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ORIGINAL ARTICLE

Pharmacokinetic-based failure of a detergent virucidal for severe acute respiratory syndrome–coronavirus-2 (SARS-CoV-2) nasal infections: A preclinical study and randomized controlled trial

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Abstract

Background: The nose is the portal for severe acute respiratory syndrome–coronavirus-2 (SARS-CoV-2) infection, suggesting the nose as a target for topical antiviral therapies. The purpose of this study was to assess both the in vivo and in vitro efficacy of a detergent-based virucidal agent, Johnson and Johnson's Baby Shampoo (J&J), in SARS-CoV-2–infected subjects.

Methods: Subjects were randomized into three treatment groups: (1) twice daily nasal irrigation with J&J in hypertonic saline, (2) hypertonic saline alone, and (3) no intervention. Complementary in vitro experiments were performed in

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cultured human nasal epithelia. The primary outcome measure in the clinical trial was change in SARS-CoV-2 viral load over 21 days. Secondary outcomes included symptom scores and change in daily temperature. Outcome measures for in vitro studies included change in viral titers.

Results: Seventy-two subjects completed the clinical study ($n = 24$ per group). Despite demonstrated safety and robust efficacy in in vitro virucidal assays, J&J irrigations had no impact on viral titers or symptom scores in treated subjects relative to controls. Similar findings were observed administering J&J to infected cultured human airway epithelia using protocols mimicking the clinical trial regimen. Additional studies of cultured human nasal epithelia demonstrated that lack of efficacy reflected pharmacokinetic failure, with the most virucidal J&J detergent components rapidly absorbed from nasal surfaces.

Conclusion: In this randomized clinical trial of subjects with SARS-CoV-2 infection, a topical detergent-based virucidal agent had no effect on viral load or symptom scores. Complementary in vitro studies confirmed a lack of efficacy, reflective of pharmacokinetic failure and rapid absorption from nasal surfaces.

KEYWORDS
epithelial cell, irrigations, surfactants, topical therapy for chronic rhinosinusitis, SARS-CoV-2, saline, virus

1 | INTRODUCTION

The nose is the primary portal for infection by severe acute respiratory syndrome–coronavirus-2 (SARS-CoV-2).[1,2] A fraction of inhaled SARS-CoV-2 impacts on nasal surfaces, which provide an angiotensin-converting enzyme 2 (ACE2) receptor-rich environment that promotes robust viral proliferation.[1] From nasal surfaces, SARS-CoV-2 can spread locally to the olfactory region and more widely to the oral cavity and lung.[1,3] Accordingly, nasal protection represents an important strategy to limit SARS-CoV-2 infection and transmission.[4] In addition to physical means, for example, masks, topical administration of a spectrum of virucidal agents to nasal surfaces has been advocated for this purpose, including iodine, metal, and detergent-based molecular entities.[5–7]

Otolaryngologists utilize high-volume normal or hypertonic saline (HTS) rinses to cleanse the nasal cavity. A common agent added to saline rinses to provide gentle detergent activity is Johnson and Johnson's (J&J) Baby Shampoo.[8] The J&J Baby Shampoo/saline (J&J/S) combination, typically ½–1 tsp/240 ml saline (8 oz), is safe and effective for treatment of chronic bacterial infections of the nasal cavity.[9] Because detergents are virucidal for enveloped viruses, including SARS-CoV-2, we tested J&J/S as a topical virucidal for the treatment of SARS-CoV-2 nasal cavity infections.[5,10,11] Our strategy was to confirm the safety and virucidal activity of clinically utilized dilutions of J&J/S[9] through in vitro studies of cultured human nasal epithelia (HNE). We then pivoted quickly to a clinical trial of the safety and efficacy of J&J/S high-volume rinses in subjects with nasal SARS-CoV-2 cavity infections given urgent need to assess potential topical therapies for coronavirus disease 2019 (COVID-19). The clinical trial was performed in parallel with further in vitro studies to better understand the pharmacokinetic (PK) and virucidal pharmacodynamic (PD) properties of J&J/S in SARS-CoV-2–infected nasal epithelia.

2 | MATERIALS AND METHODS

2.1 | In vitro safety, pharmacodynamic, and pharmacokinetic studies

2.1.1 | Safety studies in airway epithelia

Apical surfaces of well-differentiated air–liquid interface HNE cultures were exposed to 5 μl of various dilutions of J&J/S for 5 min, washed with 600 μl of cell culture media,[1] and transepithelial resistance (R_t) measured utilizing an EVOM (World Precision Instruments). Parallel cultures after J&J/S exposure were fixed in 4% paraformaldehyde (PFA) and processed for whole-mount histologic analyses.[1]

2.1.2 | Virucidal assays in vitro and cultured human nasal cells

To assess in vitro antiviral activity against SARS-CoV-2 D614G, virus stocks at 1×10^7 plaque-forming units (PFU)/ml were incubated with J&J or other similar detergents of variable chain lengths as described in Results section, for 90 min at 37°C, then serially diluted in PBS for plaque assay on Vero cells.[1] Virucidal activity of J&J Baby Shampoo was also assessed in recombinant NL63-coronavirus (NL63-CoV)[12] and respiratory syncytial virus (RSV)[13] as described in the Supplementary Material.

For experiments on airway epithelia, well differentiated HNE cultures were inoculated with 200 μl of SARS-CoV-2 D614G at a multiplicity of infection (MOI) of 0.1, incubated at 37°C for 90 min, following which the inoculum was removed and cultures lavaged 2× with 500 μl phosphate buffered saline (PBS) to remove residual virus.[14] Samples for viral quantitative polymerase chain reaction (qPCR) quantitation, utilizing the SARS-CoV-2 nucleoprotein (N1) primers employed in the clinical study, and viral titers were obtained via lavage with 200 μl PBS for 10 min at 37°C at 48, 76, and 96 h postinoculation hours post infection (h pi).[15] At 72 h pi, J&J/S or PBS lavages were administered to HNE culture surfaces, fluids aspirated 10 min later, and titering performed. The in vitro J&J/S lavage volume-to-HNE surface area ratio (200 μl/cm^2) was chosen to approximate the ratio in vivo, that is, 240 ml lavage spread over 150 cm^2 nasal surface area,[16] and the 10 min in vitro J&J/S lavage residence time mimicked the in vivo contact time of lavages with nasal surfaces.[8,17] Apical lavages were stored at −80°C until analyzed by qPCR to measure viral load and plaque assay to determine viral titer.

Individual surfactant components of cocamidopropyl betaine (CAPB) including octanoylamide propylbetaine (C8 chain length), lauroylamide propylbetaine (C12 chain length), and palmitoylamide propylbetaine (C18 chain length) were obtained from Toronto Research Chemicals (Toronto, CA). SARS-CoV-2 D614G virus stock at 10^7 PFU/ml was incubated with each surfactant individually at 1, 0.1, or 0.01 mg/ml for 90 min at 37°C. Titers of active virus posttreatment were determined by the plaque titer assay as described above.

2.1.3 | qPCR quantitation of SARS-CoV-2 viral load from HNE cultures

Apical culture lavages were inactivated with urea, and RNA was isolated using Direct-zol RNA Kits (#R2073; Zymo Research). Briefly, 100 μl apical lavage was mixed with 300 μl TRIzol (TRI) reagent followed by 400 μl 99% ethanol, transferred to the spin column, centrifuged with wash buffer, then eluted with 50 μl RNase-free water. Total RNA was reversed transcribed into cDNA with iScript Reverse Transcription Supermix for RT-qPCR (#1708841; Bio-Rad, CA, USA) and virus copy number was quantitated using the SARS-CoV-2 (2019-nCoV) Centers for Disease Control (CDC) qPCR Probe Assay kit (#10006770; Integrated DNA Technologies).

2.1.4 | Liquid chromatography–tandem mass spectrometry

Five microliters (5 μl) of a 1:100 dilution of J&J Baby Shampoo was analyzed using chromatographic and mass spectrometric conditions (liquid chromatography–tandem mass spectrometry [LC-MS/MS]) as described.[18] Full scans were run in positive mode with electrospray interface (ESI).

2.1.5 | Statistical analyses of in vitro studies

For transepithelial airway culture resistance, the Dunnett's test (control = PBS) was utilized to assess differences in R_t as a function of J&J/S concentrations at serial time points. To analyze the effects of J&J/S versus PBS on magnitudes of SARS-CoV-2 infection of HNE, the changes from 48 h at each successive time point were analyzed with a repeated measure model. Interaction of time and treatment (J&J/S vs. PBS) was explored and 48 h values were included as covariates. Post hoc comparisons of treatments at each time point were adjusted with the Holm method. Values of $p < 0.05$ were considered significant. Changes in viral titers with J&J or CAPB lipids were assessed using one-sample t tests against expected titers, with Bonferroni corrections to account for multiple testing.

2.2 | Clinical trial of J&J baby shampoo

2.2.1 | Study population and enrollment

The clinical study was approved by the Vanderbilt University Medical Center Institutional Review Board and Biosafety Committee and registered on clinicaltrials.gov (NCT 04347538). A CONSORT diagram for the study is shown in Figure 1 and demographics of study population are shown in Table 1. Inclusion criteria included a positive qualitative quantitative real-time polymerase chain reaction (qRT-PCR) test for the SARS-CoV-2 virus at Vanderbilt University Medical Center (VUMC) or affiliated

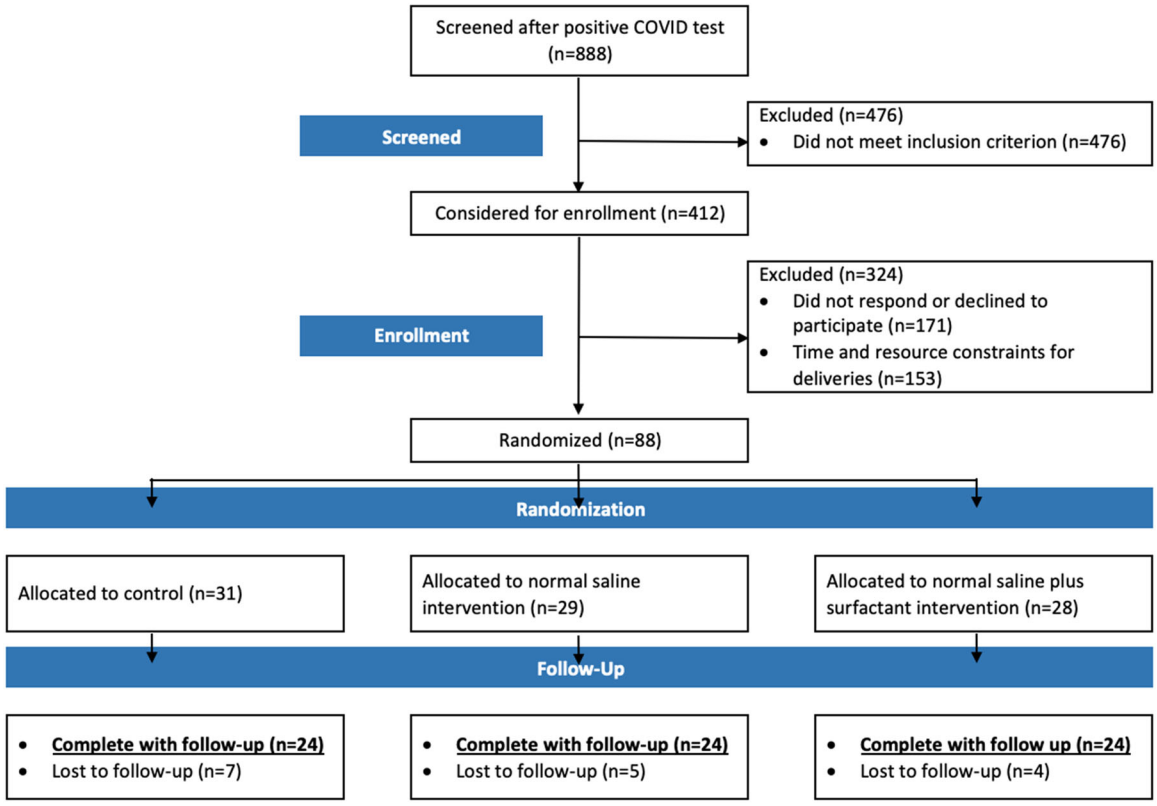

FIGURE 1 Consort diagram describing the screened and enrolled subjects for the randomized study of J&J/S, HTS, and no-intervention control groups. Diagram is based on the CONSORT transparent reporting of trials (http://www.consort-statement.org/). Abbreviations: HTS, hypertonic saline; J&J/S, Johnson & Johnson/saline solution.

TABLE 1 Clinical and demographic characteristics of study participants

Characteristic	No intervention ($n = 24$)	Hypertonic saline ($n = 24$)	Saline + surfactant ($n = 24$)	Effect size (95% CI)
Age (years), mean ± SD	39 ± 15	39 ± 15	44 ± 18	0.011 (0.001–0.151)
Sex (male), *n* (%)	14 (58)	12 (50)	9 (38)	0.171 (0.053–0.417)
BMI, mean ± SD	29.6 ± 7.2	28.5 ± 6.3	28.9 ± 5.9	0.003 (0.001–0.105)
Symptomatic days before diagnosis, median (IQR)	2.0 (1.0–3.0)	2.5 (1.0–4.8)	2.0 (1.0–3.5)	0.02 (0.002–0.177)
Smoking, *n* (%)	1 (4)	2 (8)	2 (8)	0.077 (0.03–0.325)
Comorbidities, *n* (%)				
Diabetes	3 (12)	0 (0)	3 (12)	0.213 (0.143–0.394)
Heart disease	2 (8)	0 (0)	1 (4)	0.17 (0.113–0.374)
Hypertension	4 (17)	4 (17)	6 (25)	0.099 (0.031–0.357)
Chronic lung disease	4 (17)	3 (12)	2 (8)	0.103 (0.037–0.353)

Note: Categorical data presented as *n* (%). Continuous data presented as median (IQR) or means ± SD. Following a Kruskal-Wallis test, the effect size was measured by epsilon-squared value and its 95% CI was estimated by bootstrap with 1000 replications. Following a Pearson χ^2 test, the effect size was measured by Cramér's *V* values, its 95% CI was estimated by bootstrap with 1000 replications. The rage of epsilon-squared and Cramér's *V* are both from 0 to 1, with a 1 indicting a perfect association. These values were performed using R package "rcompanion."

Abbreviations: BMI, body mass index; CI, confidence interval; IQR, interquartile range; SD, standard deviation.

testing centers, age of ≥18 years, planned self-quarantine after infection, and residence within a 30-mile radius of VUMC. Exclusion criteria included inpatient admission, current use of nasal saline irrigations or other intranasal medications, and inability to perform saline irrigations/nasal swabs in separate bathroom away from household contacts. Any patient requiring hospitalization during the course of the study terminated data collection concomitant with hospitalization. Enrollment was performed via telephone on a rolling basis between April 28 and July 31, 2020, predicated on a positive qRT-PCR test in the preceding 24 h. Patients were randomized to one of three treatment groups: (1) no intervention, (2) hypertonic nasal saline irrigations BID, and (3) hypertonic nasal saline irrigations with 0.5 teaspoon (2.5 ml) surfactant (Johnson's Baby Shampoo; Johnson & Johnson Inc., New Brunswick, NJ) twice daily (BID). Hypertonic saline solution consisted of 240 ml of distilled water with two packets of NeilMed brand buffered salt (NeilMed Pharmaceuticals, Santa Rosa, CA). Nasal lavage was performed in each nostril using NeilMed brand Sinus Rinse bottles. Randomization, enrollment, and registration took place via Research Electronic Data Capture (REDCap; Vanderbilt University, Nashville, TN).

2.2.2 | Study protocol

Patients were trained in self-performance of mid-turbinate binaral swab collection using written directions and a standardized web application. A total of seven sterile individually wrapped nasal swabs (FLOQSwabs; Copan Diagnostics, Murrieta, CA) were delivered with single-use collection and preservation vials (OMR 110; DNA Genotek Inc.; Ottawa, ON, Canada). All materials were provided to participants within 24 h of enrollment. Patients performed swab collection on days 1, 3, 5, 7, 9, 14, and 21. Swabs were performed at least 4 h after saline rinses. Patients also recorded their daily temperature using their own thermometer (same time each day) and completed a symptom questionnaire based on the validated Wisconsin Upper Respiratory Symptom Survey 21 (WURSS-21)[19] (Supplementary Figure 1). The survey was modified to capture symptoms prevalent during SARS-CoV-2 infection that may be less common during other respiratory virus infections. Added symptoms included eye redness/pain, headache, sputum production, coughing blood, shortness of breath, nausea/vomiting, muscle/joint pain, chills, and alteration of smell/taste. Each survey question is scored using a modified Likert scale with 0 indicating a lack of symptoms and 7 indicating severe symptoms. Compliance with irrigations and comfort with self-administered mid-turbinate swabs was verified by telephone 48–72 h after study enrollment. At the completion of the study, nasal swabs and symptom questionnaires were collected from participants at their residences. SARS-CoV-2 quantitative qRT-PCR testing was performed as described.[15] Both the SARS-CoV-2 nucleocapsid gene region 1 (N1) and nucleocapsid gene region 2 (N2) were amplified for detection. RNA quality and quantity were examined using ribonuclease P (RNase P).

2.2.3 | Studies of nasal swab viral load

SARS-CoV-2 viral load was measured in mid-turbinate nasal swabs collected 4 h after lavage on days 1, 3, 5, 7, 10, 14, and 21 post–study initiation using validated primers widely used for diagnostic testing (N1, N2, RP).[15] No significant differences in threshold cycle (Ct) values were identified at any time point for either the N1 or N2 primer.

2.2.4 | Power calculations and statistical analyses of the clinical trial

Power analyses utilized data from studies of nasal SARS-CoV-2 loads over time in untreated individuals[20,21] and studies of nasal viral load of non–SARS-CoV-2 coronaviruses with saline irrigation treatment[22] to estimate mean treatment effects of 1.51 and 2.23 for control[2] and treatment, respectively (units for viral load measured in log Ct PCR) with a standard deviation of 0.97. Using an alpha of 0.025 (Bonferroni correction for testing intervention 1 vs. control and intervention 2 vs. control, overall alpha = 0.05), a minimum sample size of 36 subjects was found to be necessary to generate a power of 0.80.

Participants' demographic, baseline characteristics, and outcomes measures were summarized with median and interquartile range for continuous variables, or frequency and percentage for categorical variables. Differences among the three groups were assessed using the Kruskal-Wallis test for continuous variables and Pearson chi-square test for categorical variables. The primary outcome was qRT-PCR-measured nasal SARS-CoV-2 viral load. The secondary outcomes included patient-reported symptom score, daily temperature, and viral shedding, with anosmia included as a safety signal. For calculating the viral shedding value, Ct values were capped at 40 and were converted to $\log_{10}$ values (change in values from Ct at day 1 to the maximum value of Ct/days between two values). A Ct value >50 was considered undetectable and these values were censored at 50. To account for the large number of censored values at later time points a multivariable longitudinal regression model with parametric survival was used for the primary outcome.[23] Generalized estimating

equations (GEEs) were used to account for the correlation among records collected from the same participant. Potential confounders included day, RNAse P, age, gender, body mass index (BMI), and symptom score at day 1, and were adjusted in the model. To allow nonlinear associations with the outcome and day, the restricted cubic splines with three knots was used for day. Missing values for regression model covariates were imputed using multiple imputation using chained equations (MICE). Two-sided p values ≤ 0.05 were considered statistically significant. Statistical analyses were performed using R version 4.0 in addition to rms, Hmisc, and survival packages (R Foundation for Statistical Computing; https://www.r-project.org/).

We performed a planned interim analysis after the first 45 patients completed the study protocol. The trial interventions were deemed of minimal risk by the Vanderbilt Institutional Review Board and as such did not necessitate external data safety monitoring. We, therefore, did not establish a prespecified stopping rule based on adverse events, and instead the study investigators reviewed enrollment data every 2 weeks to monitor for any adverse events and dropouts. The interim analysis permitted validation of adequate genetic material in the self-swab samples and also allowed for an analysis for futility based on the primary (change in viral load from day 1 to day 21) and secondary (time to symptom resolution) outcomes via calculation of probability of superiority and conditional power. Following interim analysis, further enrollment was discontinued based on these criteria and final data analysis was performed after all currently enrolled patients had completed the study.

3 | RESULTS

3.1 | In vitro safety and virucidal activity studies

The first component of this study investigated the in vitro safety and virucidal activity of clinically utilized dilutions of J&J/S.[9] Exposure of HNE cultures to serial dilutions of J&J/S induced transient decreases in barrier function (transepithelial resistance) at concentrations above ½ tsp J&J/240 ml saline, though no changes in cell composition (Figure 2A,B). Having identified a nontoxic concentration of J&J/S (½ tsp J&J/240 ml saline), we then showed that this concentration of J&J/S exhibited robust virucidal activity against SARS-CoV-2 at varying titers (Figure 2C). Further assays of virucidal activity demonstrated that this concentration was also virucidal in vitro against NL63 coronavirus and respiratory syncytial virus (Supplementary Figure 2A,B).

3.2 | Clinical trial

Having demonstrated in vitro virucidal activity of a concentration of J&J/S that was nontoxic in cultured HNE, an outpatient clinical trial of J&J/HTS nasal irrigation was performed in subjects with nasal swab qPCR-documented SARS-CoV-2 infection. Seventy-two subjects were randomized into three groups ($n = 24$/group): (1) twice daily nasal irrigation with ½ tsp J&J Baby Shampoo in 240 ml HTS; (2) twice daily irrigation with HTS alone; or (3) no intervention (see Table 1, Figure 1). The primary study endpoint was qPCR-measured SARS-CoV-2 nasal viral load measured 4 h after irrigation over the three-week study interval. Other endpoints included patient-reported symptom scores assessed using a modified WURSS-21 and daily temperatures.

Despite the *promising* virucidal activity measured in vitro, no reductions in the primary outcome measure, qPCR-measured SARS-CoV-2 nasal viral load, were observed in the J&J/HTS intervention group relative to HTS alone group or the control group at any time point (Figure 3A). While controlling for baseline symptom score and other covariates, there was conversely a marginally greater impact on viral load in the control group compared to the J&J/HTS group at day 1 (adjusted effect difference, −5.277; 95% CI, −9.474 to −1.080) and day 3 (adjusted effect difference, −4.727; 95% CI, −9.029 to 0.426). Similarly, no differences among groups were observed on a continuous measure of viral shedding (Control, 0.118, IQR −0.083 to 0.330; HTS 0.071, IQR −0.047 to 0.253; J&J/HTS 0.116, IQR −0.031 to 0.333, effect size 0.004 (95% CI, 0.0009–0.117) (Figure 3B) or other secondary outcome measures including nasal symptoms (Figure 3C). No safety signals were observed, for example, no changes in WURSS-21 smell indices were observed in the irrigation groups to indicate irrigation-mediated spread of SARS-CoV-2 to olfactory epithelia (Figure 3D), or daily temperature changes (not shown).

3.3 | In vitro PK/PD studies

To gain insights into the disparity between the virucidal activity of J&J/S in vitro (Figure 2C) and the absence of clinical effectiveness (Figure 3A,B), further PK/PD studies of J&J/S in HNE cultures were performed. For these studies, HNE cultures were inoculated with SARS-CoV-2, and samples for viral titers and viral qPCR quantitation were obtained via PBS lavage of HNE culture surfaces 48 h pi to verify and quantify active infection. Either J&J/S or PBS was lavaged onto HNE culture surfaces at 72 h pi, with fluids aspirated 10 min later to mimic typical

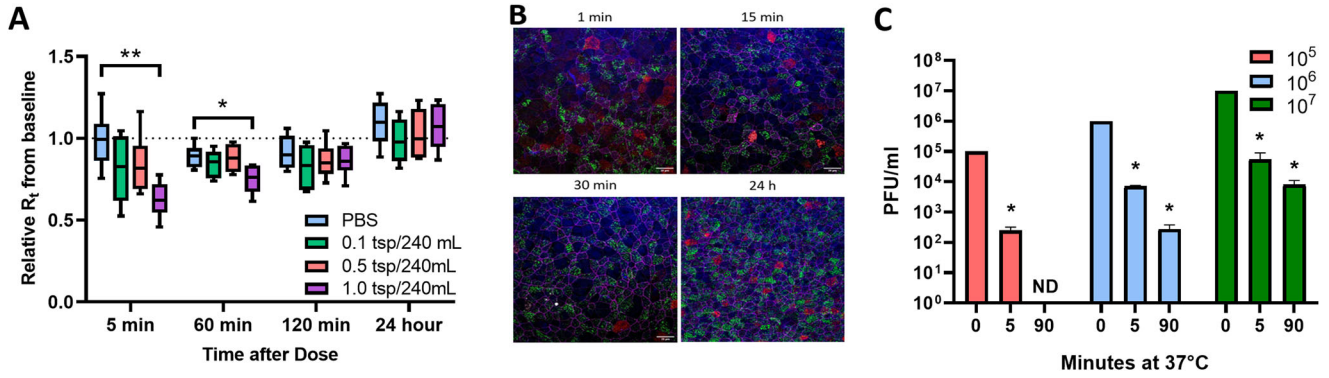

FIGURE 2 In vitro safety and efficacy data. (A) R_t relative to baseline after administration of J&J Shampoo/normal saline at three concentrations (0.1, 0.5, or 1 tsp in 240 ml of normal saline) or PBS to cultured human nasal epithelial (HNE) cells. *p <0.05 different than baseline, **p <0.01. (B) Whole-mount immunofluorescent staining images of HBE cultures. Representative fluorescent images of HBE cells after the administration of J&J Baby Shampoo at a concentration of ½ tsp/240 ml of PBS for 1, 15, or 30 min, or 24 h shown. The HBE cells were fixed and stained with α-tubulin (cilia; green), CCSP (club cell; red), phalloidin (f-actin; magenta), MUC5B protein (secretory cell; white), and DAPI (nuclei; blue). Scale bar = 20 μm. (C) Virucidal activity of J&J Shampoo (½ tsp J&J/240 ml saline) at a 1:1 dilution with SARS-CoV-2 viral stocks assayed varying viral titers. Initial SARS-CoV-2 stock titers ranged from 10^5 to 10^7 PFU/ml. *p <0.05 of measured viral titers versus starting viral titer. Abbreviations: CCSP, Clara cell secretory protein; DAPI, 4′,6-diamidino-2-phenylindole; HNE, human bronchial epithelial; ND, zero titer detected; PBS, phosphate-buffered saline; PFU, plaque-forming units; R_t, transepithelial resistance; SARS-CoV-2, severe acute respiratory syndrome–coronavirus-2.

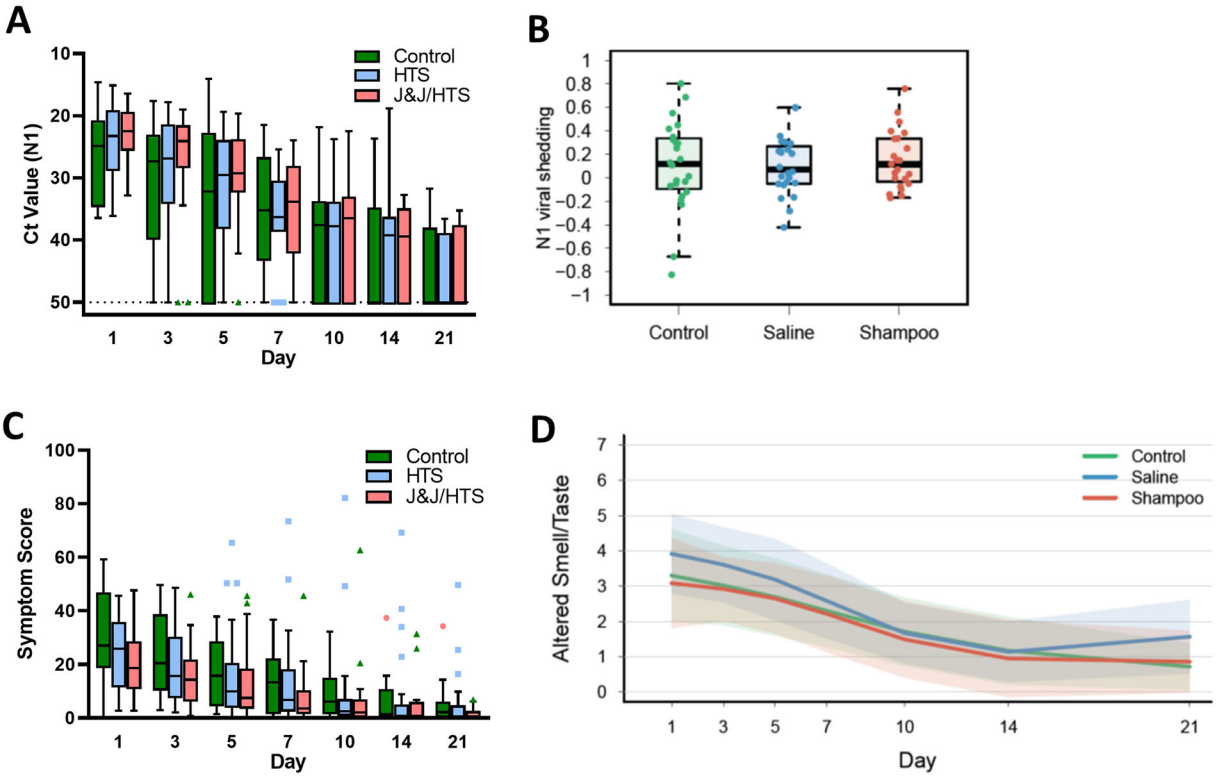

FIGURE 3 Clinical trial data. (A) Cross point (Ct) PCR-based measure of N1 primer-based viral load in the nasal cavity of SARS-CoV-2–infected subjects as a function of treatment group. n = 72; 24/group. Note, lower absolute Ct value reflects greater viral load. (B) Time-dependent change in viral shedding for each of the three treatment groups, defined as $\log_{10}$ change in Ct value between day 1 and maximum (T value) day between the two values. n = 24/treatment group. (C) Nasal WURSS-21 symptom score of SARS-CoV-2–infected subjects as a function of treatment group over the study interval. (D) WURSS-21 data describing altered smell/taste in the SARS-CoV-2–infected subjects for each of the three treatment groups over the study interval. n = 24/group. Abbreviations: Ct, threshold cycle; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome–coronavirus-2; WURSS-21, Wisconsin Upper Respiratory Symptom-21 survey.

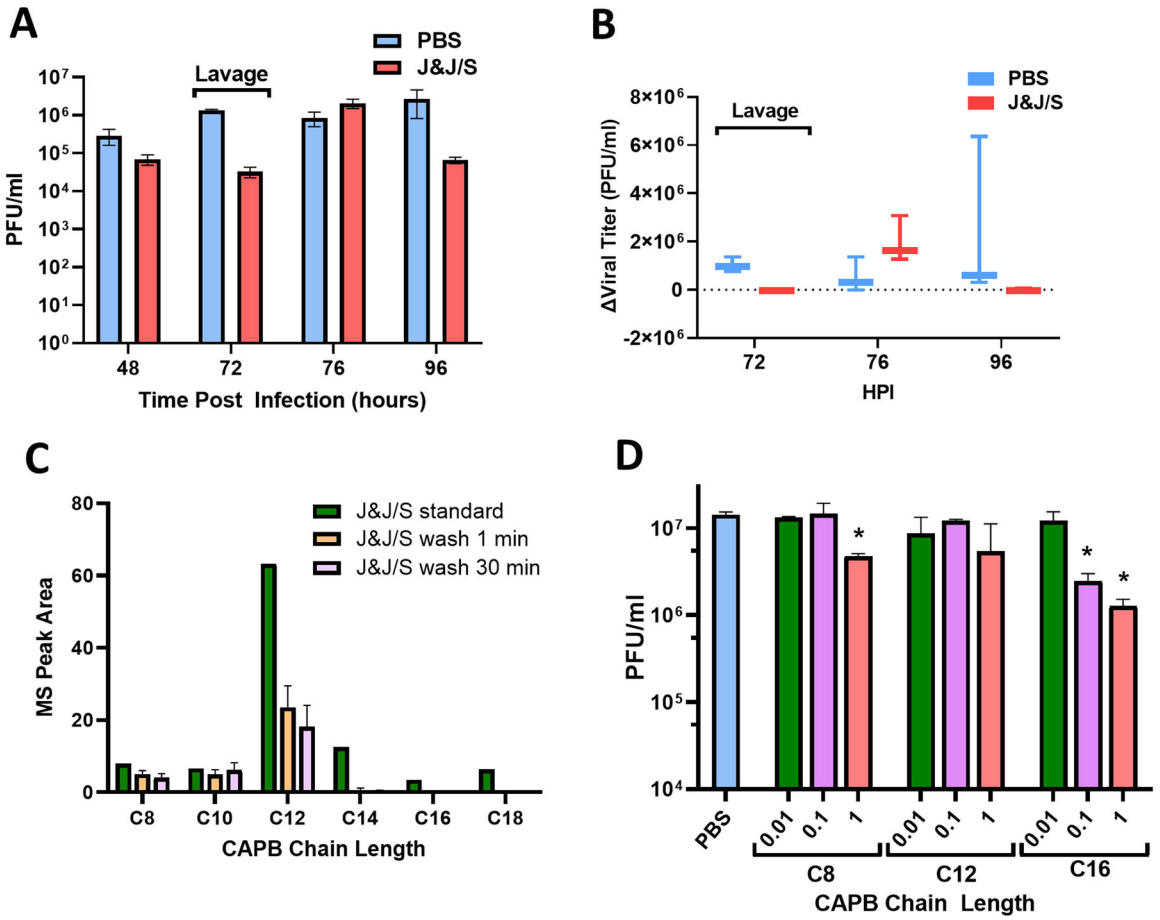

FIGURE 4 Pharmacokinetic data. (A) D614G SARS-CoV-2 viral titer data at the times post inoculum designated. Statistical analyses of changes in J&J/S and PBS 72 lavage groups compared to 48 h pi values shown in (E). (B) Change in HNE cell viral titers from the 48 h pi value at 72, 76, and 96 h pi with DG14G SARS-CoV-2 virus at an MOI = 0.1. Note, at 72 h the lavage solution was either J&J/S (½ tsp/240 ml saline) or PBS. (C) Concentrations of various chain lengths of CAPB, as determined by mass spectrometry, in J&J/S solution and after application of J&J Shampoo (200 μl, ½ tsp/240 ml normal saline concentration) to cultured HNE and harvested 1 min and 30 min later for analysis. (D) Relative virucidal activities of three detergents contained with J&J Baby Shampoo that vary by chain length. *p <0.05 versus starting viral titer (PBS). Abbreviations: CAPB, cocamidopropyl betaine; HNE, human nasal epithelial; J&J/S, J&J Baby Shampoo/saline combination; MOI, multiplicity of infection; PBS, phosphate-buffered saline; pi, postinoculation; SARS-CoV-2, severe acute respiratory syndrome–coronavirus-2.

retention time on nasal epithelia.[8,17,24] Viral titering and qPCR assays were performed on these lavages, as well as on additional PBS lavages obtained 4 h later at 76 h pi, and the next day at 96 h pi.

As expected from prior studies,[1] PBS lavage samples revealed productive SARS-CoV-2 infection of HNE at 48 h pi (Figure 4A). However, treatment with J&J/S resulted in little change in viral titer, with the titers measured in the J&J/S lavage at 72 h pi not significantly different from those measured at 48 h (Figure 4A,B). Similarly, no differences in viral titers from 48 h values were observed in the J&J/S or PBS lavaged HNE cultures over the 76–96 h pi interval (Figure 4A,B). Similar findings were observed using qPCR as a measure of viral load (Supplementary Figure 3A,B).

To better understand why J&J/S did not have an antiviral effect on infected HNE even immediately after application, we studied the retention of topically applied J&J/S on nasal epithelial surfaces. J&J Baby Shampoo is a proprietary mix of agents, and mass spectrometric analysis of J&J/S indicated that dominant detergent is cocamidopropyl betaine (CAPB), which is the second listed ingredient after water. CAPB is a mixture of detergents of varying chain lengths ranging from 8 to 18 carbons (C8–C18), which could be assessed independently by mass spectrometry (Supplementary Figure 3C). J&J/S solution included a mixture of chain lengths, primarily C12, but PBS lavages obtained 1 min or 30 min after administration of J&J/S to HNE surfaces revealed that the longer chain CAPB components were absent (Figure 4C), suggesting rapid transnasal absorption of the longer chain components of CAPB on nasal epithelia. Longer chain detergents have been shown to have greater antiviral activity in other systems,[11,25] and

to test this for SARS-CoV-2 we obtained individual components of CAPB representing short (C8), medium (C12), and long (C16) chain lengths. Consistent with prior observations, the C16 component of CAPB was a more potent antiviral against SARS-CoV-2 in vitro than C8 or C12 chain lengths (Figure 4D).

4 | DISCUSSION

Our data do not support use of J&J/S as a topical virucidal agent for treatment of active SARS-CoV-2 nasal infections. The failure of J&J/S to treat SARS-CoV-2 did not reflect a defect in the intrinsic virucidal activity of this agent. Rather, the result reflected a PK failure to maintain for prolonged intervals the concentrations of J&J virucidal ingredients on nasal surfaces required to inactivate SARS-CoV-2 virions continuously being shed onto nasal surfaces. SARS-CoV-2–infected nasal surfaces continually produce and release SARS-CoV-2 virus onto nasal surfaces over time (Figure 1E).[1] Accordingly, a virucidal agent must continually remain on nasal surfaces to produce durable and clinically meaningful virucidal activity in the nasal cavity. However, it is difficult to maintain effective concentrations of topical agents on nasal surfaces for two reasons. First, nasal surfaces are protected by a mucociliary transport system that clears nasal surfaces of topically deposited agents within 10 min.[8,17,24] Second, the nasal surfaces exhibit rapid transepithelial absorption of topically applied agents, a property accessed for drug delivery.[26–28]

These data suggest that the failure of J&J/S to reduce SARS-CoV-2 titer in the HNE studies reflected primarily the rapid transnasal absorption of the longer chain, more active virucidal components of J&J/S from the lavage fluid. Longer-chain detergents have greater antiviral activity but also readily absorb onto cell surfaces,[11,25] which we show limits their potential as antiviral agents. Although our studies focused on CAPB as the dominant detergent in J&J, similar findings likely apply to similar detergents in J&J or other products. We also cannot rule out the possibility that mucociliary transport may have also contributed to the lack of efficacy in the clinical trial. We suspect that many alternative viricidal topical agents may be similarly limited in potential efficacy against SARS-CoV-2 and/or other viral respiratory pathogens due either to rapid absorption or continuous viral shedding.

The current study is the first of its kind; however, there are a handful of possible shortcomings that are worthy of discussion. First, this is not a placebo-controlled trial and the subjects were not blinded to the intervention. This was an unavoidable characteristic of the study given the need for subjects to both formulate and deliver the irrigation themselves, and the inability of study personnel to interact directly with the subjects during the study period. Second, olfactory loss was only addressed subjectively, and thus it was difficult to assess both the incidence of COVID-19–related smell loss and any potential impacts of irrigations on smell. Additionally, the inherent and potentially long-term effect of COVID-19 on the sense of smell could potentially act as a confounder when assessing potential side-effects of irrigations on smell function. However, the study did not show any obvious differences between groups with respect to subjective reporting of the sense of smell at any time point.

This study emphasizes the need to assess a priori the PK characteristics of virucidal agents considered for topical use against SARS-CoV-2 nasal cavity infections. Cell culture systems such as the ones utilized in our study can be utilized to rapidly evaluation topical antiviral agents to determine which retain their efficacy when applied to human airway epithelia, particularly when exposure times are limited to mimic the impact of mucociliary clearance. Such studies can be utilized to inform clinical trials for SARS-CoV-2 and other respiratory viral infections.

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CONFLICT OF INTEREST

The authors have no competing interests to report.

AUTHOR CONTRIBUTIONS

Charles R. Esther Jr. wrote the manuscript and performed mass spectrometric analysis. Kyle S. Kimura, Yu Mikami, Takafumi Kato, and Scott H. Randell performed

cell culture experiments. Caitlin E. Edwards performed experiments with SARS-CoV-2, and Raymond J. Pickles performed experiments with NL63 and RSV. Justin H. Turner, Suman R. Das, Michael H. Freeman, Britton A. Strickland, Hunter M. Brown, Bronson C. Wessinger, Veerain C. Gupta, Kate Von Wahlde, Quanhu Sheng, Li Ching Huang, and Daniel R. Bacon performed the clinical trial and analysis with input from Adam J. Kimple. Agathe S. Ceppe, Quanhu Sheng, and Li Ching Huang provided biostatistical support. Ralph S. Baric, Justin H. Turner, and Richard C. Boucher provided overall oversight of the study.

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