論　文　内　容　要　旨

Virus purification highlights the high susceptibility of SARS-CoV-2 to a chlorine-based disinfectant, chlorous acid

(SARS-CoV-2 のウイルス精製により、塩素系消毒剤である亜塩素酸に対する高い感受性が明らかになった)

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Introduction: The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has posed a significant global health challenge, primarily due to its high transmission rate and potential to cause severe pneumonia and death in humans. The virus particles are stable on smooth surfaces for extended periods, allowing easy human-to-human transmission. Effective disinfectants are crucial in reducing the spread of the virus, especially in public and medical facilities. Although potent against various microbes, chlorine-based disinfectants can be unstable, particularly when exposed to organic matter. Chlorous acid water ($HClO_2$) and sodium hypochlorite water (NaOCl) have shown promise in inactivating SARS-CoV-2 due to their potent oxidizing properties. WHO has recommended that sodium hypochlorite be used at 1000 ppm for general surface eradication of SARS-CoV-2 and 5000 ppm for contaminated surfaces with large blood and body fluids spills. The Japan Ministry of Health, Labor and Welfare also recommends using 500 ppm. However, these high concentrations are concerning as they may release significant amounts of chlorine gas into the air. The Japan Ministry of Health, Labor and Welfare recommends using chlorous acid at 25 ppm or more on contaminated surfaces. This study investigates the antiviral efficacy of chlorous acid water against SARS-CoV-2, including its action against different variants, assesses its stability under various protein load conditions, and compares it to sodium hypochlorite's antiviral potency and stability.

Methods: The study employed chlorous acid water and sodium hypochlorite solutions to test their virucidal effects on SARS-CoV-2. The solutions' free available chlorine (FAC) levels were measured using the diethyl-p-phenylene diamine (DPD) method. SARS-CoV-2 strains, including the variant D614G (B.1.1), Delta (AY.29), and Omicron (BA.1.1), were cultured in VeroE6/TMPRSS2 cells. The viruses were purified using polyethylene glycol (PEG) precipitation and ultracentrifugation to remove extraneous proteins and other substances. The purified and unpurified SARS-CoV-2 viruses were exposed to the disinfectants in the presence or absence of different protein loads and were allowed to incubate. The virus titers were assessed using the $TCID_{50}$ method, and the inactivation kinetics were modeled using the Chick-Watson equation. The protein loads in this experiment included 0.03% bovine serum albumin (0.03% BSA), 5% fetal bovine serum (5% FBS), artificial saliva, 0.3% sheep red blood cells+0.3% bovine serum albumin (0.3% SRBC + 0.3% BSA), and 0.5% polypeptone. The effects of protein loads, mimicking real-world contamination scenarios, on the antiviral efficacy of chlorous acid water and sodium hypochlorite water were evaluated.

Results: Chlorous acid water demonstrated significant SARS-CoV-2 inactivation, with FAC levels correlating with reduced virus infectivity. For the D614G variant, the IC_{99} value was

9.9 ppm. Delta and Omicron variants showed similar susceptibility with IC_{99} values of 12 ppm and 5.3 ppm, respectively. Purified virus particles exhibited much higher sensitivity to chlorous acid water, with IC_{99} values dropping to 0.41 ppm and 0.74 ppm for PEG-purified and ultracentrifuge-purified viruses, respectively. The purified virus also exhibited high sensitivity to sodium hypochlorite water with an IC_{99} value of 0.54 ppm, but the disinfectant had a lower effect on the unpurified virus, which had an IC_{99} value of 97 ppm. However, protein loads reduced the inactivation efficacy of both disinfectants, more significantly in sodium hypochlorite water than in chlorous acid water. For instance, the IC_{99} for chlorous acid water in the presence of 0.3% sheep red blood cells + 0.3% bovine serum albumin, artificial saliva, 5% fetal bovine serum, and 0.5% polypeptone increased to 4.7 ppm, 21 ppm, 41 ppm, and 59 ppm, respectively. The IC_{99} for sodium hypochlorite water in the presence of 0.3% sheep red blood cells + 0.3% bovine serum albumin, artificial saliva, 5% fetal bovine serum, and 0.5% polypeptone increased to 302 ppm, 91 ppm, 58 ppm, and 499 ppm, respectively.

Discussion: The study highlights the potent antiviral activity of chlorous acid water against SARS-CoV-2, including its variants. The enhanced efficacy observed with purified viruses and reduced efficacy with various organic matters suggests that extraneous proteins and other environmental substances can reduce the effectiveness of chlorine-based disinfectants. However, chlorous acid water is more resistant to the inhibiting effects of protein loads, having 2-110 times higher potency in various protein-rich environments than sodium hypochlorite water. Chlorous acid water is more stable and requires significantly lower concentrations for its virucidal effect, even in organic-rich environments, than sodium hypochlorite water, making them less likely to release chlorine gas. These findings emphasize the importance of considering environmental factors when employing chlorine-based disinfectants for virus control, particularly in settings with high organic matter contamination.

Conclusion: Under controlled conditions, chlorous acid water is a highly effective disinfectant for inactivating SARS-CoV-2, including its variants, and may be safer for the environment than sodium hypochlorite water. The study provides valuable insights into optimizing the use of chlorine-based disinfectants to manage the spread of SARS-CoV-2 in diverse settings. Further research is recommended to explore chlorous acid water's stability and long-term efficacy in real-world settings. Additionally, studies on combining chlorous acid water with other disinfection strategies could provide a comprehensive approach to virus control.