The Laryngoscope

The Effect of Povidone-Iodine Nasal Spray on Nasopharyngeal SARS-CoV-2 Viral Load: A Randomized Control Trial

David Zarabanda, MD; Neelaysh Vukkadala, MD; Katie M. Phillips, MD; Z. Jason Qian, MD; Kenji O. Mfuh, PhD; Matthew J. Hatter, BA; Ivan T. Lee, MD, PhD; Vidya K. Rao, MD; Peter H. Hwang, MD; George Domb, MD; Zara M. Patel, MD; Benjamin A. Pinsky, MD, PhD; Jayakar V. Nayak, MD, PhD

Objectives/Hypothesis: To determine the effect of povidone-iodine (PVP-I) nasal sprays on nasopharyngeal (NP) viral load as assessed by cycle threshold (Ct) on quantitative polymerase chain reaction (qPCR) of SARS-CoV-2 in outpatients.

Study Design: Three arm, triple blinded, randomized, placebo-controlled clinical trial.

Methods: Participants were randomized within 5 days of testing positive for COVID-19 to receive nasal sprays containing placebo (0.9% saline), 0.5% PVP-I, or 2.0% PVP-I. NP swabs for qPCR analysis were taken at baseline, 1-hour post-PVP-I spray (two sprays/nostril), and 3 days post-PVP-I spray (20 sprays/nostril). Symptom and adverse event questionnaires were completed at baseline, day 3, and day 5. University of Pennsylvania Smell Identification Tests (UPSIT) were completed at baseline and day 30.

Results: Mean Ct values increased over time in all groups, indicating declining viral loads, with no statistically significant difference noted in the rate of change between placebo and PVP-I groups. The 2.0% PVP-I group showed statistically significant improvement in all symptom categories; however, it also reported a high rate of nasal burning. Olfaction via UPSIT showed improvement by at least one category in all groups. There were no hospitalizations or mortalities within 30 days of study enrollment.

Conclusions: Saline and low concentration PVP-I nasal sprays are well tolerated. Similar reductions in SARS-CoV-2 NP viral load were seen over time in all groups. All treatment groups showed improvement in olfaction over 30 days. These data suggest that dilute versions of PVP-I nasal spray are safe for topical use in the nasal cavity, but that PVP-I does not demonstrate virucidal activity in COVID-19 positive outpatients.

Key Words: Severe acute respiratory syndrome coronavirus 2, coronavirus disease 2019, povidone-iodine, nasopharyngeal swab, viral load, cycle threshold, nasal spray, clinical trial.

Level of Evidence: II

***Laryngoscope*, 00:1–7, 2021**

INTRODUCTION

The emergence and spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes the inflammatory respiratory disease termed coronavirus disease 2019 (COVID-19), has created a worldwide health crisis. Intensive research efforts have led to the formulation of effective vaccines,[1] but human-to-human transmission of SARS-CoV-2 remains widespread.[2] There is an urgent need to develop other affordable and readily available strategies to reduce viral transmission and/or mitigate symptoms of COVID-19.

Transmission of SARS-CoV-2 occurs primarily through the spread of aerosolized droplets into the nose.[3,4] The virus gains access by binding to angiotensin-converting enzyme 2 receptors present in the cilia of the upper and lower airway respiratory epithelium.[4] Recent studies have demonstrated a correlation between SARS-CoV-2 viral load and COVID-19 disease severity, mortality, and infectivity.[5,6] Therefore, direct application of topical virucidal treatment to the nasopharyngeal (NP) tissues to arrest SARS-CoV-2 replication would be expected to decrease viral burden in exposed patients.

One possible topical therapy for reducing SARS-CoV-2 viral titers is povidone-iodine (PVP-I) solution, a widely available and inexpensive antiseptic medication.

From the Department of Otolaryngology-Head and Neck Surgery (D.Z., N.V., Z.J.Q., M.J.H., I.T.L., P.H.H., Z.M.P., J.V.N.), Stanford University School of Medicine, Stanford, California, U.S.A.; Department of Otolaryngology-Head and Neck Surgery (K.M.P.), University of Cincinnati School of Medicine, Cincinnati, Ohio, U.S.A.; Clinical Virology Laboratory (K.O.M., B.A.P.), Stanford Health Care, Stanford, California, U.S.A.; Division of Allergy and Immunology, Department of Pediatrics (I.T.L.), Stanford University School of Medicine, Stanford, California, U.S.A.; Divisions of Cardiothoracic Anesthesiology and Critical Medicine, Department of Anesthesiology, Perioperative and Pain Medicine (V.K.R.), Stanford University School of Medicine, Stanford, California, U.S.A.; Shasta-ENT Medical Group (G.D.), Redding, California, U.S.A.; Department of Pathology (B.A.P.), Stanford University School of Medicine, Stanford, California, U.S.A.; Division of Infectious Diseases and Geographic Medicine, Department of Medicine (B.A.P.), Stanford University School of Medicine, Stanford, California, U.S.A.; and the Department of Otolaryngology-Head and Neck Surgery (J.V.N.), Veterans Affairs Palo Alto Health Care System, Palo Alto, California, U.S.A.

Editor's Note: This Manuscript was accepted for publication on October 25, 2021.

D.Z. and N.V. contributed equally to this work and have the right to list their name first in their CV.

This work was supported in large part by the COVID Catalyst Award, Center for Emerging and Neglected Diseases, University of California, Berkeley.

The authors have no other funding, financial relationships, or conflicts of interest to disclose.

Send correspondence to Jayakar V. Nayak, MD, PhD, 801 Welch Road, Stanford, CA 94305. E-mail: jnayak@stanford.edu

DOI: 10.1002/lary.29935

PVP-I is frequently used to treat intranasal methicillin-resistant *Staphylococcus aureus* colonization[7] and to reduce the incidence of surgical wound infections in the operating room.[8] Prior in vitro studies have demonstrated that dilute PVP-I solutions, in concentrations as low at 0.5%, produce a ≥4 $\log^{10}$ decrease in SARS-CoV-2 viral titers (inactivation of ≥99.99% of virus) within 15 seconds of exposure.[9,10] PVP-I nasal sprays of up to 4.4% concentration have also been used in healthy volunteers without adverse effects.[11]

These reports have generated intense interest in intranasal PVP-I treatments worldwide.[12] Despite the in vitro efficacy, it is unclear whether intranasal PVP-I would be effective in vivo given the surface area of the nasal mucosa, rapid mucociliary clearance, volume and viscosity of mucus present.[13] Recently, Guenezan and colleagues published the first human clinical trial comparing PVP-I mouth rinses and nasal sprays to nonintervention in COVID-19 patients and did not find significant differences in SARS-CoV-2 NP viral load between the groups.[14] This study was neither blinded nor placebo-controlled.

We report the results from our randomized, triple-blinded, placebo-control clinical trial comparing low and high concentration PVP-I and saline nasal sprays as topical treatments to reduce the NP viral load of SARS-CoV-2 in outpatients.

MATERIALS AND METHODS

Study Design

This PVP-I COVID-19 trial was a prospective, three arm, triple-blinded, placebo-controlled clinical trial conducted at Stanford University School of Medicine and the Stanford Center for Sinus and Endoscopic Skull Base Surgery. The study was approved by the Stanford Institutional Review Board (protocol #56134), and the trial was registered with clinicaltrials.gov under trial number NCT04347954.

Participant Eligibility

The eligible study population was limited to outpatients who had a documented positive NP swab for COVID-19 within 5 days prior to study enrollment. All participants were at least 18 years of age. Exclusion criteria included: 1) a history of adverse reactions to iodine supplementation and/or food dye; 2) current/active use of intranasal steroid sprays; 3) sinonasal surgery within 30 days of study enrollment; 4) pregnancy; and 5) participation in other COVID-19 trials. Study participants received a modest compensation (US$50) for their participation in this study.

Study Arms, Randomization, and Blinding

Participants were randomized 1:1:1 into three groups: 0.9% NaCl (saline group), 0.5% PVP-I in water, and 2% PVP-I in water.[15] The use of PVP-I in water rather than saline solution was based on prior publications.[9,10] Each study medication was prepared by an independent, licensed compounding pharmacy (A & O Specialty Pharmacy, Salinas, CA), and dispensed into identical, opaque nasal atomizer spray bottles that deliver standardized 0.1 mL spray volume with each pump. The spray bottles were labeled as "A," "B," or "C" and the corresponding treatment solutions were only known to the pharmacist, who was not directly involved in the study. To further ensure blinding of the study team, the pharmacist added food coloring to the 0.9% NaCl saline solution to give it a similar appearance to PVP-I. The study was thereafter triple blinded: the treatment assignment was concealed to both the participants and investigators during all aspects of data collection and analysis, until the primary outcome measure (reverse transcription polymerase chain reaction cycle threshold, or RT-PCR Ct) had been assessed.

Procedure

On treatment day 1, after providing written informed consent, participants underwent an initial baseline NP swab for RT-PCR testing for SARS-CoV-2. Next, after an educational review of nasal spray administration technique, the assigned randomized spray bottle was provided, and the first treatment of two sprays per nostril was self-administered by each participant.

A second NP swab specimen was then collected from all participants 1 hour following the first treatment. This time point was included to assess for an immediate virucidal effect to the sprays while allowing for sufficient time to sweep the nasal cavity of nonviable virus particles via mucociliary clearance. Patients were then instructed to self-administer nine additional treatments (two sprays per nostril, four times a day at home), using the assigned, randomized spray bottle. A third NP swab was collected on treatment day 3, after 10 treatments, or a total of 20 sprays per nostril, had been self-administered. All NP swabs were performed by the study principal investigator (J.V.N.) on the same side for each patient.

All patients completed baseline questionnaires, which provided demographic and clinical information, as well as a baseline olfactory assessment using the validated University of Pennsylvania Smell Identification Test (UPSIT).[16] Disease-related symptoms and potential adverse reactions related to use of the assigned nasal spray use were monitored in follow-up. On days 1, 3, and 5, participants rated the severity of their symptoms on a 4-point Likert scale with "1" representing "not experiencing that symptom" and "4" representing "severe symptoms." Additionally, on days 3 and 5, participants provided a global assessment of the trajectory of their symptoms by answering the question of "Compared with yesterday, I feel my infection is:" on a 5-point scale ranging from "much worse" to "much better." A final UPSIT was completed by participants 30 days following study enrollment and returned to Stanford by mail. Adverse events were monitored by asking participants if they experienced symptoms such as nasal pain or burning. Participants could then pick adverse event frequency as "never," "rare," "sometimes," "often," or "always." On day 5 of the study, adherence to nasal sprays was queried and participants were asked to report adherence as either "0–25%," "26–50%," "51–75%," or "76–100%" of all doses.

Quantitative PCR

All NP swab samples were placed in 2 mL of saline in 15 mL conical tubes. These tubes were kept on ice until delivered to the Stanford Clinical Virology Laboratory. Specimens were processed in the following fashion.[17] Briefly, total nucleic acids were extracted from 500 μL saline on the QIAsymphony SP using the QIAsymphony DSP Virus/Pathogen Midi Kit (both from Qiagen, Germantown, MD), and eluted in 60 μL buffer AVE as supplied by the manufacturer. SARS-CoV-2 RNA was detected using previously described primer and probe sequences targeting the *envelope* (*E*) gene.[18] These were combined in multiplex with RNase P primers and probe. Real-time RT-PCR was performed using the SuperScript III One-Step RT-PCR System with

Platinum Taq DNA Polymerase Kit (Invitrogen, Carlsbad, CA) on the Rotor-Gene Q Instrument (Qiagen).

Cycle threshold (Ct) denotes how many PCR cycles are required before the SARS-CoV-2 viral RNA reached a detectable level. Higher Ct values correspond to lower viral copy numbers. For reference, Ct values of 20 correspond to $\sim 2.12 \times 10^6$ viral copies per milliliter, while a Ct value of 40 is undetectable and is considered the lower limit of this RT-PCR assay. Viral copy number is derived using a formula which applies a logarithmic transformation to the Ct.

During the course of the study, the SARS-CoV-2 strand-specific real-time RT-PCR test for actively replicating SARS-CoV-2 virus was developed and validated at the Stanford Virology laboratory. The detection of SARS-CoV-2 minus-strand RNA was shown to correlate with the presence of active, replicating virus.[19] A post-hoc exploratory analysis was performed on our samples to assess whether there was a detectable effect on minus strand presence as a proxy for determining inactivation of viral replication following spray use.

Statistical Analyses

Because this trial was developed and conducted early in the pandemic at a time when limited information was available on the natural history of the virus and no prior data existed on PVP-I as a therapy, the sample size for this study is not based on statistical assumptions for formal hypothesis testing. We aimed to recruit 45 subjects in total. This number of participants was considered sufficient to provide a descriptive summary of the safety and primary outcome. Statistical analyses were performed using Stata 16 (StataCorp LP, TX). Ct was used as the primary unit of measurement. For our primary outcome of NP viral load, we present the data as Ct, since this utilizes the original output of the qPCR test.

The Shapiro–Wilk's test was used to determine the normality of the data. Performance of treatment arms over time was compared using a mixed effects maximum likelihood linear regression model with random intercept and random slope. Time (in hours since first swab), treatment arm, and Ct value were entered into the model, which then predicted the effect of time and treatment arm on Ct values. The model also corrected for baseline Ct values.

For symptoms, the Friedman test was used to assess changes in symptom scores for each of the tracked symptoms over time in the study arms. Changes in global symptom rating were compared using the chi-square test. Changes in olfaction, as categorized by normative values of the UPSIT were assessed using the chi-square test. Likewise, differences in the frequencies of adverse events were assessed using the chi-square test or Fischer's exact test.

RESULTS

Study Participants

Overall, 265 patients were screened for eligibility and 35 patients were included in the final analysis for the primary outcome measure (saline: 11 participants;

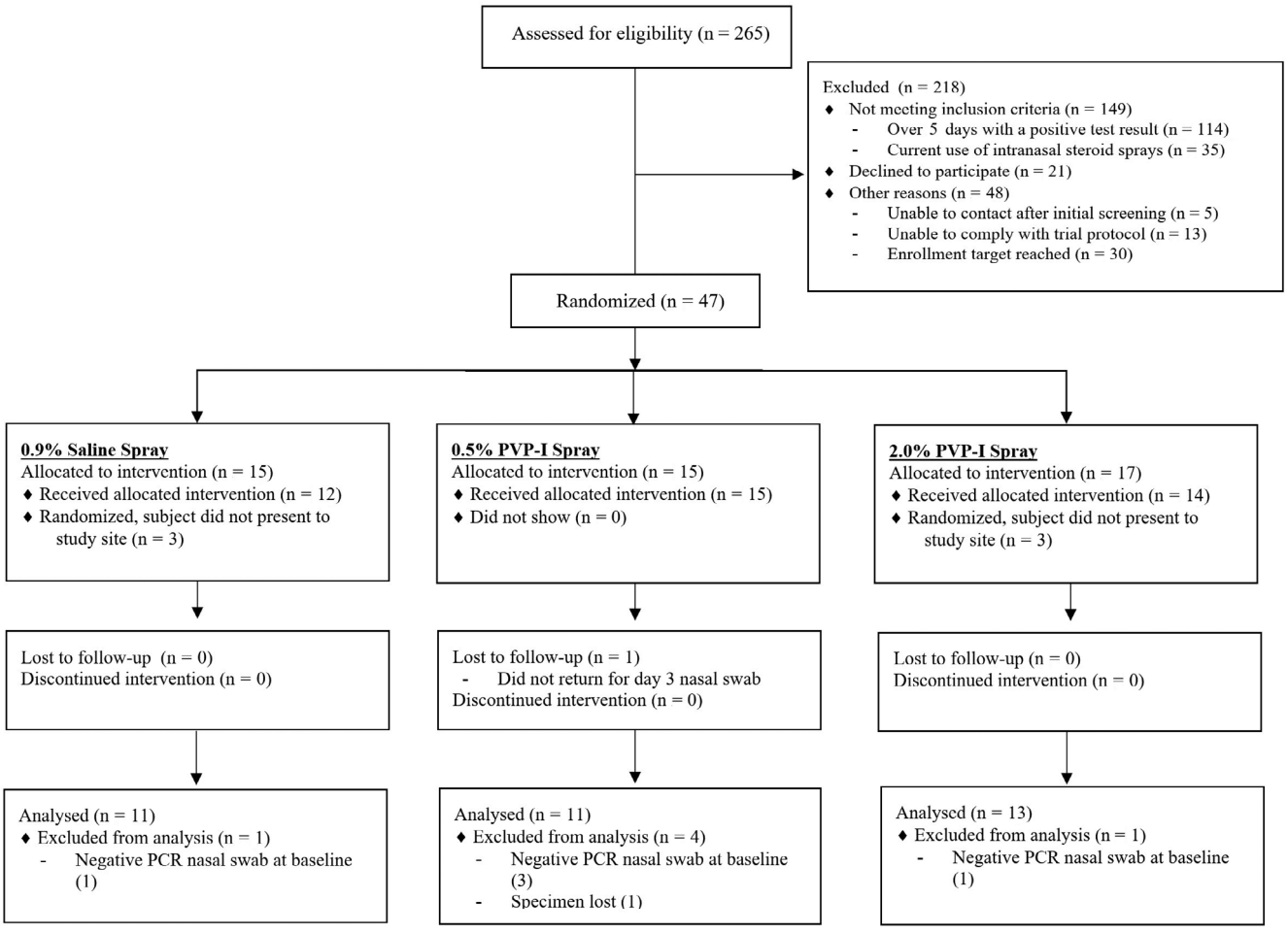

Fig. 1. CONSORT diagram.

TABLE I.
Patient Demographics.

Characteristic	Total	Saline Spray	PVP-I 0.5%	PVP-I 2.0%	*p* Value[†]
Count	35	11	11	13	
Age, mean (SD)	43.2 (18.0)	44.2 (15.6)	45.2 (12.2)	40.7 (19.9)	.758
Female, n (%)	18 (51.4)	8 (27.3)	5 (45.5)	5 (38.5)	.279
Reporting 76–100% adherence to nasal sprays, n (%)	34 (97.1)	10 (90.9)	11 (100)	13 (100)	.629
Ethnicity/Race, n (%)					
White	25 (71.4)	8 (72.3)	7 (63.6)	10 (76.9)	.564
Hispanic	7 (20.0)	1 (9.1)	3 (27.3)	3 (23.1)	
Asian	3 (8.6)	2 (18.2)	1 (9.1)	0 (0.0)	
Days since positive COVID test to enrollment, mean (SD)	3.5 (1.9)	3.9 (2.8)	3.7 (1.5)	3.0 (1.1)	.429
Days since symptoms to enrollment, mean (SD)	6.3 (2.8)	4.8 (2.2)	7.2 (2.6)	7.1 (3.2)	.081
Comorbidities, n (%)					
Sinusitis/allergy	3 (8.6)	2 (18.2)	1 (9.1)	0 (0.0)	.279
Respiratory disease/Asthma	2 (5.7)	2 (18.2)	0 (0.0)	0 (0.0)	.185
Cardiac disease	2 (5.7)	0 (0.0)	1 (9.1)	1 (7.7)	>.99
Neurodegenerative disease	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA
Prior treatment, n (%)					
Saline nasal irrigations	1 (2.9)	0 (0.0)	1 (9.1)	0 (0.0)	.869
Tylenol/nonsteroidal anti-inflammatory drugs	1 (2.9)	0 (0.0)	0 (0.0)	1 (7.7)	
None	33 (94.3)	11 (100)	10 (90.9)	12 (92.3)	
Noted change to taste or smell at enrollment, n (%)	19 (54.3)	5 (45.5)	6 (54.6)	8 (61.5)	.910
Risk factors for smell loss, n (%)					
History of head trauma	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	NA
History of sinus surgery	2 (5.7)	0 (0.0)	0 (0.0)	2 (15.4)	.316

NA = not applicable since frequencies are constant across treatment.
[†]*P*-values based on Fisher's exact test for categorical variables and Kruskal–Wallis test of ranks for continuous variables.

0.5% PVP-I: 11 participants; 2.0% PVP-I: 13 participants) (Fig. 1). The mean age was 43.2 years (standard deviation [SD] ± 18.0 years). There were 18 (51.4%) female participants. Complete patient characteristics are described in Table I. There was a mean of 3.5 days (SD ± 1.9 days) from a positive COVID test to study enrollment, and a mean of 7.0 days (SD ± 5.0 days) from symptom onset to study enrollment.

Changes in Ct Value and Viral Load via NP Swab Assessment

Mean baseline Ct values for intranasal SARS-CoV-2 were similar across all groups at 23.7 (SD ± 6.9) in the saline group, 26.0 (SD ± 4.9) in the 0.5% PVP-I group, and 28.4 (SD ± 6.4) in the 2.0% PVP-I group ($p = .179$).

Mixed effects linear regression showed that Ct values increased with time (mean difference = 0.055 cycles/hour; 95% confidence interval [CI] 0.037 to 0.074) in the overall cohort, indicating a decline in SARS-CoV-2 NP viral loads in all groups. However, there was no difference seen in the longitudinal performance between the three nasal spray cohorts over the duration of this study ([saline: reference], [0.5% PVP-I: mean difference = −0.349 cycles/hour; 95% CI −1.584 to 0.886], [2.0% PVP-I: mean difference −1.059 cycles/hour; 95% CI −2.318 to 0.201]), that is, saline and PVP-I nasal sprays were not significantly different in altering Ct in COVID-19 positive patients (Fig. 2).

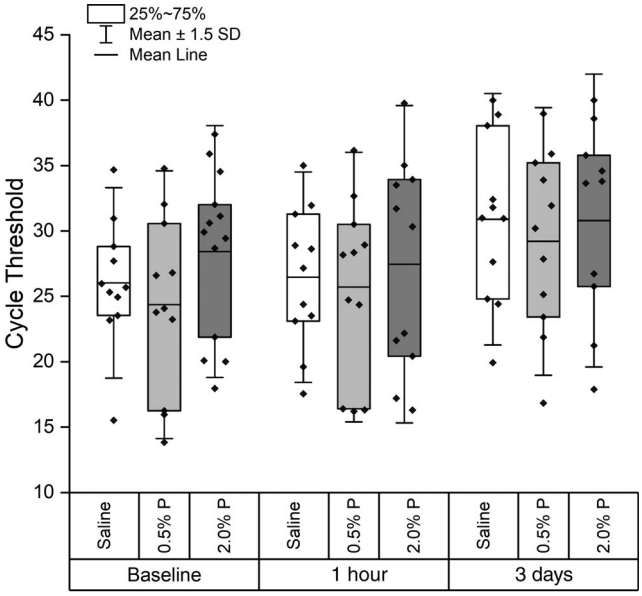

Fig. 2. SARS-CoV-2 cycle threshold levels by group and time point.

Minus Strand PCR via NP Swab Assessment

An exploratory chi-square analysis was used to compare the number of participants in each group who initially had a detectable minus-strand SARS-CoV-2 RNA (indicating active viral replication) on study day 1, who subsequently converted to an undetectable minus-strand by day 3 (suggesting viral inactivation). Given the limited sample size, the two PVP-I spray groups were combined for this analysis with seven subjects in the PVP-I and seven subjects in the saline group. In each group, five subjects converted to an undetectable minus strand by day 3, while two subjects did not. The mean change in Ct value for the minus strand between baseline and 1 hour post administration was also assessed, and again there was no significant difference between the groups (saline mean change in Ct: 0.31, PVP-I combined group mean change in Ct: 0.29, $p = .89$).

UPSIT Olfaction Outcomes

A total of 33 of 35 participants completed and returned day 30 UPSIT studies (saline: 11; 0.5% PVP-I: 11; 2.0% PVP-I: 11). When comparing the changes in the degree of anosmia from baseline to post-treatment, there was no statistically significant difference in the percentage of patients who improved in each group (saline: 70%; 0.5% PVP-I: 70%; 2.0% PVP-I: 89%; $p = .50$). No participant in this clinical trial demonstrated worsening olfaction (Table II).

Symptomatic Improvement

Mean adherence was 100% in the 2.0% PVP-I group, 100% in the 0.5% PVP-I group, and 91% in the saline group. There were no differences in the reported frequencies of baseline symptoms between groups. The 2.0% PVP-I group demonstrated statistically significant improvement in all symptoms—fevers, chills, fatigue, smell, taste, congestion, and sore throat over time. The 0.5% PVP-I group only reported significant improvements in taste and sore throat. Meanwhile the saline spray group reported significant improvements in fever, chills, fatigue, and congestion (Table III). In terms of overall subjective health status, on days 3 and 5, all participants reported feeling either the same or better than they had in the prior 24 hours. The number of participants (saline: 82%; 0.5% PVP-I: 73%; 2.0%

TABLE II.
UPSIT Results for Baseline and Day 30 Time Point.

Treatment Group	Anosmia	Severe Microsmia	Moderate Microsmia	Mild Microsmia	Normosmia
Baseline UPSIT					
Normal saline	2	3	2	2	2
0.5% PVP-I	4	1	2	0	3
2.0% PVP-I	5	1	2	3	1
30 Day UPSIT					
Normal saline	0	2	0	5	3
0.5% PVP-I	0	1	0	2	8
2.0% PVP-I	0	1	1	3	7

TABLE III.
Mean Symptom Severity for Each Group at Baseline, Day 3, and Day 5.

	Fevers	Chills	Fatigue	Smell	Taste	Congestion	Sore Throat
Saline							
Baseline	1.33	1.50	2.33	2.08	2.00	2.25	1.17
Day 3	1	1.00	1.82	1.91	1.82	1.82	1.00
Day 5	1	1.00	1.82	1.82	1.73	1.55	1.00
P-value	.050	.018	.006	.554	.738	.018	.135
0.5% PVP-I							
Baseline	1.27	1.73	2.27	2.09	1.91	2.36	1.64
Day 3	1.18	1.45	1.82	1.73	1.55	2.00	1.36
Day 5	1.27	1.64	1.64	1.64	1.18	1.82	1.09
P-value	.368	.247	.122	.504	.024	.179	.086
2.0% PVP-I							
Baseline	1.38	1.46	2.08	3.00	2.69	2.38	1.69
Day 3	1.00	1.08	1.46	2.23	1.92	2.15	1.31
Day 5	1.00	1.00	1.15	1.77	1.77	1.46	1.15
P-value	.018	.023	.002	.003	.028	.009	.037

TABLE IV.
Frequency of Adverse Events by Study Arm Reported on Day 3 and Day 5.

	Nasal burning	Headaches	Ear pain	Sneezing	Nosebleeds
Day 3					
Saline	16.7%	25.0%	0.0%	8.3%	0.0%
0.5% PVP-I	28.5%	21.4%	7.1%	28.5%	7.1%
2.0% PVP-I	92.9%	35.7%	14.3%	64.3%	7.1%
P-value	<.001	.75	.76	.009	1.00
Day 5					
Saline	16.7%	16.7%	0.0%	8.3%	0.0%
0.5% PVP-I	14.3%	14.3%	7.1%	28.5%	7.1%
2.0% PVP-I	92.9%	42.9%	14.3%	64.3%	7.1%
P-value	<.001	.22	.76	.009	1.00

PVP-I: 77%; $p = .92$) reporting improvement was not statistically different between groups.

Adverse Events

Subjects randomized to 2.0% PVP-I reported statistically higher rates of burning sensation and pain in the nose (day 3: 92.9%; day 5: 92.9%) as compared to saline (day 3: 16.7%; day 5: 16.7%) and 0.5% PVP-I (day 3: 28.5%; day 5: 14.3%) groups (Table IV). Of note, no participants in our study required visits to the emergency department or hospitalization for COVID-19, and there there were no mortalities at 30 days after study enrollment.

DISCUSSION

In this randomized, triple-blinded, placebo-controlled trial of PVP-I nasal sprays, there were similar improvements in Ct values (i.e., similar reduction in SARS-CoV-2 viral loads) between subjects who received saline nasal sprays and PVP-I nasal sprays for treatment of COVID-19. Notably, no participants in our study reported emergency department visits or hospital admissions for COVID-19-related symptoms. Participants in all three groups reported subjective improvement in their condition on days 3 and 5. Finally, no participants reported any worsening in clinical status over the course of the study.

Recently, a randomized, nonblinded, and nonplacebo controlled trial compared a 12 person intervention group of 1% PVP-I oral rinse, spray, and anterior nasal application of 10% PVP-I ointment to a 12 person nonintervention group.[14] This study did not find differences in SARS-CoV-2 viral load between the treatment and placebo groups based on RT-PCR analysis. Similarly, in our study, we found that the saline group had comparable rates of decline in SARS-CoV-2 viral load when compared to both PVP-I groups as assessed by qPCR over the 3-day study period. Although there were only 35 participants in our pilot study, there was no trend in the data toward a difference in viral load reduction between groups so it is less likely that increasing the sample size would lead to identification a clinically significant difference.

Anecdotally, many providers apply PVP-I nasal sprays or oral rinses prior to examination of patients in the outpatient setting.[20] However, our 1-hour NP swab time point after initial dosing of sprays showed no significant reduction in Ct and viral load for SARS-CoV-2 across our three groups. qPCR simply measures a specific segment of viral RNA but cannot demonstrate whether the virus is viable and capable of infecting cells. As part of our exploratory analysis, we used a minus strand SARS-CoV-2 RT-PCR test which correlates with culturable live virus to determine whether any of the treatment arms had an effect on viral replication (i.e., as a proxy for live virus). However, both the saline and PVP-I groups had minimal changes in Ct at the 1-hour time point. This indicates that neither saline nor PVP-I was effective in reducing viral replication after a single administration of therapy. The inclusion of the minus strand test lends a unique dimension to our study as it more directly assesses for changes in viral replication when compared to traditional PCR assessments of viral load. Further research will be needed to demonstrate whether these prophylactic measures indeed provide any benefit in reducing live virus and the risk of transmission.

Overall, sprays were well tolerated with excellent compliance in each group. The 2.0% PVP-I group had significantly higher reports of nasal burning and sneezing as compared to the other groups. One theoretical risk of PVP-I is chemosensory dysfunction due to in vitro cytotoxicity noted with concentrations above 2.5%.[10] Reassuringly, we did not observe any worsening of olfaction in any individual patient in any treatment arm as assessed by the UPSIT smell test. There was a trend toward greater recovery of smell function in the PVP-I groups with a higher percentage of patients improving by at least one olfaction category, and overall higher mean improvement in UPSIT scores; however, these differences did not meet the threshold for statistical significance.

Study Limitations

There was no negative control group (i.e., received no spray treatment), which limits the ability to determine

how much of the observed declines in Ct over time was due to natural decreases in viral load over 3 days' time versus decline in intranasal virus induced by the administered treatments. Participants used nasal sprays as these were felt to be easier to provide in a blinded fashion and to ensure consistent use; however, the sprays may not have reached as many surfaces as sinus irrigations. A saline nasal spray group was selected instead of a negative control group given concerns that the inconvenience of study participation would have prevented adequate enrollment if participants did not have the benefit of potentially receiving a treatment for SARS-CoV-2.

Although patients presented, on average, within 3 days following a positive test, the time from self-reported symptom onset was an average of approximately 7 days in the PVP-I groups, which may have been outside the window of peak viral load.[21] We are unable to determine whether earlier use of PVP-I might have been more efficacious. Additionally, SARS-CoV-2 RT-PCR is a proxy for and not a direct measure of the amount of viable virus present.

CONCLUSION

There is a significant need for safe and effective treatments against SARS-CoV-2. In this randomized, triple-blinded, placebo-controlled clinical trial, we compared topical nasal saline to low and high concentration PVP-I as potential therapies in COVID-19 patients. We found that saline and low dose (0.5%) PVP-I nasal sprays are overall well-tolerated, though more nasal burning symptoms were reported in the 2.0% PVP-I group. All three nasal sprays yielded similar reductions in SARS-CoV-2 NP viral load over 3 days. Olfaction improved in all groups over 30 days, and notably did not decline for any participant. Taken together, these data suggest that dilute PVP-I sprays may be safe for topical exposure on nasal mucosal tissues, but do not appear to confer increased virucidal activity in COVID-19 positive patients. Future work is needed to fully quantify the benefit of nasal sprays for the treatment of COVID-19.

Acknowledgments

The authors wish to thank the Stanford Clinical Virology Laboratory team for their assistance with testing of samples, the A&O Specialty Pharmacy for nasal spray preparation and packaging for this clinical trial, and finally all study subjects for their willingness to undergo serial NP swabs and self-administer nasal sprays for this clinical trial during the COVID-19 pandemic in the US.

BIBLIOGRAPHY

1. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med* 2020;383:2603–2615. https://doi.org/10.1056/NEJMoa2034577.
2. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med* 2021;384:403–416. https://doi.org/10.1056/NEJMoa2035389.
3. Sungnak W, Huang N, Bécavin C, et al. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. *Nat Med* 2020;26:681–687. https://doi.org/10.1038/s41591-020-0868-6.
4. Lee IT, Nakayama T, Wu C-T, et al. ACE2 localizes to the respiratory cilia and is not increased by ACE inhibitors or ARBs. *Nat Commun* 2020;11:5453. https://doi.org/10.1038/s41467-020-19145-6.
5. Fajnzylber J, Regan J, Coxen K, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nat Commun* 2020;11:5493. https://doi.org/10.1038/s41467-020-19057-5.
6. Zacharioudakis IM, Prasad PJ, Zervou FN, et al. Association of SARS-CoV-2 genomic load with COVID-19 patient outcomes. *Ann Am Thorac Soc* 2020;18:900–903. https://doi.org/10.1513/AnnalsATS.202008-931RL.
7. Anderson MJ, David ML, Scholz M, et al. Efficacy of skin and nasal povidone-iodine preparation against mupirocin-resistant methicillin-resistant *Staphylococcus aureus* and *S. aureus* within the anterior nares. *Antimicrob Agents Chemother* 2015;59:2765–2773. https://doi.org/10.1128/AAC.04624-14.
8. Mangram AJ, Horan TC, Pearson ML, Silver LC, Jarvis WR. Guideline for prevention of surgical site infection, 1999. *Infect Control Hosp Epidemiol* 1999;20:247–280. https://doi.org/10.1086/501620.
9. Anderson DE, Sivalingam V, Kang AEZ, et al. Povidone-iodine demonstrates rapid in vitro virucidal activity against SARS-CoV-2, the virus causing COVID-19 disease. *Infect Dis Ther* 2020;9:669–675. https://doi.org/10.1007/s40121-020-00316-3.
10. Frank S, Brown SM, Capriotti JA, Westover JB, Pelletier JS, Tessema B. In vitro efficacy of a povidone-iodine nasal antiseptic for rapid inactivation of SARS-CoV-2. *JAMA Otolaryngol Head Neck Surg* 2020;146:1054–1058. https://doi.org/10.1001/jamaoto.2020.3053.
11. Gluck U, Martin U, Bosse B, Reimer K, Mueller S. A clinical study on the tolerability of a liposomal povidone-iodine nasal spray: implications for further development. *ORL J Otorhinolaryngol Relat Spec* 2007;69:92–99. https://doi.org/10.1159/000097758.
12. Kirk-Bayley J, Sunkaraneni S, Challacombe S. The use of povidone iodine nasal spray and mouthwash during the current COVID-19 pandemic may reduce cross infection and protect healthcare workers. *SSRN* 2020. https://ssrn.com/abstract=3563092.
13. Yan CH, Faraji F, Prajapati DP, Boone CE, DeConde AS. Association of chemosensory dysfunction and Covid-19 in patients presenting with influenza-like symptoms. *Int Forum Allergy Rhinol* 2020;10:806–813. https://doi.org/10.1002/alr.22579.
14. Guenezan J, Garcia M, Strasters D, et al. Povidone iodine mouthwash, gargle, and nasal spray to reduce nasopharyngeal viral load in patients with COVID-19. *JAMA Otolaryngol Neck Surg* 2021;147:400–401. https://doi.org/10.1001/jamaoto.2020.5490.
15. Urbaniak G, Plous S. Research Randomizer. 2013. Available at: http://www.randomizer.org/
16. Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. *Physiol Behav* 1984;32:489–502. https://doi.org/10.1016/0031-9384(84)90269-5.
17. Hogan CA, Sahoo MK, Huang C, et al. Comparison of the Panther Fusion and a laboratory-developed test targeting the envelope gene for detection of SARS-CoV-2. *J Clin Virol* 2020;127:104383. https://doi.org/10.1016/j.jcv.2020.104383.
18. Corman VM, Landt O, Kaiser M, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveillance* 2020;25:2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045.
19. Hogan CA, Huang C, Sahoo MK, et al. Strand-specific reverse transcription PCR for detection of replicating SARS-CoV-2. *Emerg Infect Dis* 2021;27:632–635. https://doi.org/10.3201/eid2702.204168.
20. AAO-HNS Forum. More on betadine from JAMA. 2020. Available at: https://entconnect.entnet.org
21. Cevik M, Tate M, Lloyd O, Maraolo AE, Schafers J, Ho A. SARS-CoV-2, SARS-CoV, and MERS-CoV viral load dynamics, duration of viral shedding, and infectiousness: a systematic review and meta-analysis. *Lancet Microbe* 2021;2:e13–e22. https://doi.org/10.1016/S2666-5247(20)30172-5.