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Throat Lozenges and Spray Containing Chlorhexidine and Lidocaine Fixed Combination Show Virucidal Activity against Respiratory Syncytial Virus and SARS-CoV-2

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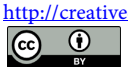

Abstract

The limitations of existing treatments for both Respiratory Syncytial Virus (RSV) and Severe Acute Respiratory Syndrome-related Coronavirus 2 (SARS-CoV-2) lie in their inability to provide universally accessible, easy-to-use, and effective solutions. A commercially available fixed combination of chlorhexidine and lidocaine in both, lozenge and spray form, were assessed for their antiviral efficacy against RSV and SARS-CoV-2 in a suspension test, the viral titres were measured by standard $TCID_{50}$. Both formulations were able to reduce the RSV titre to undetectable levels (99.9% virus inactivation, 3 log_{10} reduction) in less than 1 minute. The lozenge formulation inactivated the viral activity of SARS-CoV-2 in 5 minutes (99% virus inactivation, 2 log_{10} reduction), while the spray formulation led to a reduction of SARS-CoV-2 titre to undetectable levels in less than 1 minute (99.9%, 3 log_{10} reduction). In conclusion, our results show that preparations combining chlorhexidine and lidocaine significantly reduce certain respiratory viruses *in vitro*. In this regard, physiological effects of these preparations become more obvious potentially affecting viral transmission to other individuals and spreading to the lower respiratory tract—thereby shortening the duration and severity of symptoms.

Keywords

Throat Lozenge, Oromucosal Spray, Respiratory Syncytial Virus, SARS-CoV-2

1. Introduction

Human respiratory virus infections result in a range of respiratory symptoms with varying severity. These viruses, originating from diverse virus families, exhibit differences in how easily they spread (transmissibility) and the mechanisms of how they spread (transmission). Modes of transmission include direct (physical) contact, indirect contact (fomite), large droplets and fine aerosols [1]. Respiratory Syncytial Virus (RSV) and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) are highly contagious and spread as droplets and aerosols [2]-[4]. Both viruses are present in saliva [5] [6]; with a very high salivary viral load for SARS-CoV-2 [7] [8].

The existing therapeutic strategies for RSV and SARS-CoV-2 lack generally accessible, easy-to-use, well-tolerated and effective solutions [9]-[11]. Although there are vaccines against both diseases, they are only available for certain age groups, or are aimed at reducing the severity of the disease rather than providing sterilising immunity [10]. Furthermore, the evolving nature of the viruses raises the need for exploration of alternative therapeutic approaches [11], and, as with most respiratory viruses, the main goal is to prevent infection [9].

Our research aims to address these gaps by investigating two commercially available products, Angimed menthol compressed lozenges and Angimed menthol oromucosal spray (see **Table 1** for detailed composition; products are also available under alternative brand names in various countries). These products are part of a line up of sore throat products which all contain the same level of chlorhexidine and lidocaine available in different flavours (lozenges: menthol, lemon, honey and strawberry, spray: menthol and lemon) that cater to a diverse population, offering a more holistic and accessible approach to treatment of respiratory virus infections. The products relieve pain and are currently approved for symptomatic and local treatment of the pharynx in case of sore throat, throat and oral disease accompanied by inflammation, and as adjunctive therapy in case of bacterial infection accompanied by fever. Both products contain a fixed combination of chlorhexidine and lidocaine. While lidocaine provides a local anaesthetic effect, chlorhexidine is a cationic disinfectant biguanide exhibiting broad-spectrum antimicrobial effects by its ability to disrupt microbial cell membranes and interfere with cellular metabolism. While chlorhexidine is largely regarded as an antibacterial, it was previously demonstrated to be effective in reducing viral load in saliva samples [12] [13]. In this context, a fixed combination of chlorhexidine and lidocaine in both, lozenge and spray form, was evaluated for their ability to inactivate RSV and SARS-CoV-2.

Table 1. Test substances.

Product	Composition per lozenge/per ml
Angimed menthol compressed lozenges	Active ingredients: 5 mg chlorhexidine dihydrochloride 1 mg lidocaine hydrochloride monohydrate Excipients: sorbitol (E420), magnesium stearate, citric acid anhydrous, levomenthol

Continued

Angimed menthol oromucosal spray	Active ingredients: 2 mg chlorhexidine digluconate (0.2% m/v) 0.5 mg lidocaine chloride monohydrate (0.05% m/v) Excipients: Citric acid anhydrous, glycerol, sodium saccharin, levomenthol, cineol, ethanol 96%, purified water

2. Materials & Methods

2.1. Viruses

Viral stocks of two enveloped viruses, RSV (strain: Long, source: ATCC VR-26) and SARS-CoV-2 (strain: USA-WA1/2020, source: BEI Resources, NR-52281) were thawed. The virus stock for SARS-CoV-2 was diluted 2-fold by adding 4 mL of MEM + 5% Fetal Bovine Serum to 4 mL of stock virus (containing 5.0% organic load) to maintain 5.0% organic load of virus inoculum. The virus stock for RSV contained 5.0% organic load and was not diluted prior to use.

2.2. Preparation of Test Substances

Two commercially available products were tested: Angimed menthol compressed lozenges and Angimed menthol oromucosal spray, both containing chlorhexidine and lidocaine as active ingredients (see Table 1 for detailed composition; products are also available under alternative brand names in various countries).

For preparation of test substances, either one lozenge was dissolved, or an aliquot of 0.425 mL oromucosal spray was diluted in either 2 or 4.5 mL of artificial saliva (0.42% $NaHCO_3$, 0.05% NaCl, 0.02% K_2CO_3 and 0.3% bovine serum albumin (BSA) in sterile deionised water, pH 6.0 - 7.0), pre-equilibrated to a temperature of 33 ± 1˚C. The prepared test substances were used within 3 hours for efficacy testing.

2.3. Infectivity and Cytotoxicity Assays

Aliquots of 0.3 mL virus stocks were added to either 2.7 mL of the test substances, 5,000 ppm NaClO (sodium hypochlorite, bleach) as positive control, or dilution medium (Minimum Essential Medium [MEM] + 2% Newborn Calf Serum [NCS]) as virus recovery control. Sodium hypochlorite was chosen as positive control for the suspension assay, as it directly denatures virus particles to undetectable levels after 1 minute of exposure and does not require the presence of host cells for inhibition of the viral infection/replication stages, like other virucidal agents. The samples were incubated in triplicates (n = 3) for 1, 5 or 10 minutes (pos. control: 1 min only; virus recovery control: 10 min only) before being stopped with an equal volume of ice-cold neutraliser medium (MEM + 10% NCS). These post-neutralised samples (PNS) were considered undiluted (10^0).

To determine the quantity of infectious virus particles, serial ten-fold dilutions of the PNS were prepared in dilution medium and inoculated onto HeLa cells (source: ATCC CCL-2) for RSV or Vero E6 cells (source: ATCC CRL-1586) for SARS-CoV-2, and incubated at 36 ± 2˚C with 5 ± 3% CO_2 for 6 - 9 days (RSV) or 4 - 9 days (SARS-CoV-2).

Controls to assess whether residual active ingredient was present after neutralisation (neutraliser effectiveness control; NEC) and if the neutralised test substance interferes with virus infectivity (viral interference control, VIC) were prepared identically to the test samples except MEM + 2% NCS was used in lieu of virus inoculum. A 0.5 mL aliquot of the PNS was ten-fold serially diluted and 100 µL of virus stock with defined 50% tissue culture infective dose ($TCID_{50}$) was added individually to selected dilutions and incubated for at least 10 minutes. A sample where no virus was added to the NEC/VIC dilutions served as Cytotoxicity control.

The 50% tissue culture infective dose per mL ($TCID_{50}$/mL) was determined using published methods [14] [15]. Where due to random virus distribution a sample tested negative (no detectable virus), a statistical analysis based on Poisson distribution [16] was performed to account for the multiplicity of infection, and to determine the theoretical maximum possible titre for that sample.

Viral load was calculated as follows:

Viral Load ($\log_{10}$ $TCID_{50}$) = Titre ($\log_{10}$ $TCID_{50}$/mL) + $\log_{10}$ [Volume (mL) × Volume Correction)]

The average $\log_{10}$ virus recovery control of each experiment served as the initial viral load to calculate the $\log_{10}$ reduction factor:

$\log_{10}$ Reduction Factor = Virus Recovery Control ($\log_{10}$ $TCID_{50}$) – Output Viral Load ($\log_{10}$ $TCID_{50}$)

3. Results

3.1. Effect of the Lozenge/Spray Mixture on the Viral Load of RSV

RSV was recovered from the NEC/VIC and did not exhibit cytotoxicity. Contact of RSV with 5,000 ppm NaClO for 1 minute reduced the virus infectivity to undetectable levels, and virus recovery control retrieved a mean viral load of approx. 6.20 $\log_{10}$ $TCID_{50}$ used in the viral reduction assay. After incubation with the lozenge mixture in 4.5 ml artificial saliva, this titre was reduced to ≤ 2.61 $\log_{10}$ $TCID_{50}$ independent of the incubation time (**Table 2**), resulting in a reduction of ≥ 3.59 $\log_{10}$ (**Figure 1(a)**).

Similar results were observed with the oromucosal spray. After incubation with the spray mixture, an initial titre of approx. 6.25 $\log_{10}$ $TCID_{50}$ was reduced to ≤ 2.61 $\log_{10}$ $TCID_{50}$ independent of the incubation time (**Table 2**), resulting in a reduction of ≥ 3.64 $\log_{10}$ (**Figure 1(a)**). Both, lozenge and oromucosal spray mixture were as effective as 5,000 ppm NaClO, indicating a strong antiviral effect of a fixed combination of chlorhexidine and lidocaine against RSV.

Table 2. Inactivation of RSV infectivity of after contact with the virucidal lozenge and spray mixture.

Preparation	Contact time	Infectious titre virus recovery control ($\log_{10}$ $TCID_{50}$)	Infectious titre after incubation ($\log_{10}$ $TCID_{50}$)[a]
Lozenge	1 minute	6.20	≤ 2.61 ± 0.00
	5 minutes		≤ 2.61 ± 0.00

Continued

	10 minutes		≤ 2.61 ± 0.00
Pos. control[b]	1 minute		≤ 2.61 ± 0.00
Oromucosal spray	1 minute	6.25	≤ 2.61 ± 0.00
	5 minutes		≤ 2.61 ± 0.00
	10 minutes		≤ 2.61 ± 0.00
Pos. control[b]	1 minute		≤ 2.61 ± 0.00

[a] Mean ± standard deviation of triplicate samples are shown. [b] 5,000 ppm sodium hypochlorite (NaClO, bleach).

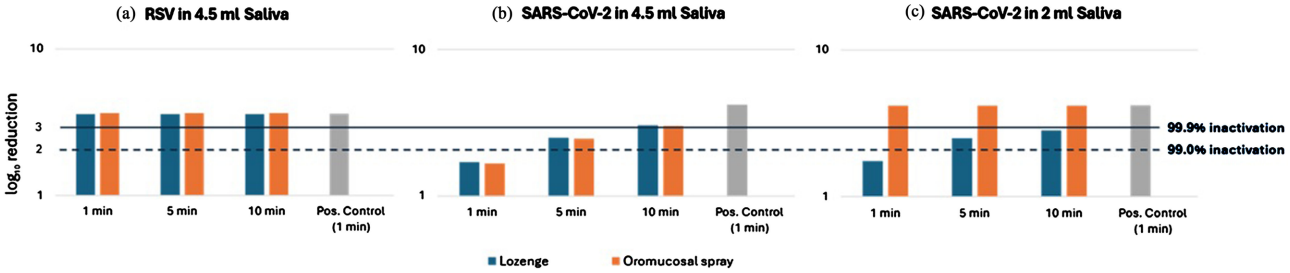

Figure 1. Reduction in infectious titre. (a) Reduction of RSV titre after incubation with lozenge or oromucosal spray solution in 4.5 ml saliva. **(b)** Reduction of SARS-CoV-2 titre after incubation with lozenge or oromucosal spray solution in 4.5 ml saliva. **(c)** Reduction of SARS-CoV-2 titre after incubation with lozenge or oromucosal spray solution in 2 ml saliva. Graphs show mean $\log_{10}$ reduction factor at three time points (n = 3). A 5,000-ppm sodium hypochlorite solution (NaClO, bleach) served as positive control and was only tested after 1 minute incubation time. For a better overview, 2 $\log_{10}$ reduction (99.0% virus inactivation) and 3 $\log_{10}$ reduction (99.9% virus inactivation) are indicated as dashed and solid line, respectively. Mean values are shown.

3.2. Effect of the Lozenge/Spray Mixture on the Viral Load of SARS-CoV-2

SARS-CoV-2 was recovered from the NEC/VIC and did not exhibit cytotoxicity. Contact of SARS-CoV-2 with 5,000 ppm NaClO for 1 minute reduced the virus infectivity to undetectable levels, and virus recovery control retrieved a mean viral load of approx. 6.83 $\log_{10}$ $TCID_{50}$ was used in the viral reduction assay. After incubation with the lozenge mixture in 4.5 ml artificial saliva, this titre was reduced to 5.12 ± 0.08 $\log_{10}$ $TCID_{50}$ after 1 minute incubation, and was further reduced to 4.33 ± 0.00 $\log_{10}$ $TCID_{50}$ after 5 minutes and 3.79 ± 0.07 $\log_{10}$ $TCID_{50}$ after 10 minutes contact time (Table 3), resulting in a mean time-dependent reduction of 1.71 ± 0.08, 2.50 ± 0.00, and 3.04 ± 0.07 $\log_{10}$, respectively (Figure 1(b)).

Again, similar results were observed with the oromucosal spray. After incubation with the spray mixture in 4.5 ml artificial saliva, an initial SARS-CoV-2 titre of approx. 6.79 $\log_{10}$ $TCID_{50}$ was reduced to ≤ 5.12 ± 0.19 $\log_{10}$ $TCID_{50}$ after 1 minute incubation, and further reduced to 2.46 ± 0.13 $\log_{10}$ $TCID_{50}$ after 5 minutes, and 3.00 ± 0.07 $\log_{10}$ $TCID_{50}$ after 10 minutes contact time (Table 3), resulting in a mean time-dependent reduction of 1.67 ± 0.19 $\log_{10}$, 2.46 ± 0.13, and 3.00 ± 0.07 $\log_{10}$ after 1, 5 and 10 minutes, respectively (Figure 1(b)). In contrast to RSV, both, lozenge and oromucosal spray mixture were less effective than 5,000 ppm NaClO, which reduced SARS-CoV-2 titre by ≥ 4.18 $\log_{10}$. These data suggest a time-dependent antiviral effectiveness of a fixed combination of chlorhexidine

and lidocaine against SARS-CoV-2.

Table 3. Inactivation of SARS-CoV-2 infectivity after contact with the virucidal lozenge and spray mixture in 4.5 ml saliva.

Preparation	Contact time	Infectious titre before incubation (log_{10} $TCID_{50}$)	Infectious titre after incubation at (log_{10} $TCID_{50}$)[a]
Lozenge	1 minute	6.83	5.12 ± 0.08
	5 minutes		4.33 ± 0.00
	10 minutes		3.79 ± 0.07
Pos. control[b]	1 minute		≤ 2.61 ± 0.00
Oromucosal spray	1 minute	6.79	5.12 ± 0.19
	5 minutes		4.33 ± 0.13
	10 minutes		3.79 ± 0.07
Pos. control[b]	1 minute		≤ 2.61 ± 0.00

[a] Mean ± standard deviation of triplicate samples are shown. [b] 5,000 ppm sodium hypochlorite (NaClO, bleach).

To exclude volume effects, the experiments were repeated in a lower amount of saliva (2 ml). Incubation with the lozenge showed a similar time-dependent reduction of SARS-CoV-2 titre – the initial titre of 6.79 log_{10} $TCID_{50}$ was reduced to 5.04 ± 0.19, 4.29 ± 0.07 and 3.96 ± 0.13 log_{10} $TCID_{50}$ after 1, 5 and 10 minutes, respectively (Table 4), yielding log_{10} reductions comparable to the previous experiment using 4.5 ml saliva. However, titre reduction after 10 min incubation with lozenge solution did not reach the effect of 1 minute incubation with 5,000 ppm NaClO (Figure 1(c)).

In contrast, SARS-CoV-2 titres were reduced to undetectable levels after 1 minute of incubation with the oromucosal spray, when a lower amount of artificial saliva was used (Table 4 and Figure 1(c)).

Table 4. Inactivation of SARS-CoV-2 infectivity of after contact with the virucidal lozenge and spray mixture in 2 ml saliva.

Preparation	Contact time	Infectious titre before incubation (log_{10} $TCID_{50}$)	Infectious titre after incubation (log_{10} $TCID_{50}$)[a]
Lozenge	1 minute	6.79	5.04 ± 0.19
	5 minutes		4.29 ± 0.07
	10 minutes		3.96 ± 0.13
Oromucosal spray	1 minute	6.79	≤ 2.61 ± 0.00
	5 minutes		≤ 2.61 ± 0.00
	10 minutes		≤ 2.61 ± 0.00
Pos. control[b]	1 minute	6.79	≤ 2.61 ± 0.00

[a] Mean ± standard deviation of triplicate samples are shown. [b] 5,000 ppm sodium hypochlorite (NaClO, bleach).

4. Discussion

This study demonstrated virucidal activity of commercially available products containing active ingredients chlorhexidine and lidocaine against RSV and SARS-CoV-2 *in vitro*. The destructive effect of both lidocaine/chlorhexidine preparations on RSV was rapid: Angimed menthol compressed lozenges as well as Angimed menthol oromucosal spray inactivated 99.9% (corresponding to a 3 $\log_{10}$ reduction) of RSV in less than 1 minute. Similar results were published by Meister *et al*. 2024 [17] where a chlorhexidine-based oral rinse showed high virucidal capacity against RSV and SARS-CoV-2 in a solution mimicking nasal secretion. The findings might be significant due to the lack of specific treatment and the unprecedented resurgence of RSV in 2022 after a period of low prevalence during SARS-CoV-2 pandemic [18] [19]. Moreover, the virus is increasingly identified as a significant etiological factor for acute morbidity in the geriatric population and is a major contributor to respiratory hospitalisations and associated mortality [20].

While data on the susceptibility of RSV to chlorhexidine are scarce, susceptibility of SARS-CoV-2 to chlorhexidine mouthwash/mouth rinse solutions was investigated in several studies. These varied not only in contact time, frequency, timepoints tested, and methods used, but also in chlorhexidine concentrations, which ranged from 0.06% to 0.2%. Although this appears to be the first experiment using artificial saliva, some randomised controlled trials have investigated the efficacy of chlorhexidine in reducing the salivary viral load in COVID-19 patients. While a single rinse with 15 ml of undiluted chlorhexidine mouthwash (0.2%) did not significantly reduce viral load compared with other commercial mouthrinses after 5 minutes in 9 patients [21], another study showed that a chlorhexidine 0.12% mouthrinse significantly reduced SARS-CoV-2 viral load after 30 and 60 min in 8 patients [22]. Compared to a control group (water), Elzein *et al*. 2021 [23] found a significant difference in viral load in patients (n = 27) before and after rinsing with chlorhexidine (0.2%) for 30 seconds.

Despite the heterogeneity of both clinical and *in vitro* study methods in scientific literature, the results generally suggest antiviral efficacy of chlorhexidine against SARS-CoV-2 oral load, as reviewed by Rahman *et al*. 2023 [24], Zhang *et al*. 2023 [25], Sbricoli *et al*. 2023 [26], and Dos Santos Fernandez *et al*. 2022 [27].

While in our experiments, 0.2% (m/v) chlorhexidine is present in the oromucosal solution, the lozenge preparation has a concentration of 0.11% (m/v) once dissolved in 4.5 ml artificial saliva. However, in 4.5 ml saliva, similar results were retrieved for both formulations. The virucidal effect against SARS-CoV-2 was dependent on exposure time but did not reach the efficacy of NaClO even after the longest contact time of 10 minutes. This is in line with the time-dependent virucidal potential of chlorhexidine that has been described in the literature [12].

Taken together, the effectiveness of virucidal formulations is contingent upon multiple variables, including the duration of incubation, the specific composition, and the concentration of active constituents [17]. Physiologically, the latter is subject to fluctuations based on the volume and flow rate of saliva. These factors are

influenced by demographic characteristics, with older age generally correlating with reduced salivary flow, and by the presence of a stimulus [28]. Stimulated saliva flow rates are subject to high intra- and interindividual variability, and thus also depend on the gustatory and mechanical stimulation due to chewing. During 20 minutes of sucking peppermint-flavoured lozenges, total volumes of 20 - 30 mL saliva were produced, suggesting a mean saliva production of 1 - 1.5 mL/min with a peak flow rate of 3 - 3.5 mL/min after 1 minute [29]. In consequence, experiments with SARS-CoV-2 were repeated in 2 mL saliva, resulting in a concentration of 0.25% (m/v) once the lozenge was dissolved. While in this experiment, the oromucosal spray was highly virucidal against SARS-CoV-2, the lozenge delivered effects in a range comparable to the higher saliva amount. Overall, in this experiment, Angimed menthol compressed lozenges inactivated 99% (corresponding to a 2 $\log_{10}$ reduction) of SARS-CoV-2 in 5 minutes and Angimed menthol oromucosal spray inactivated 99.9% (corresponding to a 3 $\log_{10}$ reduction) of SARS-CoV-2 in less than 1 minute.

The slightly lower effectiveness of the lozenge in terms of SARS-CoV-2 eradication might be explained by the variant composition of the formulations in terms of concentration and chemical form of the active ingredients, as well as excipients contained. Ethanol, which is present in higher quantities in the spray, has proven *in vitro* effectiveness in inactivating SARS-CoV-2 in concentrations of 30% (w/w) or 36.2% (v/v) [30] and might thus support virucidal activity of the spray formulation.

Limitations of current treatment options for both RSV and SARS-CoV-2 raise the need for alternative therapeutic approaches [9] [11]. Since RSV and SARS-CoV-2 are highly contagious, one possible approach is to contain transmission. Our research showed that contact of these viruses with spray or lozenge preparations could cause rapid virus inactivation. As the lozenge formulation allows for longer contact times in the mouth, it would be interesting to test this preparation at longer incubation times. Furthermore, the reduced virus titres suggest a potential decrease of viral spread to other individuals as well as to the lower respiratory tract in the patient, thus preventing more severe forms of the diseases such as bronchiolitis [20] or pneumonia [20] [31].

Virucidal substances, by their ability to directly inactivate viruses and destroy their infectious ability, might also stop the propagation of other respiratory viruses such as influenza virus, parainfluenza virus, human metapneumovirus, rhinoviruses, or adenoviruses. However, it is important to note that the effectiveness of a substance against different viruses may vary, and specific studies are required to confirm the effect of a fixed combination of chlorhexidine and lidocaine on different respiratory viruses.

Author's Contribution

AC, DW, AP and AM contributed to the design of the experiments and interpretation of the results. Microbac Laboratories conducted the experiments and helped

with the interpretation of the results. Medical writing support was provided by Ana-Marija Valland, MSc. and Eva Bauer, PhD of DREHM Pharma GmbH, Vienna, AT, and was funded by Procter & Gamble UK. Further acknowledgements go to Dr. Bonang Masilo (employee of DREHM Pharma GmbH) for critical reading of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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