Virucidal efficacy of different oral rinses against SARS-CoV-2

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Summary: Several oral rinses show significant SARS-CoV-2 inactivating properties *in vitro*, supporting the idea that oral rinsing might reduce the viral load of saliva and could thus lower the transmission of SARS-CoV-2.

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Abstract

The ongoing SARS-CoV-2 pandemic creates a significant threat to global health. Recent studies suggested the significance of throat and salivary glands as major sites of virus replication and transmission during early COVID-19 thus advocating application of oral antiseptics. However, the antiviral efficacy of oral rinsing solutions against SARS-CoV-2 has not been examined. Here, we evaluated the virucidal activity of different available oral rinses against SARS-CoV-2 under conditions mimicking nasopharyngeal secretions. Several formulations with significant SARS-CoV-2 inactivating properties *in vitro* support the idea that oral rinsing might reduce the viral load of saliva and could thus lower the transmission of SARS-CoV-2.

Introduction

The current severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has created a significant threat to global health. Since effective treatments and vaccines are currently not available, diligent attention on transmission-based precautions is essential to limit viral spread. According to current evidence, SARS-CoV-2 is mainly transmitted through respiratory droplets exhaled from infected individuals [1]. Importantly, viral loads are high in the nasal cavity, nasopharynx and oropharynx and viral shedding can be detected before, during and after the acute clinical phase of illness [2]. Aerosols produced by asymptomatic individuals during breathing, speaking and singing are therefore considered as critical drivers of the enhanced spread of SARS-CoV-2 [3]. The host cell-derived envelope of SARS-CoV-2 is highly susceptible to chemical agents (i.e. various alcohols) that disrupt lipid bio-membranes [4]. Chemical antisepsis thus provides a critical tool to decontaminate fomites and (body-) surfaces like human hands. In this context, nasal and oral antisepsis have been suggested to lower the number of active aerosolized virus particles from the nasal passages and oral cavity and consequently reduce transmission risk of SARS-CoV-2 [5]. Antiseptic mouth rinses with antimicrobial activity are used in various clinical situations for prophylactic and therapeutic purposes and have further been applied in the context of viral infections [5]. Although various commercially available dental mouthwashes contain membrane-damaging agents (i.e. ethanol, chlorhexidine, cetylpyridinium chloride, hydrogen peroxide and povidone-iodine), their ability to inactivate SARS-CoV-2 under biologically-relevant conditions has not been evaluated systematically [5]. Here, we tested the virucidal activity of eight commercially available oral rinses containing different active compounds against three different SARS-CoV-2 isolates under conditions mimicking nasopharyngeal secretions.

Methods

Virus strains and propagation

To isolate SARS-CoV-2 at the University Ulm Medical Center (Ulm, Germany), 50,000 Vero E6 cells were seeded in 24-well plates in 500 µL medium incubated overnight at 37°C. The next day, medium was replaced by 400 µL of 2.5 µg/mL amphotericin B containing medium. Then, 100 µL of throat swabs that were tested positive for SARS-CoV-2 by qRT-PCR were titrated 5-fold on the cells and incubated for 3 to 5 days. Upon visible CPE, supernatant was taken and virus expanded by inoculation of Vero E6 cell in 75 cm^2 flasks and propagated as above described. Thereby, the viral isolates BetaCoV/Germany/Ulm/01/2020 (strain 2) and BetaCoV/Germany/Ulm/02/2020 (strain 3) were obtained. In Essen, Germany, SARS-CoV-2 was isolated from a nasopharyngeal swab of a patient suffering from COVID-19 disease and named UKEssen strain (strain 1). The swab was taken using a Virocult® vial (Sigma, Germany). The Virocult® medium was then incubated on Vero E6 cells cultured in DMEM containing 10% (v/v) fetal calf serum and supplemented with penicillin (100 IU/mL), streptomycin (100 µg/mL), ciprofloxacin (10 µg/mL) and amphotericin B (2.5 µg/mL). Five days after infection, the supernatant was harvested and cell debris were removed by centrifugation. Afterwards, 100 µL of the clear supernatant was used for subsequent infection of fresh Vero E6 cells. After five days of incubation, the virus suspension was harvested and cleared from cellular debris by centrifugation and stored at -80°C. Viral titers of the three stocks were determined by endpoint dilution assay and the 50% tissue culture infective dose ($TCID_{50}$/mL) was calculated.

Quantitative Suspension Test and Virus Titration

Virucidal activity was determined with a quantitative suspension test with 30 s exposure time. Briefly, one part virus suspension was mixed with one part organic load mimicking respiratory secretions (100 µL mucin-type I-S (Sigma-Aldrich), 25 µL BSA Fraction V (Sigma-Aldrich) and 35 µL yeast extract (Sigma-Aldrich) and eight parts of the oral rinse [6] . Medium served as a control. Following 30 seconds exposure time, activity was immediately stopped by serial dilution. $TCID_{50}$/mL values were determined by crystal violet staining and subsequent scoring the amounts of wells displaying cytopathic effects. $TCID_{50}$ was calculated by the Spearman & Kärber algorithm. The titre reduction including its 95% confidence interval is calculated as the difference between the virus titre after contact with the oral rinse and the control virus titre with medium (reduction factor = RF). Cytotoxic effects of oral rinses were monitored by crystal violet staining using non-infected cells and used to determine the lower limit of quantification (LLOQ). An optical analysis for altered density and morphology of the cellular monolayer in the absence of virus was performed and was quantified analogous to the $TCID_{50}$/mL of the virus infectivity.

Results

We examined the virucidal activity of eight commercially available oral rinses based on different active compounds (Table 1) using a quantitative suspension test with three different SARS-CoV-2 isolates mixed with an interfering substance mimicking a respiratory secretion. A medium control after 30 s exposure time did not reduce viral infectivity, thus implying that the used interfering substance mimicking nasal secretions did not alter virus stability. In contrast, the different SARS-CoV-2 strains (strains 1-3) were highly susceptible to various oral rinses. Three of the eight formulations, including product c, product e and product f, significantly reduced viral infectivity to up to three orders of magnitude to background levels

(Fig. 1, Table 1). Also, for the other products containing different active compounds (Table 1) virucidal activities could be observed with log reduction factors ranging between 0.3 to 1.78 (Fig. 1, Table 1). In case of product h, which is based on polyhexamethylene biguanide, the strain 1 was only moderately reduced, whereas the other two strains were inactivated to the lower limit of quantification, which was determined by monitoring the cytotoxic effects of the products in non-infected cells. (Fig. 1). In summary, we provide evidence that SARS-CoV-2 can be efficiently inactivated by commercially available oral rinses within short exposure times of 30 seconds.

Discussion

The main route of transmission of SARS-CoV-2 is suspected to involve direct contact with respiratory aerosols or droplets of infected individuals, produced during sneezing, coughing or talking, and subsequent contact to nasal, oral or ocular mucosal membranes [1]. SARS-CoV-2 initially colonizes the upper respiratory tract of infected individuals [2]. High viral loads in the oral cavity provide a rich source of potentially infectious virus as well as an entry route for new infections. Hence, if assuming that the throat functions as a major site of viral replication during early stages (even before symptom onset), oral antisepsis could lower the number of infectious aerosolized virus particles and consequently the risk of transmission or infection. Experimental and clinical research studies on SARS-CoV-2-related viruses (e.g. SARS-CoV, MERS-CoV, and influenza virus H5N1) showed that antiseptic solutions, containing chlorhexidine gluconate (CHG), polyvinylpyrrolidone iodine (PVP-I), chlorine dioxide (ClO2), cetylpyridinium chloride (CPC), and hydrogen peroxide (H_2O_2) can indeed reduce viral loads [7]. We found that different SARS-CoV-2 strains can be efficiently inactivated with commercially available oral rinses under biologically relevant conditions mimicking respiratory secretions. In particular, we observed that three formulations (products c, e and f) containing different active compounds

significantly reduced viral infectivity to undetectable levels. In agreement with our observation, different studies using Listerine (product f) observed antiviral activities specifically against enveloped viruses, implying an impact on the viral lipid envelope [8–10]. The *in vivo* effects of the oral solutions require further analysis during clinical studies. First trials with the aim to reduce the viral load in confirmed COVID-19 patients have been registered. One study aims to compare three antiseptic mouthwash/gargling solutions compared to a control (distilled water) to reduce SARS-CoV-2 load in 120 confirmed COVID-19 individuals (https://clinicaltrials.ucsf.edu/trial/NCT04409873). Another blind, randomized controlled pilot trial plans to determine the potential of various gargling agents in reducing intraoral viral load among laboratory-confirmed COVID-19 patients (https://clinicaltrials.gov/ct2/show/NCT04341688). Our findings clearly advocate the evaluation of selected formulations in clinical context to systematically evaluate the decontamination and tissue health of the oral cavity in patients and healthcare workers to potentially prevent virus transmission.

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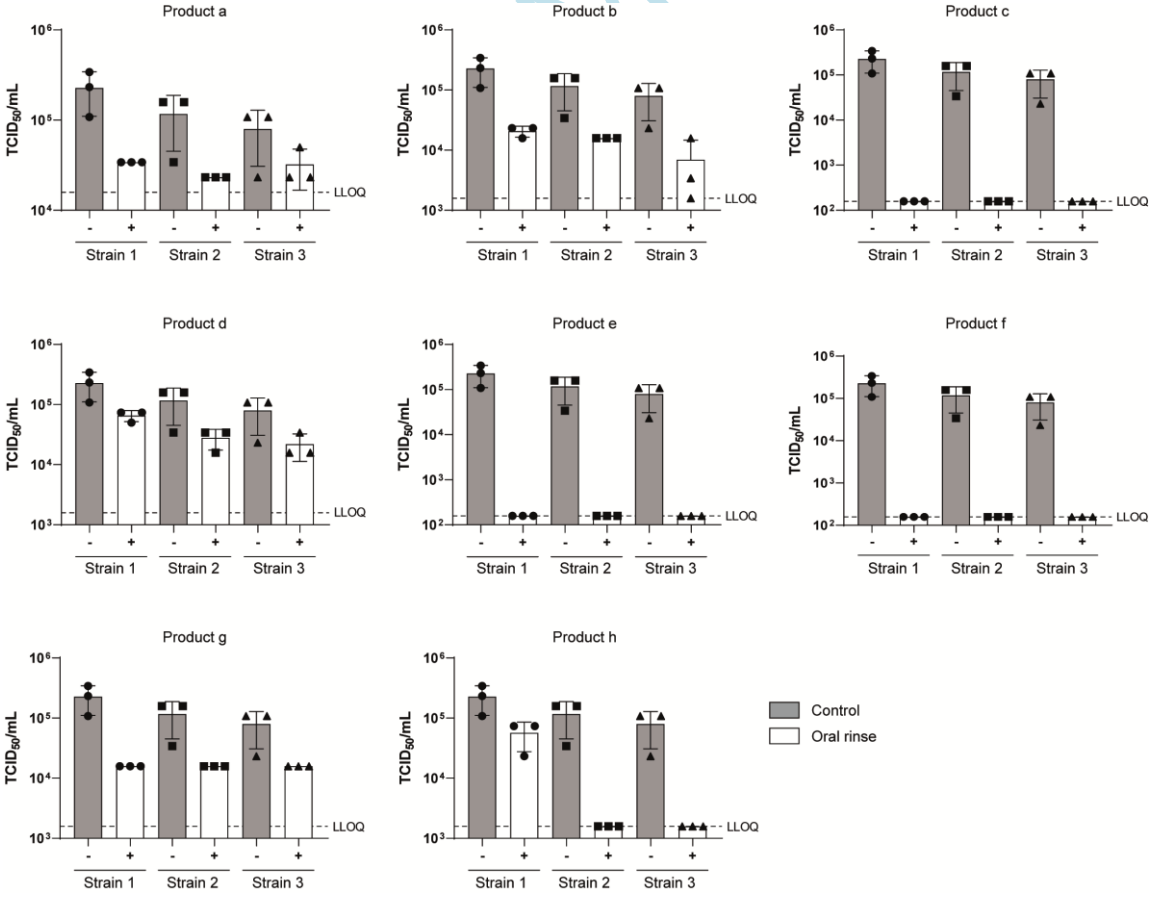
Figure legend

Figure 1. Virucidal activity of oral rinses against SARS-CoV-2. SARS-CoV-2 strain 1 (dot; UKEssen), strain 2 (square; BetaCoV/Germany/Ulm/01/2020), or strain 3 (triangle; BetaCoV/Germany/Ulm/02/2020) were incubated with medium (control) or various oral rinses for 30 s. Both conditions were supplemented with an interfering substance mimicking respiratory secretions. Viral titers were determined upon titration on Vero E6 cells. The cytotoxic effect was monitored using non-infected cells incubated with the different products, defined as lower limit of quantification (LLOQ). Tissue culture infectious dose 50 ($TCID_{50}$/mL was calculated according to Spearman-Kärber. Data indicate averages and standard deviation of three independent experiments.



Tables

Table 1: Overview of oral rinses used in the study with product name, active compounds and calculated reduction factors. The exact formulations for these oral rinses are not publicly available due to patent-related restrictions.

Product	**Trade name**	**Active compound**	**Log reduction factor (mean of n=3)**		
			Strain 1	**Strain 2**	**Strain 3**
a	Cavex Oral Pre Rinse	hydrogen peroxide	0.78	0.61	0.33
b	Chlorhexamed Forte	chlorhexidinebis (D-gluconate)	1.00	0.78	1.17
c	Dequonal	dequalinium chloride, benzalkonium chloride	≥3.11	≥2.78	≥2.61
d	Dynexidine Forte 0.2%	chlorhexidinebis (D-gluconate)	0.50	0.56	0.50
e	Iso-Betadine mouthwash 1.0%	polyvidone-iodine	≥3.11	≥2.78	≥2.61
f	Listerine cool mint	ethanol, essential oils	≥3.11	≥2.78	≥2.61
g	Octenident mouthwash	octenidine dihydrochlorid	1.11	0.78	0.61
h	ProntOral mouthwash	polyaminopropyl biguanide (polihexanide)	0.61	≥1.78	≥1.61