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ORIGINAL ARTICLE

Longitudinal effect of curcumin-photodynamic antimicrobial chemotherapy in adolescents during fixed orthodontic treatment: a single-blind randomized clinical trial study

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Abstract White spot lesions are one of the concerns during the fixed orthodontic treatment. Thus, the aim of the present study was to evaluate the antimicrobial/anti-inflammatory effect of curcumin-photodynamic antimicrobial chemotherapy (c-PACT) and chlorhexidine varnish on the plaque accumulation and gingival bleeding in adolescents under fixed orthodontic treatment. A randomized clinical trial was performed with an initial number of 45 patients being distributed into three groups: group I—chlorhexidine varnish 2 %, group II—placebo varnish, and group III—c-PACT (curcumin at 1.5 mg.mL^{-1}) exposed to blue Light-emitting diode (LED) light at 450 nm (power density=165 mW.cm^{-2}, fluency=96 J.cm^{-2}, total dose=150.7 J). The treatments were performed for four consecutive times with an interval of 1 week each. After the interventions, two calibrated examiners (*Kappa* value=0.75) analyzed the dental plaque accumulation by plaque index (PI) and gingivitis condition by gingival bleeding index (GBI) with 1 and 3 months of follow-up after the treatments comprised a final sample of 35 patients. No significant difference was found to PI between the groups during baseline and 1-month period. Group III (1.52±0.51) presented significance difference from group I (0.91±0.75) and group II (1.03±0.51) at 3 months of follow-up. In this same period, there was more plaque accumulation with significant statistical difference ($P \leq 0.05$) in comparison to the other periods to all studied groups. There was a GBI reduction statistically significant to groups I and III at 1-month follow-up in comparison to other periods. No effect was verified to dental plaque accumulation after the photodynamic application mediated with curcumin activated with a blue LED light.

Keywords Photochemotherapy · Curcumin · Orthodontic appliances

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Introduction

A positive correlation between the use of orthodontic appliances and the first stages of dental caries (white spot lesions) and gingivitis has been attested by some current investigations. Among the main reasons, the physical presence of the intra-oral devices, the need of special tools to perform an

optimal oral hygiene, and compliance of the patients could be responsible for that relationship [1, 2].

Plaque control by means of regular mechanical removal, with concomitant use of chemical agents aiming to decrease the levels of cariogenic and periodontal bacteria, represents the main prevention methods to the above-cited oral problems [3, 4].

Chlorhexidine is a broad-spectrum antimicrobial agent, with activity against streptococci, actinomyces, gram-negative species, yeasts, total aerobes, and anaerobes [5]. The high concentrations of chlorhexidine have an immediate bactericidal effect, penetrating the bacterial cell wall and leading to the precipitation of the cytoplasm, whereas lower concentrations are bacteriostatic [6]. The clinical application of chlorhexidine within the oral cavity, in the form of gel or varnishes, has proved to be effective in bacterial reduction in both saliva and dental plaque [7]. One positive property of chlorhexidine resides on its intrinsic ability to be retained by oral surfaces and gradually released into oral fluids over many hours (e.g., substantivity) [8]. An unpleasant taste, tooth and restoration staining after long-term use, and the need for frequent applications have stimulated the search for new and more appropriate alternatives with no related side effects [6, 9].

Photodynamic therapy is a clinical treatment that uses light and a photosensitizer or dyes. Its mechanism of action consists of the absorption of photons from the light source by the photosensitizer whose electrons jump to an excited state. In the presence of a substrate such as oxygen, the photosensitizer, when returning to its ground state, transfers energy to the substrate, forming highly reactive and short-lived species such as singlet oxygen and peroxide radicals, which cause damage to the cell by the irreversible oxidation of its components and consequent cell death [10, 11]. According to Wainwright [12], the most appropriate denomination to this therapy that is a result of a dye and a light source combination should be called by photodynamic antimicrobial chemotherapy (PACT). This alternative approach represents a highly effective alternative for the treatment of localized microbial infections, such as chronic ulcers, acne lesions, and a variety of oral infections. PACT seems to be effective against antibiotic-sensitive and antibiotic-resistant microorganisms. In addition, repeated applications do not result in the selection of bacteria and/or bacterial resistance [13–15].

Several studies have shown that PACT is effective against some oral gram-positive and gram-negative bacteria [16, 17] by a large variety of sensitizers [18–20] and light sources at different wavelengths [18–20]. Also, previous studies have shown that PACT is able to kill oral bacteria in planktonic cultures [21, 22], plaque scrapings [19], and organized in biofilms as well [23–26].

The dyes frequently used in PACT are represented by the phenothiazine group (methylene blue and toluidine blue), phthalocyanines (azulene and other phytotherapeutic agents), chlorins (polylysines), porphyrins (hematoporphyrins), and xanthenes (erythrosin, eosin, Rose Beng) [19–26]. Although these agents are very promising, its use is subject to lengthy clinical and legislative assessment [27]. Furthermore, potential to staining teeth, resin-based restorations, toxicity, and long illumination time for dye activation represent a clinical limitation [18].

Curcumin is the most biologically active phytochemical component of the popular Indian spice, turmeric, used as a dietary material. It displays a variety of biological properties such as anti-proliferative activity against cancer cells, antioxidant, anti-inflammatory, and antimicrobial activity [28]. Moreover, current studies have been attesting optimal outcomes in curcumin-PACT approach activated with proper wavelengths over cariogenic bacteria that could increase the related activities cited above [29, 30].

This turmeric dye shows a strong absorption of light in the spectral range of 400–450 nm that corresponds to a blue wavelength [31]. Light-emitting diodes (LEDs) represent an alternative source of light for PACT to its low cost, low thermal component, and monochromatic light [27]. Also, blue LEDs are widely used in dentistry for tooth whitening and light curing of composite resins with no need of the acquisition of newly equipments [31].

Therefore, the aim of the present study was to investigate the longitudinal antimicrobial/anti-inflammatory effect of curcumin-PACT on dental plaque accumulation and gingivitis in adolescents under orthodontic treatment.

Material and methods

Ethical aspects

The experimental protocol applied in this investigation was approved by the Bioethics Committee of Araraquara Dental School of São Paulo State University, UNESP (protocol #37/10).

Participants

Fifty-five adolescents between 13 and 18 years old that attended in a private orthodontic clinic in Araraquara, SP were recruited to this study. The sample met the following inclusion criteria: good general health, absence of systemic antibiotic intake at least 3 months prior to study, under fixed orthodontic treatment, absence of oral lesions, supragingival calculus, severe malocclusion, and evident dental plaque accumulation. Exclusion criteria were considered: the use of mouthwashes during the study and the follow-up, pregnancy, systemic disorders, patients under preventive treatment, and absence of studied evaluated teeth.

Before enrollment, the subjects were given oral and written instructions and information about the products and purpose, aim, demand of benefits, and possible harm of study participation. Both participants and guardians signed an informed consent prior to the studied procedures.

Study design

This study was designed as a single-blind, randomized clinical trial. Each subject received regular fluoride dentifrice (1500 ppm; Colgate Palmolive, New York, NY, USA), toothbrush, and instruction regarding unsupervised oral hygiene (UOH) (three times along of the day without using disclosing chemical agents). The participants followed this instruction at home for 14 consecutive days aiming sample homogenization. After the enrollment period, 10 subjects were excluded with an initial number of 45 participants to be submitted to experimental treatments. The sample received a unique trial number and were randomly assigned to three groups of 15 adolescents each receiving the following treatments: group I—2 % chlorhexidine varnish (Fórmula e Ação, Sao Paulo, SP, Brazil), group II—placebo varnish (Fórmula e Ação, Sao Paulo, SP, Brazil), and group III—curcumin-photodynamic antimicrobial chemotherapy (c-PACT). The primary investigator (MAP) was responsible for the allocation, and the subjects were instructed not to reveal their group assignment in any way to clinical examiners (FJ and JFS). Figure 1 represents the flow chart subject enrollment with the final number of participants after the treatments to constitute the final analysis.

Application of experimental methods

Varnishes application

The teeth were brushed 1.5 h prior to each varnish application (groups I and III) with the provided dentifrice performed at a private orthodontic clinic. The oral cavity of adolescents was isolated with cotton rolls, dried with compressed air, and then the varnishes were applied to all teeth with a microbrush, delivered into the interproximal areas with unwaxed dental floss. The participants of these groups were instructed not to eat, drink, or rinse after 30 min of the procedure and not to brush their teeth during the following 24 h. The varnishes were applied once a week for four consecutive weeks, resulting in four applications following a previous validated protocol [9, 32, 33].

Curcumin-photodynamic antimicrobial chemotherapy (c-PACT) application

After toothbrushing, the subjects in group II were submitted to c-PACT application. A solution of curcumin (PDT Pharma, Cravinhos, SP, Brazil) was dissolved in deionized water (final

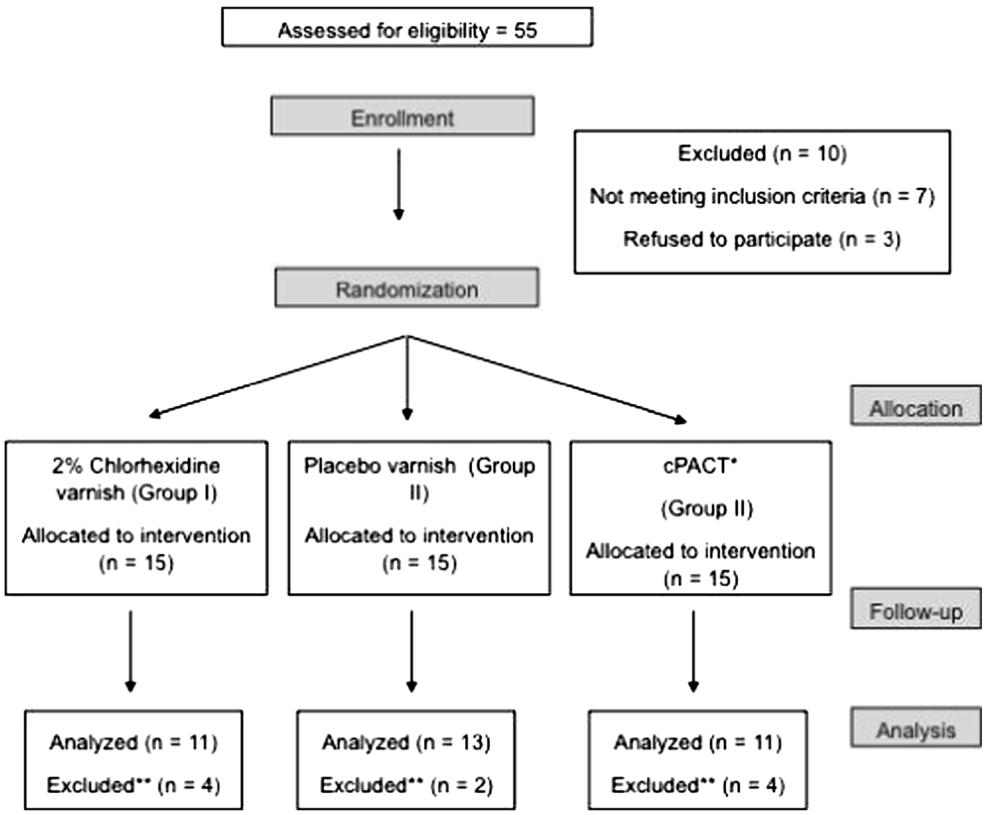

Fig. 1 Flow chart subject enrollment. *c-PACT* curcumin photodynamic antimicrobial chemotherapy. *Double asterisks* indicate five subjects did not attend the second follow-up visit (two in group I, one in group II, and two in group III) and five subjects had used mouthwashes or antibiotics (two in group I, one in group II, and two in group III)

concentration at 1.5 mg.mL^{-1}) at the same day of application to avoid photodegradation. The spectra absorption of this solution was monitored presenting a central wavelength at 430 nm [31]. A blue light-emitting diode (Prototype, Project Finep/Gnatus LED Edixeon, Edison Opto Corporation, New Taipei City, Taiwan) was used as a light source to constitute the PACT application. The device presented a power density of 165 mW.cm^{-2} with a central wavelength at 450 nm (±20 nm) that corresponds to the absorption band of the curcumin solution. The estimated fluency was 96 J.cm^{-2} where the light delivered the energy (total dose of 150.7 J) by uniform diffusion forming a semi-hemisphere illumination to each applied site [22]. A safe work distance of 5 mm from the tip of the light to the buccal surface of the teeth was established to deliver the desired fluency tested. During this period, the temperature was measured to assure the absence of dental heating (data not shown). Subjects were instructed to gargle 5 mL of the curcumin solution for 1 min, and the solution was expelled. Then, the stained teeth were illuminated into three different areas (left, right, and central area) for approximately 10 min (3.2 min per area). The c-PACT was applied once a week for four consecutive weeks, resulting in four applications in total, aiming to compare the total number sessions of varnish application as well.

Clinical measurements

Dental plaque index (PI) and gingival bleeding index (GBI)

The clinical measurements assessed in this present study were dental plaque index (PI) and gingival bleeding index (GBI) according to the criteria by Silness and Löe [34] and an adaptation by Löe e Sillness (1967) [35], respectively, using the dental elements #17, #12, #24, #36, #32, and #44 at four sites per tooth. These variables were performed by two experienced calibrated examiners and were assessed at baseline period (considered after UOH period), after 1 and 3 months of follow-up. All measurements were carried out under the same conditions and were performed by the same examiners who were blinded to the applied regimen, consistent with a design of a single-blind study.

Finally, all subjects received a questionnaire to evaluate their attitude toward the treatments and their oral perceptions. They were questioned about the presence of side effects, alteration of taste, and teeth staining.

Statistical analysis

PI and GBI mean scores of the selected teeth were used as the primary outcome variable. The analysis comparing differences between the three regimens were performed using Kruskal-Wallis non-parametric test with Newman-Keuls Student post-testing corrected for multiple comparisons. All data are presented as mean±SD per regimen. For the differences in the scores between regimens, 95 % confidence intervals were calculated, and values of $P \leq 0.05$ were considered as statistically significant. The data were analyzed using BioEstat 5.0 software for Windows (Sociedade Civil Mamiraua, Manaus, AM, Brazil) licensed from the Araraquara Dental School—UNESP. Data considering the answers related to evaluation of the subjects' appreciation and perception toward the treatments performed represented a secondary outcome.

Results

Figure 1 shows a flow chart of the subjects enrolled in this present study. A total of 55 systemically healthy adolescents were screened. Of this sample, seven subjects were excluded due to not meeting the inclusion criteria and three refused to participate. Thus, 45 subjects constituted the studied sample. During the study, 30 adolescents completed the protocol and constituted the final sample size. Five subjects were lost to follow-up because they did not attend the second follow-up visit (two in group I, one in group II, and two in group III), and five subjects had used mouthwashes or antibiotics (two in group I, one in group II, and two in group III) during the experimental period and were excluded of final analysis.

The mean values of PI and GBI scores for the evaluated periods to each studied group are reported in the Tables 1 and 2, respectively. No significant differences were found to PI to all evaluated periods between the studied groups with an exception to group III where the PI score was 1.52 (±0.51) in comparison to 0.91 (±0.75) (group I) and 1.03 (±0.51) (group II) at 3 months of follow-up. In this same period, there was more plaque accumulation with significant statistical

Table 1 Mean of plaque index (PI) values with standard deviation in parenthesis to each studied group with intra and inter-comparisons regarding the studied periods. The data were submitted to Kruskal-Wallis non-parametric test followed by Newman-Keuls Student post-testing corrected for multiple comparisons ($P \leq 0.05$)

	Baseline	1 month follow-up	3 months follow-up
Group I (2 % chlorhexidine varnish)	0.62 (±0.38)Aa	0.57 (±0.43)Aa	0.91 (±0.75)Ab
Group II (placebo varnish)	0.60 (±0.35)Aa	0.47 (±0.41)Aa	1.03 (±0.51)Ab
Group III (c-PACT)	0.80 (±0.49)Aa	0.46 (±0.39)Aa	1.52 (±0.51)Bb

Similar upper case letters mean no statistical differences between the groups. Similar lower case letters mean no statistical differences in the same group

c-PACT curcumin-photodynamic antimicrobial chemotherapy

difference ($P \leq 0.05$) in comparison to baseline and at 1-month follow-up to all groups.

In relation to GBI values, there was a reduction statistically significant to groups I and III at 1-month follow-up in comparison to other periods. The comparison to all studied groups demonstrated that the group II showed statistical difference ($P \leq 0.05$) in comparison to groups I and III just at 1-month follow-up, with no statistical difference to 3-month follow-up period.

Regarding the questionnaire, none of the participants presented side effects, alteration of taste, and teeth staining to all treatments applied. The clinical examiners performed their analysis at each follow-up with a satisfactory intra and inter *Kappa* index value (0.75).

Discussion

The present randomized single-blind clinical study aimed to investigate the longitudinal clinical antimicrobial/anti-inflammatory effects of photodynamic antimicrobial therapy mediated by curcumin (c-PACT) and chlorhexidine varnish (2 %) over dental plaque accumulation and gingivitis in a sample using fixed orthodontic appliance. The results demonstrated that both treatments were able to control gingivitis after 1 month of follow-up (Table 2), with no clinical evidence related to decreasing dental plaque accumulation to all studied periods (Table 1). Thus, the hypothesis of c-PACT was effective in controlling dental plaque, and gingivitis was partially accepted. The use of this technology as a technique to inactivate microorganisms has a considerable impact on the health field, since it can be used as a complementary therapy for controlling bacterial infection previously to prophylaxis on dental appointments, among other examples.

Table 2 Mean of gingival bleeding index (GBI) values with standard deviation in parenthesis to each studied group with intra and inter-comparisons regarding the studied periods. The data were submitted to Kruskal-Wallis non-parametric test followed by Newman-Keuls Student post-testing corrected for multiple comparisons ($P \leq 0.05$)

	Baseline	1 month follow-up	3 months follow-up
Group I (2 % chlorhexidine varnish)	0.69 (±0.17)Aa	0.19 (±0.50)Ab	0.77 (±0.39)Aa
Group II (placebo varnish)	0.71 (±0.34)Aa	0.69 (±0.42)Ba	0.73 (±0.37)Aa
Group III (c-PACT)	0.81(±0.32)Aa	0.21 (±0.52)Ab	0.79 (±0.42)Aa

Similar upper case letters mean no statistical differences between the groups. Similar lower case letters mean no statistical differences at the same group

c-PACT curcumin-photodynamic antimicrobial chemotherapy

The positive outcomes related to PACT application in dental caries and periodontal disease have been attested by some previous studies and corroborate with our findings. The study of Araujo et al. [28] achieved a decrease of *Streptococcus mutans* counting in saliva after the irradiation of oral cavity using a blue LED previously stained with curcumin solution, whereas no reduction was verified in the group submitted to illumination or dye application only. Ruhling et al. [36] compared the short-term performance of a single session of PACT application using tolonium chloride (5 %) and irradiated with a laser device for 1 min with a conventional ultrasonic debridement in the persistent pockets of maintenance patients by clinical measurements and microbiological screening.

This approach, applying PACT on this specific group (adolescents) using this protocol is new in the literature, becoming a hard task to compare with previous studies. The design study was based upon a previous investigation [28] that demonstrated no additional effects using only the drug (curcumin) or light alone. Thus, our main idea was to compare the photodynamic efficacy with a known tested *gold standard* (2 % chlorhexidine varnish) used to treat gingivitis and, at same time, decrease the accumulation of dental plaque [9, 33, 32]. Another dyes [19, 22, 24] and its association with different light sources [18, 25] have been proven to be efficient in controlling dental caries and periodontal disease organized in planktonic culture [21, 27] and biofilm counterparts [23, 24]. Our findings corroborate with published studies attesting the need for dye-light conjugation to ensure the PACT effectiveness [27, 30, 31].

A non substantial effect on dental plaque accumulation (measured by PI scores) could be explained based on the organization of biofilm structures. As known, the presence of biofilm matrix allows stability and structural integrity, limiting the diffusion of substances and, at same time, providing protection to the bacteria from inimical influences of antimicrobials and other environmental assaults [37, 38]. Furthermore, with the increase of complexity regarding the biofilm composition, it seems to be more resistant to PACT process. One of the explanations is that the interactions between the different matrix polymers, produced by different microorganism, might result in a more viscous matrix [39]. Another reason is based on reactive oxygen species (ROS) and its interaction with bacteria. Dovigo et al. [23] suggested that longer irradiation times produced lower quantities of ROS due to high photobleaching rate. They observed that the light absorption and fluorescence of curcumin decreased as a function of illumination time (light fluence). We can speculate that, taking into account the light illumination time of the present study (almost 10 min), it is expected to have a lower production of ROS; however, the quantity of this product was not measured. Furthermore, the excess of dye in the solution at

high concentrations could result in optical quenching by preventing the light from reaching the bacteria [31].

This investigation used a light-emitting diode in a blue wavelength to excite curcumin, promoting the optimization of PACT process. The advantages using LED reside on its simple manipulation, affordable price, and its availability in the dental offices with no need of new equipment acquisition. In order to evaluate the performance of different light sources, Zanin et al. [13] demonstrated that the use of a He–Ne laser or a red LED in combination with toluidine blue O (TBO) presented the same antimicrobial effect on *S. mutans* biofilm viability. To corroborate with this finding, Giusti et al. [18] achieved a preponderant bactericidal effect using a LED light at 24 J.cm^2 in combination with 2 mg.mL of Photogem© on *S. mutans* and *Lactobacillus acidophillus* presented in artificial carious bovine dentin. Similar results were obtained when *S. mutans* mature biofilms were irradiated by an energy density at 55 J.cm^2 of a red LED in the presence of TBO [40]. Hence, the utilization of light-emitting diodes at different wavelenghts that cover a broad visible spectrum of light is a trend in PACT approach.

The photosensitizer (PS) used in this present study was a yellow pigment extracted from the rhizomes of *Curcuma longa* L (turmeric plant). This dye is well known for its medicinal properties, and more recently, the role in PACT has been highlighted by several studies [27–29]. Low cost (US$5/mcg) makes the production at affordable prices possible, which, in addition to the efficacy of ROS generation and easy handling, represents advantages of the use of curcumin [27–29]. The concentration of curcumin (1.5 mg.mL^{-1}) used in this study was based on a previous study that determined a safe concentration in terms of damage to the mucosa and of discoloration of the teeth, which are important aspects to clinical applicability [28].

Our results demonstrated that c-PACT was able to control gingivitis (by GBI scores, Table 2) after 1 month of follow-up. This outcome could be explained by the anti-inflammatory properties of the PS used, stated by other studies [17, 29, 30]. In fact, this characteristic could be exacerbated upon the irradiation with proper wavelength, demonstrating a superior performance in comparison to chlorhexidine varnish, with no statistical difference to all studied follow-up periods (Table 2). In summary, this is the first investigation to verify this feedback of the studied population, stating an adjuvant option to treat gingivitis during fixed orthodontic treatment.

In relation to photodynamic parameters, success in obtaining good results is dependent on many variables. In the present investigation, the c-PACT application protocol resulted in beneficial effects even after a long illumination time. Therefore, studies with different light sources delivering high potency associated with different concentrations of the studied dye must be performed to clarify the applicability of PACT aiming to prevent gingivitis and dental plaque accumulation in population at high risk to develop dental caries and gum disease.

Conclusions

The results reported in this work demonstrated that photodynamic application mediated by curcumin and a blue LED light represents a promising antimicrobial approach for the prevention and treatment of gingivitis in adolescents under fixed orthodontic treatment. However, additional investigations should be performed aiming to clarify the actual role of PACT on the prevention of that disease and encourage the increase of the studied sample size. Moreover, studies aiming to investigate the studied parameters with the objective of decrease the light illumination time and, at same time, increase the optimization of this newly treatment approach for patients at high risk of development of oral diseases should be enocuraged.

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