The Journal of Infectious Diseases

BRIEF REPORT

Virucidal Efficacy of Different Oral Rinses Against Severe Acute Respiratory Syndrome Coronavirus 2

Toni Luise Meister,[1] Yannick Brüggemann,[1] Daniel Todt,[1,2] Carina Conzelmann,[3] Janis A. Müller,[3] Rüdiger Groß,[3] Jan Münch,[3] Adalbert Krawczyk,[4,5] Jörg Steinmann,[6,7] Jochen Steinmann,[8] Stephanie Pfaender,[1,a] and Eike Steinmann[1,a]

[1]Department for Molecular and Medical Virology, Ruhr University Bochum, Bochum, Germany, [2]European Virus Bioinformatics Center, Jena, Germany, [3]Institute of Molecular Virology, Ulm University Medical Center, Ulm, Germany, [4]Department of Infectious Diseases, West German Centre of Infectious Diseases, Universitätsmedizin Essen, University Duisburg-Essen, Essen, Germany, [5]Institute for Virology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany, [6]Institute of Clinical Hygiene, Medical Microbiology and Infectiology, General Hospital Nürnberg, Paracelsus Medical University, Nuremberg, Germany, [7]Institute of Medical Microbiology, University Hospital of Essen, Essen, Germany, and [8]Dr. Brill + Partner GmbH Institute for Hygiene and Microbiology, Bremen, Germany

The ongoing severe acute respiratory syndrome coronavirus 2 (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 coronavirus disease 2019, 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.

Keywords. SARS-CoV-2; oral rinses; inactivation; suspension test; transmission.

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 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 (ie, various alcohols) that disrupt lipid biomembranes [4]. Chemical antisepsis thus provides a critical tool to decontaminate fomites and (body) surfaces such as 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 (ie, 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 8 commercially available oral rinses containing different active compounds against 3 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 tested positive for SARS-CoV-2 by reverse-transcription quantitative polymerase chain reaction was titrated 5-fold on the cells and incubated for 3–5 days. Upon visible cytopathic effect, supernatant was taken and virus expanded by inoculation of Vero E6 cells in 75 cm^2 flasks and propagated as described above. 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 coronavirus disease 2019 (COVID-19) 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 Dulbecco's modified Eagle's medium 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 was removed by centrifugation. Afterward, 100 µL of the clear supernatant was used

Received 18 June 2020; editorial decision 21 July 2020; accepted 24 July 2020; published online July 29, 2020.

[a]S.P. and E.S. are equally contributing last authors.

Correspondence: Eike Steinmann, PhD, Department of Molecular and Medical Virology, Ruhr-University Bochum, Universitaetsstr 150, 44801 Bochum, Germany (eike.steinmann@rub.de).

The Journal of Infectious Diseases® 2020;222:1289–92

DOI: 10.1093/infdis/jiaa471

for subsequent infection of fresh Vero E6 cells. After 5 days of incubation, the virus suspension was harvested and cleared from cellular debris by centrifugation and stored at −80°C. Viral titers of the 3 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-second exposure time. In brief, 1 part virus suspension was mixed with 1 part organic load mimicking respiratory secretions (100 µL mucin type I-S, 25 µL BSA Fraction V, and 35 µL yeast extract, all Sigma-Aldrich) and 8 parts of the oral rinse [6]. Medium served as a control. Following 30 seconds of exposure time, activity was immediately stopped by serial dilution. $TCID_{50}$/mL values were determined by crystal violet staining and subsequent scoring of the amounts of wells displaying cytopathic effects. $TCID_{50}$ was calculated by the Spearman–Kärber algorithm. The titer reduction including its 95% confidence interval is calculated as the difference between the virus titer after contact with the oral rinse and the control virus titer with medium (reduction factor). Cytotoxic effects of oral rinses were monitored by crystal violet staining using noninfected 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 8 commercially available oral rinses based on different active compounds (Table 1) using a quantitative suspension test with 3 different SARS-CoV-2 isolates mixed with an interfering substance mimicking a respiratory secretion. A medium control after 30 seconds 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 8 formulations, including product C, product E, and product F, significantly reduced viral infectivity to up to 3 orders of magnitude to background levels (Figure 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 (Figure 1, Table 1). In the case of product H, which is based on polyhexamethylene biguanide, strain 1 was only moderately reduced, whereas the other 2 strains were inactivated to the LLOQ, which was determined by monitoring the cytotoxic effects of the products in noninfected cells (Figure 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 (eg, severe acute respiratory syndrome and Middle East respiratory syndrome coronaviruses and influenza virus H5N1) showed that antiseptic solutions containing chlorhexidine gluconate, polyvinylpyrrolidone iodine, chlorine dioxide, cetylpyridinium chloride, and hydrogen peroxide can indeed reduce viral loads [7]. We found that different SARS-CoV-2 strains can be

Table 1. Overview of Oral Rinses Used in the Study With Product Name, Active Compounds, and Calculated Reduction Factors

			Log Reduction Factor (Mean of n = 3)		
Product	Trade Name	Active Compound[a]	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 dihydrochloride	1.11	0.78	0.61
H	ProntOral mouthwash	Polyaminopropyl biguanide (polyhexanide)	0.61	≥1.78	≥1.61

[a]The exact formulations for these oral rinses are not publicly available due to patent-related restrictions.

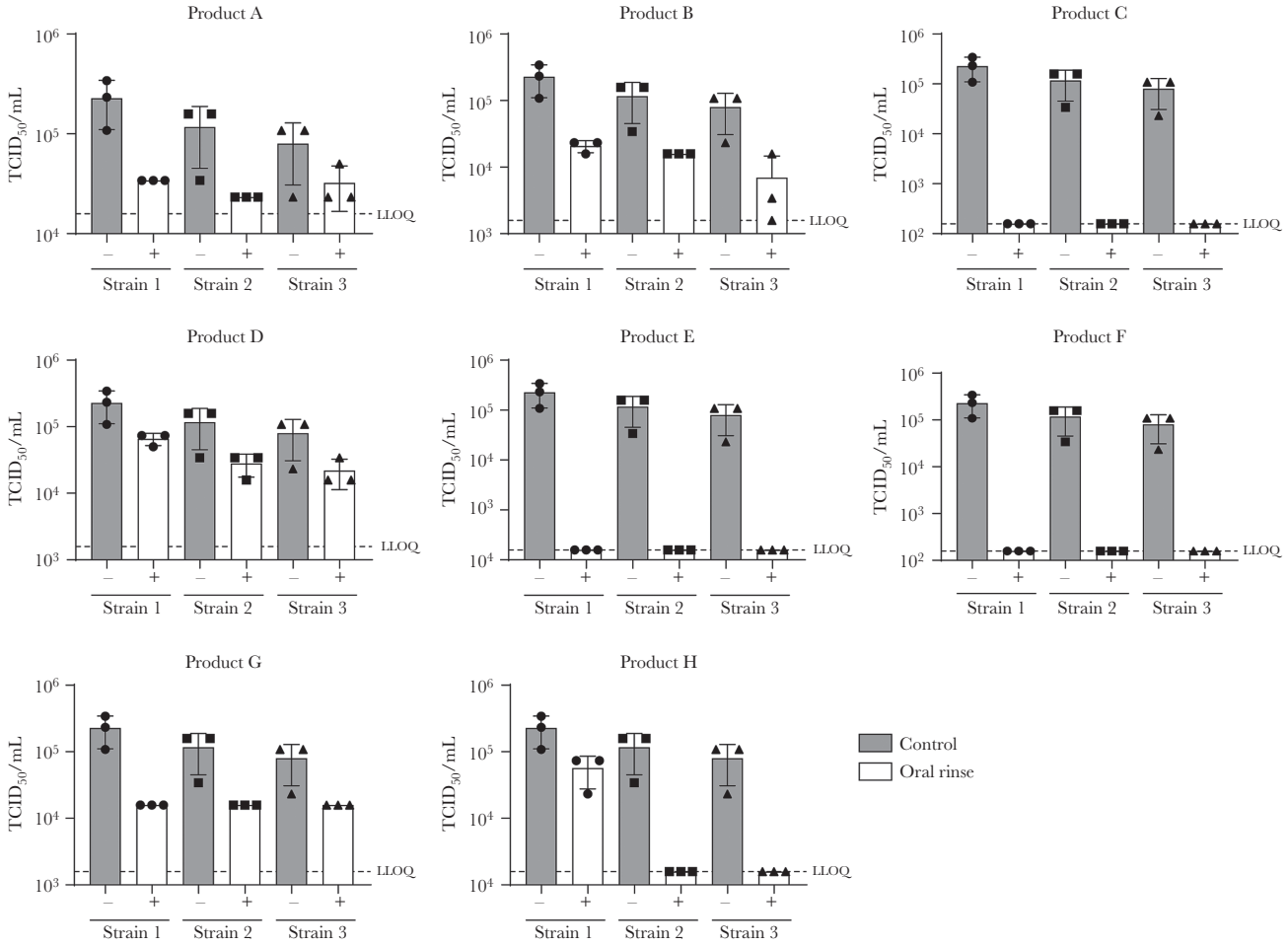

Figure 1. Virucidal activity of oral rinses against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 strains 1 (dot; UKEssen), 2 (square; BetaCoV/Germany/Ulm/01/2020), and 3 (triangle; BetaCoV/Germany/Ulm/02/2020) were incubated with medium (control) or various oral rinses for 30 seconds. 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 noninfected cells incubated with the different products, defined as the lower limit of quantification (LLOQ). The 50% tissue culture infectious dose ($TCID_{50}$/mL) was calculated according to Spearman–Kärber. Data indicate averages and standard deviation of 3 independent experiments.

efficiently inactivated with commercially available oral rinses under biologically relevant conditions mimicking respiratory secretions. In particular, we observed that 3 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 3 antiseptic mouthwash/gargling solutions compared to a control (distilled water) to reduce SARS-CoV-2 load in 120 individuals with confirmed COVID-19 (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 patients with laboratory-confirmed COVID-19 (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.

Notes

Acknowledgments. We would like to thank all members of the Department for Molecular and Medical Virology for helpful suggestions and discussions.

Financial support. This project has received funding from the European Union's Horizon 2020 research and innovation program (grant agreement number 101003555 [Fight-nCoV] to J. M.). C. C. and R. G. are part of and R. G. is funded by a scholarship from the International Graduate School in Molecular Medicine Ulm. This study was also supported by the Stiftung Universitätsmedizin Essen (awarded to A. K.).

Potential conflicts of interest. All authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Anfinrud P, Stadnytskyi V, Bax CE, Bax A. Visualizing speech-generated oral fluid droplets with laser light scattering. N Engl J Med **2020**; 382:2061–3.
2. Wölfel R, Corman VM, Guggemos W, et al. Virological assessment of hospitalized patients with COVID-2019. Nature **2020**; 581:465–9.
3. Meselson M. Droplets and aerosols in the transmission of SARS-CoV-2. N Engl J Med **2020**; 382:2063.
4. Kratzel A, Todt D, V'kovski P, et al. Inactivation of severe acute respiratory syndrome coronavirus 2 by WHO-recommended hand rub formulations and alcohols. Emerging Infect Dis **2020**; 26:1592–5.
5. O'Donnell VB, Thomas D, Stanton R, et al. Potential role of oral rinses targeting the viral lipid envelope in SARS-CoV-2 infection. Function **2020**; 1:zqaa002.
6. Eggers M, Terletskaia-Ladwig E, Enders M. How effective is washing hands against influenza viruses? [in German]. Hyg Med **2009**; 34:492–8.
7. Eggers M, Koburger-Janssen T, Eickmann M, Zorn J. In vitro bactericidal and virucidal efficacy of povidone-iodine gargle/mouthwash against respiratory and oral tract pathogens. Infect Dis Ther **2018**; 7:249–59.
8. Dennison DK, Meredith GM, Shillitoe EJ, Caffesse RG. The antiviral spectrum of Listerine antiseptic. Oral Surg Oral Med Oral Pathol Oral Radiol Endod **1995**; 79:442–8.
9. Meiller TF, Silva A, Ferreira SM, Jabra-Rizk MA, Kelley JI, DePaola LG. Efficacy of Listerine antiseptic in reducing viral contamination of saliva. J Clin Periodontol **2005**; 32:341–6.
10. Yamanaka A, Hirai K, Kato T, et al. Efficacy of Listerine antiseptic against MRSA, *Candida albicans* and HIV. Bull Tokyo Dent Coll **1994**; 35:23–6.