



Chlorine dioxide is a more potent antiviral agent against SARS-CoV-2 than sodium hypochlorite

N. Hatanaka^{a,b,c}, B. Xu^a, M. Yasugi^{a,b,c}, H. Morino^d, H. Tagishi^d, T. Miura^d, T. Shibata^d, S. Yamasaki^{a,b,c,*}

^a Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka, Japan

^b Asian Health Science Research Institute, Osaka Prefecture University, Osaka, Japan

^c Osaka International Research Center for Infectious Diseases, Osaka Prefecture University, Osaka, Japan

^d Research and Development Center, Taiko Pharmaceutical Co. Ltd, Kyoto, Japan

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SUMMARY

Background: A new coronavirus (SARS-CoV-2) abruptly emerged in Wuhan, China, in 2019 and rapidly spread globally to cause the COVID-19 pandemic.

Aim: To examine the anti-SARS-CoV-2 activity of the potent disinfectant Cleverin, the major disinfecting component of which is chlorine dioxide (ClO₂); and to compare the results with that of sodium hypochlorite in the presence or absence of 0.5% or 1.0% foetal bovine serum (FBS).

Methods: Concentrated SARS-CoV-2 viruses were treated with various concentrations of ClO₂ and sodium hypochlorite and 50% tissue culture infective dose was calculated to evaluate the antiviral activity of each chemical.

Findings: When SARS-CoV-2 viruses were treated with 0.8 ppm ClO₂ or sodium hypochlorite, viral titre was decreased only by 1 log₁₀ TCID₅₀/mL in 3 min. However, the viral titre was decreased by more than 4 log₁₀ TCID₅₀/mL when treated with 80 ppm of each chemical for 10 s regardless of presence or absence of FBS. It should be emphasized that treatment with 24 ppm of ClO₂ inactivated more than 99.99% SARS-CoV-2 within 10 s or 99.99% SARS-CoV-2 in 1 min in the presence of 0.5% or 1.0% FBS, respectively. By contrast, 24 ppm of sodium hypochlorite inactivated only 99% or 90% SARS-CoV-2 in 3 min under similar conditions. Notably, except for ClO₂, the other components of Cleverin such as sodium chlorite, decaglycerol monolaurate, and silicone showed no significant antiviral activity.

Conclusion: Altogether, the results strongly suggest that although ClO₂ and sodium hypochlorite are strong antiviral agents in absence of organic matter but in presence of organic matter, ClO₂ is a more potent antiviral agent against SARS-CoV-2 than sodium hypochlorite.

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* Corresponding author. Address: Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58, Rinku ourai-kita, Izumisano, Osaka 598-8531, Japan. Tel.: +81-72-463-5653.

E-mail address: shinji@vet.osakafu-u.ac.jp (S. Yamasaki).

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Introduction

In December 2019, a new coronavirus suddenly emerged in Wuhan, China. This virus was isolated from patients with

pneumonia and named as 'severe acute respiratory syndrome coronavirus 2' (SARS-CoV-2) [1]. The disease caused by this virus was termed COVID-19. SARS-CoV-2 quickly spread all over the world, and on March 11th, 2020, the World Health Organization (WHO) declared COVID-19 a pandemic [2]. By June 30th, 2021, confirmed cases of, and deaths from, COVID-19 were >180 million, and almost four million, respectively; the disease remains a significant threat to mankind [3].

SARS-CoV-2 is transmitted through aerosols, droplets, and fomites [4]. Therefore, it is important to inactivate virus particles on surfaces contaminated by droplets from infected persons. WHO has recommended use of 70% ethanol or 1000 ppm of sodium hypochlorite (NaClO) for disinfection [5]. NaClO is likely the most commonly used surface disinfectant in the world. However, it is well known that antiviral activity of NaClO is readily decreased by the presence of organic matter such as saliva and blood [6].

Several studies have shown that 10 ppm of chlorine dioxide (ClO₂) inactivated 99.999% of influenza A virus in the presence of 1% foetal bovine serum (FBS), whereas 100 ppm of NaClO, which is 10-fold higher concentration than that of ClO₂, was required to inactivate the same degree of influenza A virus under the same conditions [7]. However, there is no evidence as to whether ClO₂ inactivates SARS-CoV-2. If it does, there is no information available on how much ClO₂, and for how long an exposure, is needed to inactivate SARS-CoV-2. Therefore, this study examined the antiviral activity of ClO₂ against SARS-CoV-2 under various conditions in terms of its concentration, incubation time, and absence or presence of organic matter. The results were compared with those of NaClO.

Methods

Cell culture

VeroE6/TMPRSS2 cells were used for cultivation of SARS-CoV-2; the cells were incubated at 37°C in a 5% CO₂ humidified incubator (ESPEC Corp., Osaka, Japan) [8]. VeroE6/TMPRSS2 cell line was purchased from the Japanese Collection of Research Bioresources (Osaka, Japan). The cells were cultured in Dulbecco's modified Eagle medium, low glucose, pyruvate (DMEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 5% heat-inactivated foetal bovine serum (FBS; Thermo Fisher) and 1 mg/mL G418 (Nacalai Tesque, Inc., Kyoto, Japan).

Virus preparation

One hundred and forty thousand VeroE6/TMPRSS2 cells were cultured in a 25 cm² cell culture flask at 37°C for 16 h in a 5% CO₂ humidified incubator. The cells were infected with MOI = 0.001 of SARS-CoV-2 JPN/TY/WK-521 strain and incubated at 37°C in DMEM supplemented with 2% heat-inactivated FBS (Thermo Fisher) and 1 mg/mL of G418 (Nacalai Tesque) for 48 h. After a cytopathic effect had been observed, the spent culture medium was harvested and centrifuged at 1600 g for 5 min, and supernatant fraction containing virus particles was collected. One gram of polyethylene glycol 6000 and 233 mg of NaCl (Nacalai Tesque) were then added to 10 mL of collected virus solution and incubated at 4°C for 16 h. The virus solution was centrifuged at 20,000 g at 4°C for 10 min, the supernatant

was discarded, and the pellet was suspended in 1 mL of PBS (–) at pH 7.4. Experiments with the live SARS-CoV-2 virus were conducted at the bio-safety level 3 laboratory in Osaka Prefecture University after obtaining the permission from the bio-risk committee of Osaka Prefecture University.

Antiviral activity of Cleverin, sodium hypochlorite, pure ClO₂, sodium chlorite and Cleverin without ClO₂ and sodium chlorite

Cleverin (Taiko Pharmaceutical Co. Ltd, Osaka, Japan) is a mixture of 500 ppm ClO₂, 17,900 ppm sodium chlorite, 3300 ppm decaglycerol monolaurate, and 80 ppm silicone. Sixty microlitres of concentrated SARS-CoV-2 in PBS supplemented with 0, 2.5, or 5.0% FBS were mixed with 240 µL of diluted Cleverin containing several concentrations (1, 10, 30, or 100 ppm) of ClO₂ or 1, 10, 30 or 100 ppm sodium hypochlorite (Wako pure chemical industries Ltd, Osaka, Japan), 10 or 100 ppm of pure ClO₂ in which ClO₂ gas is dissolved in ultrapure water, 6000 ppm of sodium chlorite or Cleverin without ClO₂ and sodium chlorite (containing 660 ppm decaglycerol monolaurate and 16 ppm silicone). The mixture was then incubated at room temperature (25°C) for 10 or 30 s, and 1 or 3 min. After incubation, 540 µL of 5 mM sodium thiosulfate (Wako) was immediately added to 60 µL of the mixtures to neutralize the remaining activity of each chemical, after which 60 µL of 10 × DMEM (Nissui Pharmaceutical Co. Ltd, Tokyo, Japan), 12 µL of FBS and 12 µL of 50 mg/mL G418 disulfate aqueous solution were added. Subsequently, ten-fold dilution was carried out with DMEM supplemented with 2% FBS and 1 mg/mL G418 and titration was done as described below.

Titration of virus

Approximately 2.5×10^4 cells/100 µL of VeroE6/TMPRSS2 cells were seeded in a 96-well plate and cultured at 37°C for 16 h in the medium. Culture medium was removed and 100 µL of 10-fold serially diluted viral solution in DMEM supplemented with 2% FBS and 1 mg/mL G418 was added. The infected VeroE6/TMPRSS2 cells were cultured at 37°C for 72 h. Cells were fixed with methanol (Nacalai Tesque) and stained with 0.5% Crystal Violet. Fifty percent tissue culture infective dose per mL (TCID₅₀/mL) was calculated. The detection limit was confirmed to be $\leq 2.2 \log_{10}$ TCID₅₀/mL.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2016 (Microsoft, Redmond, WA, USA). Error bars show standard deviations. *P*-values were calculated with Student's *t*-test using paired, two-tailed distribution. *P* < 0.05 was considered statistically significant.

Results

To examine and compare the antiviral activity of ClO₂ in Cleverin against SARS-CoV-2 in the absence or presence of organic matter with that of sodium hypochlorite, the concentrated viruses suspended in PBS were treated with several concentrations of ClO₂ or sodium hypochlorite in the absence or presence of FBS. Antiviral activity of both chemicals was

observed in a concentration-dependent manner (Figure 1). When the viruses were treated with 80 ppm ClO₂ or sodium hypochlorite as a final concentration regardless of presence of organic matter, the viral titre was decreased to the detection limit ($\leq 2.2 \log_{10}$ TCID₅₀/mL) even in 10 s (Figure 1). When the

viruses were treated with 8 ppm ClO₂, the viral titre was decreased by 3–4 log₁₀ TCID₅₀ in the absence of organic matter (Figure 1A). In the case of 8 ppm sodium hypochlorite, the viral titre was decreased by 2–3 log₁₀ TCID₅₀ under the same conditions. However, there was no significant difference in viral

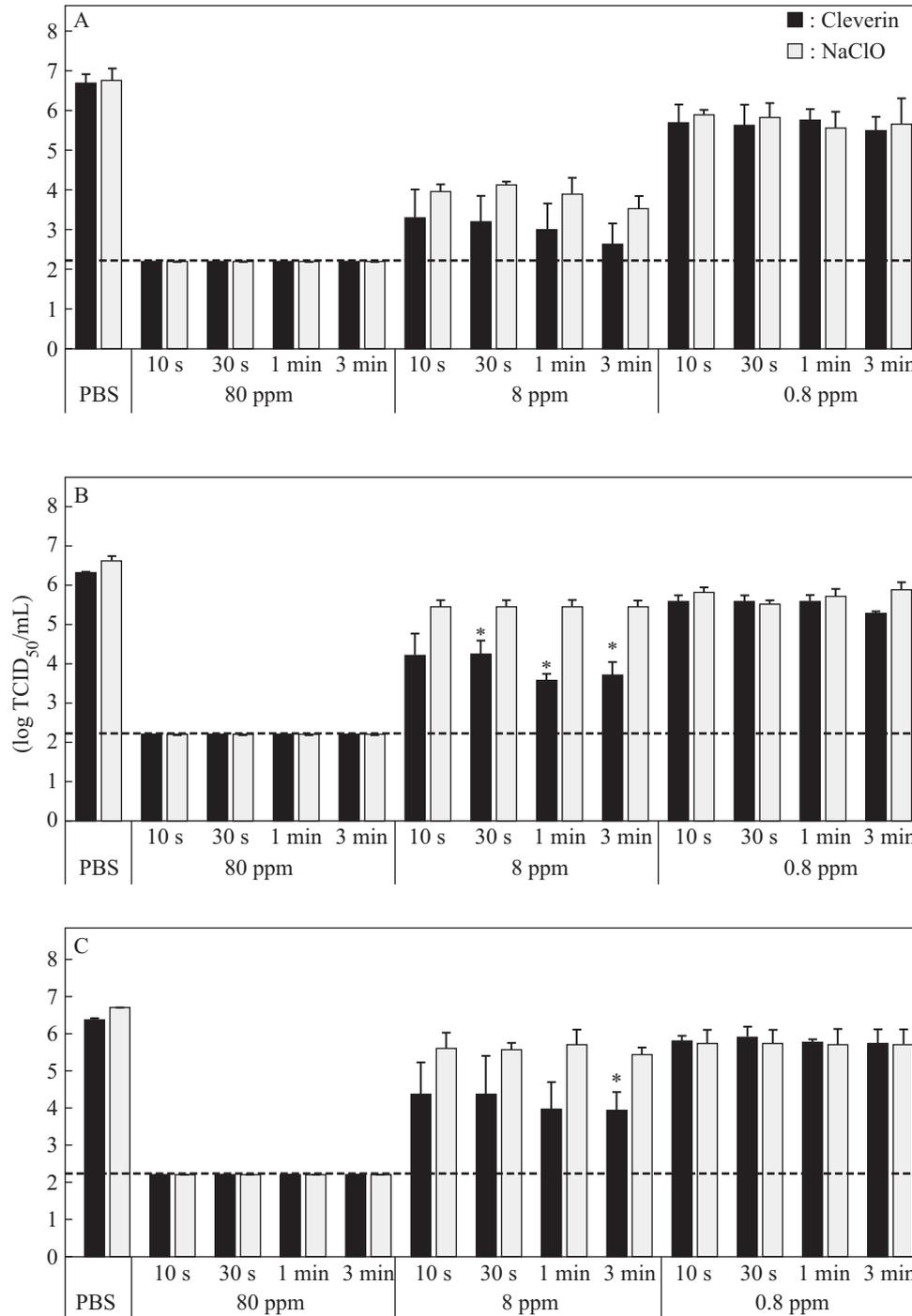


Figure 1. Antiviral activity of 0.8, 8, and 80 ppm of chlorine dioxide and sodium hypochlorite against SARS-CoV-2. Concentrated SARS-CoV-2 suspended in phosphate-buffered saline (PBS) without foetal bovine serum (FBS) (A), PBS with 0.5% FBS (B) or PBS with 1% FBS (C) was incubated with 80, 8, or 0.8 ppm of chlorine dioxide (ClO₂) or sodium hypochlorite (NaClO) for 10 s, 30 s, 1 min, or 3 min as indicated. Viral titre was determined by measurement of 50% tissue culture infective dose per mL (TCID₅₀/mL). All data represent the means \pm SD from three independent experiments. Dashed line indicates the detection limit for each experiment. *Viral titre is significantly different between ClO₂ and NaClO treatments.

titres between viruses treated with ClO_2 and sodium hypochlorite (Figure 1A). In fact, 0.8 ppm of both chemicals decreased the viral titre by only 1 \log_{10} TCID₅₀ regardless of presence of organic matter.

When the viruses were treated with 8 ppm ClO_2 in the presence of 0.5% FBS as final concentration, the viral titre was decreased by 2–3 \log_{10} TCID₅₀ whereas the titre was decreased by around 1 \log_{10} TCID₅₀ when treated with 8 ppm sodium hypochlorite under the same conditions (Figure 1B). Furthermore, when the viruses were treated with 8 ppm ClO_2 for 30 s, 1 or 3 min in the presence of 0.5% FBS, the viral titre was significantly lower than when treated with 8 ppm sodium hypochlorite (Figure 1B). Similarly, when the viruses were treated with 8 ppm ClO_2 in the presence of 1% FBS, the viral titre decreased by around 2–3 \log_{10} TCID₅₀ whereas it was decreased by only 1 \log_{10} TCID₅₀ when treated with 8 ppm sodium hypochlorite under the same conditions (Figure 1C). The viral titre was significantly lower when treated with 8 ppm ClO_2 for 3 min compared with 8 ppm sodium hypochlorite (Figure 1C).

Since 80 ppm ClO_2 inactivated the viruses completely but 8 ppm ClO_2 inactivated partially, we further examined antiviral activity of 24 ppm ClO_2 and the result was compared with that of sodium hypochlorite. When the viruses were treated with 24 ppm ClO_2 , the viral titre was decreased to below the detection limit ($\leq 2.2 \log_{10}$ TCID₅₀/mL) even in the presence of 0.5% FBS in 10 s (Figure 2); however, the same concentration of sodium hypochlorite under the same conditions was unable to inactivate the viruses completely. Furthermore, when the viruses were treated with 24 ppm ClO_2 in the presence of 1.0% FBS, the viral titre was decreased by about 4 \log_{10} TCID₅₀ even in 10 s and was significantly lower than that treated with the same concentration of sodium hypochlorite under the same conditions (Figure 2).

Cleverin is not pure ClO_2 : apart from ClO_2 it also contains sodium chlorite, decaglycerol monolaurate, and silicone. Therefore, to confirm whether the antiviral activity of Cleverin is ClO_2 dependent, the antiviral activity of pure ClO_2 , sodium chlorite, and mixture of decaglycerol monolaurate and silicone was further examined separately (Figure 3). When viruses were treated with 8 ppm of pure ClO_2 the viral titre was decreased by $>4 \log_{10}$ TCID₅₀ in the absence of FBS (Figure 3A) while the viral titre was decreased by about 2 \log_{10} TCID₅₀ only in 10 s in the presence of FBS (Figure 3B). However, when 80 ppm of pure ClO_2 was applied, the viral titre was decreased to below the detection limit ($\leq 2.2 \log_{10}$ TCID₅₀/mL) even in the presence of 1.0% FBS in 10 s (Figure 3B). On the other hand, 4800 ppm sodium chlorite was hardly able to decrease the viral titre and Cleverin devoid of 80 ppm ClO_2 and 4800 ppm sodium chlorite but containing 528 ppm decaglycerol monolaurate, and 12.8 ppm silicone decreased viral titre only by 1 \log_{10} TCID₅₀ against SARS-CoV-2 regardless of presence or absence of FBS (Figure 3). Taken together, these data clearly indicate that the main component effecting the inactivation of SARS-CoV-2 is ClO_2 but not the other components in Cleverin.

Discussion

COVID-19 is an emerging disease and to date there is no highly effective treatment, although a few medicines have been found to improve clinical outcomes in large trials [9]. Rapid development and production of vaccines in several countries has permitted large-scale vaccination of subjects in many, mostly developed, countries [10–12]. However, the majority of the world's population remains unvaccinated, and it is uncertain how effective vaccination will be in the longer term as immunity wanes, and new SARS-CoV-2 variants emerge

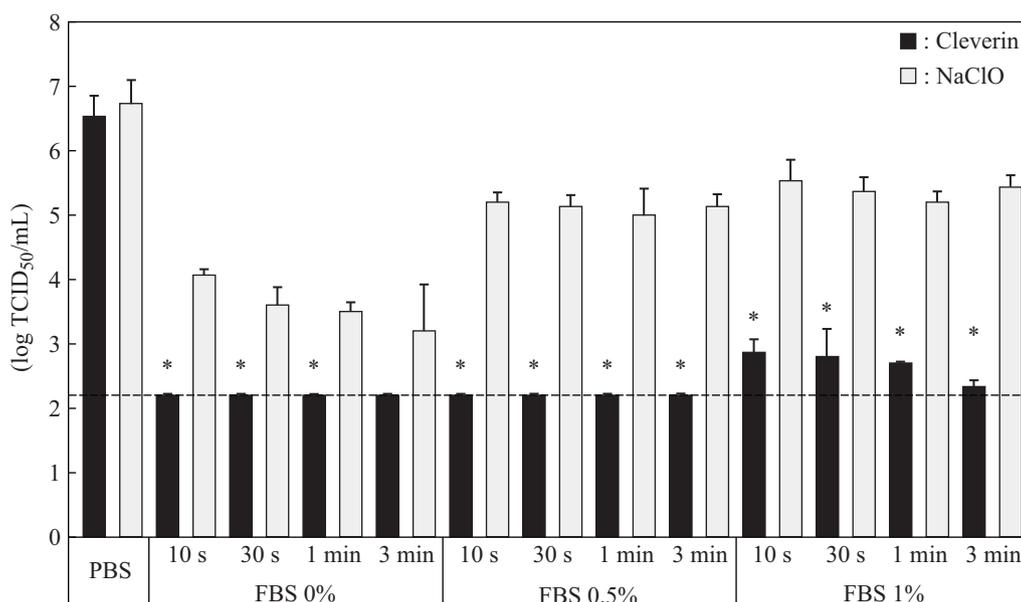


Figure 2. Antiviral activity of 24 ppm chlorine dioxide or sodium hypochlorite against SARS-CoV-2. Concentrated SARS-CoV-2 suspended in phosphate-buffered saline (PBS) without foetal bovine serum (FBS), PBS with 0.5% FBS, or PBS with 1% FBS was incubated with 24 ppm chlorine dioxide (ClO_2) or sodium hypochlorite (NaClO) for 10 s, 30 s, 1 min, or 3 min as indicated. Viral titre was determined by 50% tissue culture infective dose per mL (TCID₅₀/mL). All data represent the means \pm SD from three independent experiments. Dashed line indicates the detection limit for each experiment. *Viral titre is statistically different between ClO_2 and NaClO treatments.

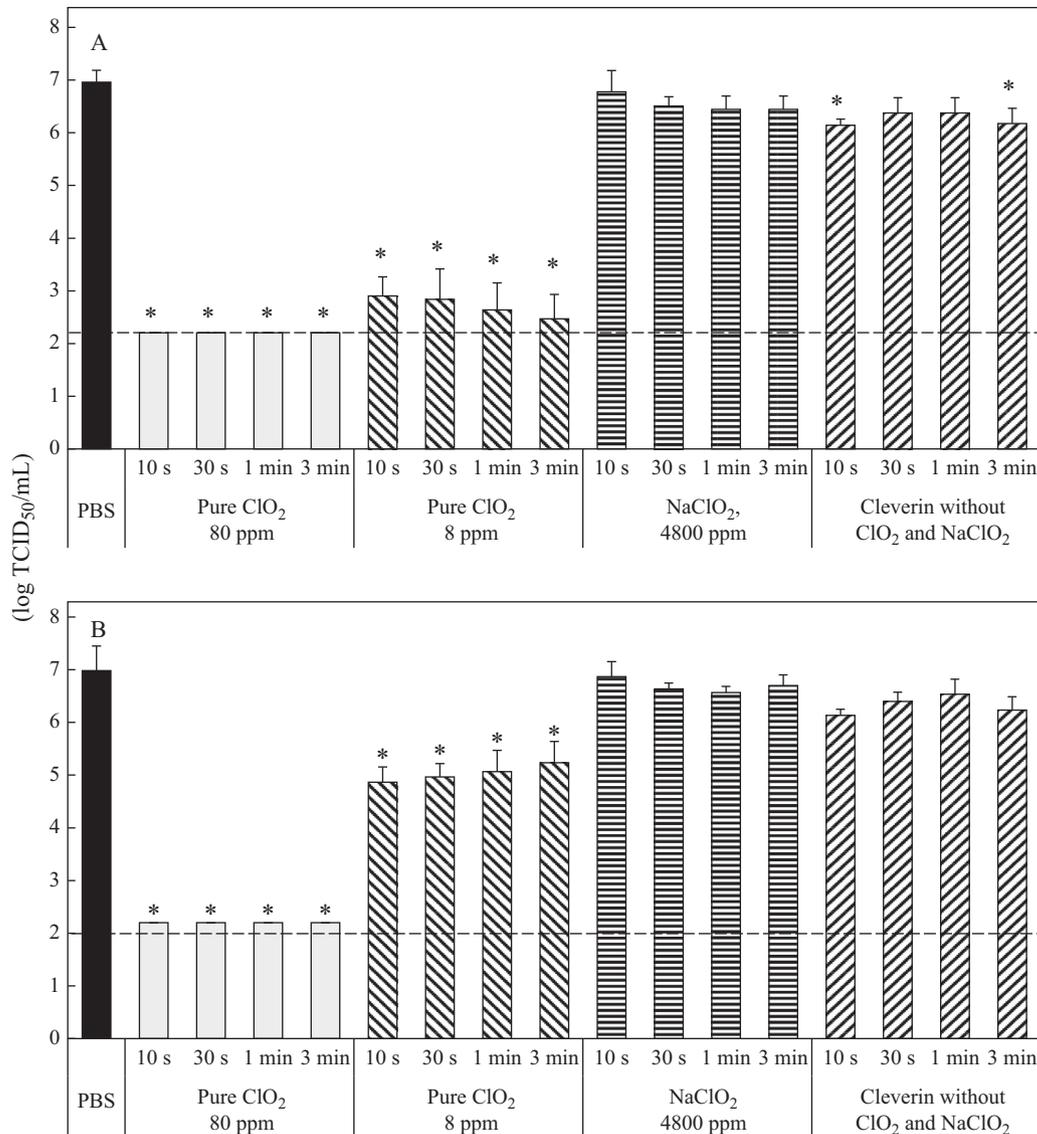


Figure 3. Antiviral activity of 80 and 8 ppm pure chlorine dioxide, 4800 ppm of sodium chlorite and Cleverin without chlorine dioxide and sodium chlorite against SARS-CoV-2. Concentrated SARS-CoV-2, which was suspended in phosphate-buffered saline (PBS) without foetal bovine serum (FBS) (A) and with 1% FBS (B), respectively, was incubated with 80 or 8 ppm pure chlorine dioxide (ClO₂), 4800 ppm sodium chlorite (NaClO₂) or Cleverin without ClO₂ and NaClO₂ but containing 528 ppm of decaglycerol monolaurate and 12.8 ppm silicone for 10 s, 30 s, 1 min or 3 min. Viral titre was determined by measurement of 50% tissue culture infective dose per mL (TCID₅₀/mL). All data represent the means ± SD from three independent experiments. Dashed line indicates detection limit for each test. *Viral titre is statistically different between PBS and each test.

[13,14]. Thus disinfectants active against SARS-CoV-2 will remain a cornerstone of control of COVID-19 globally. Sodium hypochlorite is one of the most popular disinfectants in clinical settings. However, sodium hypochlorite has some disadvantages; for example, it may produce more trihalomethane and it exhibits weak antimicrobial activity in presence of organic matters compared to ClO₂ [7,15,16]. Therefore, our study tested antiviral activity of ClO₂, which is a major component of Cleverin, against SARS-CoV-2.

Eighty parts per million of both ClO₂ and sodium hypochlorite inactivated 6–7 log₁₀ TCID₅₀/mL of SARS-CoV-2 to below the detection limit (≤ 2.2 log₁₀ TCID₅₀/mL) in just 10 s even in the presence of 1.0% FBS (Figure 1C), indicating that

both ClO₂ and sodium hypochlorite may be useful disinfectants against SARS-CoV-2 in saliva. Saliva is the most important infection source and contains ~1.1 mg/mL proteins [17]. In this study, 5.0% FBS was used as highest concentration in virus solution (1.0% FBS as final concentration), which is two times higher in protein concentration than that in saliva.

Further, the study examined the antiviral activity of pure ClO₂, sodium chlorite, and Cleverin without ClO₂ and sodium chlorite independently, since Cleverin, in addition to 100 ppm ClO₂, also contains 6000 ppm sodium chlorite, 660 ppm decaglycerol monolaurate, and 16 ppm silicone. In fact, 60 µL of 100 ppm ClO₂ (80 ppm, a final concentration) showed the same antiviral activity with Cleverin, but 60 µL of 6000 ppm sodium

chlorite (4800 ppm, a final concentration), a precursor of ClO₂, showed no antiviral activity. In addition, 60 µL mixture of 660 ppm decaglycerol monolaurate (528 ppm, a final concentration) and 16 ppm silicone (12.8 ppm, a final concentration) was also tested and showed no significant reduction of viral titre in the presence of 1.0% FBS. However, viral titre was slightly decreased (~1 log₁₀ TCID₅₀ reduction) when FBS was absent (Figure 3). Since decaglycerol monolaurate is a surfactant, this compound might affect the envelope or proteins of SARS-CoV-2 [18]. Nevertheless, we conclude that the antiviral activity of Cleverin is ClO₂ dependent.

When 24 ppm sodium hypochlorite was exposed to the virus, the viral titre decreased by 4 log₁₀ TCID₅₀ in 30 s in the absence of FBS (Figure 2); however, when 0.5% or 1.0% FBS was present, 24 ppm sodium hypochlorite reduced the viral titre by only 2 log₁₀ TCID₅₀ even in 3 min. It should be noted that 24 ppm of ClO₂ reduced the viral titre to below the detection limit (<2.2 log₁₀ TCID₅₀/mL) even in 10 s in the presence of 0.5% FBS and by >4 log₁₀ TCID₅₀ in 30 s in the presence of 1.0% FBS (Figure 2), suggesting that ClO₂ is a much more powerful disinfectant than sodium hypochlorite, especially when organic matter is present in the contaminants. This advantage was also demonstrated by other pathogens such as influenza A virus and multidrug-resistant (MDR) bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), MDR *Pseudomonas aeruginosa* (MDRP) and MDR *Acinetobacter baumannii* (MDRA) [7,16]. ClO₂ has been shown to have 10-fold higher antiviral activity than sodium hypochlorite against influenza A virus in the presence of 1% FBS [7]. When the virus was exposed to ClO₂, their major surface glycoproteins such as haemagglutinin and neuraminidase, responsible for the viral infection to and release from cells, were degraded [19]. This could be the mechanism by which ClO₂ inactivates virus infectivity. Other examples are MDR bacteria: ClO₂ had more effective antimicrobial activity than sodium hypochlorite against MRSA, MDRP, and MDRA in the presence of 3.0% BSA and 3.0% sheep erythrocyte [16]. In addition, ClO₂ is less toxic than sodium hypochlorite because of production of the carcinogen trihalomethane by the latter [15]. Taken together, these observations might point to ClO₂ being more useful than sodium hypochlorite to inactivate SARS-CoV-2, especially in clinical material.

Antiviral activity of ClO₂ against SARS-CoV-2 was expected because it has been recently demonstrated that ClO₂ may inhibit binding of recombinant spike protein of SARS-CoV-2 to its receptor molecule, angiotensin-converting enzyme 2 (ACE-2) [20]. It has also been demonstrated that ClO₂ can denature proteins by oxidative modification of tryptophan and tyrosine residues [21]. Since tyrosine at position 453, which is located in the receptor-binding domain of the spike protein, forms a hydrogen bond with histidine at position 34, located in an alpha 1 helix of the ACE-2 protein, oxidative modification of the tyrosine by ClO₂ could reduce the infectivity of the virus [22]. Indeed, infectivity of SARS-CoV-2 to the VeroE6/TMPRSS2 cells has been demonstrated to be significantly reduced by ClO₂ in this study.

Various mutant strains of SARS-CoV-2 have emerged in the UK, South Africa, and Brazil, and have spread in many countries throughout the globe [13,14]. These strains have a mutation in asparagine at position 501 to tyrosine (N501Y) in the spike protein of SARS-CoV-2, which is also responsible for receptor binding. Two new SARS-CoV-2 lineages (N501Y) reported in the

UK are more transmissible than the 501N lineage. Since ClO₂ targets tryptophan and tyrosine in proteins, ClO₂ might inactivate these novel mutants efficiently. Currently experiments are under way in our laboratory to verify whether ClO₂ can inactivate these mutant viral strains as effectively as in the case of the wild-type strain.

In conclusion, our data indicate that ClO₂ is a more effective disinfectant against SARS-CoV-2 than NaClO in presence of organic matter. Use of 24 ppm ClO₂ inactivated 6.5 log₁₀ TCID₅₀/mL of SARS-CoV-2 to below the detection limit even in the presence of 0.5% FBS in 10 s. Therefore, ClO₂ is a powerful disinfectant against SARS-CoV-2 and it may be useful for the reduction of SARS-CoV-2 infection.

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Conflict of interest statement

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References

- [1] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med* 2020;382:727–33.
- [2] World Health Organization. Archived: WHO timeline-COVID-19. Available at: <https://www.who.int/news/item/27-04-2020-who-timeline-covid-19> [last accessed June 2021].
- [3] World Health Organization. WHO coronavirus disease (COVID-19) dashboard. Available at: <https://covid19.who.int/> [last accessed June 2021].
- [4] Kumar GD, Mishra A, Dunn L, Townsend A, Oguadinma IC, Bright KR, et al. Biocides and novel antimicrobial agents for the mitigation of coronaviruses. *Front Microbiol* 2020;11:1351.
- [5] World Health Organization. Cleaning and disinfection of environmental surfaces in the context of COVID-19. Available at: <https://www.who.int/publications/i/item/cleaning-and-disinfection-of-environmental-surfaces-in-the-context-of-covid-19> [last accessed September 2021].
- [6] Fukuzaki S. Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. *Biocontrol Sci* 2006;11:147–57.
- [7] Miura T, Shibata T. Antiviral effect of chlorine dioxide against influenza virus and its application for infection control. *Open Antimicrob Agents J* 2010;2:71–8.
- [8] Matsuyama S, Nao N, Shiota K, Kawase M, Saito S, Takayama I, et al. Enhanced isolation of SARS-CoV-2 by TMPRSS2 expressing cells. *Proc Natl Acad Sci USA* 2020;117:7001–3.
- [9] Jean SS, Lee PI, Hsueh PR. Treatment options for COVID-19: the reality and challenges. *J Microbiol Immunol Infect* 2020;53:436–43.
- [10] Anderson EJ, Roupael NG, Widge AT, Jackson LA, Roberts PC, Makhene M, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1237 vaccine in older adults. *N Engl J Med* 2020;383:2427–38.

- [11] Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M, et al. COVID-19 vaccine BNT162b1 elicits human antibody and T_H1 T cell responses. *Nature* 2020;586:594–9.
- [12] Ramasamy MN, Minassian AM, Ewer KJ, Flaxman AL, Folegatti PM, Owens DR, et al. Safety and immunogenicity of ChAdOx1 nCoV-19 vaccine administered in a prime-boost regimen in young and old adults (COV002): a single-blind, randomized, controlled, phase 2/3 trial. *Lancet* 2021;396(10267):1979–93.
- [13] Leung K, Shum MHH, Leung GM, Lam TT, Wu JT. Early transmissibility assessment of the N501Y mutant strains of SARS-CoV-2 in the United Kingdom, October to November 2020. *Euro Surveill* 2021;26:2002106.
- [14] Makoni M. South Africa responds to new SARS-CoV-2 variant. *Lancet* 2021;397:267.
- [15] Sorlini S, Collivignarelli C. Trihalomethane formation during chemical oxidation with chlorine, chlorine dioxide and ozone of ten Italian natural waters. *Desalination* 2005;176:103–11.
- [16] Hinenoya A, Awasthi SP, Yasuda N, Shima A, Morino H, Koizumi T, et al. Chlorine dioxide is a better disinfectant than sodium hypochlorite against multi-drug resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Jpn J Infect Dis* 2015;68:276–9.
- [17] Jenzano JW, Hogan SL, Noyes CM, Featherstone GL, Lundblad RL, et al. Comparison of five techniques for the determination of protein content in mixed human saliva. *Anal Biochem* 1986;159:370–6.
- [18] Simon M, Veit M, Ostrrieder K, Gradzielski M. Surfactants – compounds for inactivation of SARS-CoV-2 and other enveloped viruses. *Curr Opin Colloid Interface Sci* 2021;55:101409.
- [19] Shibata T, Ogata N. Protective effect of low-concentration chlorine dioxide gas against influenza A virus infection. *J Gen Virol* 2008;89:60–7.
- [20] Ogata N, Miura T. Inhibition of the binding of spike protein of SARS-CoV-2 coronavirus to human angiotensin-converting enzyme 2 by chlorine dioxide. *Ann Pharmacol Pharmaceut* 2020;5:1195.
- [21] Ogata N. Denaturation of protein by chlorine dioxide: oxidative modification of tryptophan and tyrosine residues. *Biochemistry* 2007;46:4898–911.
- [22] Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 2020;367:1444–8.