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Effect of Chlorine Dioxide Gas on the Binding of SARS-CoV-2 Coronavirus Spike Protein to a Human Receptor

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Abstract

Aim: COVID-19, caused by a new coronavirus SARS-CoV-2, remains a serious threat to human health despite the development of several vaccines. Thus, a safe and effective disinfection system is urgently needed to prevent the further spread of COVID-19 cases in crowded and/or poorly ventilated areas. Chlorine dioxide (CD) gas is known to disinfect indoor air in hospital and healthcare settings. The aim of this work was to perform *in vitro* experiments to investigate whether CD gas could be used to block the capacity of SARS-CoV-2 to bind its receptor protein.

Materials and Methods: An assay kit was used to measure binding of the spike protein of SARS-CoV-2 virus to the human receptor protein, angiotensin-converting enzyme 2. We first measured concentration of CD that can dissolve in water. We next performed assays using spike protein that had been exposed to various concentrations of CD gas.

Results: CD gas dissolves in water in a concentration- and time-dependent manner to reach near saturation after 30 min. Almost complete inactivation of the spike protein-receptor protein binding was found at about 3 parts per million (ppmv) (volume/volume ratio) of gas with a half-Inhibition concentration (IC_{50}) of 1.2 ppmv after 30 min at 25°C.

Conclusion: CD gas can dissolve in water in a concentration- and time-dependent manner, and it can be used as an effective disinfectant against SARS-CoV-2. The mechanism of disinfection involves inhibition of binding of the virus spike protein to human receptor protein in an aqueous phase.

Keywords: Chlorine dioxide; COVID-19; SARS-CoV-2; Virus; Disinfection; IC_{50}

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Introduction

The COVID-19 pandemic began in Wuhan, China in late 2019, and then spread rapidly across the entire world [1-3]. The disease is caused by a SARS-CoV-2 virus, which is an enveloped virus with a positive single-stranded RNA genome [3]. The virus has a "spike protein" comprising 1,273 amino acids on its surface that can bind to a human receptor protein, angiotensin-converting enzyme 2 (ACE2), to facilitate entry into the host cell [3]. Although several effective vaccines have been developed against SARS-CoV-2, mostly modified mRNA vaccines [4,5], the COVID-19 pandemic has not yet ended [1]. Oral anti-virus medicines are under development in many countries but, to date, none are commercially available [6].

Chlorine dioxide (CD) is a water-soluble free radical gas with strong oxidation activity [7,8]. The gas has been shown to protect against infection from influenza virus in an experiment using mice and an aerosol suspension of viral particles [9]. CD also inactivates other viruses [10,11], at concentrations low enough to have no discernable adverse effect on human [12,13]. The corresponding mechanism of action of CD is related to the oxidation of tryptophan residues in viral proteins [14,15]. We recently demonstrated that CD dissolved in water can inhibit the binding of SARS-CoV-2 to human ACE2 [16,17]. Moreover, the inhibition of binding of SARS-CoV-2 to human ACE2 was shown to be due to inactivation of the viral spike protein of the original Wuhan strain as well as the subsequent alpha (B.1.1.7) and beta (B.1.351) variants [16,17]. Given that CD gas can prevent viral infection in animal experiments [9], it seems likely that the gas will dissolve in orinasal aerosols expelled from human. We reasoned the resulting dissolved CD may inactivate viral particles in the aerosol. The aim of this study was to investigate whether CD gas dissolved in water can inactivate the capacity of the viral spike protein to bind human ACE2 in the aqueous phase.

Material and Methods

Chemicals and software

A SARS-CoV-2 spike protein-human receptor protein binding assay kit (BPS Bioscience, San Diego, CA, USA) was used that employs the Wuhan strain spike protein. In this kit, purified and histidine tag-labeled human angiotensin-converting enzyme 2 (ACE2) was used as a receptor protein. Data points were fitted by regression analysis to a linear, an exponential increase or an exponential decay curve using GraphPad Prism software (San Diego, CA, USA). Where appropriate the half life was obtained from the exponential curves.

Solubility measurements of CD in water

The amount of dissolved CD at various concentrations of CD gas was determined at 25°C spectrophotometrically from absorption measurements at 358 nm using a molar extinction coefficient of 1570 $L{\cdot}mol^{-1}{\cdot}cm^{-1}$.

Binding assay

The binding assay kit was used according to the protocol outlined by the manufacturer (BPS Bioscience) with some modifications. Specifically, 50 µl of purified water, rather than Immuno Buffer 1 from the kit was aliquoted into each well (depth, 11.25 mm; inner diameter, 6.75 mm) of a flat bottomed 96-well microtiter plate shortly after coating with spike protein. CD gas was generated in a plastic box (dimensions: 246 mm × 175 mm × 174 mm) using a commercial table-top CD-generating canister (Cleverin, 150 g; Taiko Pharmaceutical, Osaka, Japan) (Figure 1).

The rate of release of CD gas was adjusted by covering the top of the canister with aluminum foil and then making a few pinholes in the foil. The 96-well plate containing aliquots of water was placed into the plastic box containing CD gas for 30 min at 25°C unless otherwise indicated (Figure 1). After 30 min, 20 µl of 10 mmol/L $Na_2S_2O_3$ was added to each well to terminate the reaction of CD with the spike protein. Specifically, CD reacts with $Na_2S_2O_3$ to generate ClO_2^-[14]. The extent of spike protein-ACE2 binding was measured by chemiluminescence generated by horseradish peroxidase using a luminometer (model SH-9000; Corona Electric, Hitachinaka, Japan). CD gas concentration in the plastic box was measured using a CD detector (23 M tube; GASTEC, Ayase, Japan). The concentration of CD dissolved in the 50 µl aliquot of water could also be measured using a 96-well microtiter plate without spike protein coating.

Results

CD gas dissolved in the water in a concentration- and time-dependent manner (Figure 2). As shown in Figure 2A, the amount of CD gas dissolved in the purified water increased almost linearly upon the increase of CD partial pressure.

Thus, the amount of dissolved CD in the water was dependent on the partial pressure of CD gas, and therefore obeyed Henry's law [18,19]. In the equilibrium between the gaseous and liquid phases of the gas, the amount of CD gas in the liquid phase obeys the equation $C=kP$, where C is the concentration of CD in the liquid phase, P is partial pressure of CD gas in the gaseous phase and k is the equilibrium constant. From Figure 2A, the slope k measured between the CD gas concentration from 2.0 to 6.1 ppmv after 30 min at 25°C was equivalent to approximately 2.8×10^{-6} $mol{\bullet}L^{-1}{\bullet}ppmv^{-1}$ or 2.8×10^{-5} $mol{\bullet}L^{-1}{\bullet}Pa^{-1}$. As shown in Figure 2B, the concentration of CD in water reached a near plateau after 30 min under the experimental conditions used in this study. The data points in Figure 2B were fitted to exponential increase curves, with a half increase point of 4.6 min (filled circles) and 8.3 min (open circles). Subsequent experiments were performed 30 min after exposure to the gas. Different concentrations of CD gas were used in the experimental setup to expose a 96-well microtiter plate containing 50 µl water in each well. The binding ability of the

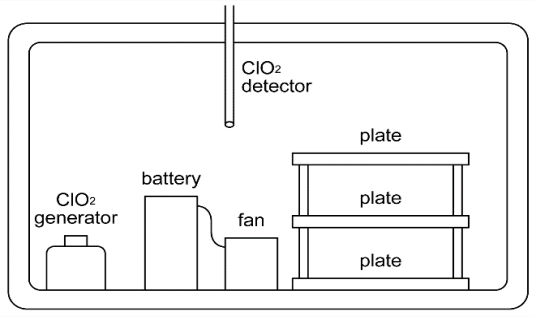

Figure 1: Illustration showing the experimental setup for chlorine dioxide generation, measurement of its concentration and evaluation of the effect of chlorine dioxide on the binding of SARS-CoV-2 spike protein to angiotensin-converting enzyme 2. Samples were placed in a plastic box (dimensions: 174 mm × 246 mm × 173 mm). A chlorine dioxide-generating canister, an electric fan, a battery box and a chlorine dioxide detector tube are shown. Three layers of spike protein-coated or non-coated 96-well microtiter plates are also shown.

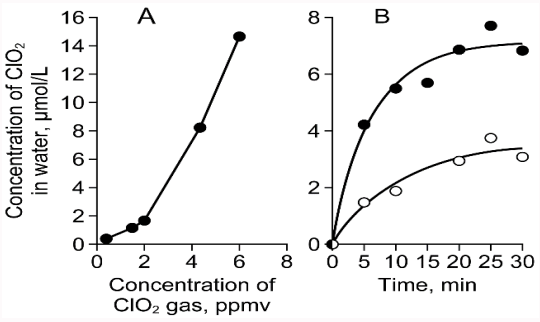

Figure 2: Concentration of chlorine dioxide in water as a function of chlorine dioxide gas concentration (A) when the microtiter plates were exposed for 30 min. Time course of the increase of chlorine dioxide concentration in water (B) when the microtiter plates were exposed to 6.2 ppmv (filled circles) or 1.5 ppmv (open circles) chlorine dioxide gas. Each data point represents a single experiment. All experiments were performed at 25°C.

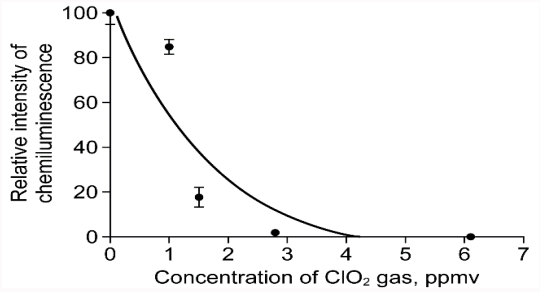

Figure 3: Effect of chlorine dioxide gas of various concentrations on the binding of the SARS-CoV-2 spike protein to angiotensin-converting enzyme 2. The spike protein-coated microtiter plate was treated with various concentrations of chlorine dioxide gas at 25°C for 30 min, and the binding ability of the spike protein to the receptor protein was subsequently measured by chemiluminescence. Each data point represents an average ± standard deviation of four independent experiments. Data points were fitted to an exponential decay curve, whose 50% decay point (IC_{50}) was found to be 1.2 ppmv.

spike protein to ACE2 was subsequently measured. Binding was completely inhibited at 3 ppmv of CD gas (Figure 3).

The 50% inhibitory concentration (IC_{50}) was 1.2 ppmv for 30 min at 25°C. Noteworthy, at a gas concentration of 3 ppmv the CD concentration in water was 4.1 μmol/L after 30 min (Figure 2A), which is consistent with the results shown in Figure 3.

Discussion

Here, we confirmed that gaseous CD dissolves in water with an estimated Henry's equilibrium constant of 2.8×10^{-5} mol•L^{-1}•Pa^{-1}. This value is close to 1.3×10^{-5} mol•L^{-1}•Pa^{-1} as reported by Ishi [19]. CD gas is known to dissolve in water with a maximal achievable concentration of 8 g/L [20]. Using a CD concentration of 6.2 ppmv the maximum concentration of CD in water was 7.2 μmol/L (Figure 2). Indeed, Ishi reported [19] that at a CD gas concentration of 6.2 ppmv dissolved CD in water was 8.1 μmol/L, which is in good agreement with the present results.

We recently reported that a CD aqueous solution added to a 96-well microtiter plate containing spike protein from the Wuhan strain of SARS-CoV-2 virus or the alpha (B.1.1.7) and beta (B.1.351) variants showed inhibition in their binding to ACE2 protein [16,17]. IC_{50} concentrations of this inhibition for the alpha (B.1.1.7) and beta (B.1.351) variants were, 7.6 and 5.8 μmol/L, respectively [16,17]. These values closely correspond to the IC_{50} values determined in the present study. These findings strongly indicate that CD gas, once dissolved in water, can inactivate binding of the spike protein to its receptor as found in the previous aqueous phase-only experiments [16,17]. Aerosols expelled from virus-infected patients may contain other components, such as proteins and salts. However, we speculate that these components do not interfere with CD from modifying spike protein to block receptor protein binding as reported in this study because their concentrations in the expelled aerosols are very low [21].

Conclusion

CD gas can inhibit the binding of the spike protein of SARS-CoV-2 virus to the human receptor protein ACE2 after it is dissolved in water in a concentration-dependent manner. The IC_{50} of CD was 1.2 ppmv after exposure to the gas at 25°C for 30 min. These findings strongly justify the use of CD gas to prevent the spread of SARS-CoV-2 virus in enclosed spaces.

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