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Clinical Comparison of Two Photosensitizers for Oral Cavity Decontamination

Hérica Adad Ricci Donato, PhD, Sebastião Pratavieira, PhD, Clovis Grecco, PhD, Aldo Brugnera-Júnior, PhD, Vanderlei Salvador Bagnato, PhD, and Cristina Kurachi, PhD

Abstract

Objective: In this study, we aim to compare the photodynamic inactivation (PDI) effects of two different photosensitizers (PS), Photogem® and Natural Curcumin, irradiated with light-emitted diodes (LED) at 630 and 450 nm, respectively. Background: The current antimicrobial mouthwash for oral hygiene has several drawbacks. In this context, PDI is an alternative technique to inactivate pathogenic microbes in mucosa and in periodontal tissue. Furthermore, there are numerous infectious diseases that may affect the oral cavity, motivating the use of PDI in dentistry. Methods: The volunteers (n=50) were randomized separated into five experimental groups (n=5) for each PS: water control, PS control, light control, and two PS concentrations (25 and 100 mg/L). Each patient underwent mouthwash solution containing the PS before illumination procedure that was performed with an LED device. For microbial decontamination evaluation, the saliva was collected three times: before (T0), immediately after (T1), and 24 h after the illumination procedure (T2). After that, the difference between the colony forming units (CFU) for each volunteer was compared. Results: The results show that regardless of PS and treatment applied, there was microbial reduction immediately after PDI, however, after 24 h only Natural Curcumin still presents a reduction. For Photogem after 24 h, the microorganism returns to the original CFU. Conclusions: Immediately after PDI, both PS have the same efficiency, nevertheless the Natural Curcumin still has an efficacy after 24 h and also is a more viable photosensitizer. In addition, the results indicate that PDI can be a promised technique used for microbial reducing for the oral cavity.

Keywords: photodynamic inactivation, in vivo, clinical, curcuminoids, hematoporphyrin

Introduction

The oral cavity is a favorable environment for the growth of various microorganisms due to its conditions such as humidity, temperature, and abundant presence of food waste. It is estimated that more than 700 species of microbes inhabit the human oral cavity, in which bacteria are predominant. In this sense, antibiotics have become routinely used in the dental office, especially in the control of postoperative infections after oral surgeries. The antibiotic therapy has provided in the field of infectious disease control the most revolutionary change throughout the history of medicine. However, microbial resistance due to the abusive and nonjudicious use of bacterial agents is now considered a global problem.

Such resistance to current antibiotics has limited the cure of diseases, leading to a growing interest in alternative therapies to combat pathogenic microorganisms. The most frequent infectious diseases that affect the oral cavity are periodontal disease, caries, endodontic infections, and oral candidiasis associated with the use of oral prostheses. A first alternative approach is the use of local methods such as the use of antimicrobial mouthwash that has been proposed as a means of reducing the levels of oral bacteria. In this sense, the chlorhexidine has been widely used in various clinical situations in dentistry. Chlorhexidine has a broad spectrum of action, acting on gram-positive bacteria, gram-negative bacteria, fungi, lipophilic yeasts, and viruses. The action of chlorhexidine mouthwash persists after more than 7 h with bacterial reduction ≥90%. Thus, it exerts a bactericidal action that starts immediately after the mouthwash, combined with a sustained bacteriostatic effect, which prevents bacterial colonization and residual inhibitor effect of the formation of dental plaque. Nevertheless, some side effects are attributed to prolonged oral use, for more than 14 days, such as brownish stains on the teeth, restorations, or in the

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back of the tongue, peeling and loss of oral sensitivity, bitter
taste, and interference with taste sensation. Among these
effects, the dental staining stands out as the main complaint
from patients, the main factor limiting the use of chlor-
hexidine for prolonged periods.5

In this context, photodynamic inactivation (PDI), anti-
microbial photodynamic therapy (aPDT), or photodynamic
antimicrobial chemotherapy (PACT) has arisen as a prom-
ising alternative to microbial control.6–7 The photodynamic
reaction is induced by the interaction of a photosensitizing
agent (PS) with light in the presence of molecular oxygen.
The PS accumulates selectively in cells or microorganisms
or binds to its outer surface (membrane or cell wall) and is
then carried the irradiation of the target tissue with light at a
suitable wavelength.7–5 The microbial reduction by PDI has
been shown effective in various organisms, from fungi,
protozoa, viruses to even undesirable bacteria.10–12

In dentistry, this treatment has been proven highly effec-
tive in endodontic treatments, stomatitis by Candida albicans,6,13,14 and periodontics.15 For conditions, such clinical
situations, which require prior microbial control, patients
undergo various forms of oral surgery to prevent contami-
nation of the surgical site and thus obtain adequate tissue
repair.16 PDI is also convenient instead of mouthwashes
such as chlorhexidine, which has proved itself ineffective in
preventing the establishment of a potentially pathogenic
microbiota.17

The present study compares the in vivo PDI effects of
two different photosensitizers (PS) as mouthwashes for re-
ducing microorganisms in the oral cavity. The PS used were
Photogem and curcuminoids.

Photogem®, a hematoporphyrin derivative, is widely used
as PS in cancerous lesion treatment with Photodynamic
Therapy (PDT).18 It also had good answers in the inactivation
of some microorganisms,19,20 as has been tested, for example,
on Streptococcus mutans and Lactobacillus acidophilus
of carious dentin,20 Candida albicans and Candida glabrata
resistant to fluconazole,21 and Staphylococcus aureus.22 All
these microorganisms can be found in the oral microbiota.

The main curcuminoids are curcumin, demethoxycur-
cumin, and bisdemethoxycurcumin. Curcuminoids are yell-
low pigments found in the rhizome of turmeric (Curcuma
longa L.).23 Many effects have been reported for curcumin,
including immunomodulatory, anti-inflammatory, antioxi-
dant, and antitumor effect.24–26 Curcuminoids also have
antibacterial effects and have been used as PS in PDI to
treat infections,27 in the inactivation of methicillin-resistant
strains of Staphylococcus aureus,28 in species of Candida
albicans,13,29 in Gram-negative and Gram-positive bacte-
ria,27,30 and in larvae of mosquitoes.31 Two similar clinical
trials were already performed with curcuminoids, one used
a salt form of curcumin and the other just explored one
curcumin concentration.32,33

Material and Methods

Selection of volunteers

In total, 50 healthy volunteers of both genders aged 18–40
years, who signed written informed consent, previously
approved by the Ethics Committee in Research (137618–
2012—Federal University of São Carlos), were enrolled in
the study. The volunteers were randomize separated into
two main groups with five experimental conditions (n = 5)
for each PS: water control, PS control, light control, and two
PS concentrations (25 and 100 mg/L). This sample size was
also based on previous studies.32,33 The exclusion criteria of
patients in this study were patients with uncontrolled sys-
temic diseases, those who underwent treatment with systemic
antibiotic therapy in the last 6 months, those who make
continuous use of some type of mouthwash, and smokers.

Photosensitizers

For this study, the following two photosensitizers, Pho-
togem (Photogem Limited Liability Company, Moscow,
Russia) and Natural Curcumin (PDT Pharma, Cravinhos,
Brazil), were used.

Natural Curcumin is a mixture of curcuminoid pigments
(60% curcumin and 40% bisdemethoxycurcumin, de-
methoxycurcumin) and was first solubilized in 99.9% of
absolute ethanol and 0.1% of dimethyl sulfoxide—DMSO.
From a stock solution at 0.15%, final solutions were ob-
tained for testing with 25 and 100 mg/L in distilled water.
Photogem was also tested at two concentrations, 25 and
100 μg/mL; to obtain such final concentrations, only dis-
tilled water was added to the stock solution of 5 mg/mL.

As for the control groups, in addition to the water con-
trol group, in which patients received only a mouthwash with
distilled water (L0C0 and L0P0), there was also a photosensitizer-only control group, which underwent three
types of mouthwash with curcumin 100 mg/L (L0C100) or
Photogem (L0P100), and no illumination of the oral cavity.
These PS concentrations were based on the literature.32,33

Before application of the light source, the patient under-
went three mouthwashes for 1 min each, with 15 mL of a
solution containing one of the PS of choice, always moni-
tored by a professional and without swallowing the solution.

Irradiation

For irradiation, immediately after mouthwash, two home-
made light-emitted diode (LED) devices were used as light
sources. A device emitting in the range of blue light at
450 nm for Natural Curcumin and the other emitting in the
range of red light at 630 nm for Photogem, with a light
intensity of ~100 W/cm². The diffuser tip of both LED
apparatus was placed in the patient’s mouth in a central
position for 6 min, the lips remained sealed for no dispersion
of light during illumination. A control group with light only
was also observed, a water mouthwash followed by the il-
 lumination with the 450 nm LED (C0L6) or 630 nm LED
was also observed, a water mouthwash followed by the il-

Saliva samples and microbiological analyses

For each patient, before the irradiation procedure (T0),
saliva was collected using a falcon-type tube (labeled with
name, date, and time of collection) where the patient spat
up to a minimum of 1.0 mL of saliva. After irradiation, new
saliva collection was made for counting of colony-forming
unit (CFU) in the same way as described in the same pro-
tocol above. The salivary collection after lighting was held
in two stages, immediately after irradiation (T1) and 24 h
postirradiation (T2).
After being collected, samples were taken to the microbiology laboratory, where they suffered six successive dilutions in previously sterile saline and then were seeded in triplicate in droplets of 25 μL on brain–heart infusion plates, a medium capable of supporting the growth of a wide range of microorganisms. The process of serial dilutions aims to reduce the concentration of CFUs, making the counting possible. Then, the plates were incubated at 37°C under aerobic conditions for about 48 h. After the incubation period, counting of CFU/mL was performed; this protocol was in accordance with similar previous studies.

Results and Discussion

The counting of microorganisms—CFU/mL of the oral cavity were expressing as the logarithm (log10). The data are presented as changes in microbial reduction to two evaluation times: immediately after PDI (T1–T0) and 24 h after PDI (T2–T0). The results are shown in Fig. 1 for Natural Curcumin.

<table>
<thead>
<tr>
<th>PS</th>
<th>Groups</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural Curcumin</td>
<td>C0L0 (n = 5)</td>
<td>Absence of light and curcumin—water mouthwash</td>
</tr>
<tr>
<td></td>
<td>C100L0 (n = 5)</td>
<td>Natural Curcumin mouthwash 100 mg/mL and absence of light</td>
</tr>
<tr>
<td></td>
<td>C0L6 (n = 5)</td>
<td>Water mouthwash and illumination of 6 min</td>
</tr>
<tr>
<td></td>
<td>C25L6 (n = 5)</td>
<td>Natural Curcumin mouthwash 25 mg/mL and illumination of 6 min</td>
</tr>
<tr>
<td></td>
<td>C100L6 (n = 5)</td>
<td>Natural Curcumin mouthwash 100 mg/mL and illumination of 6 min</td>
</tr>
<tr>
<td>Photogem</td>
<td>P0L0 (n = 5)</td>
<td>Absence of light and Photogem—water mouthwash</td>
</tr>
<tr>
<td></td>
<td>P100L0 (n = 5)</td>
<td>Photogem mouthwash 25 μg/mL and absence of light</td>
</tr>
<tr>
<td></td>
<td>P0L6 (n = 5)</td>
<td>Water mouthwash and illumination of 6 min</td>
</tr>
<tr>
<td></td>
<td>P25L6 (n = 5)</td>
<td>Photogem mouthwash 25 μg/mL and illumination of 6 min</td>
</tr>
<tr>
<td></td>
<td>P100L6 (n = 5)</td>
<td>Photogem mouthwash 100 μg/mL and illumination of 6 min</td>
</tr>
</tbody>
</table>

The analysis of the difference of microbial reduction was the choice for processing of experimental data of microbial count in different individuals with different microbial flora, allowing a comparison between patients of the same group.

After the treatment, microbial reduction was observed for all groups, even in the control group.

The average values show that post-PDI (T1–T0) occurs microbial reducing. Although the groups C0L6, C30L6, and C100L6 have promoted a high reduction rate, the control group had the greatest reduction rate. After 24 h, the post-PDI C100L6 group does not show microbial regrowth. On the contrary, remaining groups that show immediate reduction showed microbial regrowth 24 h after treatment and control curcumin (C100L0) showed the highest regrowth.

In Fig. 2 is presented the PDI for Photogem. According to the average values of reduction, it was observed that microbial reduction occurred immediately after PDI for all groups and P100L6 group shows the greatest reduction. However, 24 h after PDI, all groups showed a high rate of regrowth.

Decontamination of oral cavity becomes a relevant issue in situations ranging from routine before dental procedures...
to oral surgery. Therefore, systemic diseases have their origin in the oral cavity, which is considered the gateway to a large number of microorganisms.

The actuality of the topic justifies the variety of protocols used in this study. The concentration and the PS used, the irradiation exposure, and mouthwash time were variable parameters used because it is a new method for oral decontamination and has little support in literature.32,33

The results show that Natural Curcumin was more effective in reducing oral microflora when used at a higher concentration (100 mg/mL). Dovigo et al.,34 in a study in vivo (with mice) using curcumin for performing PDI in the treatment of oral candidiasis, showed a significant reduction in the microbial concentration of 30 mg/L. However, the brand used by the authors is different from that used in the present study, so that the purity of the product is not the same, thus justifying the concentration of curcumin required for efficiency thereof. The curcumin used by Dovigo et al. came from Sigma-Aldrich with 70% of purity, and the one used in our study is produced by PDT Pharma with 53% of purity.

In contrast, the Photogem had the highest average microbial reduction immediately after therapy when used at a concentration of 100 µg/mL followed by the concentration of 25 µg/mL. Nevertheless, in all groups, this PS maintains the microbial reduction after 24 h of PDI, an unfavorable factor when the goal of oral intervention targeting oral decontamination over a longer term, such as in certain cases such as surgical tooth extraction and dental implant in periodontal scaling procedures such as among others.

For Photogem, the experimental group of 100 µg/mL (P100L6) had the highest microbial, although only immediate reduction (T0–T1). The choice of this concentration for our study was based on the antimicrobial effect of PDI used for surface disinfection of complete dentures in vivo study, where the concentration of 100 µg/mL was more effective,35 while the Photogem concentration of 25 µg/mL tested was supported by in vitro studies,22 in which this concentration of PS associated with the light wavelength 628 nm was effective in microbial inactivation.

The control group only light (C0L6) at a wavelength of 450 nm has shown high reduction in bacterial counts immediately after its application in the oral cavity, supported in the literature in which studies have shown the potential of blue light to promote bactericidal effect on different microorganisms such as S. aureus, E. coli, and P. gingivalis.36,37 However, the red light (POL6) alone did not show such great amounts of microbial reduction, which was also according to literature in which the irradiation wavelength at 625 nm was not able to exert a bactericidal effect on microorganisms previously cited.37

Although the observation of mean microbial reduction has allowed the previous statements, the lack of statistical significance between the results, independence PS tested, can be justified by several factors among own oral anatomy plus the type of lighting performed in the study. The lighting was central type, in which the diffuser tip was kept at rest supported on the tongue and in contact with the palate. In this position, the oral anatomy is very difficult to achieve light palatine and vestibular surfaces simultaneously, which detracted from the excitement of the PS and hence the effectiveness of PDI in these areas considered shade.

Regarding symptoms, some pertinent to curcumin group patients, independent of the concentration of PS or lighting time, reported pruritus during illumination after mouthwash, but always mild. The same symptoms were observed in the in vivo study of oral decontamination using curcumin and blue LED.33 As for the patients belonging to the group Photogem, no symptoms were reported. So far, for all individuals studied, any pain sensation and late effects such as the emergence of ulcers have been reported.

An important factor to be considered when dealing with oral decontamination is to change the color of teeth as well as aesthetic materials since the completion of the mouthwash is part of the protocol. Some substances such as toluidine blue O (TBO), a PS used effectively in PDI,20,38 can cause changes in the color of the tooth surface and resinous restorations due to their nature highly of pigmentation,20,39 which its contraindicate use in mouthwashes. However, the staining observed in the bovine enamel surfaces and composites using Photogem and curcumin is considered dependent on the storage time and the concentration,40 so the concentrations of PS used in this study and the short time of mouthwash present no potential for staining of teeth and/or restorations.

Among the PS used in this study, a factor that prevents the use of Photogem for oral decontamination besides not having submitted late effect of microbial reduction in the reported period (24 h) is the costliness of the product. Photogem is a hematoporphyrin derivative, and the cost of Photogem is about four times greater than curcumin; since this microbial reduction with PS was not proportionally increased, the cost effectiveness of this product, in this case, becomes impractical.

While many in vitro studies demonstrated the efficacy of PDI in reducing oral bacteria with different PS as TBO,20 Photogem,20 and curcumin,41 in vivo studies focused on oral decontamination become necessary to adjust the proposed protocols.

When compared with other therapies, PDI offers several advantages such as high target specificity, that is, the death of the bacteria can be controlled by restricting the irradiated region, besides few adverse side effects when using proper protocols, and yet no development of resistance by microorganisms, with viable cost due to the use of PS and inexpensive light sources.12,16 However, more studies need to be done to improve the microorganism reduction and make this an efficient clinical procedure.

Conclusions

Photodynamic reactions using Photogem and Natural Curcumin followed by a specific illumination are a promising technique for reduction of microorganisms in the oral cavity. Furthermore, from the average, we can conclude that Natural Curcumin at a concentration of 100 mg/mL with 6 min illumination has greater prominence by presenting microbial reduction immediately after PDI and maintenance of this reduction even after 24 h of therapy elapsed.

The use of photosensitizers can be a promised technique used for microbial reducing of the oral cavity. Because photosensitizers can be used in an ecofriendly approach, there is a lack of bacterial resistance induced in microorganisms.
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Author Disclosure Statement

No competing financial interests exist.

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