Journal of Global Antimicrobial Resistance 28 (2022) 158–160

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

SARS CoV-2 Dispatches

In vitro evaluation of the virucidal activity of disodium citrate perhydrate (2SCP) disinfectant against SARS-CoV-2

Géraldine Dessilly[a,*], Anne-Thérèse Pâques[a], Anne-Thérèse Vandenbroucke[a], Pierre Hazée[b], Alain Gaume[c], Katia Gindro[c], Sylvain Schnée[c], Frédéric Lakaye[b], Benoît Kabamba-Mukadi[a,d]

[a] *Medical Microbiology Unit (MBLG), Brussels, Belgium*
[b] *Biorem Engineering, Nyon, Switzerland*
[c] *Agroscope, Plant Protection Department, Nyon, Switzerland*
[d] *Cliniques Universitaires Saint-Luc, Clinical Laboratory Department, Brussels, Belgium*

ARTICLE INFO

Article history:
Received 16 July 2021
Revised 27 October 2021
Accepted 9 January 2022
Available online 14 January 2022

Editor: Prof Carlo F Perno

Keywords:
SARS-CoV-2
Vero E6
Antiviral activity
Disodium citrate perhydrate
2SCP

1. Introduction

COVID-19 (coronavirus disease 2019), caused by infection with SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2), is a global pandemic and a public-health problem causing social and economic damage worldwide [1,2]. The predominant route of human-to-human transmission of SARS-CoV-2 is through respiratory droplets or aerosol particles during close, unprotected contact or through direct and indirect contact [3–5].

Manual cleaning and disinfection measures do not always sufficiently achieve surface contamination. The persistence of cleaning products is also a key variable in managing the recurrence of disinfection [4,6]. As a consequence, much attention and effort has been focused on identifying biocides, therapeutics and vaccines to contain the pandemic.

Disodium citrate perhydrate (2SCP or CovF-1) was designed (in 2017) by Biorem Engineering in partnership with Agroscope for crop protection applications in the context of the Swiss requirements aimed at reducing the use of synthetic chemicals in agriculture.

We decided to evaluate 2SCP against SARS-CoV-2 because it appears to be safe for human health and has long-lasting efficacy. At SARS-CoV-2 inhibitory concentrations, 2SCP does not present a risk to human health if ingested. It has been demonstrated according to Organisation for Economic Co-operation and Development (OECD) standards 492 and 439 that 2SCP is not cytotoxic on the skin, ocular or oral mucous membranes (data not published).

The aim of the present experimental study was to determine the virucidal activity of 2SCP disinfectant using an in vitro cell line (Vero E6) infected with SARS-CoV-2.

2. Materials and methods

2.1. Materials

2SCP, with chemical formula $Na_2HC_6H_5O_7 \cdot H_2O_2$ is a soluble, crystalline, free-flowing powder manufactured by Biorem Engineering (Switzerland) in partnership with List Technology AG (Arisdorf, Switzerland) (for the 2SCP crystal and molecular structure, see the Supplementary data).

* Corresponding author. Avenue Hippocrate 54, 1200 Brussels, Belgium.
E-mail address: Geraldine.dessilly@uclouvain.be (G. Dessilly).

https://doi.org/10.1016/j.jgar.2022.01.006

2SCP is a new crystalline form of disodium hydrogen citrate linked to hydrogen peroxide by strong hydrogen bonds. Its structure was refined using synchrotron X-ray powder diffraction data and was optimised using density functional techniques.

The product crystallises in space group *Pbca* (#61) with $a = 8.6396(25)$, $b = 12.433(4)$, $c = 17.199(5)$ Å, $V = 1847.5(16)$ Å3 and $Z = 8$ [7].

When dissolved in water and applied to human skin or any surface, the product safely recrystallises at a physiological pH of 5 with hydrogen peroxide rather than water to form a long-lasting protective biocidal barrier against a wide range of pathogens.

All experiments were performed using a SARS-CoV-2 strain (clade 20E/EU1) isolated in a biosafety level 3 (BSL-3) laboratory.

2.2. Cell line

Vero E6 cells (ATCC® CRL-1586™) were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) (Life Technologies, Belgium) supplemented with 10% heat-inactivated fetal bovine serum (Fisher Scientific, the Netherlands) and 1% gentamicin (Life Technologies) in a 5% CO_2 atmosphere at 37°C (protocol adapted from data sheet of ATCC and reference [8]).

2.3. Viability assays

One day before the experiment, 10^5 cells/well were seeded in 96-well plates in DMEM. The percentage of cell viability was investigated by loading different concentrations (0.01, 0.03, 0.09, 0.1, 0.3 and 0.9 mg/mL) of 2SCP into the cell culture plate, which was then incubated at 37°C for 120 min. One negative control (unexposed cells) and at least one positive control [cells exposed to 50% dimethyl sulfoxide (DMSO), 70% isopropanol or 0.1% Virkon S] were included in each experiment.

2.4. Antiviral assays

One day before the experiment, 5×10^5 cells/well were seeded in 12-well plates in DMEM. The next day, 1 mL of viral suspension at a multiplicity of infection (MOI) of 20 was added to each well. The MOI is the ratio defined by the number of viral particles per millilitre divided by the number of target cells present in that well.

After 2 h of exposure to viral infection, cells were washed with Dulbecco's phosphate-buffered saline (D-PBS) (Fisher Scientific) and fresh DMEM medium was added with varying concentrations of 2SCP (0.03, 0.1, 0.3 and 0.9 mg/mL). A negative control (cells unexposed to SARS-CoV-2) and a positive control (cells exposed to SARS-CoV-2, unexposed to 2SCP) were included in each experiment.

The cytopathogenic effect (CPE) was visualised 3 days after infection under an inverted microscope (Zeiss) following RAL 555 Diff-Quick staining (MLS, Belgium; fast Acting Variation of May–Grünwald Giemsa Staining). The cell-free supernatant was also collected in order to quantify viral particles in the cell culture medium. Following virus inactivation (65°C during 20 min), total RNA was extracted with a MagNA Pure Compact Nucleic Acid Isolation Kit (Roche) and was eluted in 50 μL. Extracted RNA was added to Multiplex RNA Virus Master Mix (Roche, Belgium) and was amplified by real-time RT-PCR using specific primers for SARS-CoV-2 (Egene).

Furthermore, the supernatant was collected for rapid antigen assessment with CORIS COVID-19 Ag Respi-Strip (International Medical Products, Brussels, Belgium).

Table 1
Real-time RT-PCR and antigen assays: quantification of viral particles in the cell culture medium by real-time RT-PCR and rapid antigen assessment after 3 days after SARS-CoV-2 infection

	Cq	Concentration (copies/mL)	COVID-19 Ag
2SCP (mg/mL)			
0.03	9.6	5×10^8	Positive
0.1	10.3	3.1×10^8	Positive
0.3	Negative	–	Negative
0.9	Negative	–	Negative
Positive control [a]	9.9	4.1×10^8	Positive
Negative control [b]	Negative	–	Negative

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; 2SCP, disodium citrate perhydrate; Cq, quantification cycle; Ag, antigen.
[a] Cells exposed to SARS-CoV-2, unexposed to the drug.
[b] Cells unexposed to SARS-CoV-2.

3. Results

We first assessed the viability of cells exposed to 2SCP. As expected, no cytotoxicity was observed in the presence of 2SCP compared with the positive controls (data not shown).

We further investigated the potential antiviral activity of 2SCP against SARS-CoV-2. Three days after exposure to infection, the CPE was visualised in Vero E6 cells exposed to SARS-CoV-2 as well as in Vero E6 cells exposed to SARS-CoV-2 with 0.03 and 0.1 mg/mL 2SCP. No CPE was observed in Vero E6 cells unexposed to SARS-CoV-2 or in Vero E6 exposed to SARS-CoV-2 and 0.3 mg/mL 2SCP. Slight cytotoxicity was observed at the highest concentration of 0.9 mg/mL 2SCP.

Quantification of viral particles in the cell culture medium by RT-PCR 3 days after SARS-CoV-2 infection confirmed the virucidal activity of 2SCP at 0.3 mg/mL and 0.9 mg/mL (Table 1). Vero E6 cells unexposed to SARS-CoV-2 were clearly negative for infection, while cells exposed to SARS-CoV-2 but unexposed to 2SCP were positive.

Rapid antigen assessment was also performed simultaneously (Table 1) as a rapid positive control of infection. The results were consistent to the viral quantification assays.

4. Conclusions

First, we have confirmed that the disinfectant 2SCP (from 0.03 to 0.9 mg/mL) was not cytotoxic to the Vero E6 cell line (data not shown). We then observed the virucidal activity against SARS-CoV-2 of 2SCP at 0.3 mg/mL and 0.9 mg/mL. This observation shows the real disinfectant action of 2SCP against SARS-CoV-2.

The second objective of this study would be to evaluate how long 2SCP can maintain its anti-SARS-CoV-2 virucidal activity on surfaces treated with this product. Indeed, the persistence of virucidal activity is a very important characteristic to differentiate the disinfectants on the market for efficient disinfection management.

Funding

None.

Competing interests

None declared.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.01.006.

References

[1] Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges. Int J Antimicrob Agents 2020;55:105924.
[2] World Health Organization (WHO). *Weekly epidemiological update on COVID-19*. https://www.who.int/publications/m.
[3] Tang S, Mao Y, Jones RM, Tan Q, Ji JS, Li N, et al. Aerosol transmission of SARS–CoV-2? Evidence, prevention and control. Environ Int 2020;144:106039.
[4] van Doremalen N, Bushmaker T, Morris DH, Holbrook MG, Gamble A, Williamson BN, et al. Aerosol and surface stability of SARS-CoV-2 as compared with SARS-CoV-1. N Engl J Med 2020;382:1564–7.
[5] Murbe D, Kriegel M, Lange J, Schumann L, Hartmann A, Fleischer M. Aerosol emission of adolescents voices during speaking, singing and shouting. PLoS One 2021;16:e0246819.
[6] Marquès M, Domingo JL. Contamination of inert surfaces by SARS-CoV-2: persistence, stability and infectivity. A review. Environ Res 2021;193:110559.
[7] Cigler AJ, Kaduk JA. Dilithium (citrate) crystals and their relatives. Acta Crystallogr C Struct Chem 2018;74:1160–70.
[8] Harcourt J, Tamin A, Lu X, Kamili S, Sakthivel SK, Murray J, et al. Severe acute respiratory syndrome coronavirus 2 from patient with coronavirus disease, United States. Emerg Infect Dis 2020;26:1266–73.