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Pharmacokinetics and Pharmacodynamics of Chlorine Dioxide

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Content

Abstract

Chlorine dioxide (ClO_2) is widely used as a drinking water disinfectant in many countries. Due to its antibiotic and antiviral capacity, has aroused interest as a potential therapeutic agent with respect to COVID-19 disease, AIDS and Influenza. As a result of this debate in scientific and governmental settings, it was deemed highly timely to provide an up-to-date assessment of the pharmacokinetics and pharmacodynamics of ClO_2. The main findings indicate that, due to its high chemical reactivity, ClO_2 is rapidly reduced in oral and gastric secretions, producing the chlorite ion (ClO_2^-) which becomes the active agent responsible for its systemic actions. ClO_2^- also showed potential to act as an oxidant at high concentrations and as antioxidant at low concentrations. Of particular therapeutic interest are the findings that, at low concentrations, ClO_2^- can protect erythrocytes from oxidative stress while at the same time inhibiting the excessive production of hypochlorous acid (HClO) induced by myeloperoxidase (MPO), thus reversing inflammatory responses and

macrophage activation. Finally, taurine-chloramine represents the most relevant functional product formed under the influence of ClO_2^-, said molecule activates erythroid nuclear factor 2 (Nrf2), (this transcription factor regulates the inducible expression of numerous genes for enzymes detoxifiers and antioxidants), increases the expression of heme-oxygenase (HO-1), protects cells from death caused by hydrogen peroxide (H2O2), improves the expression and activities of antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase, and contributes to the resolution of the inflammatory process.

Key words: chlorine dioxide, chlorite, hormesis, taurine-chloramine, myeloperoxidase.

1. Introduction

ClO_2 is a yellow gas that can decompose quickly in the air. Because ClO_2 is very reactive, it can inactivate viruses, bacteria and other microorganisms in the water. About 5% of the large water-treatment facilities (serving more than 100,000 people) in the United States use ClO_2 to treat drinking water. An estimated 12 million people can be exposed in this way to ClO_2 and ClO_2^-. In the communities that use ClO_2 to treat drinking water, ClO_2 and its by-product, the ClO_2^- ion, may be present at low levels in tap water (US-ASTDR (2004). EPA (Environmental Protection Agency) has established the maximum concentration in drinking water at 0.8 milligrams per liter (mg / L) for ClO_2 and 1.0 mg / L for the ClO_2^- ion. The FDA (Food and Drug Administration) of the United States of America and COFEPRIS (Federal Commission for Protection against Sanitary Risks) in México state that the consumption of ClO_2 causes kidney and liver failure, and it also destroys red blood cells.

In the paradigm of drug administration, to determine the correct dose of a drug is often a challenge. Previously, it has been observed that several drugs demonstrate contradictory effects *per se*, at high and low concentrations. This duality in the effect of one drug at different concentrations is known as hormesis (Bhakta-Guha and Efferth, 2015). For several decades it was believed that drug dosing follows a linear pattern, generating an enormous ignorance about the responses in the area of low concentrations (Calabrese and Baldwin, 2001). However, in recent years, several studies have shown an inverse response to different concentrations of a drug in the same individual, thus completely ruling out linearity and threshold response models of determination of concentration (Calabrese et al., 2010). This effect, known as the “biphasic response to concentration”, has shown importance to establish the administration of a drug (Calabrese, 2001; Huang and Zheng, 2006; Day and Suzuki, 2006; Calabrese, 2014).

It is well documented that mild environmental stress such as exposure to low doses of stressful stimuli often prompts the adaptive stress response in individuals to maintain homeostasis (Kuoda and Iki, 2010; Martins et al.,2011). It also complies with the fact that while higher levels of a toxic substance can be clearly harmful, small doses of it can promote health, governed by growth and development (Calabrese, 2001; Schumacher, 2009). External effectors (stimuli), such as stressors or aggressors that induce stress in

higher concentrations, are often referred to as hormetins (Menendez et al., 2013; Mattson, 2008). In this conjuncture, it is imperative to establish that the term "stress" can have multiple implications. In the context of this review, we mean parameter, extrinsic or intrinsic, which can induce a deviation of the normal physiological processes of the body (Bhakta-Guha and Efferth, 2015). Exposure to stress often causes pathways designed to combat the same, which are known as responses to stress (Dattilo et al., 2015). Several of these responses often require stimulation of survival pathways (Rattan, 2006). The hormetins can be of biological, physical or chemical origin (Kouda and Iki, 2010; Richardson, 2009). The generation of the adaptive response to continuous mild exposures to such stressors is an evolutionarily conserved trait, which in the long run protects an individual against future attacks of high concentration and stress (Martins et al., 2011). In this regard, an exhaustive review (Calabrese and Baldwin, 2003) compiled a series of inorganic agents (inducing hormetic dose-response relationships), including toxic agents of great environmental and public health interest (for example, arsenic, cadmium, lead, mercury, selenium and zinc).Therefore, in this article we will review the status of the experimental knowledge on toxicity, viricidal /antiviral action, pharmacokinetics and pharmacodynamics of ClO_2 / ClO_2^- with the aim of looking for hormetic mechanisms that can induce adaptive responses to stress that explain its supposed therapeutic properties.

2. Toxicity

The toxicological profile of ClO_2, and of its first reducing product, the chlorite anion (ClO_2^-), has been extensively studied in animal tests and reviewed in successive technical reports of the United States administration (US-EPA, 2000; US-ASTDR, 2004). In these studies, toxic reactions have been reported above different exposure levels, after the oral and inhalation routes. Adverse reactions consisted of oral and digestive irritation, anemia and methemoglobinemia, altered thyroid function, neurotoxicity with delayed brain development in puppies. After a comprehensive review of animal studies, the US-ASTDR (2004) concluded that the maximum dose tested among all the studies reviewed in which no adverse effects were observed (NOAEL level) should be set at 3 mg (ClO_2 / ClO_2^-) / kg / day. The lowest level of adverse effects observed in this review was concluded to be 5.7 mg / kg / day. These levels were obtained after a final US EPA-mandated animal study (Gill et al, 2000) in which toxicity was characterized in a 2-generation study to examine reproductive, developmental, and reproductive end points, neurological and hematological in rats exposed to sodium chlorite ($NaClO_2$) in drinking water, including sensitive groups.

Although few clinical reports of toxicity in humans have been reported to date, animal studies have shown effects of ClO_2 and ClO_2^- that are similar to those seen in people exposed to very high amounts of these chemicals. In a trial in non-human primates, the increasing sub-chronic toxicities of oral administration of ClO_2, $NaClO_2$, $NaClO_3$, and NH2CI were studied in African green monkeys for 30-60 days. A *reversible* inhibition of thyroid metabolism at sub-chronic doses of ClO2 (9 mg / kg / day, corresponding to 100 mg / L) in drinking water was registered, but no effects were observed at 3 mg / kg / day

(Bercz et al., 1982). The ingestion of ClO_2^- in primates at high doses caused a decrease in the erythrocyte count, as well as an increase in transaminases. Interestingly, most of the ClO2 doses induced a self-compensating oxidative stress in hematopoiesis, since a rebound phenomenon occurred in the synthesis of hemoglobin and red blood cells, suggesting an hormetic effect that will be discussed later. Furthermore, no thyroid inhibition was detected after the use of ClO_2^- at concentrations up to 60 mg / kg / day (Bercz et al., 1982). The selective thyroid effect of ClO_2 was paradoxical since ClO_2 was rapidly reduced by oral and gastric secretions to non-oxidizing species, presumably chloride (Cl^-).

In one of the first human studies ordered by the US-EPA (Lubbers et al., 1982), acute tolerance was evaluated at an increasing dose from the oral administration of different chlorinated water disinfectants. Systemic toxicity was not detected below the maximum dose of 24 mg / L of ClO_2 and 2.4 mg / L of ClO_2^-, ingested twice daily 4 hours apart. In another sub-chronic toxicity experiment, daily oral ingestion of ClO2 at a concentration of 5 mg / L for 12 consecutive weeks produced no obvious undesirable clinical adverse effects. The absence of human ClO_2^- toxicity below this US-ASTDR established NOAEL level has been reported in recent controlled clinical trials. In a phase I, placebo-controlled study of safety and tolerability in patients with Amyotrophic Lateral Sclerosis (ALS), Miller et al., (2014) tested single ascending doses of 0.2, 0.8, 1.6 and 3.2 mg / kg of intravenous $NaClO_2$. After treatment, patients were monitored for avariety of safety variables and clinical status during and for 8 h after infusions, one, four and seven days after dosing. All doses were generally safe and well tolerated, and there were no serious treatment-related adverse events. In an additional phase II study in patients with ALS, no adverse effects were observed when 2 mg / kg / day was administered in repeated sub-acute exposures monthly (3-5 days) (Miller et al, 2015).

At extreme levels of exposure, adverse events have been described due to suicide attempts when ingesting massive doses of ClO_2^-. After intoxication by a single 10 g dose of $NaClO_2$, approximately 142 mg / kg (Lin and Lim, 1993), excessive oxidative stress caused irritation in the digestive tract accompanied by nausea, vomiting and kidney failure. In another suicide attempt by an adult male who ingested about 100 ml of 28% NaClO2 solution (Gebhardtova et al., 2014), initial laboratory tests revealed 40% methemoglobin formation and acute kidney failure. The lowest observable toxicity (LOAEL) would be expected from 5.7 mg kg / day, equivalent to 420 mg / day for an average adult human. In conclusion, there is no experimental evidence available to support that administering doses lower than 3 mg / kg / day there is a risk of systemic toxicity or variations in relevant clinical parameters. This dose is equivalent to 210 mg of ClO_2 per day for an average 70 kg adult.

3. Viricidal / antiviral activity

The viricidal activity of ClO_2 in vitro has been described against different human and animal viruses (Ogata and Shibata, 2008; Sanekata et al, 2010). For example, it was

reported that ClO_2 was able to inactivate the human Influenza virus by 99.9% at 15 seconds using 1 ppm, against the Measles virus this inactivation was achieved at 30 seconds using 10 ppm and against the Herpes virus was achieved at 15 seconds using 10 ppm (Sanekata et al., 2010). Other researchers demonstrated that ClO_2 is capable of destroying the Poliomyelitis virus using 1-2 ppm, that of Hepatitis A with 7.5 ppm, Rotavirus with 0.2 ppm, 10 ppm for the HIV-1 virus, and 2.1 ppm for the coronavirus that caused SARS (cited in Miura and Shibata, 2010).

The potential use as a prophylactic against viral infections was demonstrated for influenza A by inhalation in mice (Ogata and Shibata, 2008). Regarding the direct viricidal action mechanism of ClO_2 / ClO_2-, mechanisms have been proposed based on the oxidation of key amino acid residues in the viral protein envelope, such as denaturation of virus RBD (receptor binding domain), thereby abolishing its receptor-binding ability (Ogata, 2007,2012). According to studies carried out with radioactive labeling, chlorite has a half-life of 3.5 hours (Abdel-Rahman et al., 1982), enough time to produce direct plasma antiviral effects, which would consist of the oxidation of amino acids found in the protein envelope of viruses. In particular, the oxidation of thiol residues (-SH) of tyrosine, threonine and tryptophan is important. The formation of disulfide bridges between the spike protein of SARS COV2 and the ACE2 receptors of human alveolus cells has been cited (Hati and Bhattacharya, 2020). By reducing oxidative stress as one of the pathogenic mechanisms of the virus, therapeutic doses of ClO_2 / ClO_2^- could prevent the formation of disulfide bonds and binding between the ACE2 receptor and the spike protein.

Importantly, the first in vivo trial describing an antiviral effect of ClO_2 against avian coronavirus has recently been reported (Zambrano-Estrada et al., in press). ClO_2 treatment had a marked impact on viral infection. That is, viral titers were 2.4 times lower and mortality was reduced by half in infected embryos that were treated with ClO_2. The infection caused developmental abnormalities regardless of treatment. Typical lesions of this virus infection were observed in all inoculated embryos, but gravity tended to be significantly lower in embryos treated with ClO_2. No macroscopic or microscopic evidence of toxicity caused by ClO_2 was found at the doses used in this study (Zambrano-Estrada et al., in press). In addition, the results of the first clinical study demonstrating the efficacy of ClO_2 for the treatment of COVID-19 disease have recently been published. In this research, the effect of $Cl0_2$ on the clinical evolution of 20 patients with active infection by the Sars-CoV-2 virus was analyzed. The control group consisted of 20 patients who did not receive ClO_2. The experimental group consisted of 20 patients who received an oral solution of ClO_2 at a concentration of 30 mg / L (well below the NOAEL) for 21 days. When comparing the experimental group with the control group on the seventh day after the manifestation of symptoms, a significant difference was found in the experimental group with respect to the control group for the symptoms: Fever (p: 0000), Cough (p: 0.0000) , Chills (p: 0.0000) and Dyspnea (p: 0.0006). When performing the analogous visual comparison of pain in the control group and in the experimental group, it was found that in all the items that make up the scale it decreased significantly in this group with respect to the control group (p: 0.0000; p: 00017). To the day 14, the difference was greater (p: 0.000;

p: 0.0043). When evaluating both groups (Control and Experimental) in the laboratory, a difference was found for the values of the C-reactive protein (CRP) parameters on day 7 (p: 0.0001) and LDH (Lactate Dehydrogenase) (0.0036) , with higher scores for the experimental group; D-dimer on day 7 (p: 0.0194) and day 14 (p: 0.0029); difference was found in all parameters. Overall results ($p < 0.05$) confirmed the hypothesis that chlorine dioxide is effective in the treatment of COVID-19 (Insignares-Carrione et al., 2021).

ClO_2^- also produces antiviral / viricidal effects. A study carried out in the 90s aimed to determine the effects of ClO_2^- (WF10) on the replication of HIV and on the infectivity of HIV-free particles (Raffanti et al., 1998). It was shown that the WF10 complex exhibits a significant antiviral activity against HIV. These investigators demonstrated that the ClO_2^--oxygen complex modifies the virion outer envelope (glycoprotein gp120), thus inhibiting it's binding to the CD4 molecule on CD4 + T cells. HIV inactivation is mediated by the oxidative activity of the ClO_2^--oxygen complex (Raffanti et al., 1998). This oxidative mechanism is similar to that mediated by ClO2 for the influenza virus; an oxidation of a tryptophan (W153) residue in hemagglutinin (a spike protein of the virus), thus abolishing its receptor binding capacity. In other words, this oxidative modification induces structural changes in the hemagglutinin binding site and disrupts its interaction with the host cell receptor (Ogata, 2012). The spike protein of the new coronavirus SARS CoV2 contains 54 tyrosine residues, 12 tryptophan and 40 cysteine residues (Tao et al., 2020. In a recent work, 3D reconstructions were made by computer, use of data through studies in cryo-electron microscopy and previous work based on augmented reality software (Insignares-Carrione et al, 2020). Said simulations made it possible to determine the positions of the amino acids susceptible to being oxidized by ClO_2 and which allows inferring their possible mechanism of action on the SARS-CoV-2 virus. These researchers have postulated that ClO_2 could oxidize the cysteine Cys480-Cys488, which are key to the binding between the SARS-CoV-2 spike and the ACE2 receptor (Insignares-Carrione et al., 2020).

At the time of this writing (August 2021), the Sars-Cov2 has caused the death of 4,448,408 people. In a recent survey of more than 100 immunologists, infectious-disease researchers and virologists, almost 90% said that SARS-Cov-2, the virus that causes Covid, will become endemic. That means that there could be a "constant presence and/or usual prevalence of a disease or infectious agent in a population within a geographic area," (Phillips, 2021). In this regard, it is important to mention that recent investigations demonstrated that ClO_2 at 0.25 mmol/L is effective in inactivating the binding of the spike protein to the ACE2 receptor (Ogata and Miura, 2020). Furthermore, ClO2 is also able to inhibit the binding of variants of Sars-CoV-2 spike protein to the ACE2 receptor (Ogata and Miura, 2021). These findings suggest that ClO_2 may be invaluable for inhibiting the infection of SARS-CoV-2 virus to human, and also to treat patients once infected, as mentioned above.

4. Pharmacokinetics

There are still few studies in humans that allow us to characterize the pharmacokinetics of ClO_2, that is, the processes of digestion, absorption, distribution, metabolism and excretion

to which it is subjected through its passage through the body (US-ASTDR, 2004). In this work we present a hypothesis based both on the redox properties of this gas and its metabolites, as well as on studies carried out in animals and in two controlled clinical trials published in humans (Miller et al., 2014; 2015). Chemically, ClO_2 is an unstable free radical, with an unpaired electron that makes it a strong oxidant, rapidly reducing to ClO_2^- in the presence of electron donor species, such as proteins and amino acids, that is, the processes of digestion, absorption, distribution, metabolism and excretion to which it is subjected through its passage through the body (US-ASTDR, 2004).

4.1. Degradation in the oral / gastric cavity

The most relevant experimental data to characterize pharmacokinetics come from animal studies. *In vivo* studies in mice based on the radioactive labeling of ClO_2, Abdel-Rahman et al (1979a; 1984), using radioactively labeled chlorine (Cl), determined a rapid gastrointestinal absorption and a wide distribution in tissues, with a plasma peak after one hour of ingestion. The absorption rate and half-life were estimated at 3.77 and 0.18 hours, respectively (Abdel-Rahman et al., 1982).

Bercz et al. (1982) studied the oral processing of ClO_2 and ClO_2^- solutions in non-human primates. Saliva was shown to be a powerful ClO2 reducer, with only 5-12% of the initial content remaining after 1 minute of *in vitro* reaction. Human saliva contains a wide range of biomolecules, many of which are reactive with ClO_2, including amino acids, the latter including cysteine, tryptophan, and methionine (Silwood et al., 1999). Bercz et al. (1982) also studied the gastric digestion of ClO_2 "in vivo", recovering only 8% of the initial oxidative capacity of ingested ClO_2 after 5 minutes of contact with gastric contents.

4.2. Metabolism

The absorbed ^{36}Cl is slowly removed from the blood. After 72h, 80% of the residual ^{36}Cl found in plasma was in the chloride form, and 20% in the chlorite form. After 72 hours, chlorine labeled with a radioactive isotope was mostly detected in plasma, intestine and stomach, as well as in various tissues such as lung and kidney. A small percentage of the initial radioactive chlorine (0.4-0.8%) was detected in the blood cells, in unidentified species in the body prior to its excretion, mainly as chloride and chlorite. A 72-hour urinary excretion rate of 30-35% of the ^{36}Cl that was not fixed by the tissues or remaining in the plasma was estimated, with a peak between 24 and 48 hours, and a half-life of 44 hours. The ^{36}Cl excreted was found mainly as chloride (Cl^-) (87%), chlorite (ClO_2-) (1.3%), and to a lesser degree as chlorate (ClO_3-). In this same work, potassium chlorite was reduced by in vitro plasma to chloride by 100%, and that oral ClO_2 was reduced by in vivo plasma by 82.3% to chlorite and 17.6% to chloride. Of the total eliminated, 72% was in urine and 25% in feces. In urine, the chlorine marked in ClO_2 and chlorite was mainly excreted as chloride ion, 87% and 84% respectively. The formation of trihalomethanes was not detected at the low doses tested (maximum 1.5mg / kg / day, 100 ppm). No excretion by air was detected. The absorption and elimination of ClO_2 and its metabolites was faster than for hypochlorite (HClO), the active component of common bleach.

Chlorite is also rapidly absorbed from the gastrointestinal tract. Maximum plasma levels of radiolabeled chlorine were reached 2 hours after administration of a single 100 mg/L dose of $^{36}ClO_2$- (approximately 0.13 mg/kg) to Sprague-Dawley rats. Using 72-hour urinary excretion data, 35% of the initial dose was estimated to have been absorbed (Abdel-Rahman et al., 1984). The absorption rate constant and the half-life were 0.198 / hour and 3.5 hours, respectively (Abdel-Rahman et al., 1982). Due to the extreme reactivity of ClO_2, it is rapidly reduced to chlorite in the stomach when it reacts with food, organic substances, tissues, or other materials that can serve as electron donors. Although ClO_2 is unlikely to survive the stomach environment long enough to be absorbed, the chlorite ion can be absorbed and enter the blood (Harrington et al., 1989).

These works (Abdel-Rahman et al, 1979, 1982; Bercz et al. 1984) come to support our hypothesis of the global reduction of ClO_2 to ClO_2^- and this to chloride in blood as the basis of the mechanism of action, through intermediate redox interactions with plasma proteins and cellular enzymes. Abdel-Rahman et al., 1979 found that, in aqueous solution, potassium chlorite was partly reduced to chloride (21%), but to a lesser extent oxidized to chlorate (7%), a fact to take into account when controlling the quality and stability of formulations based on chlorite as an active principle. Therefore, and depending on the redox environment and pH, a rapid interconversion between these metabolites can be triggered in initial aqueous solutions of pure ClO_2, intensified after gastric and intestinal digestion (Bercz et al, 1982). The final processing of ClO_2 and its metabolites would consist of its excretion via the urinary and fecal routes, mainly as chloride, with smaller amounts of chlorite and chlorate, as shown in the aforementioned work by Abdel-Rahman et al, (1979b; 1984).

5. Pharmacodynamics

Similarly, there is limited work that describes the pharmacodynamics of ClO_2 and ClO_2^- *in vivo*, that is, the biochemical and physiological effects of this molecule, its mechanisms of action and the relationship between the dosage of the drug solution and its therapeutic systemic effect. Taking into account the pharmacokinetic data, it can be hypothesized that orally administered ClO_2 is rapidly reduced to $ClO2^-$ in the oral cavity through saliva or in the stomach through acid secretions (Bercz et al, 1982). In the small intestine, ClO_2^- would be absorbed through the villi that are abundantly vascularized, being absorbed mainly as such anion, without ruling out minor amounts of chloride generated by partial reduction. Subsequently, both ClO_2^- absorbed by oral exposure and directly intravenously administered react by oxidizing electron donors from the complex organic matrix of plasma and blood cells, in particular proteins and amino acids.

5.1. Interactions between chlorite and peripheral blood cell heme enzymes

Schempp et al. (2001) reported that the product of macrophage hemoprotein-catalyzed chlorite reduction is a species of chloro-oxygen, probably hypochlorous acid (HClO). The common characteristic of the redox pair "H_2O_2 / hemoglobin" and "chlorite-hemoglobin" is

the initial oxidation of heme by transfer of 2 electrons, giving rise to an oxidized heme intermediate called compound I. Therefore, both oxidants are reduced, resulting in the hydroxide anion for H_2O_2, while chlorite is reduced to hypochlorous acid (HClO) that is, ClO_2 would be converted by reduction into chlorite, hypochlorous acid and finally into chloride (Henderson et al, 1999). In addition, HClO can chlorinate sulfur amino acid residues generating chloramines such as taurine-chloramine (Tau-Cl), which exert a more attenuated and stable oxidizing action than HClO, thus modulating excessive inflammation (Schempp et al., 2001).

5.1.1. Chlorite interaction with hemoglobin

Exposure to ClO_2^- can produce methemoglobinemia, but this effect was recorded only at high concentrations. For example, early reports documented that chlorite concentrations of 100 mg / L and higher in the rat caused a decrease in the number of red blood cells and hemoglobin at 30 and 60 days of exposure (Heffernan et al., 1979).

Another study (Moore and Calabrese, 1982) evaluated the toxicity of chlorite in 2 strains of mice: one with normal levels (A / J) and another with low levels (C57L / J) of Glucose-6-phosphate dehydrogenase. G6PD-deficient cells have a reduced ability to produce NADPH, thus they synthesize very little glutathione (the main defense mechanism of erythrocytes against oxidative stress). The results showed that when they were exposed to a maximum level of 100 ppm of chlorite for 30 days, there was an increase in osmotic fragility, mean corpuscular volume, and G6PD levels for both strains. However, methemoglobinemia was not reported. In the animals that were exposed to 1.0 and 10 mg / L there were none of the aforementioned anomalies. The administered doses of sodium chlorite (up to 100 mg / L) in the drinking water did not cause any toxic damage to the kidneys of the animals at 60, 90 and 180 days of exposure (Moore and Calabrese, 1982).

From these data it is clear that chlorite-mediated destruction of erythrocytes was observed only at high concentrations, revealing that erythrocytes have an efficient anti-oxidant protection system. However, when a high ClO_2^- concentration is administered, this system is no longer able to neutralize the oxidizing agent and hemolysis occurs. Normal red blood cells (RBCs) are subject to a high level of oxidative stress as a result of the continuous production of the superoxide anion that accompanies hemoglobin (Hb) autoxidation. The superoxide anion is dismutated to hydrogen peroxide (H2O2), which is further converted to the hydroxyl radical through the Fenton reaction in the presence of iron. To cope with oxidative stress, RBCs are equipped with superoxide dismutase (SOD1), catalase, glutathione peroxidase 1 (GPx1), and three isoforms of peroxiredoxin (Prx I, Prx II, and Prx VI). SOD1 converts the superoxide anion to H_2O_2, which is then removed by catalase, GPx1 and the Prxs (Rhee and Lee, 2017).

It has been reported that ClO_2, ClO_2^-; and $C1O_3^-$ in drinking water decreased blood glutathione and changed the morphology of erythrocytes in rat after 2 months (Abdel-Rahman et al., 1979). However, rats gradually adapted to $C10_2$ stress by increasing the

activity of glutathione reductase and catalase (Couri and Abdel-Rahman, 1979). These findings are consistent with the known protective role of glutation against damage by oxidants (Hill et al., 1964).

5.1.2. Chlorite is an erythrocyte protective agent

Although most investigations have reported a hemolytic effect of ClO_2^- at high concentrations, it was recently shown that ClO_2^- at low concentrations is capable of eliminating methemoglobin (Pichert and Arnhold, 2015; Flemming et al., 2016). Oxygen transport in our body is carried out by tetrameric hemoglobin in red blood cells and monomeric myoglobin in muscle tissue. In each subunit of both proteins, a protoporphyrin IX group is present with a central iron ion that can present different oxidation states. In both proteins, only the iron of the heme group in the ferrous state (Fe2 +) can bind to oxygen. Therefore, it is important to keep them in a reduced state. In erythrocytes, this is achieved primarily by methemoglobin reductase, which converts methemoglobin (containing ferric iron (Fe3 +) to ferrous hemoglobin (Fe2 +). Spontaneous methemoglobin formation generally occurs at a low rate, without generating methemoglobin yield greater than 1% (Van Slyke et al., 1946; Siggaard-Andersen et al., 1972).

From clinical trials conducted with the drug WF10, it is known that ClO_2^- interacts with hemoproteins (Schempp et al., 2001; Jakopitsch et al., 2008; Jakopitsch et al., 2014; Pichert and Arnhold, 2015), which are degraded by $ClO2^-$ with the loss of heme structure (Pichert and Arnhold, 2015). The ClO_2^--based immunomodulatory drug solution (WF10) and the more dilute form Oxoferin are applied to cushion severe inflammatory conditions and improve wound healing processes (Raffanti et al., 1998; McGrath et al., 2002; Veerasam et al., 2006; Yingsakmongkol et al., 2011).

The ClO_2^- contained in WF10 was shown to react in three different ways with hemoglobin and oxidized forms of heme (Pichert and Arnhold, 2015).

5.1.2.1. ClO_2^- oxidizes hemoglobin to methemoglobin

This can be evidenced by the change in absorbance maximum from 414 nm to 406 nm. The percentage of methemoglobin increased from 0 to 45% in the presence of WF10 when a 1: 3 dilution was used (corresponding to 21 millimoles / L of ClO_2^-, equivalent to 140 mg / L). When dilutions greater than 1: 200 (corresponding to 315 micromoles / L, equivalent to 21 mg / L) were used, methemoglobinemia did not occur (Pichert and Arhold, 2015).These results clearly demonstrate that methemoglobinemia only occurs when high concentrations of ClO_2^- are administered.

5.1.2.2. ClO_2^- degrades methemoglobin

As evidenced by the continuous decrease in the Soret band around 406 nm. This effect was obtained when a 1: 500 dilution was used (corresponding to 126 µmol of ClO_2^-, equivalent to 8.40 mg / L). This decrease in ClO_2^--mediated methemoglobin formation, which was

also later reported by Flemming et al. (2016), the ClO_2^- component of WF10, inhibited heme-induced hemolysis in a concentration-dependent manner; these authors concluded that the beneficial effects of WF10 are closely associated with the inactivation of the free heme. Furthermore, at a high concentration of WF10, iron release and degradation of the heme porphyrin ring were also observed. A drug dilution as low as 0.1%, corresponding to a ClO_2^- concentration of 62.9 µM (4.19 mg / L), completely inhibited the hemolytic effect elicited by 100 µM heme (Fleming et al., 2016).

Therefore, these data suggest that one mole of ClO_2^- is capable of inactivating around two moles of heme, by these mechanisms; heme is inactivated and loses its ability to induce hemolytic events in intact red blood cells (Flemming et al., 2016). Free heme is highly toxic to cells and tissues, especially in the spleen, liver and kidney (Schaer et al., 2013) through the activation of the Toll-like receptor 4, contributes to the activation of endothelial cells and macrophages and induces inflammatory reactions (Figueiredo et al., 2007; Belcher et al., 2014). Sepsis can evoke disseminated intravascular coagulation (such as that found in Covid-19 patients), resulting in multiple organ failure and death (Marchandot et al., 2020). Heme oxygenase-1 (HO-1) and hemopexin (HPx) can mediate cytoprotective mechanisms against these deleterious effects (Passainte et al., 2015). We suggest that ClO_2^- derived from ClO_2 metabolism could decrease the activation of disseminated intravascular coagulation in patients with Covid-19.

Other researchers (Pichert and Arnhold, 2015) found that WF10 (diluted 1: 500) is capable of efficiently reducing the production of cytotoxic hemoglobin species that can appear after excessive hemolysis of red blood cells in pathological situations. Since almost identical changes were recorded when replacing WF10 with ClO_2^- at the same concentration, these researchers concluded that ClO_2^- is the active ingredient in WF10 (Pichert and Arnhold, 2015). Very recently, a group of researchers discovered that elevated glucose levels directly induce viral replication and the expression of pro-inflammatory cytokines. Glycolysis is necessary for the replication of SARS-CoV-2 and the production of mitochondrial ROS (reactive oxygen species) induced by this virus activates the hypoxia-inducible factor (HIF-α), which in turn upregulates the glycolytic genes and IL-1b expression. Finally, they showed that SARS-CoV2-infected monocytes promote T-cell dysfunction and lung epithelial cell death. These data may explain why the uncontrolled blood glucose levels observed in diabetic patients are a significant risk for the severity of COVID-19 (Codo et al., 2020). In this sense, it was shown that the ClO_2^- -based drug (WF10) constantly reduces glycosylated hemoglobin (A1c) values and improves glucose control in diabetic patients (Maraprygsavan et al., 2016).

5.1.2.3. ClO_2^- reduces ferril hemoglobin (Fe4 +) to methemoglobin (Fe3+)

Ferril hemoglobin is capable of oxidizing numerous biological substrates such as lipids, nucleic acids, proteins, amino acids, and small molecules (Everse and Hsia, 1997; Gebicka and Banasiak, 2009). These cytotoxic reactions contribute significantly to tissue destruction in a number of diseases in which massive hemolysis occurs (Rother et al., 2005). In addition to the plasma protective components haptoglobin and hemopexin, which have the

ability to bind and eliminate free heme species (Chiabrando et al., 2011), there are other components in the blood and adjacent tissues that act as antioxidants against oxidation mediated by ferril hemoglobin.

Ascorbate, urate, and flavonoids such as catechin, quercetin, and rutin reduce ferril hemoglobin to methemoglobin (Gebicka and Banasiak, 2009; Giulivi and Davies, 1990; Jia and Alayash, 2008). Pichart and Arnhold (2015) showed that ClO_2^- (WF10) has greater potency than the natural antioxidants ascorbate and urate to reduce ferril hemoglobin, especially when it is derived from methemoglobin. This experimental evidence confirms that a strong oxidant such as ClO_2^- is capable of acting as an antioxidant when administered at low concentrations.

5.2. Inhibition of myeloperoxidase activity mediated by ClO_2^-

Klebanoff (1968) discovered that myeloperoxidase (MPO) is an enzyme that contains a heme group and is mainly found in neutrophils and macrophages. In the presence of superoxide, hydrogen peroxide, and chloride, MPO catalyzes the formation of HOCl. The MPO / HOCl system plays an important role in the destruction of microbes by phagocytes. However, myeloperoxidase can also be released outside the cell, increasing the potential for damage in atherosclerosis, Alzheimer's disease, ALS (amyotrophic lateral sclerosis), multiple sclerosis, stroke, rheumatic arthritis, Parkinson and Alzheimer's disease. (Klebanoff, 2005). HClO is kinetically the most reactive two-electron oxidant produced in appreciable amounts in our bodies. Its reactivity with most substrates exceeds that of hydrogen peroxide, hydroperoxides, and peroxynitrite by several orders of magnitude (Winterbourn, 2008). In addition to these direct toxic effects, HOCl can modulate the function and activity of various immune cells. For example, *in vitro* studies demonstrated that HOCl can activate nuclear factor kB (NF-kB) and tyrosine phosphorylation in T and B cells, increasing calcium signaling and tumor necrosis factor α production. (TNF-α) (Schieven et al., 2002; Schoonbroodt et al., 1997).

Because organizations such as the WHO and the FDA have claimed that ClO_2 / ClO^{2-} are highly toxic, hardly anyone has investigated whether they could have a therapeutic effect if administered at low concentrations. There are very few studies that have investigated the therapeutic effects of ClO_2^-. The drugs called NP001 and WF10 are formulated based on $NaClO_2$ / ClO_2^-, respectively. NP001, a highly purified, pH-adjusted stabilized form of $NaClO_2$ (sodium chlorite), is a new effector molecule that represents a new class of drug for regulating the function of inflammatory macrophages in both in vitro and in vivo studies (McGrath et al. al., 2002). ClO_2^- mediates its anti-inflammatory effect on macrophages by creating high intracellular levels of taurine chloramine, a factor known to negatively regulate inflammatory pathways induced by NF-κB (Joo et al., 2009; Giese et al., 2004). In previous clinical studies with another form of ClO_2^- (WF10), this regulation reverses inflammation and causes systemic macrophages to return to a normal phagocytic state (McGrath et al., 2002). The results of a randomized phase 2 clinical trial of a new immune regulator, NP001, in amyotrophic lateral sclerosis (ALS) (NCT01281631) were recently presented. Although the trial results were negative, they showed that NP001-treated

patients whose baseline CRP (C - reactive protein) levels were above the median of the entire randomized population had a slower progression of ALS than patients who received placebo (Miller et al., 2015).

There are several diseases related to abnormal inflammatory response and macrophage activation: ALS, AIDS, Alzheimer and multiple sclerosis (Minagar et al., 2002). Macrophage activation has been shown to require high intracellular myeloperoxidase activity (Rodrigues et al., 2002). Several studies showed that MPO-mediated HOCl formation can serve as a major source of macromolecular oxidative damage (Winterbourn et al., 1992; Hawkins et al., 2003; Kawai et al., 2004). Clearly, cellular exposure to HOCl can cause many deleterious effects by altering the cellular redox state (Woods et al., 2009), so it is important to modulate excessive MPO activity to avoid further HClO production. In this sense, a seminal study demonstrated that ClO_2^- (WF10) at low concentration (micromolar range) effectively inhibits myeloperoxidase activity (Schempp et al., 2001). This effect was later confirmed by Jakopitsch et al. (2014). This MPO inhibition is directly related to the anti-inflammatory effect reported for this drug (Schempp et al., 2001; Giese et al., 2004).

The inhibition of MPO activity with low $ClO2^-$ concentrations could have an important application in medicine. Resolution of inflammation depends on neutrophils undergoing apoptosis; however, this does not always happen and neutrophils continue to promote the inflammatory process. An unexpected role for MPO in influencing neutrophil fate and consequently the duration of inflammation has been reported. By suppressing the constitutive cell death program (apoptosis), MPO prolonged the lifespan of the neutrophils, thus delaying the resolution of inflammation (El Kebir et al., 2008). From these data, it is clear that induction of neutrophil apoptosis would improve the resolution of inflammation. As ClO_2^- is an effective MPO inhibitor, many acute and chronic inflammatory conditions could be mitigated with the use of this molecule.

Resistance to apoptosis is a key characteristic of cancer cells and is believed to be regulated by nitrosonium ion (NO) -induced S-nitrosylation of apoptotic proteins such as caspase 3. Nitric oxide (NO), produced by Inducible nitric oxide synthase (iNOS), is used by MPO to generate NO. It was shown that 65% of the analyzed invasive epithelial ovarian carcinoma samples express MPO and iNOS in neoplastic cells, without expression in normal ovarian epithelium (Saed et al., 2010). These authors also demonstrated that the genetic silencing of MPO or iNOS significantly induced apoptosis, highlighting its role as a redox switch that regulates apoptosis. ClO_2^- could induce apoptosis of cancer cells based on its demonstrated inhibitory effect on MPO activity.

5.3. Taurine-chloramine neutralizes HClO-induced toxicity

When neutrophils ingest and destroy bacteria, HClO is produced within the phagocytic vacuole that surrounds the bacteria. Although it is a powerful bactericide, it can also be toxic because it oxidizes various biological molecules (Kettle et al., 2014). To avoid this toxicity, taurine chemically reacts with HClO in leukocytes to form taurine-chloramine

(tau-clo), a more stable, less reactive, and more selective oxidant than HClO (Weiss et al., 1982; Schempp et al. ., 2001; Giese et al., 2004). It is commonly accepted that tau-clo works in biological systems as a general antioxidant: it can specifically protect cells from self-destruction during processes that generate HClO excessively (Marcinkiewicz et al., 1995). Pre-treatment of rats with taurine prior to acute ozone exposure results in a decrease in oxidant-induced lung damage (Schuller-Levis et al., 1994). In addition, taurine may prevent HOCl / OCl-induced lysis of lung epithelial cells by decreasing the chlorination of cellular components rather than preventing oxidation of sulfhydryl groups (Cantin, 1994). Tau-clo also inhibits the generation of inflammatory mediators produced by macrophages, such as macrophage inflammatory protein-2 (MIP-2), monocyte chemo-attractant protein-1 and 2 (MCP-1 and 2), nitric oxide, nitrites, prostaglandin E2 (PGE2), necrosis factor tumor alpha (TNF-α) and interleukin 6 (IL-6) (Kim et al., 1996; Schuller-Levis et al., 1994; Marcinkiewicz et al., 1995, Barua et al., 2001; Schempp et al. , 2001; Quinn et al., 2003). These down-regulation mechanisms transform monocytes and macrophages from a pro-inflammatory state to a basal phagocytic state (Lunetta et al., 2017).

In addition, tau-clo activates the nuclear factor erythroid 2 (Nrf2) (this transcription factor regulates the inducible expression of numerous genes for detoxifying and antioxidant enzymes), increases the expression of heme-oxygenase (HO-1), protects cells from death caused by hydrogen peroxide (H_2O_2), and improves enzyme expression and activities of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase (Jang et al., 2009). The tau-clo-mediated NRf2 activation mechanism involves a biphasic effect related to HClO concentration. Low to moderate HOCl concentrations elicit robust Nrf2 activation in cultured mouse macrophages that acts to restore redox balance. Much higher concentrations elicit second and third level stress responses that can potentially terminate the expression of the target Nrf2 gene (Woods et al., 2009).

Other studies demonstrated that the transcriptional effects of tau-clo in genes that regulate the expression of iNOS (inducible nitric oxide synthase) and TNF-α result, in part, from the reduction of the translocation of NF-κB to the nucleus of activated cells. . The transcription of the iNOS and TNF-α genes is critically dependent on the signaling pathway of the transcription factor NF-κB (Murphy, 1999; Liu et al., 2000; Collart et al., 1990). The genetic expressions of inflammatory cytokines are known to be regulated by NF-κB (Lloyd and Oppenheim, 1992; Cassatella, 1995). Tau-clo has been shown to inhibit the production of inflammatory cytokines (Marcinkiewicz et al., 1998; Kontny et al., 2000); the molecular mechanism of inhibition of tau-clo-induced NF-kB activation consists of the oxidation of IκBα in methionine[45].

5.4. Taurine-chloramine contributes to the resolution of inflammation

The aforementioned information allows us to conclude that the biological function of tau-clo in the neutrophil is not only the reduction of the cytotoxicity of HClO, but also the mitigation of an excessive inflammatory reaction (Kanayama et al., 2002). After destroying the microbes, mature neutrophils undergo constitutive programmed cell death (apoptosis) that renders them insensitive to chemo-attractants and allows their recognition and

elimination by scavenging macrophages (Savill et al., 1989), leading to resolution of inflammation (Savill et al., 2002). However, neutrophil survival and apoptosis are profoundly influenced by the inflammatory environment, and suppression of neutrophil apoptosis causes chronic inflammation (Simon, 2003; Gilroy et al., 2004). In fact, a marked decrease in neutrophil apoptosis was detected in patients with inflammatory diseases (Matute-Bello et al., 1997; Keel et al., 1997). The regulation of neutrophil apoptosis during the acute phase of inflammation is less well defined; however, it is essential for optimal expression and resolution of the inflammation. Recent work confirmed that tau-clo contributes to the resolution of inflammation by stimulating efferocytosis (phagocytic engulfment of apoptotic neutrophils by macrophages) (Kim et al., 2015) and inhibiting the release of MPO by neutrophils (Kim et al., 2020). The increase in the intracellular level of MPO negatively regulates the expression of MIP-2 (a chemokine involved in the recruitment of neutrophils at the site of inflammation), thus contributing to a negative feedback mechanism that ensures that neutrophils do not generate an excessive inflammatory response (Kim et al., 2020). During efferocytosis, macrophages increase their secretion of the anti-inflammatory and immunoregulatory cytokine interleukin 10 (IL-10) and decrease the secretion of pro-inflammatory cytokines, tumor necrosis factor-a (TNF-a), IL-1 and IL-12 (Voll et al. 1997). Tau-clo-stimulated efferocytosis derived from ClO_2^- metabolism should be studied in depth, as altered efferocytosis has been observed in many human autoimmune and inflammatory diseases, including cystic fibrosis, chronic obstructive pulmonary disease, asthma, fibrosis, idiopathic pulmonary disease, rheumatoid arthritis, systemic lupus erythematosus, glomerulonephritis, and atherosclerosis (Vandivier et al., 2006).

5.5. ClO_2^- restores redox balance through hormetic mechanisms

ClO_2^- is able of inducing protective effects through hormesis, which is a dose-response phenomenon characterized by stimulation at low concentrations and inhibition at high concentrations (Calabrese and Baldwin, 2001; Mattson, 2008; Calabrese and Baldwin, 2002; Calabrese and Mattson, 2017). Hormesis can occur as a result of direct stimulation or as an overcompensatory response after a disruption of homeostasis or the induction of low to moderate toxicity. (Calabrese, 2008; Calabrese, 2013). As previously mentioned, low concentrations of ClO_2^- can inhibit methemoglobin production, inactivate ferril hemoglobin, and inhibit excessive MPO activity. In general terms, these protective effects are indirectly induced by ClO_2^-, stimulating the production of antioxidant enzymes that protect erythrocytes from oxidative damage. In addition, ClO_2^- indirectly modulates the synthesis of Nrf2, by inhibiting the excessive production of HClO (Woods et al., 2009). Although we did not find biphasic effects mediated by ClO_2-, an indirect stimulation could result in strengthening of the body's antioxidant systems, thus stabilizing the redox balance to protect against oxidative damage.

6. Conclusion

We have found that the main bioactive molecule (derived from ClO_2 metabolism) within the body is ClO_2^-, since, due to its high chemical reactivity, ClO_2 is unlikely to remain in

the body unreduced for long after its ingestion. ClO_2^- has a high permanence in plasma and tissues after its absorption, before its elimination through the urinary and fecal routes, mainly in the form of chloride. The antiviral activity of ClO2 and ClO_2^- has been described in *in vitro* and *in vivo* tests, and is based on the ability to oxidize and denature virus capsid proteins. The fact that ClO_2 / ClO_2^- are not toxic if they are ingested at low concentrations suggests an interesting therapeutic application. *In vitro* studies have shown that ClO_2 can inactivate the human influenza virus, measles virus, and herpes virus (Sanekata et al., 2010). Other researchers have shown that ClO_2 is capable of destroying the polio virus, hepatitis A, HIV-1, and the coronavirus that caused SARS (cited in Miura and Shibata, 2010). Oral ingestion of aqueous solutions of ClO_2 at low concentrations (0.3-0.9 mg / kg / day) could be used for the prevention and treatment of deadly viral infections, such as influenza, COVID-19, and AIDS.

The most studied processes of immunomodulatory action are the interaction with hemoproteins of red blood cells, in particular the metabolization of excess methemoglobin and the regulation of the enzymatic activity of MPO in neutrophils and macrophages, transforming monocytes and macrophages from a pro-inflammatory to a basal phagocytic state. A key intermediate in inflammatory control is the generation of tau-clo induced by ClO_2^- (Chinake and Simoyi, 1997; Schempp et al., 2001; Giese et al., 2004) as a stable and moderate oxidizing agent, which neutralizes excess HClO, mitigates the excessive inflammatory reaction (Giese et al., 2004) and contributes to the resolution of the inflammatory response by inducing neutrophil apoptosis (Kim et al., 2020). Therefore, tau-clo represents the most relevant functional product formed under the influence of ClO_2^- (WF10) (Schempp et al., 2001; Giese et al., 2004). In fact, an in vitro experiment showed that WF10 inhibits pro-inflammatory activation of the M1 type in macrophages; the results suggested that ClO_2^- is the active principle in WF10 since it produced the same changes as WF10 (Schönberg et al., 2016). Since ClO_2^- is also the main product of ClO_2 metabolism after its oral ingestion, we suggest that the use of aqueous solutions of ClO_2 at low concentrations could induce the same therapeutic effects as WF10.

These immuno-modulatory effects could have clinical relevance for the treatment of some diseases, such as Alzheimer, Parkinson, AIDS, rheumatoid arthritis and amyotrophic lateral sclerosis. Analysis of the literature also revealed the existence of opposite effects mediated by ClO_2, a stimulating or beneficial effect at low concentrations and a toxic or harmful effect at high concentrations, according to hormetic principles. This contradicts the accepted paradigm around ClO_2 and ClO_2^-, considered only as toxic agents.

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