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Rapid In-Vitro Inactivation of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) Using Povidone-Iodine Oral Antiseptic Rinse

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Abstract

Purpose: To investigate the optimal contact time and concentration for viricidal activity of oral preparation of povidone-iodine (PVP-I) against SARS-CoV-2 ('corona virus') to mitigate the risk and transmission of the virus in the dental practice.
Materials and Methods: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) USA-WA1/2020 strain, virus stock was tested against oral antiseptic solutions consisting of aqueous povidone-iodine (PVP-I) as the sole active ingredient. The PVP-I was tested at diluted concentrations of 0.5%, 1%, and 1.5%. 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. The test solutions and virus were incubated at room temperature (22 ± 2 °C) for time periods of 15 and 30 seconds. The solution was then neutralized by a 1/10 dilution in minimum essential medium (MEM) 2% fetal bovine serum (FBS), 50 μg/mL gentamicin. Surviving virus from each sample was quantified by standard end-point dilution assay and the log reduction value (LRV) of each compound compared to the negative (water) control was calculated.
Results: PVP-I oral antiseptics at all tested concentrations of 0.5%, 1%, and 1.5%, completely inactivated SARS-CoV-2 within 15 seconds of contact. The 70% ethanol control group was unable to completely inactivate SARS-CoV-2 after 15 seconds of contact, but was able to inactivate the virus at 30 seconds of contact.
Conclusions: PVP-I oral antiseptic preparations rapidly inactivated SARS-CoV-2 virus in vitro. The viricidal activity was present at the lowest concentration of 0.5 % PVP-I and at the lowest contact time of 15 seconds. This important finding can justify the use of preprocedural oral rinsing with PVP-I (for patients and health care providers) may be useful as an adjunct to personal protective equipment, for dental and surgical specialties during the COVID-19 pandemic.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 the virus resulting in the corona virus disease 2019, COVID-19) is a novel coronavirus in the same family as the severe acute respiratory syndrome (SARS) and middle east respiratory syndrome (MERS) viruses that emerged in local outbreaks in 2003 and 2015. From the first cases recognized in late December 2019 by health care workers in China, it has rapidly spread across the globe.[1] The World health organization (WHO) declared the spread of COVID-19 a global pandemic on March 11, 2020. This has significantly changed the way that dentistry is practiced around the world. The clinical workflow of dentists, especially prosthodontists, has been significantly altered due to the fact that the viral load is highest in the nasal cavity, nasopharynx and oropharynx related to the high expression of ACE2 receptor on goblet cells and respiratory epithelium used as fist entry into the body by SARS-CoV-2.[2,3] Viral shedding can be detected from nasal swabs before, during and after the onset of acute symptomatic disease including in seropositive antibody-converted convalescent cases.[2,3] As the mouth is also part of the oropharynx,

it harbors bacteria and viruses from the nose, throat and the respiratory tract and contaminated saliva can easily result in spread of viral infections.[4,5]

In a dental setting, well recognized terms related to microbiological risk are aerosol and splatter.[4-7] Aerosols are generally defined as suspension of fine solid particles or liquid droplets in air and splatters are larger liquid particles in air that impact a surface and then break apart. In dentistry, aerosols are recognized as airborne particles smaller than 50 μm in diameter which are small enough to stay in air for extended periods and entail risk of environmental contamination, and entry into respiratory tracts.[4-7] On the other hand, splatters are denoted as airborne particles larger than 50 μm in diameter and too large to stay suspended in air for longer periods. Splatters are typically seen as droplets ejected forcefully in a ballistic manner similar to a bullet until they contact a surface.[4-7] Any dental procedure that can aerosolize contaminated saliva can significantly increase airborne contamination with microorganisms.[7] Most procedures in contemporary prosthodontics ranging from single unit restorations to complex implant surgery include aerosol production due to the use of handpieces and air-water syringes. Additional dental maintenance procedures involving the use of ultrasonic scalers and air abrasion units produce even higher visible aerosols.[4] All these procedures involve aerosol production resulting in higher risk for clinicians, dental assistants, and patients.

Due to the COVID-19 pandemic which has resulted in significant morbidity and mortality, it is imperative for clinicians to consider adjunctive protective measures that can add an additional barrier of safety in providing prosthodontics treatment. Understandably, there is no single method or agent, which can completely eliminate or minimize the risk of COVID-19 infection to dental personnel and other patients. Harrel and Molinari have described 3 layers of defense against aerosols in a dental office.[4] The first layer is personal protective equipment (PPE) such as gloves, eye glasses and masks. The second layer is regular use of oral antiseptic rinse and the third layer is the use of high volume evacuator (HEV) ("high speed suction") and adjunctive high efficiency particulate air (HEPA) filters. The first and third layers of defense have received significant attention by dentists however, the second layer of defense has not received sufficient attention.

Harrel and Molinari recommended preprocedural oral rinsing with chlorhexidine gluconate a rinse with essential oils, but both of these are known to only be efficacious against bacteria, with no viricidal properties.[4] Therefore, the American Dental Association (ADA) interim guidelines from April 2020 have suggested preprocedural oral rinsing with 1.5% hydrogen peroxide (commercially available in the United States) or 0.2% povidone-iodine PVP-I (not commercially available in the United States).[8] The Centers for Disease Control and Prevention (CDC) have also recommended preprocedural rinsing with antimicrobial rinses such as chlorhexidine gluconate, essential oils, PVP-I or cetylpyridinium chloride.[9] Presently, there are no clinical studies supporting the viricidal effects of any pre-procedural oral rinse against SARS-CoV-2.[8] Additionally, there are no in vitro studies on the viricidal effect of the commercially available 1.5% hydrogen peroxide. However, PVP-I solutions of 0.23% have effectively inactivated SARS-CoV and MERS with contact times as low as 15 seconds in vitro.[10,11] The SARS-CoV-2 virus resulting in the COVID-19 pandemic is a novel coronavirus in the same family and is expected to be inactivated by PVP-I in the same manner.

Therefore, the purpose of this study was to investigate the optimal contact time and concentration for viricidal activity of oral preparation of povidone-iodine (PVP-I) against SARS-CoV-2 ('corona virus') to mitigate the risk and transmission of the virus in the dental practice. The null hypothesis was that there would be no difference in viricidal activity of PVP-I at various concentrations and contact times as well as there would be no difference between PVP-I and the positive and negative controls.

Materials and methods

All laboratory work with SARS-CoV-2 was conducted in biosafety level 3 (BSL-3) laboratories at The Institute for Antiviral Research at Utah State University Logan, UT following established standard operating procedures approved by the Utah State University Biohazards Committee. The SARS-CoV-2, USA-WA1/2020 strain, virus stock was prepared prior to testing by growing in Vero 76 cells. Culture media for prepared stock (test media) was minimum essential medium (MEM) with 2% fetal bovine serum (FBS) and 50 μg/mL gentamicin. The oral rinse antiseptic solution consisted of various concentrations of aqueous povidone-iodine (PVP-I) as the sole active ingredient (Veloce BioPharma; Fort Lauderdale, FL). The PVP-I concentrations of each solution as supplied and after 1:1 dilution are listed in Table 1.

The test compounds were mixed directly with virus solution so that the final concentration was 50% of each individual test compound and 50% virus solution. A single concentration was tested in triplicate. Test media without 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 virus control. The test solutions and virus were incubated at room temperature (22 ± 2 °C) for 15 and 30 seconds. The solution was then neutralized by a 1/10 dilution in MEM 2% FBS, 50 μg/mL gentamicin.

Surviving virus from each sample was quantified by standard end-point dilution assay. 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 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% CO_2 for 5 days. Each well was then scored for presence or absence of infectious virus. The titers were measured using a standard endpoint dilution 50% cell culture infectious dose (CCID50) assay calculated using the Reed-Muench equation and the log reduction value (LRV) of each compound compared to the negative (water) control was calculated.[12]

Table 1 Virus titers and log reduction value of SARS-CoV-2 when incubated with various concentrations of PVP-I and the controls for 15 seconds. Each experimental sample was tested 3 times and average virus titers are reported

Test Product	PVP-I Concentration (%) After 1:1 Dilution	Incubation Time (in seconds)	Virus Titer[a]	LRV[b]
PVP-I 3.0% Oral Rinse Antiseptic	1.5	15	<0.67	3.0
PVP-I 1.5% Oral Rinse Antiseptic	0.75	15	<0.67	3.0
PVP-I 1.0% Oral Rinse Antiseptic	0.5	15	<0.67	3.0
Ethanol 70%	N/A	15	1.5	2.17
Water	N/A	15	3.67	N/A

[a]Log_{10} $CCID_{50}$ of virus per 0.1 mL. The assay lower limit of detection is 0.67 Log_{10} $CCID_{50}$/0.1 mL.

[b]LRV (log reduction value) is the reduction of virus compared to the virus control.

Table 2 Virus titers and log reduction value of SARS-CoV-2 when incubated with various concentrations of PVP-I and the controls for 30 seconds. Each experimental sample was tested 3 times and average virus titers are reported

Test Product	PVP-I Concentration (%) After 1:1 Dilution	Incubation Time (in seconds)	Virus Titer[a]	LRV[b]
PVP-I (3.0%) Oral Rinse Antiseptic	1.5	30	<0.67	3.33
PVP-I (1.5%) Oral Rinse Antiseptic	0.75	30	<0.67	3.33
PVP-I (1.0%) Oral Rinse Antiseptic	0.5	30	<0.67	3.33
Ethanol 70%	N/A	30	<0.67	3.33
Water	N/A	30	4.0	N/A

[a]Log10 CCID50 of virus per 0.1 mL. The assay lower limit of detection is 0.67 Log10 CCID50/0.1 mL.

[b]LRV (log reduction value) is the reduction of virus compared to the virus control.

Results

Virus titers and LRV of SARS-CoV-2 when incubated with various concentrations of the manufacturer's compounds for 15 seconds are shown in Table 1. After the 15-second contact time, all of the PVP-I oral rinse antiseptics tested were effective at reducing >3 log10 CCID50 infectious virus from, 3.67 log10 CCID50/0.1 mL to 0.67 log10 CCID50/0.1 mL or less. Table 2 shows the virus titers and LRV of SARS-CoV-2 when the virus was incubated for 30 seconds with each of the test compounds at 50/50 ratio. For the 30-second contact time, once again all of the PVP-I oral rinse antiseptics tested were effective at reducing >3.33 log10 CCID50 infectious virus from, 4.0 log10 CCID50/0.1 mL to 0.67 log10 CCID50/0.1 mL or less. No cytotoxicity was observed with any of the test compounds. The positive control and neutralization controls performed as expected.

Discussion

The purpose of this study was to investigate the optimal contact time and concentration for viricidal activity of oral preparation of povidone-iodine (PVP-I) against SARS-CoV-2 ('corona virus') to mitigate the risk and transmission of the virus in the dental practice. Data from this in vitro study rejected the null hypothesis, and showed the rapid viricidal activity of PVP-I oral formulation. The minimum 15 seconds contact time proved to be sufficient for PVP-I. This is similar to the results from previous studies where PVP-I solutions of 0.23% inactivated SARS-CoV and MERS with contact times as low as 15 seconds in vitro.[10,11] Oral decontamination is an important adjuvant process along with PPE to reduce viral transmission among dental patients especially during aerosol generating procedures. A majority of prosthodontic procedures involve close physical contact with the patient and generate aerosols increasing the risk of viral transmission. In the absence of clinical studies, the residual challenge in oral antisepsis is to find the effective concentration and duration of topical preparations which are safe to administer. Several different protocols have been used anecdotally to dilute PVP-I at a varying concentration ranging from 0.25% to 0.5% in an attempt to inactivate the virus from the nasal and oral cavity.[13]

Oral use of PVP-I antiseptic rinse is not novel to dentistry and has been used effectively in the past for oral decontamination,[14] periodontal therapy,[15] peri-implant therapy[16] and post-extraction therapy.[17] PVP-I has proven safe for use in the oral cavity up to 5% concentration and nasal cavities at concentrations up to 1.25%.[18-20] At these concentrations, no change in olfaction or mucociliary clearance has been noted.[21] Thyroid stimulating hormone was shown to slightly increase within normal levels after oral use of 5% PVP-I for 6 months, but no clinical thyroid disease was detected.[22] Nevertheless, at concentrations of 0.2% to 0.5% iodine absorption is minimal and below the total daily iodine intake for a healthy adult of 150 μg. Additionally, no change in taste or discoloration of teeth has been reported in the literature.[23] The substantivity (prolonged association between a topical material and a substrate) of oral PVP-I antiseptic rinse has been reported to be as long as 4 hours.[24] However PVP-I solutions are contraindicated for patients with anaphylactic allergy to iodine, pregnancy, active thyroid disease, and patients undergoing radioactive iodine therapy.[25-27]

The alternative to oral PVP-I solution is 1.5% hydrogen peroxide rinse, as recommended by the ADA interim guidelines.[8]

Presently the CDC does not recommend this rinse due to absence of sufficient scientific evidence.[9] Though 1.5% hydrogen peroxide is known to be antibacterial, it is chemically more unstable. There are no published studies showing the inactivation of SARS-CoV-2 by this solution at 1 minute contact period.[28] The potential oral toxicity of routine use of this solution for 1 minute as well as its substantivity is unknown at this time. Additionally, alcohol is present in most oral rinses and results from this study showed that it required a longer time for viral inactivation than PVP-I oral solutions tested.

Some disadvantages of the PVP-I oral solutions include the absence of clinical research such as randomized clinical trials, and the current absence of commercially available formulation at lower concentrations for intra-oral use. Therefore, dentists are required to dilute the commercially available antiseptic formulation of 10% PVP-I, by 1:20, utilizing 0.5 ccs of 10% povidone iodine and 9.5 ccs of sterile water for routine clinical use at 0.5%. This compound typically contains suds which do not cause any adverse effects for topical usage. However, dentists should make freshly diluted solutions each day and refrigerate them during the day as diluted PVP-I solutions are chemically unstable with respect to disproportionation into constituent equilibrium species. As the aqueous concentration of PVP-I decreases from 10% to less than 1%, these solutions become unstable with respect to temperature, counterions, commercial packaging and pH value. In order to ensure that a diluted solution is safe for administration to the oral or nasal cavity, there should either be an analysis of the chemical ingredients of each freshly-prepared solution or commercial preparations of PVP-I at the appropriate dose (if available) should be used.

Conclusions

PVP-I oral antiseptic preparations rapidly inactivated SARS-CoV-2 virus in vitro. The 70% ethanol control group was unable to completely inactivate SARS-CoV-2 after 15 seconds of contact, but was able to inactivate the virus at 30 seconds of contact. The viricidal activity of PVP-I oral antiseptic solution was present at the lowest concentration of 0.5 %, and at the lowest contact time of 15 seconds. This important finding warrants the use of preprocedural oral rinsing with 0.5% PVP-I for patients and health care providers. This solution serves as an adjunct to personal protective equipment for dental and surgical specialties during the COVID-19 pandemic.

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