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Inhibition of the Binding of Spike Protein of SARS-CoV-2 Coronavirus to Human Angiotensin-Converting Enzyme 2 by Chlorine Dioxide

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Abstract

Aim: The new Coronavirus (SARS-CoV-2) responsible for COVID-19 disease is still a significant threat and no effective and convenient method for decontamination of this virus exists as yet. We aimed to evaluate the effect of a novel method for the decontamination of the virus using aqueous Chlorine Dioxide (ClO_2) solution. SARS-CoV-2 virus uses its "spike protein" to adhere to human cells containing Angiotensin-Converting Enzyme 2 (ACE2), which acts as a receptor protein.

Materials and Methods: In an *in vitro* experiment, the spike protein was treated with 0.5 mmol/L chlorine dioxide solution at room temperature for 5 min, and the binding of the spike protein to ACE2 was quantitated by a chemiluminescence reaction.

Findings: The binding of the spike protein to ACE2 decreased to 1.9% of the control (no chlorine dioxide) (n=4, $p<0.001$).

Conclusion: The result strongly indicates the usefulness of chlorine dioxide solution as a decontamination method of SARS-CoV-2 virus.

Keywords: Chlorine dioxide; SARS-CoV-2 virus; COVID-19; Angiotensin-converting enzyme 2

Introduction

The worldwide emergence and rapid spread of the new Coronavirus (SARS-CoV-2) responsible for COVID-19 disease is a serious threat to human wellbeing. Powerful therapeutic approaches and methods for decontamination are needed as soon as possible to suppress the ongoing pandemic [1]. The SARS-CoV-2 virus has a positive-sense single-stranded RNA genome surrounded by a membrane with Spike Proteins (S-proteins) consisting of S1 and S2 subunits encoded by the viral genome [2]. In the case of human infection, the spike protein binds to a receptor, Angiotensin-Converting Enzyme 2 (ACE2) on host cells [3]. At this stage of virus-receptor binding, tyrosine residue 453 (Y453) located in the receptor-binding domain of the spike protein forms a hydrogen bond with Histidine residue 34 (H34) located in an α1 helix of ACE2 (Figure 1) [3].

Chlorine Dioxide (CD) has been used as a gas or aqueous solution to inactivate many different microbes [4-6]. It inactivates microbes by oxidizing their proteins; the prime targets are tryptophan and tyrosine residues [7,8]. Tyrosine residues are oxidized to dihydroxyphenylalanine or trihydroxy phenylalanine [7]. As the Y453 residue is exposed on the outside of the spike protein of the virus and is required for the hydrogen-bonding necessary for virus-receptor binding [3], we hypothesized that CD will inactivate the virus and therefore prevent the spread of viral infection.

Materials and Methods

We used a binding assay kit (SARS-CoV-2 Spike: ACE2 Inhibitor Screening Assay Kit) from BPS Bioscience (San Diego, California, USA) and followed its manual. Spike protein was first mixed with various concentrations of CD (obtained from Taiko Pharmaceutical Co., Ltd., Osaka, Japan) at room temperature for 5 min. The reaction with CD was terminated by adding two-fold molar excess of sodium thiosulfate. Next, 3.5 μg/ml of ACE2 protein was mixed with the CD-treated spike protein and left at room temperature for 30 min. The amount of ACE2 bound to the treated spike protein was next quantitated by adding horseradish peroxidase-labeled anti-ACE2 antibody. Chemiluminescence of the substrate of the horseradish peroxidase was then added to the mixture to measure the enzyme activity of the horseradish peroxidase. Chemiluminescence was immediately measured with a luminometer (model SH-9000, Corona Electric Co., Ltd., Hitachinaka, Ibaraki,

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Table 1: Effect of chlorine dioxide aqueous solution on the binging of the viral spike protein to human Angiotensin-Converting Enzyme 2.

Concentration of chlorine dioxide (mmol/L)	Chemiluminescence (count/s, × 10^3)	*p* value[a]
0	6097 ± 708	
0.1	7122 ± 967	0.14
0.25	3107 ± 709	<0.001
0.5	116 ± 17	<0.001

Each value represents an average ± standard deviation of four experiments. [a] *vs.* 0 mM chlorine dioxide

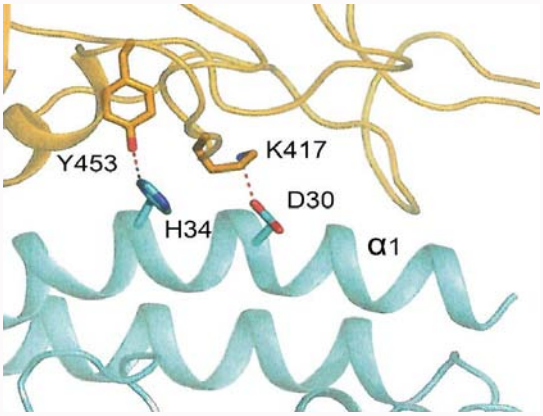

Figure 1: Binding of SARS-CoV-2 coronavirus spike protein to human Angiotensin-Converting Enzyme 2 (ACE2). The figure shows an enlarged view of the binding domain of the viral spike protein (brown) to an α1 helix of the ACE2 (blue) demonstrating hydrogen-bonding of tyrosine 453 (Y453) of the spike protein and histidine 34 (H34) (Protein Data Bank, EMD-30046).

Japan). The difference in luminescence intensity was statistically evaluated by Student t test and considered significant if $p<0.05$.

Results and Discussion

As shown in Table 1, CD at 0.25 mmol/L or 0.5 mmol/L was effective in inactivating the binding of the spike protein to ACE2 ($p<0.001$). However, CD was not effective at 0.1 mmol/L concentration. The result strongly suggests that CD in sufficient concentration is effective in inactivating the spike protein of SARS-CoV-2 coronavirus and thus preventing its binding to human cells. CD aqueous solution will be a useful disinfection measure to prevent the spread of the virus.

Conclusion

The spike protein of SARS-CoV-2 virus was treated with CD solution. It inactivated the biding ability of the viral protein to human ACE2 protein, suggesting that the virus cannot bind to human cells. The result strongly indicates the usefulness of this solution to decontaminate SARS-CoV-2 virus.

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