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Effects of Photodynamic Therapy with Blue Light and Curcumin as Mouth Rinse for Oral Disinfection: A Randomized Controlled Trial

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Abstract

Objective: The purpose of this study was to evaluate the effects of the antimicrobial photodynamic therapy (a-PDT) with blue light and curcumin on oral disinfection during the 2 h after treatment. ***Background data:*** a-PDT is a technique that can potentially affect the viability of bacterial cells, with selective action targeting only areas with photosensitizer accumulation. ***Materials and methods:*** A randomized controlled trial was undertaken. Twenty-seven adults were randomly divided into three groups: (1) the PDT group, which was treated with the drug, curcumin, and blue light ($n=9$); (2) the light group, which was treated only with the blue light, and no drug ($n=9$) and; (3) the curcumin group, which was treated only with the drug, curcumin, and no light ($n=9$). The irradiation parameters were: blue light-emitting diode (LED) illumination (455 ± 30 nm), 400 mW of average optical power, 5 min of application, illumination area of 0.6 cm^2, 600 mW/cm^2 of intensity, and 200 J/cm^2 of fluence. A curcumin concentration of 30 mg/L was used. The saliva samples were collected for bacterial counts at baseline and after the experimental phases (immediately after treatment, and 1 and 2 h after treatment). Serial dilutions were performed, and the resulting samples were cultured on blood agar plates in microaerophilic conditions. The number of colony-forming units (CFU) was determined. ***Results:*** The PDT group showed a significant reduction of CFU immediately after treatment (post-treatment) with PDT (5.71 ± 0.48, $p=0.001$), and 1 h (5.14 ± 0.92, $p=0.001$) and 2 h (5.35 ± 0.76, $p=0.001$) after treatment, compared with pretreatment (6.61 ± 0.82). There were no significant changes for the light group. The curcumin group showed a significant increase of CFU 1 h after treatment (6.77 ± 0.40, $p=0.02$) compared with pretreatment (5.57 ± 0.91) falling to baseline values at 2 h after treatment (5.58 ± 0.70). ***Conclusions:*** The PDT group showed significant difference in microbial reduction ($p<0.05$) compared with both the light and curcumin groups until 2 h post-treatment. The new blue LED device for PDT using curcumin may be used for reduction of salivary microorganisms, leading to overall disinfection of the mouth (e.g., mucosa, tongue, and saliva), but new protocols should be explored.

Introduction

THE NORMAL MOUTH HAS A LARGE NUMBER OF BACTERIA. It results in increased risk of infection when many types of surgical procedures are performed, mainly intraoral surgery. In these cases, prophylactic systemic antibiotics are used, but these drugs may be associated with unfavorable side effects. Oral antiseptics (for example, chlorhexidine) can also be used in these cases, but the reduction of intraoral bacterial counts is temporary.[1] In this context, new procedures for oral disinfection should be investigated.

Blue light (405–470 nm) without the addition of exogenous photosensitizers (PS) has intrinsic antimicrobial effect and shows fewer deleterious effects to mammalian cells than ultraviolet irradiation.[2] According Soukos et al.,[3] the amount of endogenous porphyrin and/or other cell pigments produced in *Prevotella intermedia*, *Porphyromonas gingivalis*, *Prevotella melaninogenica*, and *Prevotella nigrescens* can explain a susceptibility to blue light resulting in oral disinfection.

Moreover, curcumin [1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5,-dione] shows antimicrobial activity as

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well. Curcumin is the principal yellow pigment isolated from turmeric (*Curcuma longa Linn*).[4] Several pharmacological properties of curcumin have been reported, such as antioxidant and anti-inflammatory,[4,5] antibacterial,[6] antifungal,[7] and anticarcinogenic[5] effects, mainly with high doses of curcumin alone.[8] Because of the extended antimicrobial activity of curcumin and its safety assessed by clinical trials in humans,[9] it was used as a structural sample to design the new antimicrobial agents with modified and increased antimicrobial activities through the synthesis of various derivatives related to curcumin.[10] Moreover, curcumin-mediated antimicrobial photodynamic therapy (a-PDT) can be used at low doses in combination with light exposure, with considerable antibacterial effect. In this context, curcumin has a rather broad absorption peak (range 300–500 nm), with a maximum absorption band at wavelength 430 nm, and it can be used as PS in a-PDT.[11]

PDT uses a nontoxic drug called a PS, which is activated by exposure to light of a specific wavelength in the presence of oxygen. This results in the production of reactive oxygen species (ROS), which can potentially affect the viability of bacterial cells with selective action targeting only areas with PS accumulation.[12,13] In this context, PDT has no side effects, and bacteria do not develop resistance to ROS.[1]

Several *in vitro* studies and clinical trials were performed to investigate bactericidal action and oral disinfection using blue light[3,14,15] or curcumin,[16,17] or by performing PDT with blue light and curcumin.[18–22] In dentistry, a-PDT may reduce both dental plaque and the risk of developing caries, as well as contributing to treating gingivitis, periodontitis, peri-implantitis and endodontic diseases.[23]

Previous clinical trials from our group have found that PDT with blue light and curcumin significantly reduced salivary microorganisms pre- and post-treatment.[1] However, oral disinfection as a function of time, to our knowledge, has not been investigated. Therefore, the aim of this study was to evaluate the effects of the PDT with blue light and curcumin on oral disinfection during 2 h after treatment. Our target was overall oral flora (mucosa, tongue, saliva), conducting analyses of salivary pathogens before and after antimicrobial photodynamic therapy. Our hypothesis was that blue light with or without curcumin, as well as only curcumin, could reduce colony-forming units (CFU).

Materials and Methods

The current research has been approved by the Ethics Committee of the Federal University of São Carlos (UFSCar) in São Carlos, Brazil (N. 258.461). The study was registered with the National Institutes of Health (NIH) ClinicalTrials (NCT02152475). All subjects signed written informed consent forms before their participation in the study.

A randomized controlled trial was undertaken. The inclusion criteria were healthy adults not using any antibiotic therapy who did not perform any oral hygiene, such as flossing, brushing, or use of antiseptic mouthwash, and who had fasted for 12 h prior the treatment and measurements. The exclusion criteria were having had oral cancer, smoking, pregnancy, or wearing partial or total dentures or orthodontic brackets. We performed simple randomization by a computer program. Twenty-seven healthy adult females and males between 20 and 35 years of age were randomly divided into three groups: (1) the PDT group, which was treated with the drug, curcumin, and blue light ($n=9$); (2) the light group, which was treated only with the blue light, and no drug ($n=9$); and (3) the curcumin group, which was treated only with the drug, curcumin, and no light ($n=9$).

Instrumentation to perform PDT

In order to perform PDT on the oral cavity, a device based on blue light-emitting diode (LED) (455 ± 30 nm) with transparent acrylic diffuser tip and cylindrical shape (89 mm length and 6.73 mm diameter) was developed by researchers of the industry (MM Optics, São Carlos, SP, Brazil) and the Optics Group of the Physics Institute of São Carlos (IFSC), University of São Paulo (USP). An optical power meter model FieldMaster TO-II (Coherent Inc., Santa Clara, CA) linked to a photodetector was used to calibrate this device, and to reveal a 400 mW average optical power, a 0.6 cm^2 illumination area, and a 600 mW/cm^2 intensity. The light was applied for 5 min, which led to an energy density (radiation dose) delivered of $\sim$200 J/cm^2. We considered a total energy per unit of area reaching the surface as the delivered dose, but this was not necessarily uniformly absorbed. The dose delivery was approximated, because different areas and several distances were irradiated. The new blue LED device in the oral cavity can be seen in Fig. 1. Although we applied the blue LED for 5 min as in the clinical trial of Araújo and collaborators,[1] the device

FIG. 1. New blue light-emitting diode (LED) device for photodynamic therapy (PDT) in the oral cavity.

TABLE 1. EFFECTS OF THE BLUE LED ILLUMINATION WITH OR WITHOUT CURCUMIN

	Pretreatment	*Post-treatment*	*Post 1 h*	*Post 2 h*
PDT group	6.61 ± 0.82	5.71 ± 0.48[a]	5.14 ± 0.92[a]	5.35 ± 0.76[a]
Light group	5.67 ± 0.82	6.10 ± 0.62	5.51 ± 0.93	5.84 ± 0.65
Curcumin group	5.57 ± 0.91	6.21 ± 0.58	6.77 ± 0.40[a]	5.58 ± 0.70[b]

Data represent the $\log_{10}$ CFU/mL.
[a]Significant intragroup difference compared with pretreatment (two way ANOVA with post-hoc Tukey, $p < 0.01$).
[b]Significant intragroup difference compared with period immediately before (two way ANOVA with post-hoc Tukey, $p < 0.05$).
LED, light-emitting diode; PDT, photodynamic therapy; CFU, colony-forming units.

geometry used in our study was different; therefore, the parameters also were different.

Curcumin

A stock solution (1.5 g/L) of curcumin (PDT Pharma, Cravinhos, SP, Brazil) was prepared in dimethylsulfoxide (DMSO) (0.1%) and then diluted in autoclaved distilled water (980 mL) to obtain the concentration used (30 mg/L). The literature explores different concentrations.[18–20,22] In the clinical trial of Araújo and collaborators,[1] the curcumin salt used had 1 g of salt containing 0.654 g of the cucumin plus curcuminoid, but in our study, natural curcumin (curcumin 53.4% and curcuminoid 46.16%) was used.

Treatment for oral disinfection

The volunteers in the PDT group used mouthwash with 20 mL of curcumin solution for 5 min, after which the solution was expelled and a blue light was introduced to activate the curcumin for 5 min. In the same way, the oral cavity of the light group was illuminated with blue light for 5 min and the curcumin group used mouthwash with 20 mL of curcumin solution for 5 min.[1] We did not use similar parameters of the blue illumination and curcumin concentration to those applied in *in vitro* studies, because *in vivo* studies show complexity regarding variety of biological tissues in the oral cavity and in immunological response.

Microbiological analyses

Two saliva samples from each volunteer were collected at each time point (pretreatment, post-treatment, post 1 h, and post 2 h) and stored in sterile containers. The saliva samples underwent serial dilutions and 100 μL aliquots were plated on Brain Heart Infusion Agar (BHIA) with 10% Sheep Blood (Difco Laboratories, Detroit, MI) plates (in duplicate) and then incubated under microaerophilic conditions for 48 h at ~36°C. After incubation, the total number of CFUs was determined.[1]

Statistical analysis

The data were expressed as means and standard deviations. In order to assess the effect of the treatments, CFU/mL values were transformed to logarithm ($\log_{10}$). The Shapiro–Wilk test was used to analyze data normality and the homogeneity of variances using Levene's test. Two way repeated measures ANOVA with post-hoc Tukey tests were used to compare changes in CFUs as a function of time. The independent factors were group (with three levels: PDT, light, and curcumin groups) and time (with four levels: pretreatment, post-treatment, post 1 h, and post 2 h), which was also considered a repeated measurement (intragroup differences). The survival fraction normalized and the delta CFU between the situations before and after the treatments (post-treatment, post 1 h, and post 2 h) was performed for intergroup comparisons using a one-way ANOVA with post-hoc Tukey tests. The Statistica for Windows Release 7 software (Statsoft Inc., Tulsa, OK) was used for the statistical analysis, and the significance level was set at 5% ($p < 0.05$).

Results

The PDT group showed a significant reduction in CFUs (1 log reduction) at post-treatment, post 1 h, and post 2 h ($p < 0.01$) compared with instance pretreatment. There were no significant changes for the light group. The curcumin group showed a significant increase in CFUs at post 1 h ($p < 0.05$) compared with pretreatment, falling to baseline values at post 2 h. These intragroup differences can be seen in the Table 1. The PDT group showed a significant difference ($p < 0.05$) in both normalized CFUs (Fig. 2) and microbial reduction (Fig. 3) compared with both the light and curcumin groups.

Discussion

The main finding of this study was that the PDT group showed reduction in CFUs immediately post-treatment.

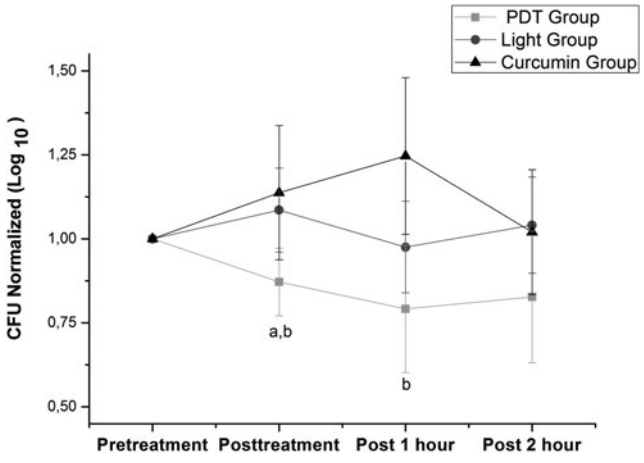

FIG. 2. Normalized colony-forming units (CFU). [a]Significant intergroup difference compared with light group (one way ANOVA with post-hoc Tukey, $p < 0.05$). [b]Significant intergroup difference compared with curcumin group (one way ANOVA with post-hoc Tukey, $p < 0.01$).

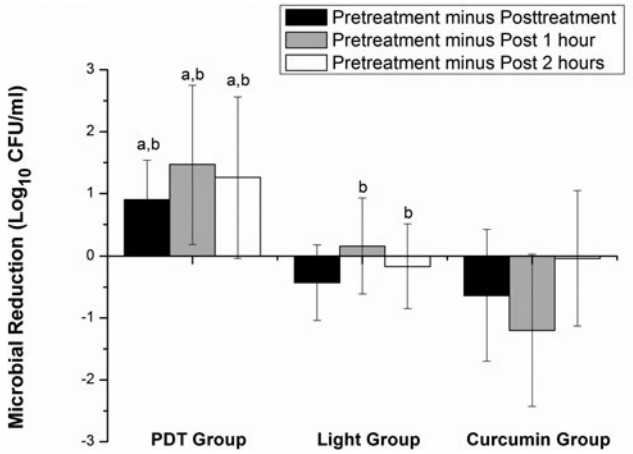

FIG. 3. Viable counts of colony-forming units (CFU) between pretreatment and after treatment. Significant intergroup difference (one way ANOVA with post-hoc Tukey, $p<0.05$). The photodynamic therapy (PDT) group showed significant microbial reduction compared with both the light[a] and curcumin[b] groups at pretreatment minus posttreatment ($p=0.02$ and $p=0.03$), pretreatment minus post 1 h ($p=0.01$ and $p=0.04$), and pretreatment minus post 2 h ($p=0.04$ and $p=0.03$). The light group showed significant difference compared with the curcumin[b] group ($p=0.01$).

Surprisingly, this antimicrobial effect was observed for 1 h after PDT (~1 log reduction). These findings corroborate study of Araújo et al.,[1] which investigated the immediate effects of PDT with blue light and curcumin in a clinical trial. Other studies also showed the positive effects of a-PDT. However, these studies used first and second generation photosensitizers such as porphyrin derivative,[24] phthalocyanines,[13] chlorine,[25] toluidine blue,[26] and methylene blue,[27] which may target both gram-negative and gram-positive bacteria.

Regarding a-PDT with curcumin and blue light, several *in vitro* studies were performed. Dovigo et al.[18,19] showed that low curcumin concentrations were effective for inactivating *Candida albicans* when associated with blue LED (450 nm) excitation. In similar studies, Araújo et al.[22,28] found reduction of *Streptococcus mutans* and *Lactobacillus acidophilus* on planktonic cultures,[28] and this reduction was more effective in biofilm compared with carious dentine conditions.[22] In a recent study, Pileggi et al.[20] showed that curcumin associated with a dental quartz-tungsten-halogen light source, emitting blue light (380–500 nm) inactivated *Enterococcus faecalis* on planktonic cultures or in biofilm cultures. In another recent study, Panhóca et al.[29] performed PDT with blue LED and curcumin associated with surfactant (sodium dodecyl sulfate 0.1%), and showed inactivation of *S. mutans* in biofilm, optimizing a-PDT. In the same line, Paschoal et al.[21] showed that a low concentration of curcumin associated with white light (400–700 nm with a central wavelength of 550 nm) illumination lead to inactivation of *S. mutans*.

These results with regard to a-PDT are limited to an *in vitro* model; however, they may support our clinical trial results. Moreover, these *in vitro* model results also showed that blue light irradiation alone or curcumin alone did not reduce CFU.

Unlike other *in vitro* studies, the work of Lipovsky et al.[30] showed bacterial reduction (*Staphylococcus aureus* and *Escherichia coli*) with blue light (415 and 455 nm) alone, mainly at higher fluences (120 J/cm^2); however, at low fluences, blue light enhanced bacterial proliferation.[31] In addition, Feuerstein et al.[14] showed bacterial reduction (*P. gingivalis* and *Fusobacterium nucleaturn*) with blue light (450 nm) at fluences of 62, 78, and 94 J/cm^2 under aerobic condition; however, this phototoxic effect was not observed when the bacteria were exposed to light under anaerobic conditions. Considering blue LED applications, several clinical trials for treatment of acne showed positive effects of this wavelength.[31,32] It is also evidenced by decrease numbers of *Propionibacterium acnes in vitro*.[32] Therefore, the antibacterial effect of blue light is dose dependent as well as dependent upon the response of an organism to O_2 in its environment.

Regarding use of curcumin alone, our study showed that CFU increased significantly, suggesting that curcumin probably caused disaggregation of dental plaque clumps on tooth enamel leading to saliva. Curcumin has been tested as a compound to inhibit fibril formation. Rabbie et al.[33] observed disaggregation of preformed fibrils upon addition of curcumin. Overall, this compound appears to be able to interact with native, intermediate, and fibrillar forms.[34,35] There is a fair amount of support from oral research to suggest that bacteria fibrils are made of protein, and some evidence that suggests that some are even made of glycoprotein. They are difficult to remove, and some strains of oral streptococci have tufts of fibrils (that were grouped together into a new species and given the name ''*Streptococcus cristae*'').[36] There is evidence that fibril tufts and coaggregation may also be involved in adhesion, this time to rod-shaped bacteria, to make the structures commonly found in mature dental plaque called ''corncob-configuration.''[37]

Considering the action on adhesion, our result in which curcumin alone increased CFUs significantly, suggests that this mouth rinse disrupts coaggregation bacteria attachment to a tooth surface, leading bacteria to saliva. However, several studies have shown that curcumin and other oral disinfectants reduced CFU. Curcumin mouth rinse may be dependent upon several factors, including duration of fasting[28] and time of mouth rinse.[38] Although there is a growing number of publications about the effect of curcumin on bacterial reduction,[16,18,28,39,41] few publications[28,39] such as this study observed the magnitude of curcumin as a mouth rinse.

In an *in vitro* model, Hegde and Kesaria[17] showed that curcumin reduced only *C. albicans*, and that sodium hypochlorite and neem (*Azadirachta indica*) were more effective in microbial inactivation, because it reduced both *E. faecalis* and *C. albicans*. In a clinical trial, Bhat et al.[16] showed that the antimicrobial efficacy of neem (3%) was highest, followed by cetylpyridinium chloride (0.5%), curcumin (5%), and chlorhexidine gluconate (0.2%) when the CFU (*S. mutans*) reduction was measured. Chlorhexidine is widely used in dentistry for decontamination. In the study by Hayek et al.,[41] *Prevotella sp.*, *Fusobacterium sp.*, and *Streptococcus beta-haemolyticus* were significantly reduced in ligature-induced peri-implantitis in dogs; however, no significant differences were observed between chlorhexidine and PDT with paste-based azulene and GaAlAs laser ($\lambda=660$ nm).

The action of curcumin alone as mouth rinse is quite encouraging for a further development of the technique, as prewash plaque remover and the reduction of 1 log for 2 h after PDT is an excellent result, considering the advantages of curcumin being a natural substance and harmless to the oral tissues. Overall, decontamination using a simple procedure is desirable for general use in dentistry. In this context, the reduction of bacterial counts and its maintenance during 2 h are important for several intraoral surgical procedures during a single session. Moreover, bacterial reduction is initiated on superficial surfaces of the oral environment, and after multiples sessions of PDT, deeper layers can be achieved. PDT is cumulatively bactericidal.[42] However, some limitations of our study were the small sample size per group, and not having used different procedures for optimizing a-PDT. Future studies should explore these aspects.

Conclusions

In conclusion, in this study, the results indicate that curcumin has a potential to disaggregate oral plaque, and the new blue LED device for PDT-curcumin may be used for reduction of salivary microorganisms lasting 2 h, leading to overall disinfection of the mouth for several intraoral surgical procedures during a single session of dentistry. However, new protocols should be explored to optimize a-PDT.

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Author Disclosure Statement

No competing financial interests exist.

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