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Mouthwashes with CPC Reduce the Infectivity of SARS-CoV-2 Variants In Vitro

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Abstract

Oral mouthwashes decrease the infectivity of several respiratory viruses including SARS-CoV-2. However, the precise agents with antiviral activity in these oral rinses and their exact mechanism of action remain unknown. Here we show that cetylpyridinium chloride (CPC), a quaternary ammonium compound in many oral mouthwashes, reduces SARS-CoV-2 infectivity by inhibiting the viral fusion step with target cells after disrupting the integrity of the viral envelope. We also found that CPC-containing mouth rinses decreased more than a thousand times the infectivity of SARS-CoV-2 in vitro, while the corresponding vehicles had no effect. This activity was effective for different SARS-CoV-2 variants, including the B.1.1.7 or Alpha variant originally identified in United Kingdom, and in the presence of sterilized saliva. CPC-containing mouth rinses could therefore represent a cost-effective measure to reduce SARS-CoV-2 infectivity in saliva, aiding to reduce viral transmission from infected individuals regardless of the variants they are infected with.

Keywords: oral hygiene, virucide, airborne transmission, COVID-19, cellular infection, coronaviruses

Background

Several studies have shown the antiviral potential of mouthwashes, which decrease the infectivity of airborne-transmitted viruses such as influenza and distinct coronavirus, including SARS-CoV-2 (Popkin et al. 2017; Meister et al. 2020; O'Donnell et al. 2020; Statkute et al. 2020). If proven effective in the oral cavity, this antiviral strategy could represent a globally accessible and affordable measure that could easily be implemented worldwide to reduce the infectivity of SARS-CoV-2 in saliva and cut the viral transmission chain. Higher viral loads found in index cases are associated with a higher transmission rate among their contacts (Marks et al. 2021). Thus, any intervention aimed at reducing viral loads in the saliva, exhaled as aerosols by infected individuals, could help decrease viral transmission and even prevent superspreading events. As mouthwashes are also available in oral spray formats, they represent a promising strategy for vulnerable populations such as the elderly.

Novel SARS-CoV-2 variants have appeared in different geographic areas, raising concerns about their higher viral transmission potential, the severity of the associated symptoms upon infection, and their ability to escape from preestablished neutralizing responses in vaccinated individuals (Challen et al. 2021; Davies et al. 2021; Lythgoe et al. 2021; Wang et al. 2021). The documented broad antiviral efficacy of certain mouthwashes could be instrumental in tackling different SARS-CoV-2 variants and reducing the impact of their transmission. Yet, despite the universal applicability of this antiviral approach and the diverse reports proving the activity of various oral rinses in vitro, we still do not know which active components in these mouthwashes exert the antiviral effect and what their precise mechanism of action is. Moreover, we do not know if mouth rinses could be active against SARS-CoV-2 variants.

Here we focused on the effect of cetylpyridinium chloride (CPC), a quaternary ammonium compound used in many oral mouthwashes and breath sprays with broad antiseptic and microbicide activity. CPC has antiviral activity against different enveloped viruses, including influenza and several coronaviruses such as MERS (Popkin et al. 2017; Shen et al. 2019). In contrast, other chemical components

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prevalent in mouthwashes, such as hydrogen peroxide and chlorhexidine, have shown very limited activity against SARS-CoV-2 (Meister et al. 2020). We compared the anti–SARS-CoV-2 activity of CPC and CPC-containing mouthwashes against their respective vehicles and found that CPC-containing mouthwashes inhibit SARS-CoV-2 entry into target cells after disrupting the integrity of the viral membrane. CPC-containing mouthwashes decreased more than a thousand times the infectivity of replication-competent SARS-CoV-2, were active in the presence of sterilized saliva, and were effective against SARS-CoV-2 variants.

Material and Methods

Biosafety Approval

The biologic biosafety committee of Germans Trias i Pujol Research Institute approved the execution of SARS-CoV-2 experiments at the BSL3 laboratory of the Center for Bioimaging and Comparative Medicine.

Cells and Pseudoviruses

Vero E6 cells (ATCC CRL-1586) and HEK-293T cells overexpressing the human ACE2 were obtained and cultured as previously described (Rodon et al. 2021). The generation of HIV-1 luciferase reporter pseudoviruses expressing SARS-CoV-2 spike was performed as detailed earlier (Rodon et al. 2021). Pseudoviruses expressing the spike containing either the single D614G mutation or the full B.1.1.7 variant were generated as detailed before (Pradenas et al. 2021; Trinité et al. 2021). Pseudoviruses were titrated in ACE2 HEK-293T to use equal amounts of fusogenic viruses.

Pseudovirus Assays

ACE2 HEK-293T cells were used to test mouth rinses and their vehicles. A constant pseudoviral titer was used to pulse cells in the presence of the mouth rinses. After 48 h postinoculation, cells were lysed with the Bright Glo Luciferase Assay System (Promega). To detect any associated cytotoxic effect, mouth rinse formulations were mixed with media and equally cultured on cells but in the absence of virus. Cytotoxic effects of these products were measured 48 h postinoculation with the CellTiter-Glo Luminescent Cell Viability Assay (Promega). Luminescence was measured with an EnSight Multimode Plate Reader (PerkinElmer). Response curves of CPC-containing mouthwashes were adjusted to a nonlinear fit regression model, with a 4-parameter logistic curve with variable slope to calculate the IC_{50} as previously described (Rodon et al. 2021). Cells exposed to the pseudovirus in the absence of products were set as 100% of viral fusion to calculate the percentage of viral entry inhibition. Cells not exposed to the mouthwashes or to the pseudovirus were set as 100% to calculate the percentage of cytopathic effect. All analyses were generated with Prism 8.0b (GraphPad).

Virus Isolation, Titration, and Sequencing

SARS-CoV-2 was isolated in March 2020 from a nasopharyngeal swab as previously described (Rodon et al. 2021). Genomic sequence was deposited at the GISAID repository (http://gisaid.org; accession EPI_ISL_510689). When compared with the Wuhan/Hu-1/2019 strain, this isolate has the following point mutations: 376 D614G (spike), R682L (spike), C16X (NSP13), and 12 in NSP3 (M1376X, P1377X, 377 T1378X, T1379X, I1380X, A1381X, K1382X, N1383X, T1384X, V1385X, K1386X, S1387X). The SARS-CoV-2 B.1.1.7 variant was identified during sequencing of a clinical nasopharyngeal swab in Spain in January 2021 and subsequently isolated on Vero E6 cells. The SARS-CoV-2 B.1.1.7 sequence is deposited at the GISAID database (accession EPI_ISL_1663567).

CPC-Containing Mouthwashes Employed

Here we tested 3 CPC-containing formulations from Dentaid SL with different intended uses. Vitis Encias (0.05% CPC) is a daily-use mouthwash indicated for people with delicate gums, and it offers a formulation that has an antiplaque antiseptic and other components that aid in gum care (panthenol, allantoin, and zinc lactate). Perio Aid Intensive Care (0.05% CPC) contains 0.12% chlorhexidine and is for limited-term use as a coadjuvant for patients undergoing periodontal treatment and after surgery in the oral cavity. Vitis CPC Protect (0.07% CPC) is recommended as a daily-use product for intensive care of the oral cavity to prevent and reduce dental plaque formation.

Viral Treatment with CPC-Containing Mouthwashes and Nucleocapsid Detection by ELISA

SARS-CoV-2 B.1.1.7 variant and the D614G variant from March 2020 were assayed with Vitis CPC Protect (Dentaid SL) with 2.063mM CPC. A total of 250 µL of mouth rinse was mixed with 250 µL of viruses for 2 min. Untreated viruses were mixed with 250 µL of media for 2 min. Mixes with viruses were diluted in phosphate-buffered saline (PBS) and filtered for 10 min at 1,000*g* in macrosept advance centrifugal devices of 100K MWCO of exclusion (Pall Corporation) to wash away mouth rinses twice. Washed viruses were resuspended in 1.5 mL of media. The amount of SARS-CoV-2 nucleoprotein in these supernatants was measured with SARS-CoV-2 nucleocapsid protein High-Sensitivity Quantitative ELISA (ImmunoDiagnostics) according to the manufacturer's protocol but with a 0.1% bovine serum albumin buffer instead of the assay buffer of the kit, which contains a detergent to lyse viral membranes and release nucleocapsid content.

Viral Treatment with CPC and Dynamic Light-Scattering Analysis

A total of 100 µL of SARS-CoV-2 B.1.1.7 variant or the D614G variant from March 2020 was mixed with 100 µL of CPC

(10 mM) or 100 µL of H_2O for 2 min. These samples were fixed with 1.2 mL of paraformaldehyde 4% (Biotium) for 30 min. Particle-size distributions of the viral preparations and control vehicles were determined with a dynamic light-scattering analyzer combined with noninvasive backscatter technology (Malvern Zetasizer; Malvern Instruments). Three measurements were used to calculate the mean diameter and SD. The effective electric charge on the viral surface was examined by measuring the zeta potential with an electrophoretic mobility and light-scattering analyzer (Malvern Zetasizer). Three measurements were used to calculate the mean zeta potential and SD of the dispersed system.

Antiviral Activity

Activity against SARS-CoV-2 D614G variant from March 2020 was tested against 3 oral formulations from Dentaid SL containing CPC: Vitis Encias (with 1.47mM CPC), Perio Aid Intensive Care (with 1.47mM CPC plus 1.33mM chlorhexidine), and Vitis CPC Protect (with 2.063mM CPC). Vehicles containing the same formulation without CPC were tested in parallel. We also assayed 10mM CPC diluted in distilled water. Colorants were removed from all formulations to avoid any interference with luciferase reactions. One milliliter of mouth rinses or their corresponding vehicles were mixed with 1 mL of SARS-CoV-2 D614G variant for 2 min. Virus was mixed with 1 mL of media as positive control. After 2 min of incubation, mixes with viruses were diluted in PBS and filtered for 10 min with macrosept centrifugal devices to wash away mouth rinses twice. Washed viruses were resuspended in 2 mL of media and titrated in triplicates on Vero E6 cells through 10 serial dilutions. After 3 d postinfection, cells were assayed in a microscope for a viral-induced cytopathic effect. To detect any associated cytotoxic effect, mouth rinse formulations were mixed with media, washed, and centrifuged as previously described and were equally cultured on Vero E6 but in the absence of virus. The cytotoxic effects of these products were measured 3 d after infection with the CellTiter-Glo Luminescent Cell Viability Assay (Promega). Luminescence was measured in a Fluoroskan Ascent FL Luminometer (ThermoFisher Scientific). The cytotoxicity obtained for each compound tested determined the limit of detection of the assay.

Activity against the SARS-CoV-2 B.1.1.7 variant with the D614G variant from March 2020 was assayed with Vitis CPC Protect following the same procedure described. This time, however, 250 µL of mouth rinse was mixed with 250 µL of viruses for 1 or 2 min. Untreated viruses were mixed with 250 µL of media and left for 2 min. Washed viruses were resuspended in 1.5 mL of media and titrated as previously described. Again, the cytotoxicity obtained for this experiment was used to determine the limit of detection. Finally, antiviral activity against the SARS-CoV-2 B.1.1.7 variant in the presence or absence of saliva was assayed with Vitis CPC Protect. A total of 800 µL of mouth rinse or cell media was mixed with 200 µL of SARS-CoV-2 B.1.1.7 for 30 s in the presence or absence of 200 µL of sterilized saliva obtained from a nonvaccinated donor with a negative COVID-19 antigen test (Panbio; Abbott) 2 d prior to saliva donation. Saliva was centrifuged for 20 min at 25,000*g* and sterilized first with a 0.45-µm filter and then through a 0.22-µm filter (Millex Millipore). After 30 s of incubation, mixes with viruses were diluted in PBS and filtered for 10 min at 1,000*g* in macrosept centrifugal devices to wash away mouth rinses twice. Washed viruses were resuspended in 1.5 mL of media and titrated as previously described.

Results

We first tested the capacity of CPC-containing mouth rinses to inhibit SARS-CoV-2 entry into target cells. We employed a luciferase-based assay using a reporter lentivirus pseudotyped with the spike protein of SARS-CoV-2, which allows the detection of viral fusion with target HEK-293T cells expressing human ACE2 receptor. Of note, this system provides high sensitivity to detect viral fusion activity and is widely used to search for antivirals and neutralizing antibodies (Pradenas et al. 2021; Rodon et al. 2021; Trinité et al. 2021). A constant concentration of the reporter pseudovirus containing the SARS-CoV-2 original Wuhan spike protein was mixed with increasing concentrations of the indicated CPC-containing mouth rinses or their corresponding vehicles and added to the target cells. To control for any mouthwash-induced cytotoxicity, target cells were cultured with increasing concentrations of the indicated products in the absence of pseudoviruses. By these means, we calculated the concentration at which certain mouth rinses blocked viral entry and achieved a 50% maximal inhibitory capacity (IC_{50}). CPC-containing mouth rinses were able to inhibit viral fusion in a dose-dependent manner (Fig. 1A, C, E, red lines) at concentrations where no cytotoxic effects of the mouth rinses were observed (Fig. 1A, C, E, gray lines). No obvious inhibitory activity was detected on vehicles (Fig. 1B, D, F, red lines), clearly pointing to CPC as the antiviral compound contained in the oral formulations. To confirm the specific antiviral activity of CPC, we directly tested this compound resuspended in water and found that it inhibited SARS-CoV-2 pseudoviral fusion and entry into target cells (Fig. 1G). We also assayed the capacity of CPC-containing mouth rinses to reduce the fusion of pseudoviruses displaying different SARS-CoV-2 spikes, including the D614G mutation and the full B.1.1.7 variant, which was originally identified in United Kingdom and whose higher transmissibility and pathogenicity are a global concern (Davies et al. 2021). Yet, a CPC-containing mouthwash was equally effective in abrogating the entry of pseudoviruses expressing each spike (Fig. 1H). These results indicate that CPC-containing mouth rinses are able to block SARS-CoV-2 viral entry into target cells due to the activity of CPC, which is efficacious against different variants.

To understand if CPC-containing mouth rinses abrogated viral fusion by disrupting the viral envelope of SARS-CoV-2, we next worked with 2 SARS-CoV-2 clinical isolate variants: the virus circulating in March 2020 in Spain containing the D614G mutation and the B.1.1.7 variant originally detected in United Kingdom. Each SARS-CoV-2 variant was mixed at a

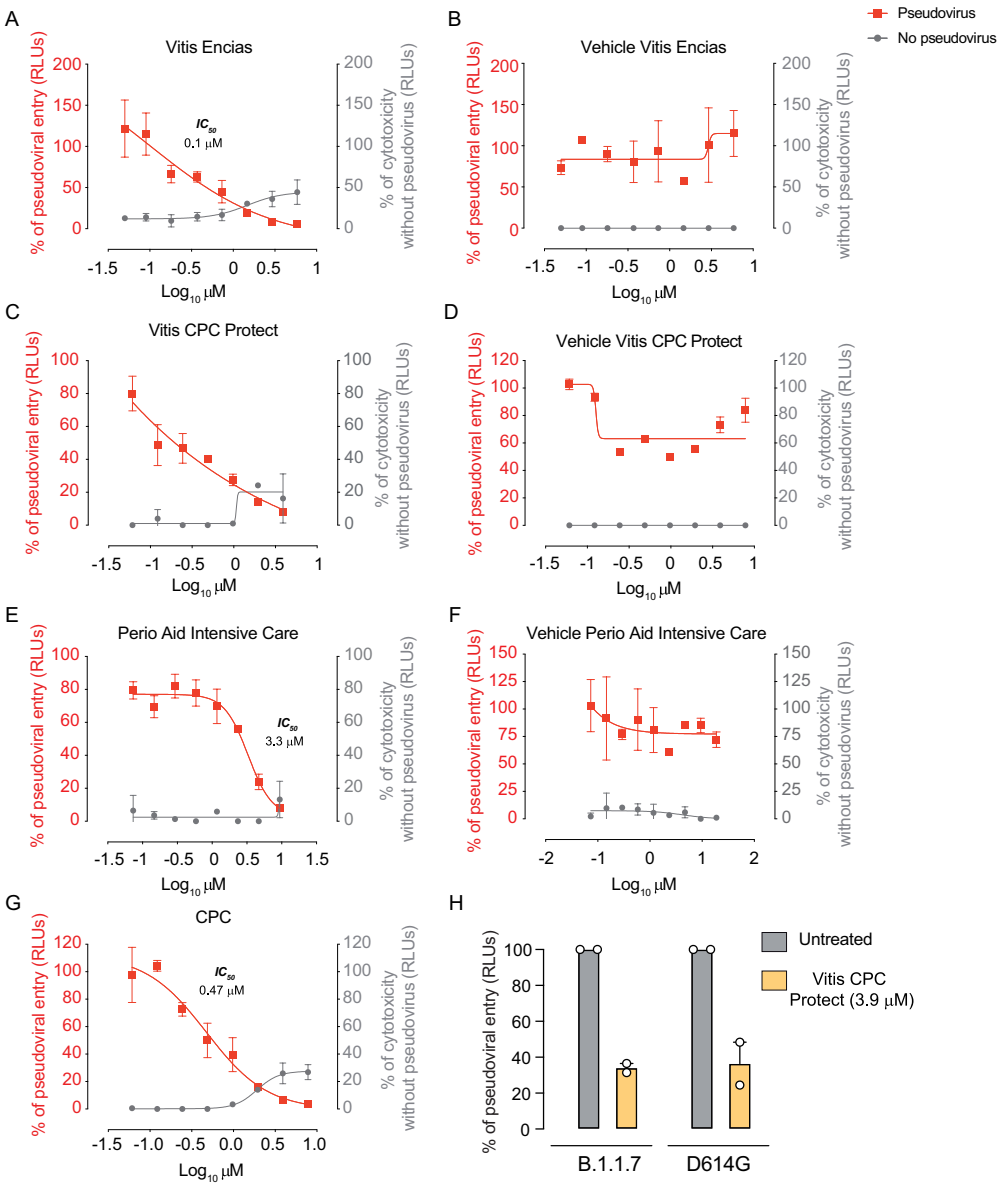

Figure 1. Antiviral activity of CPC-containing mouthwashes inhibiting SARS-CoV-2 entry. Percentage of viral entry inhibition on target HEK-293T cells expressing ACE2 exposed to a fixed concentration of SARS-CoV-2 pseudoviruses in the presence of increasing concentrations of (**A**, **C**, **E**) oral formulations, (**B**, **D**, **F**) their vehicles, and (**G**) CPC diluted in water. Nonlinear fit to a variable response curve from 1 experiment with 2 replicates is shown (red lines), excluding data from drug concentrations with associated toxicity. When calculated, the particular IC_{50} value of the graph is indicated. Cytotoxic effect on HEK-293T cells expressing ACE2 cells exposed to increasing concentrations of mouthwashes or vehicles in the absence of virus is also shown (gray lines). (**H**) Percentage of viral entry inhibition on target HEK-293T cells expressing ACE2 exposed to a fixed concentration of SARS-CoV-2 pseudoviral variants (D614G and B.1.1.7) in the presence of a final concentration of 3.9µM Vitis CPC Protect, which had no cell-associated cytotoxicity. Values are presented as mean ± SD. CPC, cetylpyridinium chloride; RLUs, relative light units.

1:1 volume ratio with a CPC-containing mouth rinse or left untreated for 2 min. To remove the oral rinse, these samples were washed twice with PBS by ultrafiltration with a macrosept centrifugal device. Collected viruses were assayed with an ELISA that detects SARS-CoV-2 nucleocapsid but in the absence of the detergent used to lyse the viruses in the conventional protocol (Fig. 2A). Without this lysis buffer, viruses treated with CPC-containing mouthwash were detected to a much higher extent than untreated viruses, regardless of the variant tested (Fig. 2B). Addition of the ELISA buffer containing detergents in untreated viruses increased nucleoprotein detection (Fig. 2C) but not to the extent of CPC-containing mouthwashes. In addition, we measured the impact of CPC treatment on the zeta potential and size distribution of viral particles. Each viral variant was treated with CPC (10 mM) at a 1:1 volume ratio for 2 min, fixed with paraformaldehyde, and

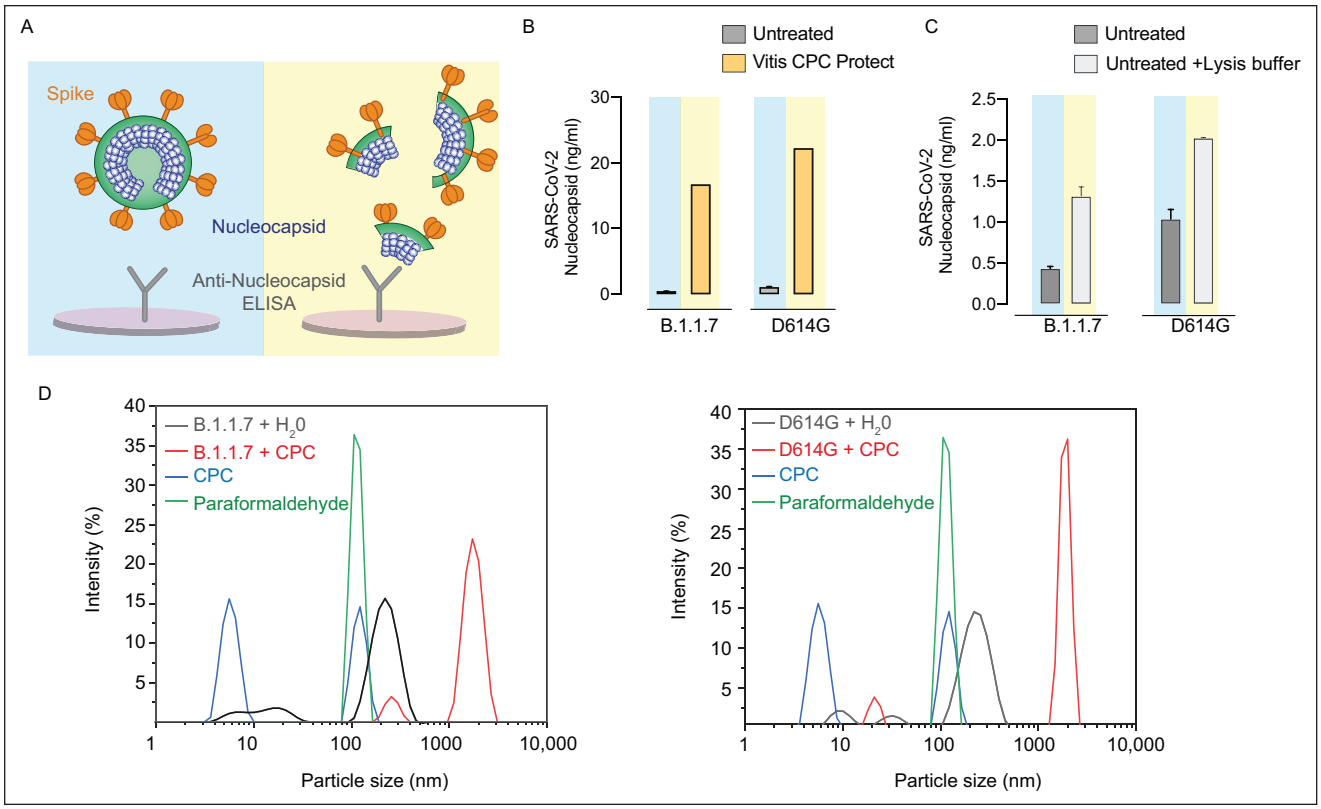

Figure 2. ELISA and dynamic light-scattering analysis of SARS-CoV-2 variants treated with CPC. (**A**) Schematic representation of the expected outcome of an ELISA performed in the absence of lysis buffer for untreated viruses (shaded in blue) and those treated with CPC-containing mouth rinse (shaded in yellow). (**B**) Amount of nucleocapsid measured after 250 µL of SARS-CoV-2 B.1.1.7 or the D614G variant isolated in March 2020 was left untreated (gray bars) or mixed with 250 µL of CPC-containing mouth rinse (yellow bars) for 2 min. Viruses were washed right after treatment by ultrafiltration to remove mouthwashes and assayed with an ELISA detecting SARS-CoV-2 nucleocapsid performed in the absence of lysis buffer. (**C**) Amount of nucleocapsid measured on untreated viruses (gray bars) with the same ELISA in the absence (shaded in blue) or presence (shaded in yellow) of the lysis assay buffer of the kit. (**D**) Hydrodynamic size (intensity averaged) of different SARS-CoV-2 variants (B.1.1.7 and D614G) in the presence of CPC obtained by dynamic light scattering. Viruses were mixed at a 1:1 volume ratio with CPC (10 mM) or H_2O for 2 min and fixed with paraformaldehyde. Values are presented as mean ± SD. CPC, cetylpyridinium chloride.

analyzed by 2 light-scattering methods. Using electrophoretic light scattering, we found that upon CPC treatment, the zeta potential of untreated viruses that was originally electronegative increased exponentially (Table). Moreover, this treatment broadened the viral size distribution in both types of SARS-CoV-2 variants, while CPC or paraformaldehyde alone had a narrower and smaller distinctive profile (Fig. 2D), as detected with dynamic light scattering. The complementary approaches of ELISA and electrophoretic/dynamic light-scattering analyses point to the effective disruption of viral membranes and loss of electrostatic repulsion, resulting in the emulsion and aggregation of the viral membrane lipids.

Next, we tested the capacity of CPC to reduce the infectivity of the clinical isolate of the SARS-CoV-2 D614G variant from March 2020. A 1:1 volume ratio of SARS-CoV-2 was mixed with CPC, CPC-containing mouth rinses, or their vehicles for 2 min and washed twice with PBS to remove the formulations by ultrafiltration with a macrosept centrifugal device. Collected viruses were titrated on Vero E6 cells to calculate the tissue culture infectious dose 50% ($TCID_{50}$) per milliliter after each treatment. While water used to dilute CPC had no effect on SARS-CoV-2 infectivity, high doses of CPC effectively suppressed viral infection on Vero E6 (Fig. 3A). Analogously, 2-min treatment with CPC-containing mouthwashes decreased about 1,000 times the $TCID_{50}$ per milliliter of SARS-CoV-2, while vehicles had no impact on SARS-CoV-2 infectivity when compared with untreated virus. To control for the presence of mouthwash remaining in the viral preparations that could induce cytotoxic effects, the indicated products were washed in ultrafiltration centrifugal devices but in the absence of SARS-CoV-2 and equally cultured with target cells for 3 d. By these means, we confirmed that the observed SARS-CoV-2–induced cytopathic effect was effectively inhibited at concentrations where the CPC-containing mouthwashes that could possibly remain after filtration were not toxic for the cells. Similar inhibition was observed when viral stocks were treated with a 10-fold excess volume of CPC-containing mouthwashes for 2 min. Thus, CPC exerts an antiviral activity against replicative-competent SARS-CoV-2, and CPC-containing mouthwashes have the capacity to reduce 1,000 times the infectivity of a viral stock when treated at least at a 1:1 volume ratio for 2 min.

Table. Hydrodynamic Size Change and Increase in Zeta Potential of SARS-CoV-2 Variants B.1.1.7 and D614G in the Presence of CPC Obtained by Dynamic Light Scattering.

	Particle Size				
Sample	Peak 1, nm	Intensity, %	Peak 2, nm	Intensity, %	Zeta Potential, mV
CPC paraformaldehyde	5.8±1.1	56	121.2±19.3	44	15.5±0.9
D614G					
+H_2O	16.1±5.5	12	226.6±63.7	82	−12.8±1.8
+CPC	260.4±40.2	9	1780±369.4	91	8.9±0.6
B.1.1.7					
+H_2O	9.2±2.4	3	237±72.3	84	−11.9±1.4
+CPC	21.2±2.51	9	1888±231.2	91	7.87±0.2

Viruses were mixed with CPC (10 mM) or H_2O for 2 min and fixed with paraformaldehyde. The mean ± SD from 3 acquisitions was used.
CPC, cetylpyridinium chloride.

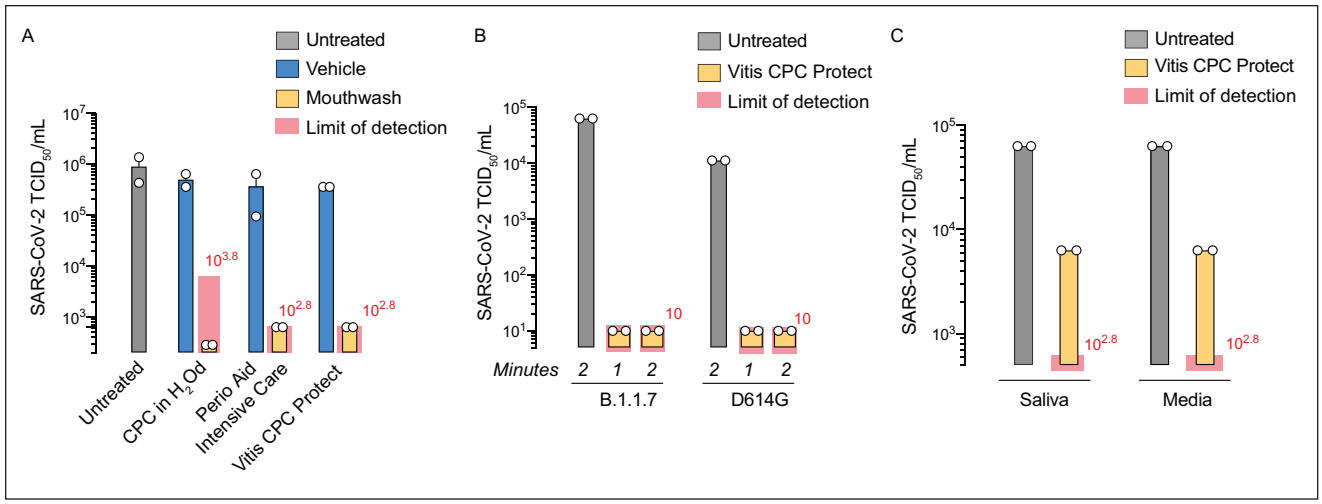

Figure 3. Infectivity of different SARS-CoV-2 variants treated with CPC-containing mouthwashes for different time frames and with or without sterilized saliva. (**A**) SARS-CoV-2 D614G variant (1 mL) isolated in March 2020 with $10^{5.8}$ $TCID_{50}$ was treated with CPC (10 mM) or CPC-containing mouth rinses (2 mM) and their respective vehicles for 2 min at a 1:1 volume ratio. Untreated virus was used as positive control. Infectivity of treated viruses washed right after treatment by ultrafiltration to remove cytotoxic mouthwashes was assayed on Vero E6 cells 3 d postinfection. In parallel, we confirmed that the inhibitory effect was not due to any remaining cytotoxic effect of the mouthwashes, as tested on Vero E6 cells exposed to the media left from washed mouth rinses that were equally centrifuged in the absence of virus. The cytotoxicity obtained for each compound tested determined the detection limit of the assay, which is represented with a shaded red area. An equivalent decrease in $TCID_{50}$ was obtained when viral stocks were mixed at a 1:10 volume ratio with CPC-containing mouthwashes (data not shown). (**B**) SARS-CoV-2 B.1.1.7 (250 µL) with $10^{4.8}$ $TCID_{50}$ or SARS-CoV-2 D614G variant (250 µL) isolated in March 2020 was treated with a CPC-containing mouth rinse (2 mM) or left untreated for 1 to 2 min at a 1:1 ratio. Infectivity of treated viruses washed right after treatment by ultrafiltration was performed as described in panel A. The cytotoxicity obtained for this mouthwash determined the detection limit of the assay, which is represented with a shaded red area. (**C**) SARS-CoV-2 B.1.1.7 (200 µL) was treated with a CPC-containing mouth rinse (2 mM) or left untreated for 30 s at a 1:10 volume ratio in the presence or absence of 200 µL of sterilized saliva. Infectivity of treated viruses washed right after treatment by ultrafiltration was performed as described in panel A. The cytotoxicity obtained for this mouthwash determined the detection limit of the assay, which is represented with a shaded red area. Values are presented as mean ± SD. CPC, cetylpyridinium chloride.

To confirm if CPC could reduce the infectivity of different clinical isolates of SARS-CoV-2, we performed an additional experiment including the B.1.1.7 SARS-CoV-2 variant along the D614G circulating variant in March 2020 (Fig. 3B). Additionally, we tested a 1-min treatment as mouthwashes are usually recommended to be used for that time. After treatment, viruses were titrated as described, controlling again for the possible presence of mouthwash remaining in the viral preparations that could induce cytotoxic effects. Once again, we could see a reduction of infectivity >1,000 times regardless of the variant employed or the duration of exposure.

Finally, we tested the capacity of a CPC-containing mouth rinse to exert its activity in the presence of sterilized saliva and with a very restrictive treatment duration of 30 s. A 1:10 volume ratio of the B.1.1.7 SARS-CoV-2 variant was mixed with CPC-containing mouth rinse or media in the presence or absence of a 1:1 volume ratio of sterilized saliva. Viruses were washed twice with PBS to remove the formulations by ultrafiltration and assayed on Vero E6 as previously described. Treatment for 30 s with CPC-containing mouthwashes decreased 10-fold the $TCID_{50}$ per milliliter of the B.1.1.7 SARS-CoV-2 variant as compared with untreated virus (Fig. 3C). Moreover,

the presence or absence of saliva did not alter the inhibition, showing that the CPC-containing mouth rinse has the same antiviral activity in the presence or absence of saliva. Collectively, these results support the potential effectiveness of CPC-containing mouth rinses to decrease viral loads in the oral cavity of infected individuals, regardless of the SARS-CoV-2 variant with which they are infected.

Discussion

All CPC-containing mouthwashes tested herein displayed virucidal activity, but further work should address if any particular formulation is more effective than others. CPC has antiviral activity against different variants of SARS-CoV-2, and this compound exerts its activity by blocking viral entry by inhibiting viral fusion on target cells. CPC acts by disrupting the integrity of the viral envelope, as previously shown for influenza virus (Popkin et al. 2017), and it equally affects distinct SARS-CoV-2 variants. Our results indicate that CPC destabilizes the membrane of the different variants, as detected with ELISA, via electrostatic interactions where the cationic amino groups of CPC cover the negatively charged viral membranes, as detected by the shift in zeta potential. Surfactant CPC activity triggers viral membrane aggregation and colloidal stabilization of solubilized viral membranes that tend to fuse with oppositely charged CPC-bound membranes, increasing the size distribution of treated viruses by dynamic light-scattering analysis. This mechanism therefore has the potential to reduce viral infectivity regardless of the variant tested.

Although CPC-containing mouthwashes could protect the oral mucosa from infection, SARS-CoV-2 most likely infects cells via the upper respiratory tract. Thus, further strategies should consider the use of CPC in nasal sprays to fully achieve the prophylactic potential of this approach. Our results point to the utility of CPC-containing oral rinses to decrease viral load in saliva. We have showed that in a very restrictive experiment, where we mixed equal volumes of highly infectious SARS-CoV-2 viral variants with CPC-containing mouthwashes, these treatments reduced >1,000 times the $TCID_{50}$/mL while corresponding vehicles had no impact. Since virucidal activity with a CPC-containing oral rinse was equally effective when saliva was added, this suggests that CPC-containing mouthwashes will most likely be active in the oral cavity.

CPC-containing mouthwashes could be a cost-effective measure to reduce SARS-CoV-2 infectivity in saliva, aiding to reduce viral transmission from infected individuals. Performing oral washes for 1 to 2 min should be enough to effectively decrease the infectivity of viruses in the saliva, especially during the first 2 wk after infection, when higher viral titers are detected and individuals are more contagious (Wölfel et al. 2020). Several events where numerous people were infected at the same time, which are considered superspreading events, are related to activities where people were talking, shouting, or singing (Hamner et al. 2020; Lemieux et al. 2021). Indeed, viable viruses were isolated from the saliva of COVID-19–infected individuals (Jeong et al. 2020), proving that exhaled saliva microdroplets and aerosols are infectious. Future work should address if CPC-containing mouth rinses are able to decrease the viral load and infectivity of viruses found in the oral cavity of SARS-CoV-2–infected individuals. If proven effective, CPC-containing mouthwashes should be active against those variants that pose a threat to vaccine efficacy, which may increase transmissibility rates and could even worsen clinical outcome. While prior studies have shown that CPC has an antibacterial activity that lasts for 3 to 5 h in saliva (Elworthy et al. 1996), forthcoming studies should address the duration of the CPC antiviral activity in the oral cavity. This information will be key to effectively validate this approach as a mean to maintain a reduced infectious capacity of SARS-CoV-2 in the saliva with this cost-effective intervention.

Author Contributions

J. Muñoz-Basagoiti, D. Perez-Zsolt, B. Trinité, contributed to data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; R. León, V. Blanc, J. Gispert, contributed to data conception, design, and data analysis, critically revised the manuscript; D. Raïch-Regué, M. Cano-Sarabia, E. Pradenas, contributed to data acquisition, analysis, and interpretation, critically revised the manuscript; J. Blanco, contributed to data analysis, critically revised the manuscript; B. Clotet, contributed to conception, design, and data interpretation, critically revised the manuscript; N. Izquierdo-Useros, contributed to conception, design, and data interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

Declaration of Conflicting Interests

The authors declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: R. León, V. Blanc, and J. Gispert are researchers working for the Dentaid Research Center. The authors declare that no other competing interest exists. Unrelated to the submitted work: J. Blanco and B. Clotet are founders and shareholders of AlbaJuna Therapeutics SL. B. Clotet is founder and shareholder of AELIX Therapeutics SL. N. I-U. reports institutional grants from Pharma Mar and Palobiofarma.

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Data Availability

Data are available from the corresponding author upon reasonable request.

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