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By Hidemasa Nakaminami

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Virucidal activity of olanexidine gluconate against SARS-CoV-2

--Manuscript Draft--

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Short Communication

Virucidal activity of olanexidine gluconate against SARS-CoV-2

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Abstract

Introduction.

Antiseptics have been used for infection control against SARS-CoV-2. Ethanol (EtOH) was effective against SARS-CoV-2, while chlorhexidine gluconate (CHG) was less effective. Therefore, there may be differences in virucidal activity between classes of antiseptic agents.

Aim.

In this study, we evaluated the efficacy of antiseptics against SARS-CoV-2 and identified effective agents for infection control.

Methods.

The following antiseptics were used in this study: 1.5% olanexidine gluconate (OLG); 80% EtOH; 1% sodium hypochlorite (NaClO); 0.2% benzalkonium chloride (BKC); 1% povidone-iodine (PVP-I); 0.5%, 1%, and 1.5% chlorhexidine gluconate (CHG); and 0.5% alkyldiaminoethylglycine hydrochloride (AEG). Virucidal activity was evaluated at 0, 30 s, 1, 2, and 3 min according to EN14476.

Results.

After 30 s of exposure, 1.5% OLG, 80% EtOH, 1% NaClO, 0.2% BKC, and 1% PVP-I inactivated SARS-CoV-2 below the detection limit. In contrast, the virus was survived

in 0.5% CHG, 1% CHG, and 0.5% AEG after 3 min of exposure. However, the virucidal activity of 1.5% CHG was insufficient after 30 s of exposure.

Conclusion.

This study showed that the virucidal activity against SARS-CoV-2 differs depending on the class of antiseptic agent. Despite belonging to the same class of biguanide antiseptics, OLG was more effective against SARS-CoV-2 than CHG.

Keywords.

SARS-CoV-2, antiseptics, olanexidine gluconate, virucidal activity

Abbreviations.

SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; OLG, olanexidine gluconate; SARS-CoV-2^{WK-521}, SARS-CoV-2 JPN/TY/WK-521/2020 strain; EtOH, ethanol; NaClO, sodium hypochlorite; BKC, benzalkonium chloride; PVP-I, povidone iodine; CHG, chlorhexidine gluconate; AEG, alkyldiaminoetylglycine hydrochloride; $TCID_{50}$, Median Tissue Culture Infectious Dose; CPE, cytopathic effect.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was isolated from Wuhan, China, in 2019 [1]. SARS-CoV-2 is thought to have spread through contact and droplet transmission. Therefore, the appropriate use of an effective antiseptic agents is important for infection control [2, 3]. Antiseptic agents are classified based on their antimicrobial spectra as having high, medium, or low virucidal activity. High-level antiseptics are used to disinfect medical devices, such as endoscopes, and medium-level antiseptics are used to disinfect the environment, skin, and hands. Low-level antiseptics are used to disinfect wounds, mucous membranes, and the environment. In 2015, oranexidine gluconate (OLG) was launched in Japan as a biguanide surgical field disinfectant [4]. OLG has strong virucidal activity against bacteria, including *Staphylococcus aureus, Pseudomonas aeruginosa*, human coronaviruses and influenza viruses [4, 5]. The SARS-CoV-2 has a lipid envelope that renders it susceptible to alcohol-based antiseptic agents [2]. In contrast, chlorhexidine gluconate (CHG), a biguanide disinfectant, was less effective against SARS-CoV-2 [2]. Therefore, there may be differences in virucidal activity between classes of antiseptic agents. In this study, we evaluated the efficacy of antiseptics against SARS-CoV-2 and identified effective agents for infection control.

Methods

SARS-CoV-2 JPN/TY/WK-521/2020 strain (SARS-CoV-2^{WK-521}) was obtained from the National Institute of Infectious Diseases (Tokyo, Japan). VeroE6/TMPRSS2 cell (JCRB1819) were purchased from the Institute of Biomedical Innovation, Health, and Nutrition (Osaka, Japan). VeroE6/TMPRSS2 cells were maintained in VP-SFM (GIBCO, Nebraska, USA) supplemented with 200 mM L-glutamine, 100 units/mL penicillin and 100 μg/mL streptomycin. The following antiseptics were included in this study at clinically used concentrations: 1.5% OLG (Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan), 80% ethanol (EtOH; FUJIFILM Wako Pure Chemical Corp.), 1% sodium hypochlorite (NaClO; FUJIFILM Wako Pure Chemical Corp.), 0.2% benzalkonium chloride (BKC; FUJIFILM Wako Pure Chemical Corp.), 1% povidone iodine (PVP-I; Sigma-Aldrich Co. LLC, Missouri, USA), 0.5%, 1%, and 1.5% chlorhexidine gluconate (CHG; FUJIFILM Wako Pure Chemical Corp.) and 0.5% alkyldiaminoetylglycine hydrochloride (AEG; FUJIFILM Wako Pure Chemical Corp.).

The virucidal test was conducted under clean conditions without interfering substances according to EN14476 [6]. An aliquot of 50 μL of test virus was mixed with 450 μL of the test substances (or HEPES buffer as a negative control), and the samples were incubated for 0, 30 sec, 1, 2 and 3 min at room temperature. No interfering substances were used in this study, and the test substances were diluted with distilled water. After incubation, the antiseptics were removed by gel filtration [6]. For the gel filtration method, 100 µL of the samples were diluted with 900 µL of PBS and filtered through a gel filtration column (Columns filled with Sephacryl S-400; Cytiva, Tokyo, Japan). The filtrates were then serially diluted 10-fold with the culture media. Viral titers [$\log_{10}$ Median Tissue Culture Infectious Dose ($TCID_{50}$)/mL] in all antiseptics were measured by quantitative assays of six wells per dilution and three biological replicates and were determined by the Spearman–Kärber method [6]. The $\log_{10}$ reductions in viral titers were calculated, and the results were expressed as the mean of the $\log_{10}$ $TCID_{50}$/mL ± standard deviation.

Results

A comparison of the virucidal activities of the antiseptic agents showed that 1.5% OLG, 80% EtOH, 1% NaClO, 0.2% BKC, and 1% PVP-I inactivated SARS-CoV-2 to below the detection limit after 30 s of exposure (Fig. 1). In contrast, the virus survived in 0.5% CHG, 1% CHG, and 0.5% AEG after 3 min of exposure. Although the virucidal activity of 1.5% CHG was insufficient after 30 s of exposure, the virus was reduced below the detection limit after 1 min of exposure.

Cell morphology was maintained by inactivating the virus with 1.5% OLG, 80% EtOH, 1% NaClO, 0.2% BKC, and 1% PVP-I for 30 s (Fig. 2A, B). In contrast, cytotoxic effects (CPE) were observed when the virus was exposed to 0.5% CHG, 1% CHG, and 0.5% AEG for 30 s and 3 min, respectively, owing to insufficient virucidal activity. Consistent with virucidal activity, cell morphology was maintained by 1.5% CHG with 3 min exposure to the virus, whereas 30 s exposure was insufficient, and CPE was observed.

Discussion

This study showed that OLG, EtOH, NaClO, BKC, and PVP-I were highly effective antiseptic agents against SARS-CoV-2. EtOH is an effective antiseptic agent against SARS-CoV-2 [7]. EtOH is most commonly used for hand hygiene because of its fast-acting properties and is also used in hospitals to disinfect medical equipment, although it is not expected to have a long-lasting effect owing to its high volatility. NaClO, which is mainly used to disinfect medical devices, volatilizes its components in a short time. SARS-CoV-2 has been reported to survive for long periods on various surfaces, including human skin and materials [8, 9]. Therefore, disinfection with EtOH or NaClO is likely to allow SARS-CoV-2 to survive if it is dried over time. In contrast, BKC and PVP-I are reported to be highly persistent and have long-lasting effect [10]. Thus, the effectiveness of disinfectants varies greatly depending on their persistence on object surfaces. Further studies should be conducted to determine the effectiveness of disinfectants on a variety of surfaces.

The virucidal activities of CHG and AEG were lower than those of other antiseptic agents used in this study. Low virucidal activity of CHG against SARS-CoV-2 has been reported [2]. This study is consistent with a previous study and shows that AEG has low virucidal activity against SARS-CoV-2. The virucidal activity of CHG increased in a concentration-dependent manner, although the virus survived even at 1.5% CHG, which was the maximum concentration used in this study. Despite belonging to the same class of biguanide antiseptics, OLG was more effective against SARS-CoV-2 than CHG. However, they are structurally slightly different; CHG is a divalent cationic compound, whereas OLG is a monovalent cationic compound [4]. Therefore, it is possible that the difference in the charges of the compounds affects their virucidal activity. Further studies are required to clarify the differences in the mechanisms of action of CHG and OLG against SARS-CoV-2 infection.

In this study, various antiseptic agents were evaluated at the maximum concentrations used in clinical settings. The results showed that most antiseptic agents inactivated SARS-CoV-2 below the detection limit. The appropriate concentrations of antiseptic agents depend on their intended use. Therefore, the evaluation of virucidal activity at low concentrations may reveal more detailed differences between antiseptic agents.

Conclusion

The virucidal activity against SARS-CoV-2 differs depending on the class of antiseptic agents. Despite belonging to the same class of biguanide antiseptics, OLG was more effective against SARS-CoV-2 than CHG. Therefore, appropriate antiseptic agents should be selected to SARS-CoV-2 infection control.

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Conflicts of interest.

The authors (T. Y and H. N) have financial conflicts of interest with Otsuka Pharmaceutical Factory, Inc. as joint research funders.

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Figure legends

Fig. 1. Virucidal activity of antiseptic agents against SARS-CoV-2$^{\text{WK-521}}$.

Fig. 2. Phase contrast image of VeroE6/TMPRSS2 after 30 s (A) and 3 min (B) of antiseptic exposure.

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