Scientific Research Publishing

Advances in Microbiology, 2020, 10, 194-201
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Comparative Analysis *in Vitro* of the Application of blue®m Oral Gel versus Chlorhexidine on *Porphyromonas gingivalis*: A Pilot Study

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How to cite this paper: Deliberador, T.M., Weiss, S.G., Rychuv, F., Cordeiro, G., Cate, M.C.L.T., Leonardi, L., Brancher, J.A. and Scariot, R. (2020) Comparative Analysis *in Vitro* of the Application of blue®m Oral Gel versus Chlorhexidine on *Porphyromonas gingivalis*: A Pilot Study. *Advances in Microbiology*, **10**, 194-201.
https://doi.org/10.4236/aim.2020.104015

Received: March 28, 2020
Accepted: April 21, 2020
Published: April 24, 2020

Abstract

Oxygen is an essential nutrient for cellular metabolism, especially energy production. The substance is involved in multiple processes including oxidative killing of bacteria, reepithelialization, angiogenesis, and collagen synthesis. In order to test and compare the effects of the oxygen gel blue®m *in vitro* on *Porphyromonas gingivalis*, four groups were evaluated: 100% oxygen gel (B1), 75% oxygen gel (B2), 50% oxygen gel (B3), and 100% 0.12% chlorhexidine digluconate solution (C1). For this purpose, evaluations of the proportion of bacterial growth were performed, using the Agar diffusion test. The results demonstrated that blue®m at a dose of 100% and 75% is similar to chlorhexidine ($p > 0.05$); however blue®m at a concentration of 50% showed a lower inhibition halo when compared to chlorhexidine ($p = 0.024$). blue®m at higher concentrations provided inhibitory halo of *Porphyromonas gingivalis* similar to chlorhexidine digluconate, while blue®m at lower concentration had a lower bacterial inhibition halo compared to chlorhexidine.

Keywords

Porphyromonas gingivalis, Periodontics, Chlorhexidine, Oxygen

1. Introduction

Periodontitis is a multifactorial chronic inflammatory disease associated with an altered dental biofilm that causes the progressive destruction of the supporting tissues of the teeth [1]. Some disorders and systemic conditions can affect the periodontium and cause loss of periodontal insertion and alveolar bone, influen-

cing periodontal inflammation or through mechanisms other than periodontitis (such as genetic, immunological factors or some medications) [2].

Periodontitis is treated with basic periodontal procedures and respective maintenance and sometimes may require standard administration of amoxicillin and metronidazole and of topical use of chlorhexidine digluconate [3] [4] [5]. This is necessary due to the high resistance of periodontopathogenic bacteria, such as *Porphyromonas gingivalis* (PG). PG is a Gram-negative oral anaerobic bacterium, involved in the pathogenesis of periodontitis, which has numerous virulence factors, capable of inducing intense tissue destruction in periodontal infections [6].

Oxygen is an essential nutrient for cellular metabolism, especially energy production. The substance is involved in multiple processes including oxidative killing of bacteria, reepithelialization, angiogenesis, and collagen synthesis [7] [8]. Maybe the main function attributed to oxygen is energy production, both in eukaryotic cells and in eukaryotic cells; however there is a paradox since oxygen is indispensable for the maintenance of cell life and on the other hand, it can cause cell death when the cell is exposed to high concentrations of it [9]. A team of dental surgeons led by Dr. Peter Blijdorp in the Netherlands, developed a product based on active oxygen (blue®m), with the intention of putting all the desirable properties of mouthwashes in just one product. blue®m has in its composition sodium perborate, the glucose oxidase enzyme derived from honey, xylitol and lactoferrin. For dental applications, a new product that releases oxygen has recently been brought to market. The company's main products are oral gel, toothpaste, mouthwash and mouth foam. Inclusion in everyday hygienic oral care of toothpaste and mouthwash blue®m reduced the severity of inflammatory changes and improved the hygienic condition of the oral cavity in cardiology patients suffering from periodontal disease [10]. A Randomized Controlled Clinical Trial showed that toothpastes containing active oxygen and lactoferrin have comparable antiplaque and antigingivitis efficacies with triclosan-containing toothpastes [11]. However, there is lack of studies regarding oxygen therapy for topical antibacterial treatment in periodontitis in the scientific community.

Considering the benefits that slow oxygen release seems to induce in wound healing [12] [13] and its beneficial effects in the few clinical studies related to periodontal diseases, more studies are needed to investigate the effect of this new gel on periodontitis and on *Porphyromonas gingivalis*.

This pilot study aims to test and compare the *in vitro* effects of the product in gel with slow release of oxygen (blue®m) and chlorhexidine on *Porphyromonas gingivalis.*

2. Materials and Method

2.1. Microbiological Assay

The raising technique is a simple and traditional method used in the maintenance of cultures in the laboratory. To verify the viability of the bacterium, the

technique by seeding exhaustion was performed, in which a loop of nickel chrome flamed by fire and cooled then collects a portion of bacteria and transfers it to the plates of Agar Muller-Hinton, disposed in a Gaspak jar, in the presence of an anaerobic sachet, incubated in an oven for 72 hours at a temperature of 36˚C. After the incubation period, bacterial colonies grew (**Figure 1**), confirming their viability.

2.2. Agar Diffusion Test

For the agar diffusion test, 6 petri dishes with 20 mL Mueller-Hinton culture medium were inoculated with *Porphyromonas gingivalis* (ATCC 33277). With a pipette, 0.2 mL of bacteria was inoculated into 5 mL of brain heart infusion (BHI) broth (Oxoid Brazil Ltd). The test tube containing the inoculated culture medium was vortexed until the medium became homogenous. The inoculated BHI was adjusted to a turbidity reading of 0.5 on the McFarland scale, corresponding to a bacterial concentration of approximately 1.5 × 108 cells/mL. Then, 200 mL of this bacterial broth was deposited onto the agar surface and uniformly spread using a sterile Drigalski loop.

In order to test and compare the effects of oxygen on *Porphyromonas gingivalis*, 3 plates were evaluated with samples of blue®m and 0.12% chlorhexidine digluconate. For this, 12 sterile filter paper disks with a diameter of 5 mm were soaked with the solutions described in **Table 1** for 30 seconds. With the aid of a tweezers, the discs were deposited with a slight pressure on the Petri dish on the surface of the Agar in an equidistant manner as determined on the back face of the plate (**Figure 2**), under aseptic conditions. A single plate was divided into 4 equal parts and blue®m gel was applied in three different concentrations (B1 100%, B2 75% and B3 50%) besides 0.12% chlorhexidine digluconate in a concentration of 100% (C1). Three identical plates were repeated for the same bacteria. This was performed on the posterior face of the plate and under aseptic conditions.

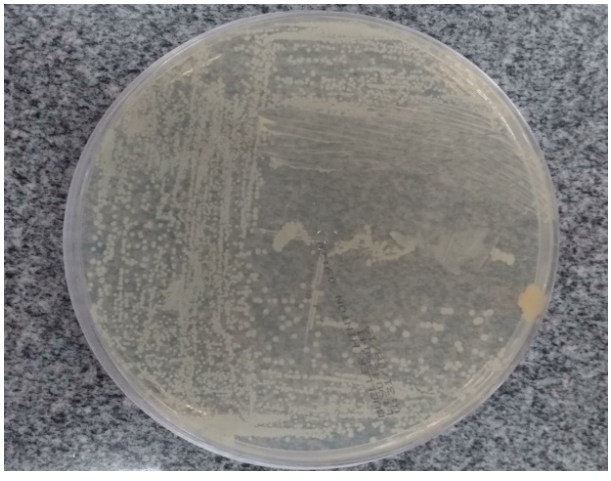

Figure 1. Growth of *Porphyromonas gingivalis* colonies in a Petri dish.

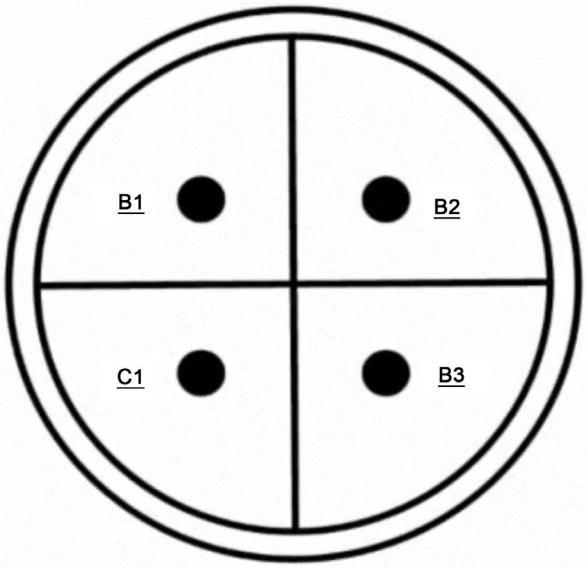

Figure 2. Arrangement of the groups tested in the Petri dish under the culture medium.

Table 1. Samples distribution according to the tested concentrations.

Groups	Substance
B1	blue®m 100%
B2	blue®m 75%
B3	blue®m 50%
C1	0.12% Chlorhexidine Digluconate (100%)

After carrying out the experiment, the plates were kept at room temperature for 30 minutes to allow the substances to diffuse before microbial development. Then, the plates were incubated in an oven at 37˚C for 72 hours. At the end of this period, the diameter of the halos of inhibition of bacterial growth around the paper discs promoted by the tested solutions was measured by two different examiners and recorded in centimeters using a transparent millimeter ruler. For this, four measurements perpendicular to each other were used, obtaining the average of their sizes.

2.3. Statistical Analysis

To assess the reliability of the data obtained between the examiners, the intraclass correlation coefficient (ICC) test was performed. All analysis was performed with a significance level of 0.05 in the Statistical Package for Social Science Software (SPSS 21.0). In order to assess the normality of the data, the Shapiro Wilk test was used. To perform the comparison between the groups, Kruskall Wallis and Mann Whitney tests were performed.

3. Results

Bacterial growth *in vitro* of the bacterium *Porphyromonas gingivalis* was ob-

served in all groups, but in a very reduced way in group C1 and slightly accentuated in groups B1, B2 and B3 compared to the group C1 (Figure 3). The median of the diameter of the inhibitory halos obtained by the calibrated examiners (CCI 0.901) is shown in Table 2.

The blue®m at a concentration of 100% and 75% is similar to chlorhexidine ($p > 0.05$), however blue®m at a concentration of 50% showed a lower inhibition halo when compared to chlorhexidine ($p = 0.024$).

4. Discussion

After the text edit has been completed, the paper is ready for the template. Duplicate the Chlorhexidine Digluconate is a chemical substance with antibacterial action against Gram-positive and Gram-negative bacteria. Studies prove that toothpastes and mouthwashes that have chlorhexidine digluconate in their composition decrease the metabolic activity of microorganisms, paralyzing their metabolic components [14]. However, the prolonged use of chlorhexidine should be avoided, due to its adverse effects, such as staining on teeth , restorations and prostheses, altered taste and peeling of the mucosa [15]. Therefore, it is necessary to search for products with the same antimicrobial efficacy and less side effects. Recently, the blue®m products, a slow oxygen release product, has been used by clinical dentists for the treatment of gum disease and as an aid in the healing of oral wounds in the post-surgical period. Some studies show they oxygen may act in several cellular mechanisms involved in cicatrization such as angiogenesis [7] [16].

In the present study, the Agar diffusion test was performed in order to verify the *in vitro* action of the new product blue®m gel compared to 0.12% chlorhexidine digluconate in inhibiting the growth of the bacterium PG, which is considered

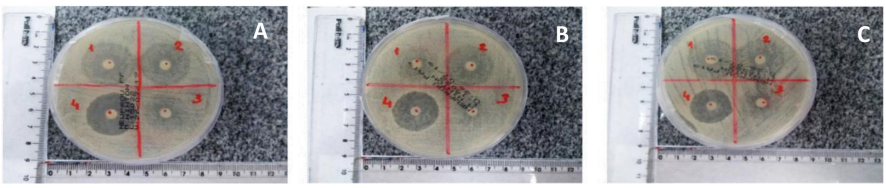

Figure 3. Petri dishes containing the *Porphyromonas gingivalis* bacteria and the inhibition halos caused by the solutions (1, 2, 3, 4): blue®m 100%, 75% and 50% and chlorhexidine diguclunate, respectively.

Table 2. Measurement of the diameters (in mm) of the growth inhibition zones of *Porphyromonas gingivalis.*

Group	Median (min - max)	p value
B1	11.5 (7 - 13)^{ab}	0.041
B2	11.5 (9 - 13)^{ab}	
B3	7.5 (6 - 11)^{a}	
C1	24 (21 - 26)^{c}	

Kruskall Wallis test, $p = 0.041$. Different letters demonstrate statistically significant difference: Mann Whitney test, $p = 0.024$.

one of the most periodontopathogenic bacteria. In this test, it was observed that all solutions presented an inhibitory halo against the tested microorganism, confirming its antimicrobial property. The 0.12% chlorhexidine digluconate, the most used solution today, resulted in an equal bacterial inhibition halo as blue®m in concentrations of 75% and 100%, significantly reducing the concentration of bacteria.

It is also important to note that the main solution to the global antibiotic resistance crisis is to reduce the volume of antibiotic use. One of the novel agents is using reactive oxygen species (ROS), oxygen radicals, as an antimicrobial mechanism [17]. ROS is highly antimicrobial against Gram-positive and negative bacteria and prevents the formation of biofilms caused by a range of bacterial species. Thus, ROS is highly suitable for chronic inflammatory conditions, such as periodontitis. Its effects have been demonstrated, among others, in chronic wounds, mucosal infections in the respiratory tracts and in prosthetic device [18]. ROS has also demonstrated greater anti-MRSA (methicillin-resistant Staphylococcus aureus) biofilm efficacy than mupirocin *in vitro*, suggesting a possible role for topical clearance of MRSA colonized patients [19]. Since, ROS can be delivered to the site in many ways, such as topical application, thus providing an alternative to systemic antibiotics, the results of the present study are promising.

This pilot study has the limitation of testing only one bacterium related to periodontitits (PG); however, a wide range of bacteria has been described to be associated to the condition [20]. Thus, the evaluation of this product in the biofilm is necessary to demonstrate its effects on other pathogen microorganisms in order to determine if it can be as effective in controlling periodontitis, with less adverse effects. In addition, further microbiological testing is necessary to understand the mechanism of action of the blue®m oral gel.

5. Conclusion

blue®m at higher concentrations provided inhibitory halo of *Porphyromonas gingivalis* similar to 0.12% chlorhexidine digluconate, while blue®m at lower concentration had a lower bacterial inhibition halo compared to chlorhexidine.

Conflicts of Interest

The authors declare there are no conflicts of interest.

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