

Comparison of In Vitro Inactivation of SARS CoV-2 with Hydrogen Peroxide and Povidone-Iodine Oral Antiseptic Rinses

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Abstract

Purpose: To evaluate the in vitro inactivation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with hydrogen peroxide (H₂O₂) and povidone-iodine (PVP-I) oral antiseptic rinses at clinically recommended concentrations and contact times.

Materials and Methods: SARS-CoV-2, USA-WA1/2020 strain virus stock was prepared prior to testing by growing in Vero 76 cells. The culture media for prepared virus stock was minimum essential medium (MEM) with 2% fetal bovine serum (FBS) and 50 µg/mL gentamicin. Test compounds consisting of PVP-I oral rinse solutions and H₂O₂ aqueous solutions were mixed directly with the virus solution so that the final concentration was 50% of the test compound and 50% of the virus solution. Thus PVP-I was tested at concentrations of 0.5%, 1.25%, and 1.5%, and H₂O₂ was tested at 3% and 1.5% concentrations to represent clinically recommended concentrations. Ethanol and water were evaluated in parallel as standard positive and negative controls. All samples were tested at contact periods of 15 seconds and 30 seconds. Surviving virus from each sample was then quantified by standard endpoint dilution assay and the log reduction value of each compound compared to the negative control was calculated.

Results: After the 15-second and 30-second contact times, PVP-I oral antiseptic rinse at all 3 concentrations of 0.5%, 1.25%, and 1.5% completely inactivated SARS-CoV-2. The H₂O₂ solutions at concentrations of 1.5% and 3.0% showed minimal viricidal activity after 15 seconds and 30 seconds of contact time.

Conclusions: SARS-CoV-2 virus was completely inactivated by PVP-I oral antiseptic rinse in vitro, at the lowest concentration of 0.5 % and at the lowest contact time of 15 seconds. Hydrogen peroxide at the recommended oral rinse concentrations of 1.5% and 3.0% was minimally effective as a viricidal agent after contact times as long as 30 seconds. Therefore, preprocedural rinsing with diluted PVP-I in the range of 0.5% to 1.5% may be preferred over hydrogen peroxide during the COVID-19 pandemic.

The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has resulted in exceptional challenges to infection control procedures in the dental office.^{1,2} Human modes of transmission of SARS-CoV-2 from infected individuals can occur by simple procedures such as breathing, talking, coughing or sneezing.^{2,3} It has been accepted that

the oral cavity including oropharynx, and the nasopharynx have the highest viral loads.²⁻⁴ Adding to the risk is the nature of dental care, which involves close physical contact of dental professionals with patients, and constant exposure to saliva which is known to have a high load of oral microbes including virus.²⁻⁴ Therefore, any dental procedure, especially prosthodontic procedures, which can aerosolize contaminated

saliva (using handpieces, air-water syringes, and ultrasonic instrumentation), has the potential to significantly increase airborne transmission of the virus.⁵⁻⁷ All of this places dental professionals at a significantly higher risk for infection, and be a source for further transmission of infection. This behooves dental professionals to consider additional and augmented protocols for infection control in the dental office.^{6,7}

The nasal and oral cavities are considered a major portal of entry for SARS-CoV-2, and are directly associated with the evolutionary process of COVID-19. They are involved in inhalation of virus containing droplet particles and aerosols in the ambient regions, as well as in expectorations of virus containing droplets.^{8,9} Therefore, a significant amount of attention has been paid during the COVID-19 pandemic towards enhancing personal protection equipment (PPE) for dental professionals.⁹ Some examples include higher particulate filtration masks (N-95), respirators and face shields.⁹⁻¹¹ Additional equipment such as routine use of high efficiency particulate air (HEPA) filters and improved designs for high volume evacuator (HEV) have also been advocated.^{10,11}

An additional component of protection that has recently begun to receive increased attention is the use of preprocedural oral rinses.^{2,3,7,12} Due to the knowledge that the oropharyngeal region is a major site of viral replication during the early asymptomatic stages of COVID-19, it has been suggested that oral antiseptic rinses that target the lipid envelope of SARS-CoV-2, has the potential to reduce viral load in the oropharynx.¹² There is increasing recognition that oral antiseptic rinses with significant viricidal activity may have a role as potential therapeutic agents to inactivate infective particles generated in the oropharyngeal region.^{7,12,13} Recent articles have discussed the importance of preprocedural oral antiseptic rinses for use on patients as well as dental and medical professionals, in order to reduce the risk of transmission associated with viral shedding from asymptomatic individuals.^{6,7,14}

A variety of oral antiseptic rinses have been suggested in recent literature for preprocedural use to reduce viral transmission.¹² Oral rinses ranging from chlorhexidine gluconate, ethanol, essential oils, povidone-iodine (PVP-I), hydrogen peroxide (H₂O₂) chlorinated water, hypertonic saline, bioflavonoids, cyclodextrins, cetylpyridinium chloride have been recommended.¹⁰⁻¹² Presently, there are no clinical studies reported on any oral antiseptic rinses specifically against SARS-CoV-2. A recent in vitro study of PVP-I oral rinses has demonstrated complete inactivation of SARS-CoV-2 at concentrations between 0.5% and 1.5% and contact times as little as 15 seconds.⁷ Though PVP-I solutions at concentrations below 2.5% have been demonstrated to be safe for routine, repeated use in the oral cavity, they are not recommended for patients with active thyroid disease, pregnancy, anaphylactic allergy, and in patients undergoing radioactive iodine therapy.¹⁵⁻¹⁷

Therefore, it would be helpful to study alternatives to PVP-I to mitigate these contraindications to allow a broader population to seek the benefits of preprocedural oral rinsing. Hydrogen peroxide oral rinse is a popular rinse anecdotally used by dentists due to its long history of use in teeth whitening procedures.^{18,19} It has also been recommended by the American Dental Association (ADA) as a preprocedural rinse op-

tion during the COVID-19 pandemic.¹⁰ Some of the advantages of H₂O₂ include easy accessibility, low cost, and long-track record in dentistry.^{18,19} However its disadvantages include its potential for toxicity under routine use by dental professionals,¹⁸ gastric and colon disturbances,²⁰ inactivation in the mouth due to host catalase activity in the saliva,²¹ and absence of any clinical or in vitro evidence for viricidal activity against SARS-CoV-2.¹²

The purpose of this study was to compare in vitro inactivation of SARS CoV-2 with hydrogen peroxide (H₂O₂) and povidone-iodine (PVP-I) oral antiseptic rinses at clinically recommended concentrations and clinically convenient timescales. The null hypotheses were that there would be no difference between viricidal activity of H₂O₂ and PVP-I and that there would be no difference in viricidal activity at varied concentrations and contact times for both types of oral rinses.

Materials and methods

All laboratory procedures with SARS-CoV-2 was conducted in biosafety level 3 (BSL-3) laboratories at The Institute for Antiviral Research at Utah State University at Logan, Utah, following established standard operating procedures approved by the Utah State University Biohazards Committee. Hydrogen peroxide 6% (w/w) and 3% (w/w) oral rinse solutions were prepared from dilution of purified United States Pharmacopeia (USP) grade hydrogen peroxide 30% aqueous solutions (Sigma-Aldrich; St. Louis, MO) with sterile deionized water. Povidone-iodine (PVP-I) oral rinse antiseptic solutions (Veloce BioPharma; Fort Lauderdale, FL) at concentrations of 3.0%, 2.5%, and 1.0% were prepared as well. SARS-CoV-2, USA-WA1/2020 strain, virus stock was prepared prior to testing by growing in Vero 76 cells. Culture media for the prepared stock (test media) was minimum essential medium (MEM) with 2% fetal bovine serum (FBS) and 50 µg/mL gentamicin.

The 5 test compounds were then incubated in a 1:1 ratio with the virus solution so that the final concentration of each individual test compound was 50% of the starting concentration. The test solutions were chosen to represent the recommended PVP-I and peroxide rinse concentrations after 1:1 dilution with virus stock solution, in order to evaluate the clinically significant concentrations. A single concentration of each sample was tested in triplicate. Test media without any virus was added to 2 tubes of the compounds to serve as toxicity and neutralization controls. Ethanol (70%) was tested in parallel as a positive control and water only, as a negative control. Incubation times of 15 seconds and 30 seconds were chosen to represent reasonable and conveniently achievable rinse and gargle times for both patients and providers in clinical settings. The test solutions and virus were incubated at room temperature (22 ± 2°C) for 15 seconds and for 30 seconds per standard testing protocols. The solutions were then neutralized by a 1/10 dilution in MEM with 2% FBS, 50 µg/mL gentamicin.

The surviving virus from each sample was quantified by standard end-point dilution assay. Briefly, the neutralized samples were pooled and serially diluted using eight log dilutions in test medium. Then 100 µL of each dilution was plated into quadruplicate wells of 96-well plates containing 80% to 90% confluent Vero 76 cells. The toxicity controls were added to

Table 1 Viricidal efficacy of test compounds against SARS-CoV-2 after a 15-second incubation with virus at 22 ± 2°C

Test product	Percent concentration of PVP-I as tested	Percent concentration of H ₂ O ₂ as tested	Incubation time (in seconds)	Virus ^a titer	LRV ^b
PVP-I 1.0% Oral Rinse	0.5	0	15	<0.67	>4.33
PVP-I 2.5% Oral Rinse	1.25	0	15	<0.67	>4.33
PVP-I 3.0% Oral rinse	1.5	0	15	<0.67	>4.33
H ₂ O ₂ 3.0%	0	1.5	15	≤3.67	1.33
H ₂ O ₂ 6.0%	0	3.0	15	≤4.0	1.00
Ethanol Control	N/A	N/A	15	<0.67	>4.33
Virus Control	N/A	N/A	15	5.0	N/A

^aLog₁₀ CCID₅₀ of virus per 0.1 mL. The assay lower limit of detection is 0.67 Log₁₀ CCID₅₀/0.1 mL.

^bLRV (log reduction value) is the reduction of virus compared to the virus control.

Table 2 Viricidal efficacy of test compounds against SARS-CoV-2 after a 30-second incubation with virus at 22°C to 2°C

Test product	Percent concentration of PVP-I as tested	Percent concentration of H ₂ O ₂ as tested	Incubation time (in seconds)	Virus ^a titer	LRV ^b
PVP-I 1.0% Oral Rinse	0.5	0	30	<0.67	>3.63
PVP-I 2.5% Oral Rinse	1.25	0	30	<0.67	>3.63
PVP-I 3.0% Oral rinse	1.5	0	30	<0.67	>3.63
H ₂ O ₂ 3.0%	0	1.5	30	≤3.33	1.0
H ₂ O ₂ 6.0%	0	3.0	30	≤2.5	1.8
Ethanol Control	N/A	N/A	30	<0.67	>3.63
Virus Control	N/A	N/A	30	4.3	N/A

^aLog₁₀ CCID₅₀ of virus per 0.1 mL. The assay lower limit of detection is 0.67 Log₁₀ CCID₅₀/0.1 mL.

^bLRV (log reduction value) is the reduction of virus compared to the virus control.

an additional 4 wells of Vero 76 cells and 2 of those wells at each dilution were infected with virus to serve as neutralization controls, ensuring that residual sample in the titer assay plate did not inhibit growth and detection of surviving virus. Plates were incubated at 37 ± 2°C with 5% CO₂ for 5 days. Each well was then scored for presence or absence of infectious virus by examining for any cytopathic effect (CPE) in the wells. Briefly, after incubation in wells of known dilution containing susceptible cells, if any active virus was present at the start of the incubation, the virus grew resulting in a CPE in the wells where it was plated. Using the CPE count and the dilution of that well, the concentration that allowed the virus to grow in the cells and cause the CPE, were used for calculation. The titers were then measured using a standard endpoint dilution 50% cell culture infectious dose (CCID₅₀) assay calculated using the Reed-Muench equation.²² Subsequently, the log reduction value (LRV) of each compound compared to the negative (water) control was calculated.

Results

The virus titers and log reduction value of SARS-CoV-2 when incubated with a single concentration of the test compounds for 15 seconds are shown in Table 1. After the 15-second contact period, all 3 of the PVP-I tested solutions were effective at reducing >4.33 log₁₀ CCID₅₀ infectious virus from

5.0 log₁₀ CCID₅₀/0.1 mL to <0.67 log₁₀ CCID₅₀/0.1 mL. The H₂O₂ solutions at the clinically recommended and commercially available concentrations of 3.0% and 1.5% had minimal viricidal activity after 15 seconds. Table 2 shows the virus titers and LRV of SARS-CoV-2 when the virus was incubated for 30 seconds with each of the 5 test compounds at 50/50 ratio. For the 30-second second contact time, the compounds had nearly the same effect as the 15 second contact time, with PVP-I showing more effective viricidal activity than H₂O₂. Both the positive control (ethanol) and neutralization controls (water) performed as anticipated.

Discussion

The purpose of this study was to compare in vitro inactivation of SARS CoV-2 with hydrogen peroxide (H₂O₂) and povidone-iodine (PVP-I) oral antiseptic rinses at different clinically recommended concentrations and contact times of 15 seconds and 30 seconds. The first null hypothesis related to the comparison of the 2 tested oral rinses was rejected and the second null hypothesis related to contact times for PVP-I was not rejected. The findings from this study confirm the results of a recent study by Bidra *et al*⁷ which also showed that PVP-I oral rinses completely inactivated SARS CoV-2 with a concentration as low as 0.5% and contact time as low as 15 seconds.

The Centers for Disease Control and Prevention (CDC) has suggested chlorhexidine gluconate, essential oils, PVP-I or cetylpyridinium chloride as options for preprocedural rinsing before dental procedures.¹¹ The American Dental Association (ADA) interim guidelines has suggested only 1.5% H₂O₂ or 0.2% PVP-I as options for preprocedural oral rinsing.¹⁰ Findings from this study showed that H₂O₂ had weak viricidal activity and the log reduction value for low-concentrations of PVP-I were three times higher than H₂O₂ at the tested concentrations and contact times. It is possible that commercially available H₂O₂ oral rinses may have additional ingredients incorporated to improve the viricidal activity and that the viricidal activity may be better or worse at increased contact times. However, the 15- and 30-second contact times were chosen to represent convenient, routinely achievable and recommended time periods for oral rinsing in the clinical setting.²³ In light of the findings of this study, the recommended practice of preprocedural rinsing with hydrogen peroxide in solutions at concentrations between 1.5% and 3% may not be effective and therefore the current guidelines from the ADA may need to be updated. Additional concerns related to local toxicity of hard and soft tissues from routine use of H₂O₂ also requires further investigation. Furthermore, additional alternatives for patients unable to use PVP-I oral rinses should be investigated soon.

PVP-I oral rinses at non-toxic dilute concentrations have been shown to inactivate viruses related to severe acute respiratory syndrome (SARS), middle-east respiratory syndrome (MERS) and now SARS-CoV-2 as well.^{7,24,25} Randomized clinical trials on samples of COVID-19 positive patients comparing dilute PVP-I oral rinses as the standard agent against other oral rinses is the next important next step in further determination of the ideal pre-procedural rinse strategy for routine dental care. In the interim, dental professionals and patients can benefit from routine preprocedural rinsing with at least 0.5% PVP-I as it is inexpensive, safe for use in oral cavity up to 2.5%, rarely allergic, easily accessible, and has also been listed as a broad-spectrum antimicrobial agent for topical uses on the World Health Organization's List of Essential Medicines.^{7,13,26,27} PVP-I at 0.23% concentration is routinely used in Japan and has also been recommended by the Japanese Ministry of Health, Labor and Welfare for daily gargling to prevent upper respiratory tract infections.^{12,24,25} Despite its brown color PVP-I oral rinse has not been shown to stain teeth or cause a change in gustatory function.²⁸⁻³⁰ One clinical trial showed significantly less staining of PVP-I and higher preference among patients compared to chlorhexidine gluconate.³⁰ The predictable and verifiable in vitro viricidal effect of PVP-I at very low concentrations and contact times may open up new possibilities not only in the field of management of viral infectious diseases in medicine, but also in improvement of dental and medical equipment and related water lines.

Conclusions

SARS-CoV-2 virus was completely inactivated by PVP-I oral antiseptic rinse in vitro, at all concentrations tested in as little as 15 seconds. Hydrogen peroxide solutions at the recommended oral rinse concentrations of 1.5% and 3.0% showed minimal viricidal effect after contact times as long as

30 seconds. Until further clinical research is available, preprocedural oral rinsing using dilute PVP-I in the range of 0.5% to 1.5% (for patients and health care providers) should be the preferred choice over hydrogen peroxide, during the COVID-19 pandemic. This additional procedure has the ability to be an important adjunctive to personal protective equipment, masks and hand hygiene procedures.

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