

Use of Ocudox™ or hypochlorous acid to inactivate SARS-CoV-2 as an alternative to alcohol-based products

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ABSTRACT

Objective: The main route of entry of SARS-CoV-2 which responsible of COVID-19 is through the nasal, oral, or conjunctival mucosa and droplets or aerosols transmit the virus. Even if transmission through fomites is considered unlikely, hand hygiene is important to minimize this and other infections, especially in certain scenarios as healthcare facilities and crowded places as public transport or social events.

Material and methods: In this study, we assess and compare the virucidal efficacy of HOCl solutions against SARS-CoV-2 at different time-points. a nasopharyngeal swab collected from an 89-year-old male patient during March 2020 in Catalonia, Spain (GISEAD ID EPI_ISL_510689). The virus was propagated in Vero E6 cells (ATCC CRL-1586) cultured in Dulbecco's modified Eagle medium (DMEM). A total of 24 plastic plates (35 mm SPL Ref. PLC20035) for the first study, and 12 plates for the second one, were prepared. One day before titration, 96-well plates were prepared with Vero E6 cells. The day of titration, two replicates of five 10-fold serial dilutions of each sample were performed. Plates were incubated at 37°C and 5% CO₂ for 7 days, and afterward, SARS-CoV-2-induced cytopathic effect (CPE) was determined under light microscope

Results: The virucidal effect by means of TCID₅₀/mL found after HOCl treatment at different concentrations and times tested. Logarithmic reduction (logR) and the correspondent percentage of inactivation (%) were calculated compared to the untreated controls (desiccated SARS-CoV-2 with water or media). All tested concentrations were able to partially inhibit SARS-CoV-2

Conclusion: Our studies showed that HOCl products represent a valid virucidal alternative to inactivate SARS-CoV-2 potentially present in skin or surfaces.

Keywords: HOCl, SARS-CoV-2, disinfection

INTRODUCTION

The available vaccines reduce the risk of getting COVID-19, and in case of infection, immunization minimizes the symptoms and severity of the disease. However, issues like the emerging variants of concern and the remaining unvaccinated population could hinder the ideal herd immunity. At the beginning of the COVID-19 pandemic, proper hand hygiene was considered a relevant measure to reduce SARS-CoV-2 transmission as other infectious microorganisms, and it is especially important in public places and health care facilities. Nowadays, we know that transmission of SARS-CoV-2 is through respiratory fluids as saliva droplets or aerosols, and rarely through contaminated surfaces. However, transmission through fomites cannot be discarded entirely, and initial recommended alcohol-based products and other antiseptic solutions with virucidal capacity against SARS-CoV-2 might help stop transmission.

Up to now, different disinfectants and detergents have demonstrated their ability to eliminate SARS-CoV-2 from skin and surfaces (1). However, continuous usage of disinfection products may cause skin damage and alteration of the integrity and functions of this natural barrier. Dry skin, irritation, contact dermatitis, and biofilm of pathogenic bacteria can result from overuse of this type of product (2). Hypochlorous acid (HOCl) has been used for more than 100 years for wound treatment and is now frequently used in odontology as disinfectant for oral-maxillofacial surgical practices (3).

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Moreover, it is one of the recommended solutions against SARS-CoV-2 by the US Environmental Protection Agency (4), and at a concentration of 20 ppm, HOCl shows virucidal activity, and concentrations of 50-200 ppm have been described as effective against SARS-CoV-2 (3).

The aim of the present study is to evaluate the virucidal efficacy of a HOCl solution against SARS-CoV-2.

MATERIAL and METHODS

Study design: The experimental designs were adapted from UNE EN14476:2013+A2:2019 norm and performed at the IRTA-CReSA High Biocontainment Unit using the appropriate personal protective equipment (double glove, double gown and Sundström Powered Air Purifying Respirator equipment). SARS-CoV-2 isolate was obtained from a nasopharyngeal swab collected from an 89-year-old male patient during March 2020 in Catalonia, Spain (GISEAD ID EPI_ISL_510689). The virus was propagated in Vero E6 cells (ATCC CRL-1586) cultured in Dulbecco's modified Eagle medium (DMEM), supplemented with 5% fetal calf serum (FCS), 100 U/mL penicillin, 100 µg/mL streptomycin, and 2 mM glutamine. After propagation of the virus, a virus stock was prepared collecting the supernatant. For this study, samples of 100 µL of the virus stock at 10⁶ 50% tissue culture infectious dose (TCID₅₀/mL) were desiccated in a period of 90-100 minutes on sterile plastic cell culture dishes (SPL Ref. PLC20035, Labclinics). After, SARS-CoV-2 was exposed to different concentrations of HOCl in two independent experiments. In the first study, 0.005% (50 ppm; Ocudox™) and 0.02% (200 ppm) of HOCl were used, and in the second study, a concentration of 0.01% (100 ppm) was tested. A total of 24 plastic plates (35 mm SPL Ref. PLC20035) for the first study, and 12 plates for the second one, were prepared.

Sample collection: After the exposure time, HOCl was carefully removed and residual virus was gently eluted with 1 mL of media (DMEM, 1% FBS). All samples were stored at -75°C until the virus titration assay.

Sample analysis: One day before titration, 96-well plates were prepared with Vero E6 cells. The day of titration, two replicates of five 10-fold serial dilutions of each sample were performed. Plates were incubated at 37°C and 5% CO₂ for 7 days, and afterward, SARS-CoV-2-induced cytopathic effect (CPE) was determined under light microscope.

Data analysis: All replicates for each time point were considered independently. To analyze the differences between treatments and untreated control, Tukey's multiple comparisons test (ANOVA) was used and p-value of less than 0.05 was accepted as statistically significant. Calculations were done using Graphpad Prism 9.

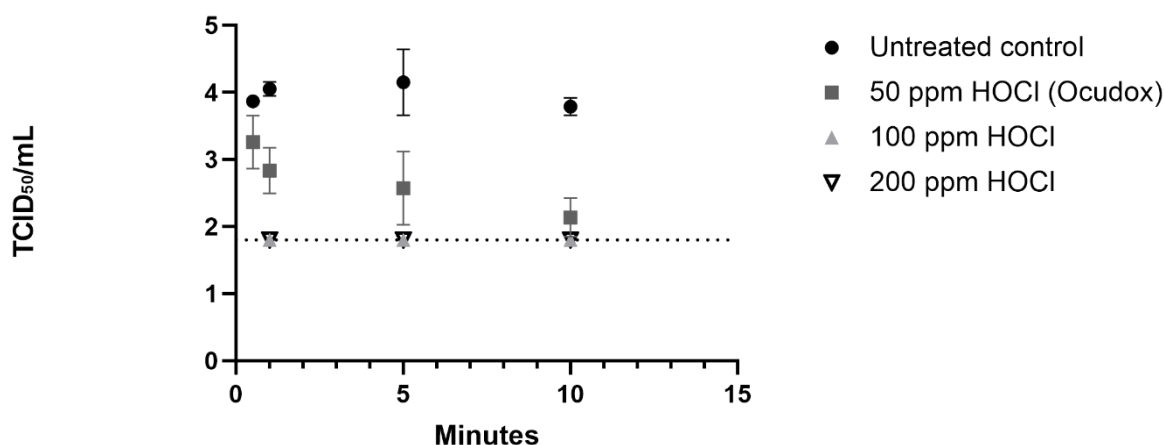
RESULTS

Table 1 shows the virucidal effect by means of TCID₅₀/mL found after HOCl treatment at different concentrations and times tested. Logarithmic reduction (logR) and the correspondent percentage of inactivation (%) were calculated compared to the untreated controls (desiccated SARS-CoV-2 with water or media). All tested concentrations were able to partially inhibit SARS-CoV-2. Concentrations of 100 and 200 ppm were able to inactivate SARS-CoV-2 from 1 minute onwards, below the detection limit of the viral titration technique (1.8 TCID₅₀/mL). Figure 1 shows the residual viral titers detected after HOCl treatment. Differences between all tested concentrations compared to the untreated control were statistically significant (P<0.01).

Table 1: Inactivation results of three different HOCl concentrations under the test conditions of the two studies.

Study	Hypochlorous acid concentration (ppm)	Exposition Time (minutes)	Mean TCID ₅₀ /mL and standard deviation (SD)	LogR	Inactivation (%)
1	Ocudox™ (50)	0.5	3.2 ± 0.4	0.7	80.1
	Ocudox™ (50)	1	2.8 ± 0.4	1.1	92.1
	Ocudox™ (50)	5	2.6 ± 0.6	1.4	96.0
	Ocudox™ (50)	10	2.4 ± 0.2	1.5	96.8
	Hypochlorous acid solution (200)	1	≤1.8 ± 0.0	≥2.1	≥99.2
	Hypochlorous acid solution (200)	10	≤1.8 ± 0.0	≥2.1	≥99.2
	Untreated Control (DMEM)	0.5	3.9 ± 0.0	-	-
	Untreated Control (DMEM)	1	3.9 ± 0.0	-	-
	Untreated Control (DMEM)	5	4.0 ± 0.3	-	-
	Untreated Control (DMEM)	10	3.9 ± 0.1	-	-
2	Hypochlorous acid solution (100)	1	≤1.8 ± 0.0	≥2.1	≥99.2
	Hypochlorous acid solution (100)	5	≤1.8 ± 0.0	≥2.1	≥99.4
	Hypochlorous acid solution (100)	10	≤1.8 ± 0.0	≥2.1	≥99.2
	Untreated Control (sterile water)	1	3.9 ± 0.1	-	-
	Untreated Control (sterile water)	5	3.9 ± 0.1	-	-
	Untreated Control (sterile water)	10	3.9 ± 0.1	-	-

Figure 1. Results of viral titration after treatment with HOCl (50, 100 and 200 ppm) during different contact times (30 seconds, 1, 5 and 10 minutes). Sample points on the dots line (100 and 200 ppm) are below the detection limit of the technique (1.8log TCID₅₀/mL).



DISCUSSION

This study provides an alternative solution to inactivate SARS-CoV-2 from skin and surfaces instead of alcohol-based products and demonstrates high effectiveness from 1 minute onwards at ≥ 100 ppm of HOCl. The principal advantage of HOCl compared to hydro-alcoholic antiseptics is that HOCl is innocuous at the effective concentrations and no skin damage effects have been reported. In fact, Ocudox™ is a non-toxic, non-irritating and non-sensitizing ocular antiseptic based in HOCl, which is a potent inorganic compound produced by all mammals and is part of the immunity system. HOCl can be obtained in vitro by electrolyzing NaCl, which releases mainly NaCl and HOCl, is effective against many pathogens and is cheap to produce (3). Moreover, according to our results, HOCl could be a potential disinfectant of non-porous surfaces for the indicated contact times, as food bricks, envelopes or containers. Up to now, we agree with other authors that there is a little risk of SARS-CoV-2 transmission through fomites (5), although different environmental stability has been reported regarding Omicron variant among previously reported variants (6). Thus, proper hand-washing and disinfection are still recommended since it is an effective way to avoid infectious pathogen transmission.

CONCLUSION

Our studies showed that the HOCl products represent a valid virucidal alternative to inactivate SARS-CoV-2 potentially present in skin or surfaces.

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Conflict of interest: CG a is full time employee of Brill Engines S.L. The other authors do not declare any conflict of interest.

Author Contributions: CLO, NR, CGM, XA: Study design, Literature review, Data collection and processing, CGM: Writing, Revisions

Ethical approval: All procedures performed in studies involving human participants were in accordance with the institutional and/or national research committee's ethical standards and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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