Virucidal activity of the antiseptic mouthwash and dental gel containing anionic phthalocyanine derivative: in vitro study

Caique Andrade Santos
Bauru School of Dentistry of University of São Paulo https://orcid.org/0000-0001-5646-3424
Bernardo da Fonseca Orcina
Bauru School of Dentistry of University of São Paulo https://orcid.org/0000-0003-3367-483X
Verônica Brito Reia
Bauru School of Dentistry of University of São Paulo https://orcid.org/0000-0003-1352-5474
Lucas Gabriel Ribeiro
Botucatu Medical School, São Paulo State University (UNESP) https://orcid.org/0000-0002-1109-0385
Rejane Maria Tommasini Grotto
Botucatu Medical School, São Paulo State University (UNESP) https://orcid.org/0000-0002-4035-9486
Aruã Mastrangelo Prudenciatti
Botucatu Medical School, São Paulo State University (UNESP) https://orcid.org/0000-0002-7896-0453
Leonardo Nazario de Moraes
Botucatu Medical School, São Paulo State University, (UNESP) https://orcid.org/0000-0003-4515-4137
Mariana Schutzer Ragghianti Zangrando
Bauru School of Dentistry of University of São Paulo https://orcid.org/0000-0003-0286-7575
Fabiano Vieira Vilhena
Trials Technology https://orcid.org/0000-0003-3840-3633
Paulo Sérgio da Silva Santos (✉ paulosss@fob.usp.br)
Bauru School of Dentistry of University of São Paulo https://orcid.org/0000-0002-0674-3759

Short Report

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Abstract

Aim: This research suggested an *in vitro* virucidal action of a dental gel and a mouthwash with phthalocyanine derivative.

Purpose: The aim of this study was to report an *in vitro* study evaluating the virucidal capacity of mouthwash and dental gel containing anionic phtalocyanine derivate (APD).

Methods: The research followed the recommendations of the National Health Surveillance Agency (ANVISA) and adapted methodology, described in the standards EN14776: 2015; ASTM E1053-11 and the Robert Koch Institute - RKI, in addition to Good Laboratory Practices (GLP). The determination of the percentage of inactivation of the SARS-CoV-2 virus particles was carried out by imposing the viral solution in contact with the respective tested products, with intervals of 30 seconds, 1 and 5 minutes, with subsequent submission of the aliquots, recovered in cell culture microplates following virus titration using the TCID50 (50% Median Tissue Culture Infectious Dose).

Results: The Mouthwash APD presented 90% of viral inactivation percentage while the dental gel APD demonstrated 99.99% of viral inactivation.

Conclusion: *In vitro* analyzes showed that mouthwash and dental gel APD can reduce the viability of SARS-CoV-2 virus particles.

Introduction

Phthalocyanines are chemical compounds widely used as photosensitizing dyes in photodynamic therapy (PDT). The antimicrobial property of phthalocyanines has been demonstrated in several studies in the literature, whether associated with PDT or in its free form, also including antibiofilm action.[1,2,3] This characteristic is based on the good adhesion of this molecule to microbial cells, as well as the changes caused in the cellular components of microorganisms. Recently, our research group found *in vitro* intense antiviral activity of anionic phthalocyanine derivative (APD) against SARS-CoV-2 in non-cytotoxic concentrations.[4]

The infection by SARS-CoV-2 occurs by transmission through human saliva possibly when talking, coughing, sneezing or even breathing and droplets formed may contain microorganisms. Each cough can produce 3,000 droplets of saliva, which is similar to 5 minutes of conversation with another person.[5] This is the reason why the oral cavity is linked not only in the infection but also in the transmission of COVID-19.[6] The most common receptor involved in this relationship is the Angiotensin-Converting Enzyme 2 (ACE-2) which is present in high concentrations in the lungs, myocardial and renal cells, as well as in the oral mucosa, especially in the tongue and salivary glands, the latter being identified as a virus reservoir.[7,8]

The pathophysiology of the COVID-19 itself highlights the importance of effective measures in the early stages of the disease, considering that in the first week of infection, individuals present their symptoms in

the upper respiratory tract.[9,10] On the other hand, many of the infected individuals showed symptoms and even neurological sequels, and this is suggested by the entry of SARS-CoV-2, present in the oral or nasal mucosa, in the central nervous system, showing how the severity of the disease.[11]

Therefore, the objective of this in vitro study was to test a mouthwash and a dental gel both containing a phthalocyanine derivative in the possible inactivation of SARS-CoV-2.

Material And Methods

1. Materials

Aliquots of SARS-CoV-2 obtained from oral samples isolated from patients diagnosed with the new coronavirus and expanded in cell culture Vero ATCC ® CCL-81TM (Fiocruz, BR) were used for tests.

Vero ATCC ® CCL-81TM (Fiocruz, BR) cells were maintained in culture with Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum in an incubator at 37°C and 5% CO2. As for the cultivation of SARS-CoV-2, titration was initially carried out according to the plaque assays quantitative method to quantify the plaques formed in cell culture upon SARS-CoV-2 infection with serial dilutions of the virus, in addition to the determination of viral load through a calibration curve by RT- qPCR.

The products used to assess the virucide effect were: mouthwash APD and dental gel APD (Rabbit Corp, BR).

2.1 – Measuring maximum non-cytotoxic concentration of products

To determine the concentration of each product to be tested, which does not cause toxicity to the cells, the end-point-dilution method was used. The methodology involves the inoculation of successive dilutions (pure compound until 10^{-8} dilutions) of the compounds, in Vero ATCC ® CCL-81™, in which it is possible to determine the cytotoxic effect in 50% of the cells in culture (DCTI50). The assay was conducted in 96-well, flat-bottomed and sterile microplates, in which $2x10^5$ Vero 81 cells were added per well and 200 µL of DMEM supplemented with 2% fetal bovine serum and 1% antibiotic-antimycotic. Thus, the microplates were incubated at 37°C in an incubator with 5% CO_2 for 72 hours.

In order to determine product concentrations that would not cause cytotoxic effects and that could not provide incongruous interpretations to distinguish artifacts from cytopathic and cytotoxic effects, it was observed that for the dental gel APD a dilution factor of 10^{-4} would be necessary. In addition, for mouthwash APD it was not necessary to perform the dilutions because no toxic effect was verified for the used cell line.

2.2 – Virucidal activity tests

The experiment was performed following good laboratory practices (GLP), methodologies described and adapted according to the standards, EM 14476.[12]

The experimental groups were:

1. Positive Control: Only the viral solution and cellular system

2. Negative Control: Cellular system only

3. Treatment 1: Cellular system, Viral solution and Dental Gel APD (dilution without cytotoxic effect)

4. Treatment 2: Cellular system, Viral solution and Mouthwash APD (dilution without cytotoxic effect)

Virucidal activity tests were performed in three times (30 seconds, 1 and 5 minutes) of exposure and contact of the compounds with the SARS-CoV-2 viral solution. It should be noted that a 96-well microplate was used for each compound and the respective contact times. From these samples, dilutions of the viral solution in base 10 were made in replicates in the 96-well sterile microplates, in the dilution factors from 10^{-1} to 10^{-8}, and the dilutions were carried out with DMEM with 2% fetal bovine serum (inactivated). Microplates at the desired confluence of Vero ATCC ® CCL-81™ cell lines with a concentration of $2x10^5$ cells / well with DMEM with 2% fetal bovine serum (inactivated) were prepared so that the dilutions of the assays of different times of contact of each product. The microplates were incubated in an incubator with 5% CO2 for 1 hour under agitation, then the viral particle can get into the host cell. Subsequently, each well of the microplates was washed with 200 µL of buffered saline solution (PBS), with the consequent addition of 200 µL of DMEM with 2% fetal bovine serum (inactivated) and 1% of antibiotic-antimycotic. For the positive control, only the viral solution and the cell concentration specified above were used. The microplates containing the viral solution after contact with the products and the cellular system were incubated at 37°C in an incubator with 5% CO_2 for 72 hours. After this interval, it was evaluated by means of optical microscopy whether there was a cytopathic effect, based on Spearman & Karber method.[13] To determine the viral inhibition index, a logarithmic difference between the control groups and experimental groups was used, through the endpoint calculation method.[14]

Results

According to the experimental design used, the results are presented in percentage of viral inactivation (Table 1) in comparison with the positive control. The mouthwash APD presented 90% of viral inactivation percentage, while the dental gel APD demonstrated 99.99% of viral inactivation in 30 seconds, 1 and 5 minutes.

Table 1
Inactivation percentual of Mouthwash APD and Dental Gel APD.

Products	Viable copies per mL / SARS-CoV-2	Viral inactivation (%)	Incubation time
Positive Control	10E + 5,5	-	-
Mouthwash APD	10E + 4,5	90%	30 s
			1 min
			5 mi
Dental Gel APD	10E + 1,5	99,99%	30 s
			1 min
			5 min
Abbreviations:			
APD, Anionic phthalocyanine derivative			

Discussion

According to this *in vitro* study, the use of mouthwash APD and dental gel APD, in maximum and non-cytotoxic concentration, at different times of exposure and contact with the SARS-CoV-2 viral solution, inactivated viral load by 90% and 99.99%, respectively, compared to the positive control, which corroborates the excellent results of previous studies.[4,10,17]

Considered as a gateway for infectious agents, the oral cavity is directly related to COVID-19 disease, since the spread of the SARS-CoV-2 virus can occur through saliva.[15,16] The viral load of the oral microbiota has been used as a potent indicator for the increase in the severity of the disease and as a consequence, its transmission on a larger scale.[17] Thus, mouthwashes containing oxidizing agents work as a viable option for reducing viral load in the oral cavity and oropharynx and are widely recommended.[4]

Phthalocyanine derivatives have the principle of promoting self-activation and continuous production of reactive oxygen in the presence of molecular oxygen.[2] Two case series studies showed that solutions used for gargling/rinsing with the phthalocyanine derivative contributed to the clinical improvement and general health of individuals infected with COVID-19.[10] An in vitro study, demonstrated a promising antiviral action against SARS-CoV-2 in non-cytotoxic concentrations with efficiency between 92% and 99%. The same study also reported a clinical evaluation with significant decrease in hospital stays, to 4 days, with no evolution in the severity of cases. The individuals contaminated by COVID-19 included in this clinical study used 5 ml of the solution with the phthalocyanine derivative for 1 minute, 5 times a day. This evidence raises the possibility of the benefit for the world population that lacks preventive and

therapeutic treatments in the face of this virus and would further enhance its indication as complementary therapy, even helping hospitalized patients.[4]

Promising results are found in this study with the use of phthalocyanine derivatives in the form of a mouthwash and dental gel to reduce the *in vitro* viability of virus particles, suggesting that they may be useful in reducing transmission, aid in adjuvant treatment and prevention of contamination by COVID-19. Studies *in vivo* are necessary to demonstrate the action of phthalocyanine derivates in oral hygiene products as an adjunctive strategy against SARS-CoV2.

Conclusion

Testing the Mouthwash APD against SARS-CoV-2 demonstrated virucidal activity within 30 seconds. According to in vitro study and after the exposure time was shown that Moutwash APD reduce about 90% of SARS-CoV-2 viral particles. In addition, the Dental Gel APD was also active against SARS-CoV-2 as 99,99% of viral inactivation within 30 seconds.

Declarations

Competing interests:
The research received financial support to perform the laboratorial tests by Golden Technology. There are no potential competing interests of the authors, except of the Fabiano Vieira Vilhena. The other authors are researchers of their respective universities."

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