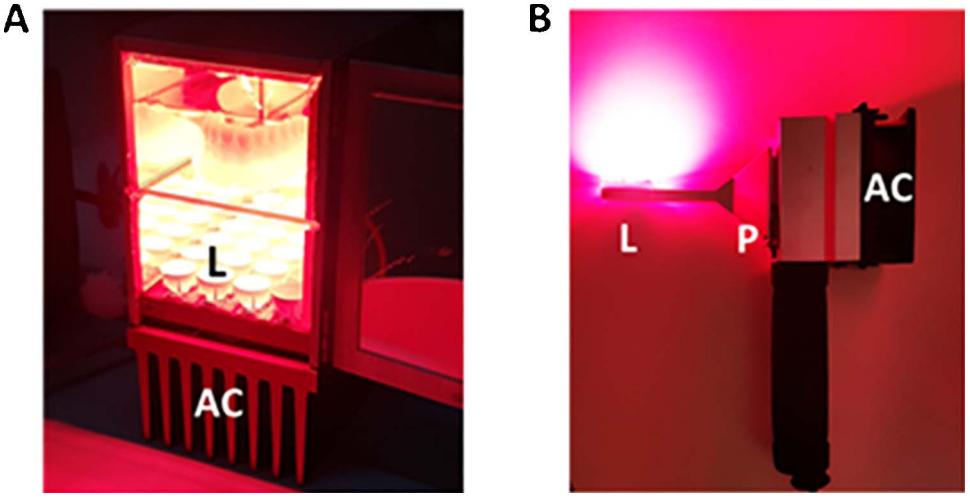

Fig. 1. A – LED device used to illuminate the dentures. B – LED device used to illuminate the patients' palate. L = LEDs; P = Peltier chip; AC = air coolers.

Table 1
Mean values of $\log_{10}$ (CFU/mL) of *Candida* spp. (cultured in **SDA**) and total microorganisms (cultured in Blood Agar – **BA**) recovered from dentures (**D**) and palates (**P**) of each subject, before (Initial) and after (Final) treatment and at follow-up time intervals (days 15, 30 and 45).

	Initial		Final		Day 15		Day 30		Day 45	
	BA	SDA	BA	SDA	BA	SDA	BA	SDA	BA	SDA
Patient 1										
P	5.61	2.64	2.30	1.71	4.85	1.32	4.60	0.00	5.38	0.00
D	6.76	4.90	4.30	3.20	4.90	3.43	6.23	4.32	5.54	4.07
Patient 2										
P	5.32	1.04	1.04	0.00	4.30	0.00	4.60	0.00	5.04	0.00
D	6.46	2.88	4.60	1.91	5.59	1.49	4.00	2.12	4.60	1.49
Patient 3										
P	5.32	1.04	1.32	0.00	5.18	0.00	5.48	0.00	5.18	0.00
D	6.28	3.76	4.18	2.18	5.38	0.00	5.00	0.00	5.72	0.00
Patient 4										
P	4.18	0.00	3.85	0.00	3.66	0.00	3.85	0.00	3.76	0.00
D	6.08	6.00	4.00	2.94	3.73	2.81	3.79	2.94	3.96	3.17
Patient 5										
P	4.48	0.00	1.04	0.00	5.00	1.79	1.32	0.00	4.60	0.00
D	5.60	3.89	4.60	2.08	6.64	3.18	5.78	3.89	6.63	4.19

Table 2
Clinical classification of the DS of the palates before (initial) and after (final) treatment and at follow-up time intervals (days 15, 30 and 45).

Patient	Initial	Final	Day 15	Day 30	Day 45
1	I	I	No clinical signal of DS	No clinical signal of DS	I
2	II	I	No clinical signal of DS	II	I
3	I	I	I	I	I
4	II	I	No clinical signal of DS	I	I
5	II	I	II	II	II

can be found in Table 2. Three patients presented clinical resolution of DS (no DS signal) after aPDT treatment. One individual demonstrated reduction in palatal inflammation and another one did not show improvement in the oral lesion. Recurrence of DS was observed in all individuals in the follow-up period. The Fig. 2 illustrates a patient's palate during all periods evaluated.

3. Discussion

The treatment of DS can be considered difficult and complex due to its multifactorial etiology, common recurrences, lack of antifungal drug efficacy, as well as the development of antifungal resistant by *Candida* species. Therefore, aPDT has been suggested as a promising treatment for this infection. The present study reports 5 patients with DS submitted to aPDT and the follow-up of them. All participants were positive for *Candida* species in the dentures before the treatment, and aPDT was able to reduce the number of *Candida* spp. as well as the total microbiota of all patients. These results are in accordance with those found by Mima et al. [13], who observed reduction in the CFU/mL values of *Candida* spp. after 6 sessions of aPDT mediated by Photogem. This result may be explained by the fact that the interaction of the reactive oxygen species (ROS) generated by aPDT with organic molecules is not specific, for this reason, any organelle of the cell can be a potential target of the ROS [14]. Thus, aPDT can cause cell damage for both fungal and bacterial cells. Although *Candida* species are considered important pathogens in the occurrence of DS, bacteria may contribute to the colonization and proliferation of *Candida* strains in the oral cavity [1]. On the oral microbiota, fungal and bacterial species are living in harmony with each other and forming a polymicrobial biofilm. For this reason, the ability of aPDT to kill both fungal and bacterial species is another advantage over conventional drugs for the management of DS. It is important to highlight that, in the present investigation, the patients were followed by the periods of 15, 30 and 45 days after the conclusion of the treatment, and during these periods none of them presented neither worsening nor complication of the DS nor another type of infection. Therefore, it is possible to suggest that this reduction on the oral microbiota was not harmful for the commensal microbiota.

In the present study, it was observed that three patients presented clinical resolution of DS (no DS signal) after aPDT treatment. One individual demonstrated reduction in palatal inflammation and another

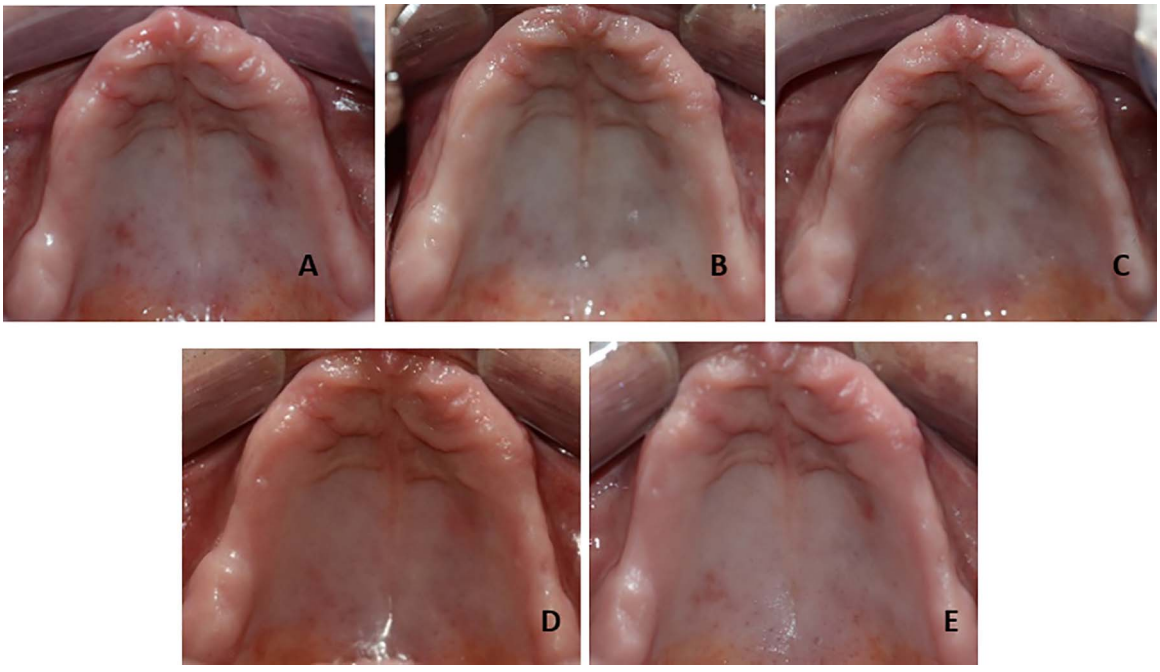

Fig. 2. Palate of patient 4. A: denture stomatitis (DS) type II (Initial), B: DS type I (Final), C: resolution of the DS (follow-up, day 15), D: DS type I (follow-up, day 30) and E: DS type I (follow-up, day 45).

one did not show improvement in the oral lesion. In the literature, there is a study that evaluated 6 sessions of aPDT mediated by Photogem (500 mg/L) for the treatment of 5 patients with DS [13]. In this study it was observed that four patients showed clinical resolution of DS (no clinical signal of DS) after aPDT sessions, and only 1 subject demonstrated reduction in palatal inflammation [13]. In another study, Mima et al. [6] evaluated aPDT in the treatment of DS, using Photogem in comparison with the topical antifungal Nystatin. In this clinical trial, the palate and denture of patients were treated with Photogem at 500 mg/L (30 min), then, they were irradiated with LED light at 37.5 and 122 J/cm^2, respectively. Authors observed that aPDT was as effective as Nystatin [6]. In these studies [6,13], the Photogem was used in a high concentration, compared with the concentration of the PDZ employed in the present study. Therefore, the use of second generation PSs, such as PDZ, may be a better choice, once this class of PSs can be used at low concentrations. The results obtained in the present investigation show that aPDT mediated by PDZ is a promising treatment for DS. However, this is an initial clinical study, and the conduction of a clinical trial is required for safe applications of aPDT in the future.

The Infectious Diseases Society of America (IDSA) recommends the prescription of Nystatin suspension for the treatment of mild oropharyngeal candidosis [4]. However, it is possible to suggest that PDZ-mediated aPDT shows important advantages over traditional treatments for DS. The dilution effect of saliva and cleansing action of the tongue and oral musculature may decrease the concentration of topical drugs, such as Nystatin, to sub therapeutic levels, requiring multiple doses, which can lower patient's compliance. Moreover, high recurrence rates after antifungal treatment have been reported [15]. In addition, during a clinical session of aPDT, the procedure is performed and controlled by the professional, who can reinforce the importance of the oral hygiene. Besides that, none of the patients complained about pain, discomfort nor find it uncomfortable. For this reason, the aPDT is as feasible as Nystatin and could be considered as a DS treatment by the patients and the health professional.

At baseline (before treatment), patients showed DS type I or II, and clinical improvement of the inflammation after 6 sessions of aPDT was observed. Clinical resolution was observed on day 15 of three patientes (1, 2 and 4). Concomitantly, reductions in the CFU/mL values were also observed after treatment compared with baseline (Initial). Both *Candida* spp. and total microbiota were decreased after aPDT treatment. During the follow-up period, all subjects presented recurrence of the inflammation. Mima et al. [6,13], also observed recurrence of DS in patients treated with aPDT mediated by Photogem. The recurrence of DS has been often reported [15] and it may be attributed to the inner surface of the acrylic resin denture that retains microorganisms [16] and facilitate the reinfection of the palate. In the present work, it was observed a higher growth of *Candida* spp. on the denture of the patients than that in the palate (Table 1). Besides that, most of the patients had worn the same prosthesis for more than 9 years, and only one was wearing the same denture for 4 years. In the cases that denture is very old and there is a lack of adaptation and retention, the appropriate approach would be the confection of new denture. Therefore, the condition of the denture should also be considered when treating DS.

This first clinical investigation using PDZ-mediated PDT has the following limitations: low number of patients evaluated, and only DS type I and II were assessed. A randomized clinical trial should be performed to better elucidate the potential of aPDT for the treatment of DS.

Ethical approval

This study was permitted by the Ethics Committee of the Araraquara School of Dentistry, São Paulo State University (Unesp) (Permit number – CAAE: 23558614.8.0000.5416).

Conflicts of interest

None.

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