Correspondence

DOI: 10.5582/bst.2022.01495

Can nasal irrigation with chlorine dioxide be considered as a potential alternative therapy for respiratory infectious diseases? The example of COVID-19

Jing Cao[1,§], Yirong Shi[1,§], Min Wen[1,§], Yuanyuan Peng[1], Qiqi Miao[1], Xiaoning Liu[1], Mingbin Zheng[2], Tetsuya Asakawa[2,*], Hongzhou Lu[2,3,*]

[1] Department of Nursing, National Clinical Research Center for Infectious Diseases, Third People's Hospital of Shenzhen, Shenzhen, Guangdong, China;
[2] Institute of Neurology, National Clinical Research Center for Infectious Diseases, Third People's Hospital of Shenzhen, Shenzhen, Guangdong, China;
[3] Department of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Third People's Hospital of Shenzhen, Shenzhen, Guangdong, China.

SUMMARY Chlorine dioxide (ClO_2) is a high-level disinfectant that is safe and widely used for sterilization. Due to the limitations on preparing a stable solution, direct use of ClO_2 in the human body is limited. Nasal irrigation is an alternative therapy used to treat respiratory infectious diseases. This study briefly summarizes the available evidence regarding the safety/efficacy of directly using ClO_2 on the human body as well as the approach of nasal irrigation to treat COVID-19. Based on the available information, as well as a preliminary experiment that comprehensively evaluated the efficacy and safety of ClO_2, 25-50 ppm was deemed to be an appropriate concentration of ClO_2 for nasal irrigation to treat COVID-19. This finding requires further verification. Nasal irrigation with ClO_2 can be considered as a potential alternative therapy to treat respiratory infectious diseases, and COVID-19 in particular.

Keywords chlorine dioxide (ClO_2), nasal irrigation, COVID-19, SARS-CoV-2, respiratory infectious diseases

Chlorine dioxide (ClO_2) is an oxidizing agent that is commonly used as a high-level disinfectant. It is effective at killing pathogenic microorganisms including bacteria, viruses, fungi, and spores, and it has almost no toxic effects on human or animal cells in daily use (*1*). ClO_2 has a molecular structure with 19 electrons in the outer layer, which contributes to its oxidizing action and penetration. It can adsorb to and penetrate the surface of microorganism without markedly destroying the integrity of the microbial shell (such as the cytoderm or protein capsid), and it markedly acts on enzymes containing sulfhydryl groups. The mechanism of disinfection by ClO_2 is *via*: i) Rapid damage to tyrosine on the capsid of the bacterium or virus, thereby suppressing their specific adsorption; ii) Suppression of protein synthesis; and iii) Killing these microorganisms, which account for its sterilizing action (*2*). In the context of SARS-CoV-2, ClO_2 directly affects the spike protein and RNA of the virus, ultimately killing the virus (*3*). Hence, ClO_2 has been long used for sterilization, both for sterilization of equipment and environments as well as for human disinfection, such as dental oral cleaning (*4-6*) and wound cleaning (*7*). Its disinfecting action in home environments, the water supply, environmental surfaces, and medical equipment have been well documented. However, there is a limitation to directly using ClO_2 on human body, namely the limited availability of a stable ClO_2 solution that can be stored for a prolonged period. A ClO_2 solution often needs to be prepared before using *via* a chemical reaction of precursors such as sodium chlorite ($NaClO_2$) or use of an effervescent tablet. Such "activation" procedures are inconvenient. Importantly, the concentration and stability of the obtained ClO_2 solution are not easily controlled, thereby limiting the use of ClO_2 to disinfect the human body. Fortunately, a stable ClO_2 solution (free of activation) has recently become available, and this offers hope for the direct use of ClO_2 in the human body.

1. Use of ClO_2 for human disinfection

Many previous animal studies have demonstrated the safety of ClO_2 as a sanitizer. Ma *et al*. verified the efficacy, toxicity, and safety of ClO_2 *in vitro* and *in vivo* (*8*). Their *in vitro* experiments found that ClO_2 at 5-20 ppm resulted in a 98.2% reduction in bacteria and fungi. ClO_2 at 200 ppm (37℃, 2 min) killed most strains of influenza A and B and enterovirus 71. In terms of

toxicity, cellular viability was 74.0% at 600 ppm, and 40.3% at 800 ppm. In *in vivo* experiments, inhalation of ClO_2 at 0-20 ppm (24 h) or oral administration of ClO_2 at 0-40 ppm (90 days) did not cause any pathological changes in the heart, lungs, liver, kidneys, or spleen of mice. Oral administration of ClO_2 at 0-40 ppm also did not cause any pathological changes in these organs. Use of 0.1 mL of ClO_2 at 50 ppm did not lead to ocular irritation in rabbits (*8*). These experiments verified the biosafety of ClO_2 in different animals.

However, evidence regarding the direct use of ClO_2 in humans is limited due to the aforementioned limitation. By far, the most common context is dental disinfection. Early in 2008, a Japanese team used 0.1% ClO_2 (1,000 ppm) mouthwash to treat healthy subjects with halitosis (*4*). They found that halitosis was alleviated, and no adverse events were reported (*5*). Later, the same team used ClO_2 at 1,000 ppm (7 days of mouthwash) in 15 subjects with halitosis. They found that accumulation of plaque, coating of the tongue, and the count of *Fusobacterium nucleatum* in saliva decreased. Only three subjects reported "dislike of the smell and taste of ClO_2". Recently, an Indian team also used ClO_2 at 1,000 ppm for disinfection in patients who underwent periodontal surgery (mouthwash bid for 14 days). They found that all of the patients were able to tolerate the ClO_2 mouthwash. No discomfort was reported (*6*). Noszticzius *et al*. used ClO_2 at 300 ppm as an antimicrobial agent for the wounds of patients with deep venous thrombosis or diabetic foot (*7*). They found that ClO_2 at 300 ppm displayed efficacy in killing all bacteria. It helped with wound healing without causing any toxic reactions. They contended that ClO_2 might be a good disinfectant for use in all living organisms (Table 1).

In terms of using ClO_2 in the context of COVID-19, most studies similarly concerning the environment. There are only limited studies in humans (Table 1). Aparicio-Alonso *et al*. orally administered ClO_2 at 3 ppm as a prophylactic agent to family members living with COVID-19 patients in Mexico (*9*). They found that ClO_2 was effective at preventing COVID-19, and no adverse events were reported. In another study, Aparicio-Alonso *et al*. orally administered a mean dose of 1.41 mg/kg to treat COVID-19 patients (*10*). They found that CIO2 helped to resolve COVID-19 symptoms and reduce the duration of treatment. Only 6.78% of patients reported mild and sporadic uncomfortable reactions such as headaches, dizziness, vomiting, diarrhea, and nausea. They hence concluded that ClO_2 might be considered as a safe alternative therapy with which to treat COVID-19.

There are only 2 studies reporting toxic reactions. Bathina *et al*. reported an unusual case of reversible acute kidney injury due to chlorine dioxide poisoning due to consumption of 250 mL of stable ClO_2 (*11*). Recently, Medina-Avitia *et al*. reported a 55-year male who developed acute kidney injury and disseminated intravascular coagulation due to the oral administration of CIO2 to prevent and treat COVID-19. After treatment with hemodialysis, the kidney injury was reversed (*12*). These cases imply that: i) Oral administration of CIO2 in a short period, in a large dose, or to patients with underlying illnesses might be risk factors for the development of acute kidney injury and ii) this ClO_2-related kidney injury is reversible.

2. Using nasal irrigation as an alternative therapy for COVID-19

Since there is no specific treatment for COVID-19, many alternative therapies have been considered. High titers

Table 1. The concentrations of ClO_2 in representative studies directly using ClO_2 in the human body

Studies/Country	Subjects	Intervention	Concentration of ClO_2 (ppm)	Safety	Efficacy
Shinada *et al*., 2008/Japan	15 healthy subjects	7 days of mouthwash	1,000	No adverse events reported	Relief of halitosis
Shinada *et al*., 2010/Japan	15 healthy subjects	7 days of mouthwash	1,000	Three subjects reported "dislike of the smell and taste"	Accumulation of plaque, coating of the tongue, and the count of Fusobacterium nucleatum in saliva decreased
Noszticzius *et al*., 2013/Hungary	One patient with thrombosis and two patients with diabetic foot	Direct administration of ClO_2 to the wound	300	No adverse events reported	Effective at wound disinfection and helped with wound healing
Kale *et al*., 2020/India	Patients who underwent periodontal surgery	14 days of mouthwash	1,000-2,000	No adverse events reported	ClO_2 contributed to the promotion of early wound healing after periodontal surgery
Aparicio-Alonso *et al*., 2021/ Mexico	Family members living with patients with COVID-19	14-day oral administration	3 ppm	No obvious adverse reactions reported	ClO_2 reduced COVID-19-related symptoms and contributed to prevention of the COVID-19
Aparicio-Alonso *et al*., 2021/ Mexico	Family members living with patients with COVID-19	14-day oral administration	3 ppm (1.41 mg/kg)	Only 6.78% of patients reported mild and sporadic uncomfortable reactions such as headaches, dizziness, vomiting, diarrhea, and nausea.	ClO_2 reduced COVID-19-related symptoms and contributed to prevention of the COVID-19

of SARS-CoV-2 can be detected in the upper airways of asymptomatic/symptomatic COVID-19 patients (*13*), with higher viral loads found in nasal swabs compared to pharyngeal swabs. Nasal irrigation has hence been considered as an alternative therapy to treat COVID-19. During the early days of the COVID-19 pandemic, Casale *et al*. (*14*), Ramalingam *et al*. (*15*), and Panta *et al*. (*16*) proposed that nasal irrigation may be a potential treatment for COVID-19. Later, Huijghebaert *et al*. reported that early nasal irrigation with saline may ameliorate COVID-19 symptoms (*17*). Yilmaz *et al*. found that nasal irrigation with hypertonic alkaline significantly reduced the viral load in patients with COVID-19 (*18*). Later, Yildiz *et al*. found that nasal saline irrigation with triamcinolone acetonide may relieve COVID-19-related hyposmia (*19*). Baxter *et al*. found that nasal irrigation with povidone-iodine or sodium bicarbonate helped to reduce disease severity and the duration of hospitalization in patients with COVID-19 (*20*). These studies seem to prove the efficacy of nasal irrigation to treat COVID-19. However, whether nasal irrigation can be used as a potential alternative therapy for COVID-19 requires further investigation because of the small samples in those studies. Moreover, those studies involved the early beta and delta strains; whether nasal irrigation is effective against the omicron strain warrants investigation.

Based on the aforementioned evidence, ClO_2, is a safe and efficient disinfectant, and it is particularly useful as an agent for nasal irrigation to treat respiratory infectious diseases, and COVID-19 in particular.

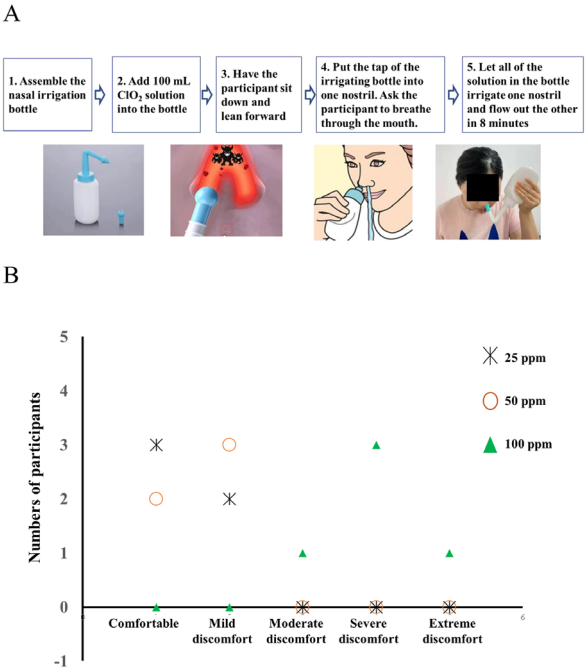

Figure 1. Results of a preliminary experiment exploring the doses of ClO_2 for nasal irrigation in 5 healthy participants. (A) Schematic diagram of nasal irrigation used in a preliminary experiment. **(B)** Results of the preliminary experiment with regard to discomfort. At a ClO_2 concentration of 25 ppm, 3 participants felt comfort and 2 participants felt mild discomfort. At a ClO_2 concentration of 50 ppm, two participants felt comfort and three participants felt mild discomfort. At a ClO_2 concentration of 100 ppm, one participant felt moderate discomfort, three participants felt severe discomfort, and one participant felt extreme discomfort. Hence, 25-50 ppm was considered to be an appropriate concentration range for nasal irrigation and was used in subsequent experiments.

3. Deduction of an appropriate dose of ClO_2 for nasal irrigation

The first consideration is safety. The dose, concentration, and method of administration are known to be the most crucial factors associated with the safety of ClO_2 in the context of COVID-19 (*3*). Hence, the dose/concentration of ClO_2 must be carefully and comprehensively determined by balancing efficacy, safety, and ease of solution preparation. Several aspects need to be considered to deduce the appropriate dose:

1). Hatanaka *et al*. reported that exposure to ClO_2 at 24 ppm for 10 s can kill 99.99% of SARS-CoV-2 (*1*). This suggests that SARS-CoV-2 is extremely sensitive to ClO_2.

2). Aparicio-Alonso *et al*. reported that oral administration of ClO_2 at 3 ppm in a dose of 0.3 mg/kg/day was safe (*9*). Assuming the body weight of an adult is 50 kg, oral consumption of 15 mg/day ClO_2 is safe. This is 20 times lower than the lowest observed adverse effect level (LOAEL) and 300 times lower than the LD_{50}.

3). Ma *et al*. found that ClO_2 at 50 ppm did not cause ocular irritation in rabbits, which proved that 50 ppm causes no mucosal irritation. Thus, it is safe for the nasal mucosa.

4). In a preliminary experiment performing nasal irrigation in 5 healthy subjects, 25 and 50 ppm did not cause any intolerable discomfort, whereas 100 ppm may cause discomfort due to the smell (Figure 1).

5). If using ClO_2 at 50 ppm for nasal irrigation (100 mL, bid), the nasal irrigation dose is 10 mg (for a 50 kg adult), about 2/3 of the dose in the study by Aparicio-Alonso *et al*. (3). This is 30 times lower than the LOAEL and 450 times lower than the LD_{50}

6). When preparing a ClO_2 solution, concentrations of 25 and 50 ppm are easily handled and stored.

Accordingly, 25-50 ppm was deemed to be an appropriate concentration range for nasal irrigation with ClO_2 in terms of safety and efficacy. Indeed, the current authors are now conducting a subsequent study to evaluate the safety and efficacy of nasal irrigation with ClO_2 at 25 or 50 ppm. The forthcoming results should help to provide evidence regarding whether nasal irrigation with ClO_2 can be used as an alternative therapy to treat COVID-19, as well as the other respiratory infectious diseases such as influenza.

Acknowledgments

The authors would like to thank Shenzhen Caseche

Biotech Co., Ltd. for providing the stable ClO_2 solution for the preliminary experiment.

This experiment was approved and supervised by the Ethics Committee of The Third People's Hospital of Shenzhen (approval number 2022-182-03). The study protocol was explained to all of the participants who then provided written informed consent to participation in this study.

Funding: This work was supported by the Shenzhen Science and Technological Foundation (No. JSGG20220301090005007), the Third People's Hospital of Shenzhen Foundation (No. G2021027), and the Third People's Hospital of Shenzhen Foundation (No. G2022062)

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Hatanaka N, Xu B, Yasugi M, Morino H, Tagishi H, Miura T, Shibata T, Yamasaki S. Chlorine dioxide is a more potent antiviral agent against SARS-CoV-2 than sodium hypochlorite. J Hosp Infect. 2021; 118:20-26.
2. Abdighahroudi MS, Jütte M, Hupperich K, Mutke XA, Schmidt TC, Lutze HV. Mechanisms and byproduct formation in the application of chlorine dioxide. In: Comprehensive Analytical Chemistry (Elsevier, 2021; pp. 51-83.
3. Eduardo I-C, Blanca BG, Yohanny A, Patricia C, Maria SA, San Martín ABA, Gonzales CO. Determination of the effectiveness of chlorine dioxide in the treatment of COVID 19. Mol Gen Med. 2021;1-11.
4. Shinada K, Ueno M, Konishi C, Takehara S, Yokoyama S, Zaitsu T, Ohnuki M, Wright FA, Kawaguchi Y. Effects of a mouthwash with chlorine dioxide on oral malodor and salivary bacteria: A randomized placebo-controlled 7-day trial. Trials. 2010; 11:14.
5. Shinada K, Ueno M, Konishi C, Takehara S, Yokoyama S, Kawaguchi Y. A randomized double blind crossover placebo-controlled clinical trial to assess the effects of a mouthwash containing chlorine dioxide on oral malodor. Trials. 2008; 9:71.
6. Kale A, Mahale S, Sethi K, Karde P. Clinical and microbial comparative evaluation of 0.1% chlorine dioxide mouthwash versus 0.2% chlorhexidine mouthwash after periodontal surgery: A randomized clinical trial. Int J Innov Res Sci Eng Techno. 2020; 6:935-939.
7. Noszticzius Z, Wittmann M, Kály-Kullai K, Beregvári Z, Kiss I, Rosivall L, Szegedi J. Demonstrating that chlorine dioxide is a size-selective antimicrobial agent and high purity ClO_2 can be used as a local antiseptic. 2013; https://doi.org/10.48550/arXiv.1304.5163
8. Ma JW, Huang BS, Hsu CW, Peng CW, Cheng ML, Kao JY, Way TD, Yin HC, Wang SS. Efficacy and safety evaluation of a chlorine dioxide solution. Int J Environ Res Public Health. 2017; 14.
9. Aparicio-Alonso M, Domínguez-Sánchez CA, Banuet-Martínez M. A retrospective observational study of chlorine dioxide effectiveness to COVID19-like symptoms prophylaxis in relatives living with COVID19 patients. Int J Multidiscip Res Anal. 2021; 4:1062-1071.
10. Aparicio-Alonso M, Domínguez-Sánchez C, Banuet-Martínez M. Chlorine Dioxide as an alternative treatment for COVID19. J Infect Dis Ther. 2021; 9:1-8
11. Bathina G, Yadla M, Burri S, Enganti R, Prasad Ch R, Deshpande P, Ch R, Prayaga A, Uppin M. An unusual case of reversible acute kidney injury due to chlorine dioxide poisoning. Ren Fail. 2013; 35:1176-1178.
12. Medina-Avitia E, Tella-Vega P, Garcia-Estrada C. Acute kidney injury secondary to chlorine dioxide use for COVID-19 prevention. Hemodial Int. 2021; 25:E40-E43.
13. Zou L, Ruan F, Huang M, *et al*. SARS-CoV-2 viral load in upper respiratory specimens of infected patients. N Engl J Med. 2020; 382:1177-1179.
14. Casale M, Rinaldi V, Sabatino L, Moffa A, Ciccozzi M. Could nasal irrigation and oral rinse reduce the risk for COVID-19 infection? Int J Immunopathol Pharmacol. 2020; 34:2058738420941757.
15. Ramalingam S, Graham C, Dove J, Morrice L, Sheikh A. Hypertonic saline nasal irrigation and gargling should be considered as a treatment option for COVID-19. J Glob Health. 2020; 10:010332.
16. Panta P, Chatti K, Andhavarapu A. Do saline water gargling and nasal irrigation confer protection against COVID-19? Explore (NY). 2021; 17:127-129.
17. Huijghebaert S, Hoste L, Vanham G. Essentials in saline pharmacology for nasal or respiratory hygiene in times of COVID-19. Eur J Clin Pharmacol. 2021; 77:1275-1293.
18. Yilmaz YZ, Yilmaz BB, Ozdemir YE, Kocazeybek BS, Karaali R, Cakan D, Ozdogan HA, Batioglu-Karaaltin A. Effects of hypertonic alkaline nasal irrigation on COVID-19. Laryngoscope Investig Otolaryngol. 2021; 6:1240-1247.
19. Yildiz E, Koca Yildiz S, Kuzu S, Gunebakan C, Bucak A, Kahveci OK. Comparison of the healing effect of nasal saline irrigation with triamcinolone acetonide versus nasal saline irrigation alone in COVID-19 related olfactory dysfunction: A randomized controlled study. Indian J Otolaryngol Head Neck Surg. 2021;1-6.
20. Baxter AL, Schwartz KR, Johnson RW, Kuchinski AM, Swartout KM, Srinivasa Rao ASR, Gibson RW, Cherian E, Giller T, Boomer H, Lyon M, Schwartz R. Rapid initiation of nasal saline irrigation to reduce severity in high-risk COVID+ outpatients. Ear Nose Throat J. 2022;1455613221123737.

Received November 19, 2022; Revised November 30, 2022; Accepted December 6, 2022.

[§]These authors contributed equally to this work.
**Address correspondence to:*
Hongzhou Lu, Department of Infectious Diseases, National Clinical Research Center for Infectious Diseases, the Third People's Hospital of Shenzhen, 29 Buji Bulan Road, Shenzhen 518112, Guangdong, China.
E-mail: luhongzhou@fudan.edu.cn

Tetsuya Asakawa, Institute of Neurology, the Third People's Hospital of Shenzhen, 29 Buji Bulan Road, Shenzhen 518112, Guangdong, China.
E-mail: asakawat1971@gmail.com

Released online in J-STAGE as advance publication December 9, 2022.