

Chlorine Dioxide in COVID-19: Hypothesis about the Possible Mechanism of Molecular Action in SARS-CoV-2

Eduardo Insignares-Carrione^{1*}, Blanca Bolano Gómez² and Andreas Ludwig Kalcker³

¹LVWWG Global Research Director, Liechtensteiner Verein für Wissenschaft und Gesundheit, Liechtenstein, Switzerland

²Director of the Research Department, Genesis Foundation, Colombia

³Swiss SVNB Biophysics Researcher, Managing Director, Liechtensteiner Verein für die Wissenschaft und Gesundheit, Switzerland

Abstract

Introduction: The aim of this review is to hypothesize the mechanism of action of chlorine dioxide in COVID-19 by studying its mechanism of action in the structure of SARS-CoV-2.

Methods: Reviews of research on the mechanism of action of chlorine dioxide in viruses, particularly SARS-CoV-2 and influenza viruses at the amino acid level in the viral spike were conducted and these data were transferred to the same structural amino acids of SARS-CoV-2. We used 3D computer reconstructions, use of data through cryo-electronic studies, and previous work based on ChimeraX (UCSF) augmented reality software.

Results: The projection and simulation of chlorine dioxide oxidation in structural amino acids of SARS-CoV-2 allows inferring the sites in which chlorine dioxide exerts a denaturalizing action on viral structure and on human ACE2 as well as it is possible to understand the extreme speed with which it acts, which could explain the first findings of clinical observational studies of chlorine dioxide use in COVID-19 carried out by the authors in Bolivia under strict compliance of ethics committee.

Conclusion: The oxidation by chlorine dioxide of critical amino acids in the spike of the coronavirus SARS-CoV-2 and in the structure of ACE2 allows us to understand the potentially therapeutic actions of chlorine dioxide dissolved in water by oral way in the COVID-19. We hope to publish clinical application trials of this promising systemic virucide soon.

Keywords: SARS-CoV-2 • COVID-19 • Aminoacids • Chlorine dioxide

Introduction

COVID-19 is an infectious disease caused by the SARS-CoV-2 virus. It was first detected in the Chinese city of Wuhan (Hubei province) in December 2019. In three months it spread to basically all countries in the world, which is why the World Health Organization declared it a pandemic. (WHO, March 11, 2020).

There is no specific treatment; the main therapeutic measures are to relieve symptoms and maintain vital functions. Research to find an effective treatment began since the pandemic scale of the disease was verified. The central problem is that, eleven months after its official onset, an effective treatment for the disease is still unknown. In the absence of an effective treatment, we studied new therapeutic possibilities with the intention of finding an effective and safe treatment for COVID-19.

In accordance with the above, this research addresses current results and previous research adding the possible therapeutic action as a virucidal of chlorine dioxide in aqueous solution and without the presence of sodium chlorite using the concepts of translational medicine based on knowledge about the structure of the virus and the mechanism of action of chlorine dioxide in viruses, to propose a possible treatment of choice for COVID-19 [1,2].

***Address for Correspondence:** Dr. Eduardo Insignares-Carrione, LVWWG Global Research Director, Liechtensteiner Verein für Wissenschaft und Gesundheit, Liechtenstein, Switzerland, E-mail: eduardoinsignarescarrione@gmail.com/ eic@lvwg.org

Copyright: © 2020 Insignares-Carrione E, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Received 10 November 2020; **Accepted** 22 November 2020; **Published** 30 November 2020

Chlorine dioxide

The action of chlorine dioxide is given by its selectivity for pH and by the area or size where it generates its action. It means that this molecule dissociates and releases oxygen when it comes into contact with another acid [3]. Upon reacting, its chlorine atom binds to sodium in the medium and turns into sodium chloride (common salt) releasing oxygen, which oxidizes the acidic pH pathogens present, converting them to alkaline oxides. Therefore, when chlorine dioxide dissociates, it releases oxygen into the blood, as erythrocytes (red blood cells) do by the same principle (known as the Bohr effect), which is to be selective for acidity.

As it normally happens in blood, chlorine dioxide releases oxygen when it encounters acidic soil, be it lactic acid or the acidity of the pathogen. Its possible therapeutic effect is postulated due, among other effects, to the fact that it creates an alkaline environment, while eliminating small acid pathogens, by oxidation, with an electromagnetic overload that is impossible to dissipate by unicellular organisms. The death time in a virus must be analogous to the lag time caused by the chemical reaction, due to the times required to cover the entire volume. We can expect that in a virus with a diameter of 120 nanometers, the destruction time will be much shorter due to its geometric factor.

According to studies by Zoltán Noszticzius, chlorine dioxide is a size-selective antimicrobial agent that can quickly kill micrometer-sized organisms, but cannot cause real harm to much larger organisms such as animals or humans, as it cannot penetrate deep into their tissues.

It is known that multicellular tissue has the highest capacity to dissipate electrical charges and therefore is not affected in the same way by the voltages of the oxidation-reduction process (ORP) as is the case of unicellular organisms and therefore there is biochemically speaking, a greater cell protection because of size.

Chlorine dioxide, which is the most effective non-cytotoxic disinfectant known after ozone, and used as an aqueous solution has immense possibilities

of being used therapeutically since it is also capable of penetrating and eliminating biofilm, which ozone does not do [3]. The great advantage of the possible therapeutic use of chlorine dioxide in infections is the impossibility of a bacterial or viral resistance to ClO_2 since it has an oxidation mechanism unlike chlorine (Cl_2) which acts by chlorination [3].

Although ozone is stronger in antiseptic terms, its high oxidative potential of 2.07 and its short half-life of only 15 minutes at 25°C with a pH value of 7.0 makes it less effective than ClO_2 for therapeutic applications *in vivo*. Chlorine dioxide is pH (-) and a size selective oxidant and, unlike other substances, it does not react with most components of living tissues (3). Chlorine dioxide reacts rapidly with phenols and thiols essential for bacterial life.

In phenols, the mechanism consists in the attack of the benzene ring, eliminating odor, taste and other intermediate compounds [4]. Chlorine dioxide kills viruses effectively and is up to 10 times more effective than sodium hypochlorite (bleach or bleach). It was also shown to be very effective against small parasites, protozoa [5]. One topic that has been reviewed a lot lately is the reactivity of chlorine dioxide with amino acids. In tests for reactivity of chlorine dioxide with 21 amino acids, only cysteine [4], tryptophan [5], tyrosine [6], proline and hydroxyproline reacted at a pH of around 6.

Cysteine and methionine (4) are two aromatic amino acids that contain sulfur, tryptophan and tyrosine and the two inorganic ions Fe^{2+} and Mn^{2+} [3]. Cysteine, because it belongs to the group of thiols, is an amino acid up to 50 times more reactive with all microbial systems than the other four amino acids and, therefore, it is impossible for it to create resistance against chlorine dioxide.

The hypothesis we propose here is that the cause of the antiviral effect of chlorine dioxide can be explained by its actions on at least five amino acids listed above or on peptide residues.

Chlorine dioxide (ClO_2) has been used since 1944 in the treatment of drinking water due to its biocidal power, as well as in most bottled waters suitable for human consumption due to its almost zero lack of toxicity in an aqueous solution being used systematically in the disinfection and conservation of blood transfusion bags [3,4]. As it is a selective oxidant, its mode of action is very similar to that of phagocytosis, where a mild oxidation process is used to eliminate all types of pathogens [3,4].

Chlorine dioxide (ClO_2) is a yellowish gas that to date is not part of the conventional pharmacopoeia as a medicine despite its proven effectiveness in denaturing viruses, with multiple patents for use in different treatments such as disinfection or sterilization of blood components (blood cells, blood proteins, etc.) 4, the parenteral treatment (intravenous route) of HIV infections, or for the treatment of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Alzheimer's and other patents for uses such as patents for: apoptosis induction cancer treatment (CN 103720709 A) tumor treatment (US 10, 105, 389 B1) Sinusitis antiviral treatment (US 2016/0074432 A1), System stimulation immunological (US 5,830,511), Stem cell initiation and differentiation (WO2014082514A1), Vaginal treatment method (US 6280716B1), Skin treatment against viruses and bacteria (US 4,737,307), Human amoebiasis treatment method (US 4,296,102), Treatment against candidiasis infections (US 2015/0320794 A1), Wound treatment (US 87.3106), Oral cavity treatment (US 100015251), (US4689215), Against inflammations (US53841134), Nail fungus treatments (US 20100159031) and Against inflammations (US53841134), Treatments against nail fungus (US 20100159031) and Against inflammations (US53841134), Treatments against nail fungus (US 20100159031) and Swiss patent pending/11136-CH. (Kalcker, A.) [4].

Based on the above, three premises can be established:

1. Chlorine dioxide can fight viruses through the selective oxidation process by denaturing capsid proteins and subsequent oxidation of the virus's genetic material, rendering it disabled. As there is no possible adaptation to the oxidation process, it prevents the development of resistance by the virus, making chlorine dioxide (ClO_2) a promising treatment for any viral subspecies.
2. There is scientific evidence that chlorine dioxide is effective against

the SARS-CoV-2 [4] and SARS-CoV-2 coronavirus, such as the work carried out at the University of Queretaro in Mexico and published in November 2020 COVID-19, called "*In vivo* evaluation of the antiviral effect of ClO_2 (chlorine dioxide) in chicken embryos inoculated with avian coronavirus

(IBV), in which ClO_2 treatment had a marked impact on IBV infection. Namely, viral titers were 2.4 times lower and mortality was halved in infected embryos that were treated with ClO_2 . The infection caused developmental abnormalities regardless of treatment. Lesions typical of IBV infections were observed in all inoculated embryos, but the severity tended to be significantly less in ClO_2 -treated embryos. No macro or microscopic evidence of toxicity caused by ClO_2 was found at the doses used.

3. Toxicity: The biggest problems that arise with drugs or substances that can be considered as such in general are due to their toxicity and side effects. There is toxicity with chlorine dioxide in case of respiratory inhalation, but there are no reports of toxicity at the recommended dose of 30 mg or 30 ppm in aqueous solution when taken orally and no clinically proven death even at high doses by oral ingestion. The lethal dose (LD50, acute toxicity ratio) is estimated at 292 mg per kilo for 14 days, where its equivalent in a 50 kg adult would be 15,000 mg administered over two weeks. The sub-toxic oral doses that can be used are approximately 50 ppm dissolved in 100 ml of water 10 times a day, which is equivalent to 500 mg. Furthermore, chlorine dioxide, by dissociation, it decomposes into a chlorine ion that immediately associates with the sodium ion, forming common salt NaCl and oxygen O_2 within the human body. In summary, chlorine dioxide at the recommended doses in COVID-19 of 30 mg or 30 ppm per day is not toxic [5-8].

Virucidal effects of chlorine dioxide

Chlorine dioxide is an effective antimicrobial agent that kills bacteria, viruses, and some parasites [9]. Its broad spectrum germicidal profile is derived from the action of this compound as a non-cytotoxic oxidant.

Viruses generally consist of an outer layer or a protein coat that encapsulates a nucleic acid, which can be DNA or RNA. When chlorine dioxide comes in contact with a virus, a single, highly reactive nascent oxygen atom is released on the target virus. This oxygen binds to specific amino acids in the protein coat of the virus, denaturing the proteins and rendering the virus inactive. Additionally, nascent oxygen atoms bind to guanine, one of the four nucleic acid bases found in RNA and DNA, forming 8-oxoguanine. This oxidation of guanine residues prevents viral nucleic acid replication [10].

In the published scientific literature there are reports that chlorine dioxide inactivates a wide variety of viruses, including influenza A, human adenovirus, human rotavirus, echovirus, bacteriophage f2, and poliovirus [11-16].

Influenza A viruses are spherical, negative-sense, single-stranded RNA viruses that possess a lipid membrane that contains peaks composed of glycoproteins known as HA (hemagglutinin) and NA (neuraminidase). Within the virus there are eight single strands of RNA [17]. A preclinical study found that chlorine dioxide gas is effective in preventing aerosol-induced influenza A virus infection. This study used low concentrations of chlorine dioxide gas (ie 0.03 ppm) in a mouse cage. This level is below the OSHA long-term exposure level (8 hours) for chlorine dioxide gas in ambient air in a human workplace, which is 0.1 ppm [18]. Chlorine dioxide gas effectively reduced the number of infectious viruses in the lungs of mice and markedly reduced mortality. Mortality was 70% (7/10) on day 16 in the group not treated with chlorine dioxide and 0% (0/10) in the group treated with chlorine dioxide. The authors confirmed these results by repeating their experiment. The results of the repeat study were 50% (5/10) mortality in the untreated group and 0% (0/10) in the treated group.

The authors concluded that low levels of chlorine dioxide gas (i.e., 0.03 ppm), which are below the permissible exposure level in human workplaces, "could be used in the presence of humans to prevent their infection by influenza A virus and possibly other viruses associated with tract infections respiratory (p. 65). They suggested that "chlorine dioxide gas could be used in places

like offices, theaters, hotels, schools and airport buildings without evacuating people, without disrupting their normal activities." The authors suggested that their method "opens a new path for the prevention of pandemic influenza" (p. 65) after conducting a study in a school with favorable results in this regard.

The infectivity of the virus was found to be reduced *in vitro* by the application of chlorine dioxide, and higher concentrations produce even greater reductions. This inhibition of infectivity was correlated with alterations in viral proteins. These alterations resulted from the incorporation of oxygen atoms in the tryptophan and tyrosine residues located in the HA and NA proteins [11]. These proteins are denatured by the addition of oxygen atoms, which eliminates the ability of the virus to infect other cells [19]. A later study found that influenza A virus inactivation is caused by the transfer of 2 oxygen atoms from chlorine dioxide to a specific tryptophan (W153) residue in the hemagglutinin (HA) tip protein [20].

Adenoviruses are non-enveloped viruses with an icosahedral capsid containing a double-stranded DNA genome. Seven groups of human adenoviruses have been classified [21]. A recent study found that chlorine dioxide can help reduce adenovirus levels in drinking water [12]. This study examined the effects of chlorine dioxide and ultraviolet light on adenovirus levels in drinking water in the Netherlands. The authors found that the application of chlorine dioxide in low concentrations (0.05 - 0.1 ppm) reduced adenoviruses in drinking water, while UV disinfection was insufficient without chlorine dioxide disinfection.

Rotaviruses are double-stranded RNA viruses that consist of 11 unique double-stranded RNA molecules surrounded by a three-layered icosahedral protein capsid [22]. These viruses, which are the leading cause of severe diarrheal diseases in infants and young children worldwide, are inactivated by chlorine dioxide. In fact, at concentrations of chloride dioxide ranging from 0.05 to 0.2 ppm, they are inactivated within 20 seconds *in vitro* [23,24].

Bacteriophage f2 is a positive sense single-stranded RNA virus that infects *Escherichia coli* bacteria. An *in vitro* study found that 0.6 mg/liter of chlorine dioxide rapidly (i.e., within 30 seconds) inactivated bacteriophage f2 and interfered with its ability to bind to its host, *E. coli* [15]. Both the inactivation of the virus and the inhibition of its ability to bind to its host increased with higher pH and with increasing concentrations of chlorine dioxide. Additionally, the authors found that chlorine dioxide denatures virus capsid proteins by reacting with tyrosine, tryptophan, and cysteine residues. These amino acids were almost completely degraded within 2 minutes of exposure to chlorine dioxide.

Poliovirus is a positive-sense, positive-strand RNA virus [25]. Ridenour and Ingerson found that chlorine dioxide can inactivate polio virus *in vitro*.

Later, Álvarez and O'Brien expanded this work by showing that treatment with 1 ppm of chlorine dioxide *in vitro* results in the separation of RNA from the capsid and also produces alterations in the RNA [16,26].

In addition to the studies mentioned above, the US Environmental Protection Agency (EPA), which on April 10, 2020 listed chlorine dioxide as an EPA-registered disinfectant to kill the SARS-CoV-2 virus, provides additional support for the virucidal effects of chlorine [27]. The EPA website indicates that this product is for surface use and not for human use.

Human studies on the effects of chlorine dioxide on the SARS-CoV-2 virus have not yet been conducted. Currently, two of the authors (Insignares and Bolano) are conducting the first multicenter clinical trial in the world on the effectiveness of oral chlorine dioxide in humans in COVID-19 (ClinicalTrials.gov identifier: NCT04343742). An *in vitro* study found that chlorine dioxide inactivates the genetically related SARS-CoV-2 virus [28]. A concentration of 2.19 mg/liter of chlorine dioxide was found to cause complete inactivation of SARS-CoV-2 in wastewater. A branch of our group is in the process of conducting an *in vitro* investigation of the action of chlorine dioxide on SARS-CoV-2 in India and we are in the process of publishing a report on the simulation of the mechanism of action of chlorine dioxide in SARS-CoV-2 using the *in silico* method, carried out in Japan.

In Ecuador (Aememi) for Chlorine dioxide, an effective therapy for the treatment of COVID-19; 51) A preliminary trial was carried out with the administration of oral chlorine dioxide on 104 COVID-19 patients who had variable profiles in terms of age, sex and severity of the disease, the minority diagnosed by testing and the majority by screening according to typical symptoms of the illness. Therefore, the data were managed using a symptomatic scoring scale, with 10 being the maximum perception and 0 being the minimum of the symptom: fever, chills, muscle pain, dry cough, headache, back pain, difficulty breathing, vomiting, diarrhea, sore throat, loss of smell, loss of taste, poor appetite.

Chlorine dioxide in a concentration of 3000 ppm was recommended at a dose of ten cc diluted in one liter of water, taken throughout the day, divided into 10 daily doses, taken every hour and a half for 20 days. The results were distributed according to the symptoms after the first, second, third and fourth treatment days. They were segmented between men and women, and common results were also presented. The following tables show the symptoms, and in the first and last graph the behavior in relation to the symptomatological scale between the first and fourth day of oral chlorine dioxide intake (Figures 3 and 4).

From this preliminary study the following conclusions can be drawn: Chlorine dioxide is definitely harmless - not toxic at all - in the recommended

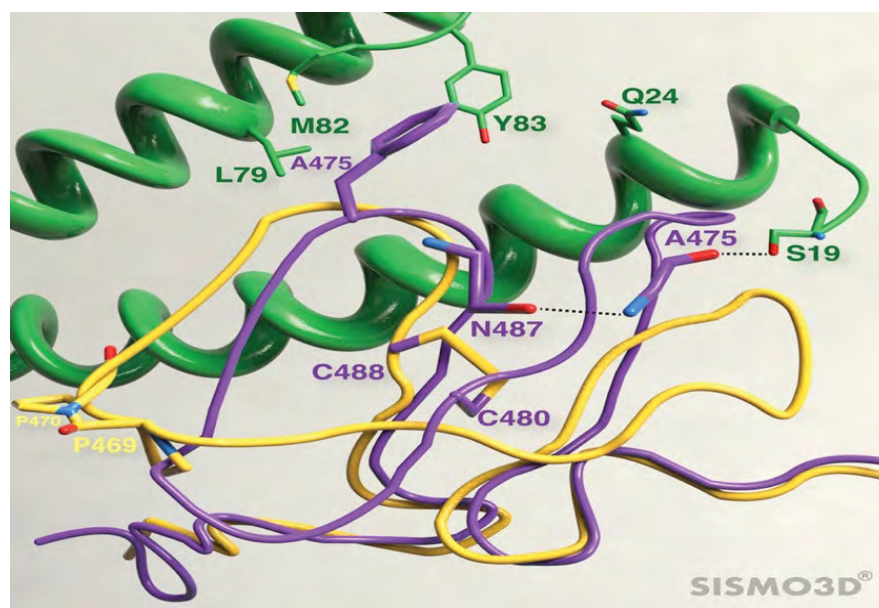


Figure 1. The structural differences between the RBMs of SARS-CoV-2 and SARS-CoV.

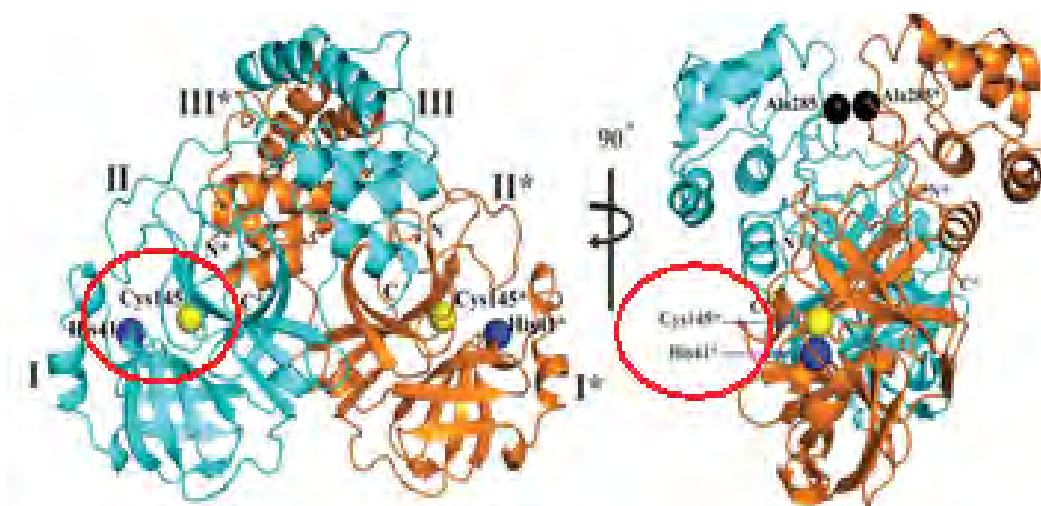


Figure 2. Three-dimensional structure of SARS-CoV-2 Mpro in two different views.

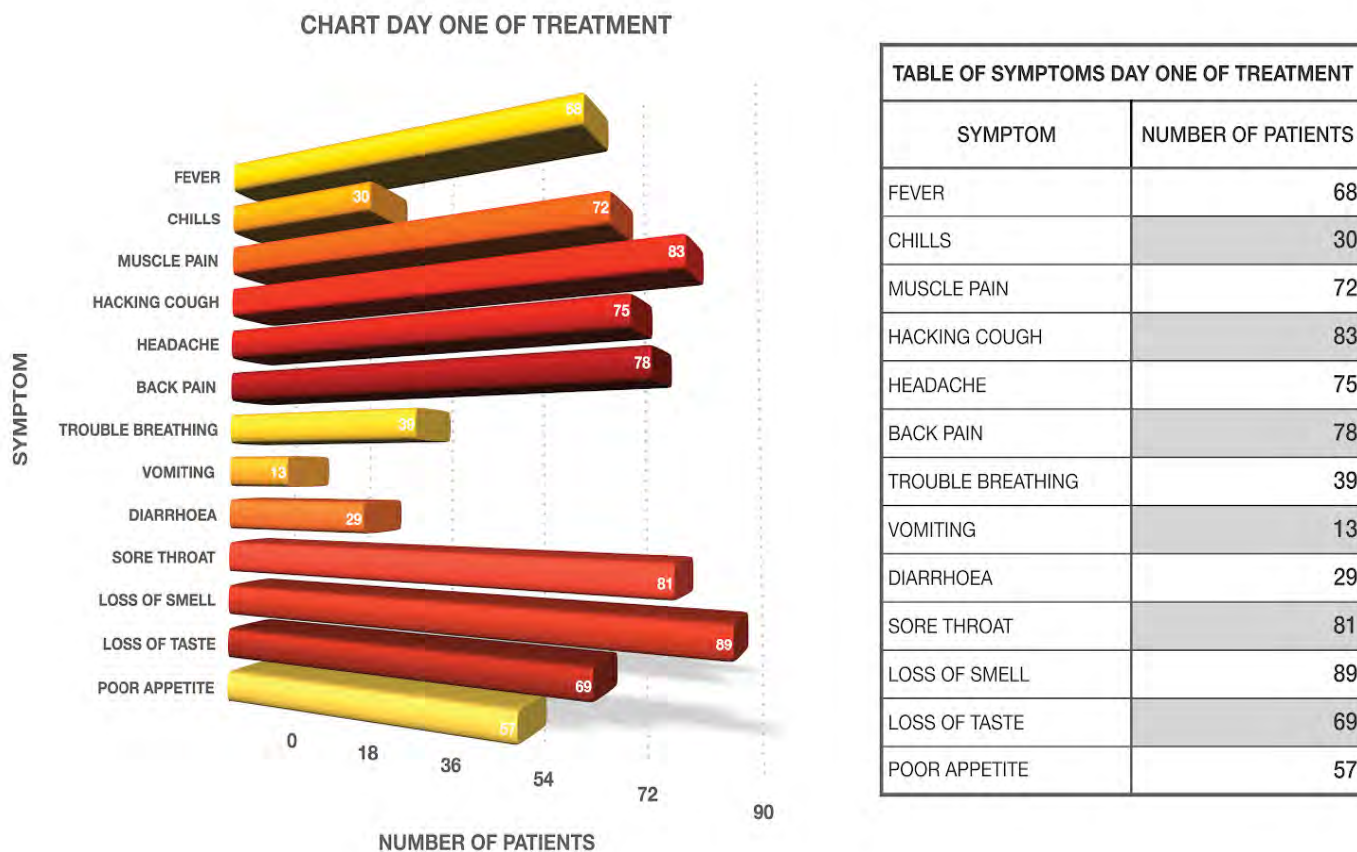


Figure 3. Results of chlorine dioxide on day 1 of its administration.

and ingested doses and all initial symptoms began to decrease from the first day of treatment, the decrease being totally evident on the fourth day. Specifically, symptoms most indicative of an ongoing infection, such as fever, chills, headache, sore throat, loss of appetite, and loss of the senses of taste and smell, were dramatically decreased. Other symptoms, such as muscle pain and cough, remained somewhat common, as they tend to remain residual for longer after the illness has ended.

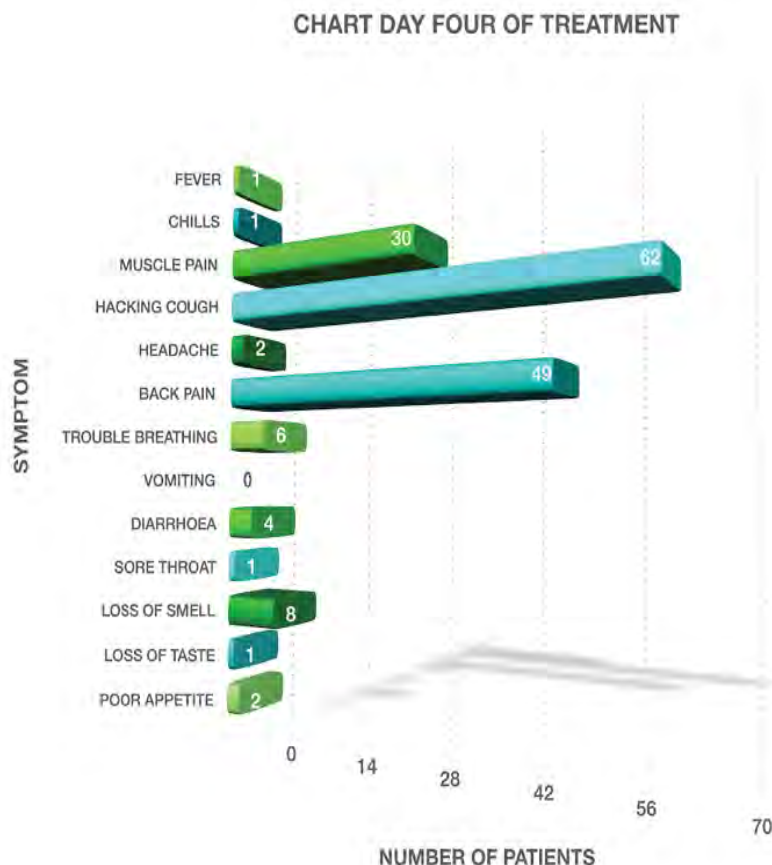
Materials and Methods

To search for the reference information used in this article, the web search engines were reviewed using the MeSH criteria, in accordance with the search strategy indicated in subsequent lines in the periods between January and

April 2020, finding the following results: 1. PubMed (Medline): 4 references, 2. LILACS: 18 references, 3. Cochrane Library: 56 references, 4. Science: 1,168 references, 5. Scielo: 61 references, 6. MedScape: 19 references for a total of 1,326 scientific publications whose contents were on the use of chlorine dioxide in different applications and on the mechanism of action of chlorine dioxide in the SARS-CoV-2 virus. Finally, we reviewed the registries at www.clinicaltrials.gov and those of the WHO International Clinical Trials Registry Platform (ICTRP) in order to identify ongoing or unpublished clinical trials.

Search strategy

"Chlorine dioxide" OR "Chlorine dioxide protocol" OR Chlorine dioxide AND virus; Chlorine dioxide AND SARS-COV-2; OR "COVID-19 drug treatment" OR "spike glycoprotein, COVID-19 virus" OR "severe acute respiratory syndrome



SYMPTOM	NUMBER OF PATIENTS
FEVER	1
CHILLS	1
MUSCLE PAIN	30
HACKING COUGH	62
HEADACHE	2
BACK PAIN	49
TROUBLE BREATHING	6
VOMITING	0
DIARRHOEA	4
SORE THROAT	1
LOSS OF SMELL	8
LOSS OF TASTE	1
POOR APPETITE	2

Figure 4: Results of chlorine dioxide on day 4 of its administration.

coronavirus 2" OR "COVID-19" OR "2019-nCoV" OR "SARS-CoV-2" OR "2019 new coronavirus" OR "2019 coronavirus disease" OR (pneumonia).

From the search results, we selected those that made reference to the virucidal action of chlorine dioxide on various microorganisms, in particular on viruses and, among these, SARS-CoV-2 or SARS-CoV.

We also reviewed the studies carried out on the action of chlorine dioxide on amino acids, especially those that are part of viral capsids. From the findings we highlight that in 1986, Noss et al. demonstrated that the inactivation of bacterial virus (bacteriophage) f2 by ClO₂ was due to its reactions with viral capsid proteins. Additionally, they found that three amino acids of the viral protein, namely cysteine, tyrosine and tryptophan, could react with ClO₂ rapidly [15]. In 1987, Tan and others tested the reactivity of ClO₂ at 21 free amino acids [29]. The ClO₂ reacted with only six amino acids dissolved in 0.1 M sodium phosphate buffer at pH 6.0. The reaction with cysteine, tryptophan and tyrosine was too rapid to be followed by his technique.

The reactivity of the three fast-reacting amino acids (cysteine, tyrosine and tryptophan) were studied in the laboratory between 2005 and 2008, finding that cysteine had the highest reactivity among these three amino acids [30,31].

In 2007, Ogata discovered that the antimicrobial activity of ClO₂ is based on the denaturation of certain proteins, which is mainly due to the oxidative modification of the tryptophan and tyrosine residues of the two model proteins (bovine serum albumin and glucose - 6- phosphate dehydrogenase) used in their experiments [32]. In 2012, it was again Ogata who demonstrated that the inactivation of the influenza virus by ClO₂ was caused by the oxidation of a tryptophan (W153) residue into hemagglutinin (a protein from the spike of the virus), thus suppressing its ability to bind to receptors [20].

In this context, it is interesting to note that the spike protein of the new coronavirus SARS-CoV-2 contains 54 tyrosine residues, 12 tryptophan and 40 cysteine [33].

If we assume that in an aqueous solution all these amino acid residues are

capable of reacting with ClO₂ as well as with free amino acids, the inactivation of the virus can be extremely rapid even in a solution of 0.1 mg/L of ClO₂.

On the other hand, we selected the articles that describe the action of SARS-CoV-2 in cells, in its interaction with ACE2 and, in particular, we investigated augmented reality videos or simulation videos based on Silico, for three-dimensional representation. From action sites like videos in which the spicular protein and the ACE2 receptor, among others, are manipulated with ChimeraX (UCSF) augmented reality software [34-41].

In the same way, we reviewed the structure of the virus spike and based on research from Daniel Wrapp and Jason S. McLellan at the University of Texas.

The three-dimensional image of the spicular S glycoprotein of the SARS-CoV-2 betacoronavirus has been seen with electron cryomicroscopy in record time. Thanks to this image with a resolution of 3.5 Å, it is confirmed that this S protein is coupled to the hACE2 protein of human cells with a higher affinity than that of the SARS-CoV-2 coronavirus. Protein S is the target of the antibodies that immunize us. Its 3D structure makes it possible to understand why published monoclonal antibodies against SARS-CoV-2 are not effective against SARS-CoV-2. It will undoubtedly help accelerate the development of vaccines and therapies against COVID-19 infection [42].

In these simulation and virtual reality videos, it is observed that protein S is a trimer made up of three peptides, each with two subunits S1 and S2. The S1 subunit acts as a hinge with two conformations called "down" (RBD down) and "up" (RBD up). Electron cryomicroscopy imaging shows that only one of the peptides is in the "up" state, while the other two are in the "down" state. Binding to the cellular receptor occurs in the "upstream" configuration. After binding, the three protein S peptides are cleaved at the S1/S2 site; a second split then occurs at the S2 'point, unfolding the key fusion peptide (FP) at the junction between the membranes.

The spicular protein (S) is a type I transmembrane trimeric protein with between 1,160 and 1,400 amino acids, depending on the type of coronavirus.

This protein forms the coronavirus corona; it is composed of three repeating peptides and is highly glycosylated, which facilitates its binding to proteins and sugars. Each peptide is made up of two domains called S1 and S2. In beta coronaviruses like SARS-CoV-2, cleavage of the S1 and S2 subunits occurs during fusion between the membranes.

The S1 domain has two subdomains, one N-terminal (NTD), which ends with an amino acid that has a free amino group (-NH₂), and another C-terminal (CTD), which ends with a carboxyl group (-COOH); both bind to the host cell's ACE2 receptor, then they are receptor-binding domains (RBD). The S2 domain is C-terminal in type and is highly conserved among all coronaviruses, which differ much more in the S1 subunit. The S2 domain contains two regions, HR1 and HR2, in which groups of seven amino acids (called heptides) repeat, in abcdefg form, that contain a and d hydrophobic residues that participate in the fusion between the membranes. The HR1 and HR2 domains are therapeutic targets, since drugs are known that inhibit their action, preventing or hindering fusion.

The infection of the epithelial cells of the respiratory tract is orchestrated by the S protein of the virus. In the general steps of the fusion process first, the S1 domain recognizes and binds to the host cell receptor. Second, there is a first split at the S1 and S2 domains, and a second split at the S2 'point'; the latter allows the fusion peptide (FP) that connects the membranes of the host and the virus to be activated (this stage is called the intermediate stage of fusion or intermediate stage of fusion). And third, the region between HR1 and HR2 remodels (folds) giving rise to a heptamer (6-HB) that joins both membranes allowing the entry of the virus.

The S protein of coronaviruses is key in the development of vaccines (antigens that induce an immune response to the presence of the S1 domain) and for the development of antivirals (inhibitors of some of the fusion stages between membranes, normally attacking specific regions of the domain S2). Knowing the three-dimensional structure of protein S is essential to combat the COVID-19 epidemic.

The sequence of the protein S of SARS-CoV-2 coincides 98% with the protein S of the coronavirus Bat-RaTG13, with the great difference that it has four RRAR amino acids (arginine-arginine-alanine-arginine) instead of just one arginine (R). Furthermore, they differ in 29 residues, 17 of which are in the RBD region. The comparison made between the 61 complete SARS-CoV-2 genomes available in GISAID (Global Initiative to Share All Influenza Data) shows that there are only 9 different amino acids between all of them; and all these variants are found in very well preserved places, which does not seem to affect the lethality of the coronavirus.

First, it was possible to characterize the 3D structure of the spicular S glycoprotein of the SARS-CoV-2 coronavirus and its RBD receptor binding domain. Then that of the host cell receptor, the human angiotensin-converting enzyme hACE2. The next step for the researchers was to determine the structure of the SARS-CoV-2 RBD/hACE2 complex, which were obtained by X-ray crystallography, reaching resolutions of 2.45 Å and 2.68 Å. Among the findings, it was determined that very subtle structural changes explain the higher infectivity and pathogenesis of SARS-CoV-2 (COVID-19) compared to SARS-CoV-2 (SARS).

These findings are of great relevance for the development of drugs to combat COVID-19. *In silico* reconstructions have been carried out (using theoretical models using computers), but observation of the real crystallographic structure by X-ray diffraction is essential. As noted at the outset, the authors are in the process of publishing the Japan-based *in silico* study they conducted on the mechanism of action of chlorine dioxide on the SARS-CoV-2 spike and hemoglobin.

The first problem that arises in the research process is how to form the SARS-CoV-2 RBD/hACE2 complex with sufficient stability for its observation; Previous experience in the formation of the SARS-CoV-2RBD/hACE2 complex (evidenced in 2005) has been key, in which a salt bridge between Arg426 of RBD and Glu329 of hACE2 is used to reinforce the binding of the complex. A very important observation is that cysteine at positions Cys336-Cys361, Cys379-Cys432 and Cys391-Cys525 stabilize the five beta sheets (β 1, β 2,

β 3, β 4 and β 7), and Cys480-Cys488 is key in the junction between the ridge SARS-CoV-2 RBM and the N-terminal helix of hACE2 [43-45].

When the simulation of the action of dioxide on these amino acids (Cys) is placed, it is easy to understand the fabulous direct virucidal effect of dioxide on viruses and in particular on SARS-CoV-2. The image that is revealed is of a devastating effect of chlorine dioxide on the virus, degrading and denaturing it. Comparison between SARS-CoV-2RBD/hACE2 and SARS-CoV-2 RBD/hACE2 complexes provides insight into why COVID-19 is more infectious than SARS-CoV.

SARS-CoV-2 RBM forms a larger and more highly contacted junction interface with hACE2 than SARS-CoV-2RBM; the salt bridge between SARS-CoV-2RBD and hACE2 is weaker than between SARS-CoV-2 RBD and hACE2. The crystal structure of the complex also contains glucans coupled to the four hACE2 sites and the RBD site. The glucan coupled to Asn90 from hACE2 forms a hydrogen bond with Arg408 in the core of RBD; this interaction is conserved between SARS-CoV-2 and SARS-CoV.

The structural differences between the RBMs of SARS-CoV-2 and SARS-CoV are subtle, but affect the conformations of the loops in the receptor-binding ridges. In both RBMs, one of the ridge bonds contains a disulfide bond that is critical for bonding. SARS-CoV-2 and bat-CoV Rs3367 contain a motif with three Pro-Pro-Ala residues in said loop; but in SARS-CoV-2 and bat-CoV RaTG13 show a motif of four Gly-Val/Gln-Glu/Thr-Gly residues; therefore, the conformation of the loop changes because the glycines are more flexible. This change favors the RBD/hACE2 binding. Furthermore, the ridge has a more compact conformation thanks to the Asn487 and Ala475 hydrogen bonds in SARS-CoV-2 RBM, bringing the loop containing Ala475 closer to hACE2.

The contact of the crest of SARS-CoV-2 RBM with the N-terminal helix of hACE2 is greater than for SARS-CoV-2RBM. For example, the N-terminal residue Ser19 of hACE2 forms a new hydrogen bond with the Ala475 backbone of SARS-CoV-2 RBM, and the Gln24 of the N-terminal helix of hACE2 also forms a new contact with SARS-CoV-2 RBM. When compared to Leu472 from SARS-CoV-2RBM, Phe486 from SARS-CoV-2 RBM points in a different direction and forms a hydrophobic region involving Met82, Leu79 and Tyr83 from hACE2 (Figure 1).

Comparison with SARS-CoV-2RBM shows that these small structural changes of SARS-CoV-2 RBM are more favorable for hACE2 binding. They are subtle differences, but very relevant from a functional point of view. Two critical binding sites (virus binding hotspots) have been revealed, the hotspot-31 critical point on the Lys31 and Glu35 salt bridge, and the 353 hotspot on another salt bridge between Lys353 and Asp38. These two salt bridges are weak, due to the great distance in the interaction, but being enclosed in a hydrophobic environment, which reduces the effective dielectric constant, their binding energy is higher (Figure 2).

To confirm these structural findings, biochemical studies of the RBD/hACE2 binding affinity have been performed after introducing certain mutations in SARS-CoV-2 RBD. These mutations suggest that the bat coronavirus RaTG13 could infect humans (supporting the zoonotic origin of the epidemic). Furthermore, the RBMs of SARS-CoV-2 and bat-CoV RaTG13 contain a similar motif of four residues in the ACE2-binding ridge, supporting that one has evolved from the other. In addition, to enhance hACE2 recognition, SARS-CoV-2 exhibits two changes in the L486F and Y493Q residues of RaTG13, which appear to have facilitated the transmission of SARS-CoV-2 from bat to humans. Therefore, there may not be an intermediate host between the bat and the human in COVID-19, unlike what happened with SARS and MERS. Of course, for now it is impossible to rule out the existence of a mediator, which could well be a pangolin or other wild animal sold in the Wuhan market; In the case of the pangolin, it is necessary to sequence more genomes of the pangolin coronavirus to clarify the issue, but so far a genomic similarity of more than 99% has been evidenced between them [46].

Results and Discussion

The SARS-CoV-2 spike is strongly glycosylated and glycosylation is

believed to play an important role in detecting the virus against our own immune systems. A section of alpha helices runs the length of the spike protein. For the most part, beta sheets are concentrated at this end, which is where the spike protein fuses with a cell to infect it. The interesting thing is that the helices are made up of amino acids sensitive to the action of chlorine dioxide (at the Cysteine level).

The spike protein is actually made up of three intertwined chains that have identical amino acid sequences; each of these chains is called a protomer. However, protomers do not have identical three-dimensional conformations.

We can see the difference in conformation in the protomers by examining a section of the spike protein that is critical to the life cycle of the virus, the receptor-binding domain or RBD. RBD is where the virus binds to an enzyme on the surface of host cells, allowing it to fuse with the cell and transport viral genetic material within. Two of these RBDs are in a lower conformation in the structure. However, one of these RBDs flips up. This "upward" conformation is higher energy, ready to bind to the cellular receptor and lead to fusion. It is believed that when the spike protein binds, each of these RBDs is changed to this less stable conformation.

Our own enzymes, the ones that break peptide bonds called proteases, can cut the spike protein at specific sites and conformational changes in the spike protein fusion occur. RBD is bound to ACE2, which is the receptor on the surface of our cell to which the coronavirus binds to cause fusion. These structures are also strongly glycosylated. If we hide the sugars to create a model for understanding the RBD-ACE2 interaction, and put chlorine dioxide there acting on the amino acids, we can focus on some of the weak interactions that hold RBD and ACE2 together.

For example, we have an extensive network of hydrogen bonds at the RBD-ACE2 interface that invades two tyrosine residues (Tyr-489 and Tyr-83). This tyrosine side chain is also bonded to the carbonyl hydrogen of the asparagine side chain (Asn-487), which in turn bonds through its NH hydrogen atom to the glutamine carbonyl in ACE2 (Gln-24). Chlorine dioxide, we postulate, oxidizes these residues Tyr-489 and Tyr-83, among others, with which the RBD-ACE2 interface is denatured and the virus can no longer bind or is already oxidized. Additionally, chlorine dioxide also oxidizes the proline present in ACE2 which completes the oxidation and deformation of ACE2.

Moving along, the alpha helix of ACE2, we have the glutamate side chain that is deprotonated at a pH of 7.4, and a lysine residue that carries a positive charge at that pH.

If the virus fuses, viral genetic material is released into the cell. In the case of coronaviruses, this piece of RNA travels to the ribosomes of our cell and holds it hostage to create its own viral proteins. One interesting thing is that this viral RNA is capable of changing the three-letter frame of the RNA bases that is read by the ribosome; this essentially duplicates the peptide sequence that can be made from a viral replica using our ribosomes; the proteins the virus needs to assemble additional copies of itself, which will eventually be released from the cell and infect others. There is an important protein that is transferred in this process, and it is the main protease that cuts the chain of viral polypeptides in the functional proteins necessary to assemble new viruses. This is another therapeutic objective, if an individual is already infected with the virus; a drug that joins the protease can be administered avoiding the development of mature viral proteins, stopping thus the viral replication.

This major SAR-CoV-2 protease is a dimer made up of two identical protein chains, and must dimerize to become a functional protease. There are many amino acid interactions at the dimer interface, but the researchers who published this crystal structure suggest that ionic interactions between the side chain of this arginine residue and this glutamate drive dimerization. This interaction is present on both sides of the dimer. Moving towards the active site, the important residues are constituted by cysteine chain (Cys-145) and histidine (His-41).

This enzyme is a cysteine protease, so it uses nucleophilic cysteine to attack the amide bond of a peptide. In the mechanism, the histidine nitrogen grabs the proton of the cysteine side chain allowing it to attack the peptide bond.

The peptide bond breaks, and then a water molecule can enter, releasing cysteine so that the protease can break another polypeptide chain. Enzymes containing nucleophilic catalytic residues are excellent targets for irreversible inhibition. Because they contain a nucleophilic amino acid side chain - cysteine in this case - inhibitors can be designed that bind to the enzyme with a permanent covalent bond. Chlorine dioxide also acts here, oxidizing cysteine, so this mechanism is blocked by it. Unlike reversible inhibitors that can move in and out of an active site, these irreversible inhibitors - also called suicide inhibitors - permanently inactivate the protein, preventing it from doing its job and creating more viral proteins. These researchers had previously designed inhibitors for other coronavirus proteases. They were able to bind one of these inhibitors to the active site of the SARS-CoV-2 protease. Serine is clearly involved in a covalent bond with the inhibitor ketone. Now this is a reversible reaction, so it is not a suicide inhibitor in itself, with the presence of the cysteine covalently bound at this active site. Over here, this carbonyl from the inhibitor is hydrogen bonding with three NH groups on the protein. The protease catalytic histidine is also involved in hydrogen bonding. This ring is involved in an extensive hydrogen bonding network that involves both the backbone atoms of the structure and the side chains. Knowing the contacts that an inhibitor makes with an enzyme allows chemists and biologists to consider the interactions and potentially design even better inhibitors. Beyond enzymatic inhibition, which would be an effective strategy to control the virus, the appearance of chlorine dioxide as a substance that does not inhibit but "dissolves" by oxidation the key structures of the virus, allows an action with almost a "surgical" molecular precision, being therefore much more effective as a viral infection control mechanism [47].

Conclusion

In conclusion, knowing the disposition of the areas where the amino acids sensitive to oxidation by chlorine dioxide are located, highlighting that the spike protein of the SARS-CoV-2 coronavirus contains 54 tyrosine, 12 tryptophan, 40 residues of cysteine, in addition to proline, which in turn is present in the structure of ACE2 in connection with RBD, allows projecting the actions of chlorine dioxide on the viral spike. The best pedagogical example is that the spike is the key and the ACE2 the lock. The deformation of the key by oxidation of chlorine dioxide in the amino acids cysteine, tyrosine, tryptophan and proline, of the helix chains and of the oxidation of the lock (ACE2) prevent not only the union, but also dissolve the existing union between the spike (RBD) and ACE, very quickly.

Recognition

We want to express our gratitude for your collaboration and contributions to the doctor Dr. Mitchell B. Liester, University of Colorado School of Medicine, Colorado Springs Branch, Monument, CO 80132.

Funding

This work was supported with the researchers' own resources.

Conflict of Interest

Kalcker, Andreas declares a possible financial interest as he is the inventor of the Swiss patent pending/11136-CH. The other two authors have no competing economic interests. This does not alter the authors' adherence to all policies on the exchange of data and materials.

References

1. Guo, Yan-Rong, Qing-Dong Cao, Zhong-Si Hong and Yuan-Yang Tan, et al. "The Origin, Transmission and Clinical Therapies on Coronavirus Disease 2019 (COVID-19) Outbreak—An Update on the Status." *Mil Med Res* 7 (2020): 1-10.

2. Oyarzún, Gómez Manuel. "Translational medicine: a silver bridge between basic sciences and Clinical Medicine." *Rev Chil I breathed* 33 (2017): 81-84.
3. <https://www.lenntech.es/procesos/desinfeccion/quimica/desinfectantes-diocopio-de-cloro.html>
4. Ison A, Odeh IN and Margerum DW (2006) Kinetics and Mechanisms of Oxidations of Chlorine Dioxide and Cysteine Chlorite and Glutathione. *Inorg Chem* 45: 8768-8775.
5. Stewart DJ, Napolitano MJ, Bakhmutova-Albert EV and Margerum DW (2008) Kinetics and Mechanisms of Oxidation of Tryptophan with Chlorine Dioxide. *Inorg Chem* 47: 1639-1647.
6. Napolitano, Michael J, Brandon J Green, Jeffrey S Nicoson and Dale W. Margerum. "Chlorine Dioxide Oxidations of Tyrosine, N-Acetyltyrosine, and Dopa." *Chem Res Toxicol* 18 (2005): 501-508.
7. Tan, Hsiou-Kun, Willis B Wheeler and Cheng-I Wei. "Reaction of Chlorine Dioxide with Amino Acids and Peptides: Kinetics and Mutagenicity Studies." *Mutation Res* 188 (1987): 259-266.
8. Logjnova, IV, SA Rubtsova and AV Kuchin. "Oxidation by Chlorine Dioxide of Methionine and Cysteine Derivatives to Sulfoxides." *Chem Nat Compd* 44 (2008): 752-754.
9. Gray, Nicholas F. "Free and Combined Chlorine." In: *Microbiology of Waterborne Diseases*, (2nd Edn) Academic Press, London.
10. Young, RO. "Chlorine Dioxide (ClO₂) As a Non-Toxic Antimicrobial Agent for Virus, Bacteria and Yeast (Candida Albicans)." *Int J Vaccines Vaccin* 2 (2016): 00052.
11. Ogata, Norio and Takashi Shibata. "Protective Effect of Low-Concentration Chlorine Dioxide Gas against Influenza a Virus Infection." *J Gen Virol* 89 (2008): 60-67.
12. Schijven, Jack, Peter Teunis, Trudy Suylen and Henk Ketelaars, et al. "QMRA of Adenovirus in Drinking Water at a Drinking Water Treatment Plant Using UV and Chlorine Dioxide Disinfection." *Water Res* 158 (2019): 34-45.
13. Harakeh, M and M Butler. "Inactivation of Human Rotavirus, SA11 and other Enteric Viruses in Effluent by Disinfectants." *Epidemiol Infect* 93 (1984): 157-163.
14. Zhong, Qingxia, Anna Carratalà, Rachele Ossola and Virginie Bachmann, et al. "Cross-Resistance of UV-or Chlorine Dioxide-Resistant Echovirus 11 to Other Disinfectants." *Frontiers Microbiol* 8 (2017): 1928.
15. Noss, Charles I, Fred S Hauchman and Vincent P Olivieri. "Chlorine Dioxide Reactivity with Proteins." *Water Res* 20 (1986): 351-356.
16. Alvarez, Maria E and RT O'Brien. "Mechanisms of Inactivation of Poliovirus by Chlorine Dioxide and Iodine." *Apl Reign Microbiol* 44 (1982): 1064-1071.
17. <https://Microbeonline.Com/Influenza-Flu-Virus-Structure-Classification/>
18. <https://www.Osha.Gov/Dts/Sltc/Methods/Inorganic/ld202/ld202bkr.html>
19. Ogata, Norio. "Denaturation of Protein by Chlorine Dioxide: Oxidative Modification of Tryptophan and Tyrosine Residues." *Biochem* 46 (2007): 4898-4911.
20. Ogata, Norio. "Inactivation of Influenza Virus Haemagglutinin by Chlorine Dioxide: Oxidation of the Conserved Tryptophan 153 Residue in the Receptor-Binding Site." *J Gen Virol* 93 (2012): 2558-2563.
21. Nemerow, Glen R, Phoebe L Stewart and Vijay S. Reddy. "Structure of Human Adenovirus." *Curr Opin Virol* 2 (2012): 115-121.
22. Pesavento, JB, SE Crawford, MK Estes and BV Venkataram Prasad. "Rotavirus Proteins: Structure and Assembly." In *Reoviruses: Entry, Assembly And Morphogenesis*, Springer, Berlin, Heidelberg, 2006.
23. Dennehy, Penelope H. "Rotavirus Infection: A Disease of the Past?" *Infect Dis Clin* 29 (2015): 617-635.
24. Chen, Yu-Shiaw, and JAMES M. Vaughn. "Inactivation of Human and Simian Rotaviruses by Chlorine Dioxide." *Appl Environ Microbiol* 56 (1990): 1363-1366.
25. Hogle, James M "Poliovirus Cell Entry: Common Structural Themes in Viral Cell Entry Pathways." *Annu Rev Microbiol* 56 (2002): 677-702.
26. Ridenour, GM and RS Ingols. "Inactivation of Poliomyelitis Virus by "Free" Chlorine." *Am J Public Health Nations Health* 36 (1946): 639-644.
27. <https://www.Epa.Gov/Pesticide-Registration/List-N-Disinfectants-Use-Against-Sars-CoV-2>
28. Wang, Xin-Wei, Jin-Song Li, Min Jin and Bei Zhen, et al. "Study on the Resistance of Severe Acute Respiratory Syndrome-Associated Coronavirus." *J Virol Methods* 126 (2005): 171-177.
29. Tan, Hsiou-Kun, Willis B Wheeler and Cheng-I Wei. "Reaction of Chlorine Dioxide with Amino Acids and Peptides: Kinetics and Mutagenicity Studies." *Mutat Res* 188 (1987): 259-266.
30. Ison, Ana, Ihab N Odeh and Dale W Margerum. "Kinetics and Mechanisms of Chlorine Dioxide and Chlorite Oxidations of Cysteine And Glutathione." *Inorg Chem* 45 (2006): 8768-8775.
31. Napolitano, Michael J, Brandon J Green, Jeffrey S Nicoson and Dale W Margerum. "Chlorine Dioxide Oxidations of Tyrosine, N-Acetyltyrosine, and Dopa." *Chem Res Toxicol* 18 (2005): 501-508.
32. Stewart, David J, Michael J Napolitano, Ekaterina V Bakhmutova-Albert and Dale W Margerum. "Kinetics and Mechanisms of Chlorine Dioxide Oxidation of Tryptophan." *Inorg Chem* 47 (2008): 1639-1647.
33. Tao, Y, K Queen, CR Paden And J Zhang, et al. Severe Acute Respiratory Syndrome Coronavirus 2 Isolate 2019-Ncov. USA-IL1/2020, Complete Genome. NCBI Genbank, 2020.
34. Song, Wenfei, Miao Gui, Xinquan Wang And Ye Xiang. "Cryo-EM Structure of the SARS Coronavirus Spike Glycoprotein in Complex with its Host Cell Receptor ACE2." *Plos Pathogens* 14 (2018): E1007236.
35. Jaimes, Javier A, Nicole M André, Jean K Millet and Gary R Whittaker. "Structural Modeling of 2019-Novel Coronavirus (Ncov) Spike Protein Reveals a Proteolytically-Sensitive Activation Loop As A Distinguishing Feature Compared to SARS-CoV-2 and Related SARS-Like Coronaviruses." *Arxiv* 2002.06196 (2020): 2-4.
36. Grifoni, Alba, John Sidney, Yun Zhang and Richard H Scheuermann, et al. "Candidate Targets for Immune Responses to 2019-Novel Coronavirus (Ncov): Sequence Homology and Bioinformatic-Based Predictions." *Cell-Host-Microbe-D-20-00119* (2020).
37. Xie, Liangzhi, Chunyun Sun, Chunxia Luo, and Yanjing Zhang, et al. "SARS-CoV-2 and SARS-CoV-2 Spike-RBD Structure and Receptor Binding Comparison and Potential Implications on Neutralizing Antibody and Vaccine Development." *Biorxiv* (2020).
38. Yan, Renhong, Yuanyuan Zhang, Yingying Guo and Lu Xia, et al. "Structural Basis for the Recognition of the 2019-Ncov by Human ACE2." *Biorxiv* (2020).
39. Lan, Jun, Jiwan Ge, Jinfang Yu And Sisi Shan, et al. "Structure of the SARS-CoV-2 Spike Receptor-Binding Domain Bound to the ACE2 Receptor." *Nature* 581 (2020): 215-220.
40. Zhang, Linlin, Daizong Lin, Xinyuanyuan Sun and Katharina Rox, et al. "X-Ray Structure of Main Protease of the Novel Coronavirus SARS-CoV-2 Enables Design of α -Ketoamide Inhibitors." *Biorxiv* (2020).
41. Walls, Alexandra C, Young-Jun Park, M Alejandra Tortorici and Abigail Wall, et al. "Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein." *Cell* (2020).
42. Wrapp, Daniel, Nianshuang Wang, Kizzmekia S Corbett and Jory A. Goldsmith, et al. "Cryo-EM Structure of the 2019-Ncov Spike in the Prefusion Conformation." *Science* 367 (2020): 1260-1263.
43. Shang, Jian, Gang Ye, Ke Shi and Yushun Wan, et al. "Structural Basis of Receptor Recognition by SARS-CoV-2." *Nature* 581 (2020): 221-224.
44. Xiaoli, Xiong, Kun Qu and John AG Briggs "A Closed and Thermostable SARS-CoV-2 Peak Protein Trimer", *Nature Structural Mol Biol. Biorxiv* (2020).
45. Lam, Tommy Tsan-Yuk, Na Jia, Ya-Wei Zhang And Marcus Ho-Hin Shum, et al. "Identifying SARS-CoV-2-Related Coronaviruses In Malayan Pangolins." *Nature* (2020): 1-4.
46. Zambrano-Estrada, Xochitl, Carlos A Dominguez-Sanchez, Marina Banuet-Martinez and Fabiola Guerrero-De La Rosa, et al. "Evaluation of the Antiviral Effect of Chlorine Dioxide (ClO₂) Using A Vertebrate Model Inoculated With Avian Coronavirus." *Biorxiv* (2020).
47. García, R. Preliminary Results Use of Oral Chlorine Dioxide in Patients with COVID-19. Direct Report, 2020.

How to cite this article: Insignares- Carrione E, Bolano Gomez B and Kalcker Andreas. "Chlorine Dioxide in COVID-19: Hypothesis about the Possible Mechanism of Molecular Action in SARS-CoV-2." *J Mol Genet Med* 14 (2020): 468