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Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology 33 (2021) 475–477

Contents lists available at ScienceDirect

Journal of Oral and Maxillofacial Surgery, Medicine, and Pathology

journal homepage: www.elsevier.com/locate/jomsmp

Original Research

Virucidal activity of oral care products against SARS-CoV-2 *in vitro*

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ARTICLE INFO

Article history:
Received 27 November 2020
Received in revised form
27 December 2020
Accepted 3 February 2021
Available online 22 February 2021

Keywords:
SARS-CoV-2
Virucidal activity
CPC (cetylpyridinium chloride)
CHX (chlorhexidine gluconate)
Delmopinol hydrochloride

ABSTRACT

Objective: Coronavirus disease 2019 (COVID-19) caused by infection by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide. Since reducing the amount of virus in saliva is considered to prevent broader infection, the Center for Disease Control (CDC) and American Dental Hygienists' Association (ADHA) have recommended use of CPC- or CHX-containing oral care products before the dental procedure. However, there is no certified evidence. So, we examined inactivation of SARS-CoV-2 by oral care products in several countries *in vitro*.

Methods: 0.05 % Cetylpyridinium chloride (CPC) mouthwash, 0.05 % CPC toothpaste and 0.30 % CPC spray in Japan; 0.06 % chlorhexidine gluconate (CHX) + 0.05 % CPC mouthwash and 0.12 % CHX + 0.05 % CPC mouthwash in Europe; 0.075 % CPC mouthwash, 0.12 % CHX mouthwash, and 0.20 % delmopinol hydrochloride mouthwash in the USA; and 0.04 % CPC mouthwash in China were assessed for their virucidal activity with ASTM E1052.

Results: The virus was inactivated *in vitro* by the contact time in directions for use of all oral care products containing CPC or delmopinol hydrochloride as anticeptics.

Conclusions: These results suggest that these oral care products in each country may reduce the viral load in the mouth.

1. Introduction

Coronavirus disease 2019 (COVID-19) due to infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide from December 2019, and COVID-19 was declared a pandemic by the World Health Organization (WHO) in March 2020. On November 18th, 2020 the WHO reported that 54,771,888 people had been infected, with the result of 1,324,249 deaths [1].

SARS-CoV-2 is a beta coronavirus that is about 50–200 nm in size, has an envelope as a lipid bilayer, and a single-stranded positive-sense RNA genome of about 30,000 bases [2]. Urgent research and development on cell models of SARS-CoV-2 infection, therapeutic agents, and vaccines is underway. The infection route is contact infection or droplet infection by saliva, with infection by saliva droplets being most common [3,4]. The oral cavity is considered to be a reservoir for viruses, and reducing the amount of virus in the oral cavity can lead to prevention of infection in other individuals [4]. Povidone-iodine (PVP-I), Cetylpyridinium chloride (CPC), chlorhexidine gluconate (CHX), and delmopinol hydrochloride are often added to oral care products as antiseptics. PVP-I is an excellent fungicide that has an inactivating effect on SARS-CoV-2 *in vitro* and *in vivo* [5–8]. However, PVP-I and CHX carry a risk of anaphylactic shock and also PVP-I cannot be used by people with impaired thyroid function [9,10].

The Center for Disease Control (CDC) and American Dental Hygienists' Association (ADHA) have recommended use of CPC- or CHX-containing mouthwash before the dental procedure against COVID-19 [11,12]. However, only a few reports have examined an inactivating effect of CHX on SARS-CoV-2 [7,13] and some have suggested effects of CPC on other viruses [3,14–17], but with no certified data on direct inactivation effects of CPC on SARS-CoV-2 [18]. On the other hands, in previous studies, virucidal activities were tested against some Coronaviruses [19,20]. There are some different anti-viral sensitivities depend on Coronavirus species, for example Beazalkonium chloride and hydrogen peroxide [7,19].

Therefore, in this study, we evaluated SARS-CoV-2 inactivation *in vitro* by oral care products made under the regulations of quasi-drugs, drugs, medical device or cosmetics standards of several different countries.

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https://doi.org/10.1016/j.ajoms.2021.02.002

2. Materials & methods

All tests in the study were performed by QTEC (Japan Textile Products Quality and Technology Center, https://www.qtec.or.jp/en) as a test contract facility.

2.1. Virus and cells

SARS-CoV-2 (JPN/TY/WK-521 strain) was obtained from the National Institute of Infectious Disease (Tokyo, Japan) and VeroE6/TMPRSS2 (JCRB1819) cells were obtained from the JCRB Cell Bank (National Institutes of Biomedical Innovation, Health and Nutrition; Osaka, Japan) [21]. The cells were cultured in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA) and washed with Minimum Essential Medium Eagle (EMEM, Sigma-Aldrich). The cells were then inoculated with SARS-CoV-2 for 1 h and an appropriate amount of EMEM was added. After 1–3 days, the culture soup was harvested and centrifuged at 4°C and 1000 *g* for 15 min. The supernatant was combined with Fetal Bovine Serum (FBS, Sigma-Aldrich) at a final concentration of 5 %. This solution was used as the test virus suspension.

2.2. Test solutions

The test solutions are shown in Table 1. The oral care products were 0.05 % CPC mouthwash [Product No.2: GUM® WELL PLUS Dental rinse (alcoholic type) (Sunstar, Osaka, Japan), No.3: GUM® WELL PLUS Dental rinse (non-alcoholic type) (Sunstar, Osaka, Japan)], 0.05 % CPC toothpaste [No. 1: GUM® WELL PLUS Dental paste (Sunstar, Osaka, Japan) (1/4 slurry with ultrapure water: 0.0125 % CPC)] and 0.3 % CPC spray [No. 4: GUM® Disinfection spray for mouth/throat (Sunstar, Osaka, Japan)] in Japan; 0.06 % CHX + 0.05 % CPC mouthwash [No. 5: GUM® PAROEX (0.06 % CHX) (Sunstar Suisse S.A., Etoy, Switzerland)] and 0.12 % CHX + 0.05 % CPC mouthwash [No. 6: GUM® PAROEX (0.12 % CHX) (Sunstar Suisse S.A., Etoy, Switzerland)] in Europe; 0.075 % CPC mouthwash [No. 7: GUM® Oral Rinse (Sunstar Americas Inc., Schaumburg, USA)], 0.12 % CHX mouthwash [No. 8: GUM® PAROEX (0.12 % CHX) (Sunstar Americas Inc., Schaumburg, USA)], and 0.20 % Delmopinol mouthwash [No. 9: GUM® PerioShield] (Sunstar Americas Inc., Schaumburg, USA) in the USA; and 0.04 % CPC mouthwash [No. 10: GUM® MOUTHWASH HERB 2020 (Sunstar Co. Ltd., Shanghai, China)] in China. All products were evaluated for cytotoxicity, interference and virucidal activity.

2.3. Cytotoxicity and interference tests

To evaluate damage to cells, the cytotoxicity of the test solutions was investigated. A test mixture was made with 0.1 mL 5 % FBS containing EMEM and 0.9 mL of test solution. A sample of 0.1 mL of the test mixture was added to a test tube containing 0.9 mL of neutralizer (1/10 SCDLP [Fujifilm, Osaka, Japan] diluted with 2 % FBS containing DMEM) and mixed well. A 10-fold serial dilution series was prepared with 2 % FBS containing DMEM. Cell damage was examined by plaque assay. To verify cell sensitivity to the virus and inactivation of antiviral activity, an interference test was conducted. A sample of 0.5 mL of the test mixture was mixed with 4.5 mL of neutralizer. A 10-fold serial dilution series was prepared with 2 % FBS containing DMEM. Each diluted mixture was taken at 4.5 mL and added to 45 μL of virus suspension prepared at a concentration of 4.0–6.0 × 10^4 PFU/mL. After 10 min at 25°C, the infected titer was determined.

2.4. Virucidal activity test and virus titration (Plaque assay)

Virucidal activity was evaluated with an ASTM E1052-20 [22]. The virus suspension (viral titer of 8.49 Log_{10} PFU/mL) of 0.1 mL was added to 0.9 mL of a test solution and mixed well with a vortex mixer. This suspension was incubated for 20 s, 30 s or 3 min at 25°C (Table 1). Then, 0.1 mL of the suspension was added to a tube containing 0.9 mL of neutralizer and mixed well to form an inactivated mixture. A series of 10-fold dilutions of this mixture was made using 2 % FBS containing DMEM and the viral infectivity titer was measured per 0.1 mL of inactivated mixture and calculated per 1.0 mL. Three independent experiments were performed (n = 3).

3. Results

We tested the anti-viral activity of eleven test solutions including CPC, CHX or delmopinol hydrochloride in several countries.

Before the virucidal activity test, we examined the cytotoxicity and interference tests. There were no cytotoxic or interference effects at dilutions ranging from 1 to 1/100 (Table 1).

The virucidal activity of the oral care products against SARS-CoV-2 was evaluated using the recommended use time by the manufacturer's instructions. All the products containing 0.0125 to 0.30 % CPC inactivated SARS-CoV-2 with a reduction of 3.3 to >4.4 Log_{10} PFU/mL regardless of dosage form. Mouthwash containing 0.20 % delmopinol hydrochloride inactivated SARS-CoV-2 with a >5.4 Log_{10} PFU/mL reduction. However, the mouthwash containing only 0.12 % CHX as antiseptic did not show a sufficient inactivation effect against SARS-CoV-2 in this study.

4. Discussion

A recent study showed that mouthwash containing dequalinium chloride and benzalkonium chloride had virucidal activity against SARS-CoV-2 [7], which suggested that a quaternary ammonium cation has activity against SARS-CoV-2. CPC is known to be a quaternary ammonium cation and delmopinol hydrochloride is known to be a cationic surfactant. So, our findings also suggest that cationic surfactants may be effective against SARS-CoV-2, although octenidine dihydrochloride did not have activity in the previous study [7].

In this study and the previous study, mouthwash including CHX had no sufficient virucidal effect against SARS-CoV-2 *in vitro* [7]. However, a previous clinical case study showed that CHX mouthwash transiently decreased the SARS-CoV-2 viral load in the saliva [13]. Since CHX can retain on a surface of the oral cavity for several hours due to its cationic feature, the long-lasting effect *in vivo* might lead to reduce the viral load compared to neutralize the antiseptic in a short contact time *in vitro* [23]. But, there are few researches about CHX mouthwash against SARS-CoV-2, so more *in vivo* investigations of the antiviral effect of CHX mouthwash are necessary.

5. Conclusions

This study showed that oral care products containing CPC or delmopinol hydrochloride have antiviral activity against SARS-CoV-2. This supports the recommendation for a preprocedual use of CPC-containing mouthwash for SARS-CoV-2 reduction in aerosol by the CDC and ADHA. We note that the data in this report were collected *in vitro*, and a further evaluation of the anti-SARS-CoV-2 activity of the products is required *in vivo*.

Table 1
Virucidal activity of several oral care products against SARS-CoV-2.

Product No.	Country	Dosage Form	Bactericidal Ingredient		Contact Time	Neutralization of Bactericidal Ingredient		Viral Titer [Mean of n = 3] (Log_{10} PFU/mL)	Reduction in Viral Titer [Mean of n = 3]	
			Ingredient Name	Compounding Amount		Dilution rate with 1/10 SCDLP	Dilution rate with 2 %FBS-containing DMEM		Log_{10} PFU/mL	%
Negative Control	–	Aqueous Solution	PBS	–	20 s	1/10	1	7.35	–	–
	–	Aqueous Solution	PBS	–	30 s	1/10	1	7.34	–	–
	–	Aqueous Solution	PBS	–	3 min	1/10	1	7.35	–	–
Positive Control	–	Aqueous Solution	EtOH*1	70 %	20 s	1/10	1	< 2.00	> 5.4	> 99.9996
1	JP	Toothpaste	CPC*2	0.05 % (1/4 slurry : 0.0125 %)	3 min	1/10	1	4.10	3.3	99.94
2	JP	Mouthwash	CPC	0.05 %	20 s	1/10	1/10	3.13	4.2	99.994
3	JP	Mouthwash	CPC	0.05 %	20 s	1/10	1/10	3.23	4.1	99.992
4	JP	Spray	CPC	0.30 %	20 s	1/10	1/100	< 4.00	> 3.4	> 99.96
5	EU	Mouthwash	CHX*3+CPC	CHX:0.06 %, CPC:0.05 %	30 s	1/10	1/10	< 3.00	> 4.3	> 99.995
6	EU	Mouthwash	CHX + CPC	CHX:0.12 %, CPC:0.05 %	30 s	1/10	1/10	< 3.00	> 4.3	> 99.995
7	US	Mouthwash	CPC	0.075 %	30 s	1/10	1/10	< 3.00	> 4.3	> 99.995
8	US	Mouthwash	CHX	0.12 %	30 s	1/10	1	7.10	0.2	42.5
9	US	Mouthwash	Delmopinol Hydrochloride	0.20 %	30 s	1/10	1	< 2.00	> 5.3	> 99.9995
10	CN	Mouthwash	CPC	0.04 %	20 s	1/10	1/10	< 3.00	> 4.4	> 99.996

*1) EtOH : Ethanol, *2) CPC : Cetylpyridinium chloride, *3) CHX : Chlorhexidine gluconate.

Funding sources

This work was supported by Sunstar Inc. and Sunstar Suisse SA. QTEC (Japan Textile Products Quality and Technology Center) (https://www.qtec.or.jp/en) performed the tests in the study at the request of Sunstar Inc.

Declaration of Competing Interest

All authors are employees of Sunstar Inc.

Acknowledgements

We would like to thank QTEC for performance of virucidal tests.

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