Immunome Research
ISSN: 1745-7580
OPEN ACCESS Freely available online
Hypothesis

Nebulized saline produced by ultrasonic humidifiers should be considered as an option for COVID-19

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ABSTRACT

Saline-based therapies had been considered as promising options for COVID-19 due to the augment of innate responses. SARS-CoV-2 may be present in lower respiratory tracts (LRT) during early stage of the infection, therefore inhaled therapies are ideal to supply saline for the entire airway tract. This is possible with ultrasonic humidifiers that can aerosolize any mineral content in the input water, continuously producing micron- and submicron-sized aerosols that may reach both the upper respiratory tract and LRT. The virucidal action of saline has been demonstrated with not only hypertonic but also with isotonic and hypotonic saline. Therefore, we recommend investigation of the antiviral activity of hypotonic saline for long-term administration as starting points for future experimental designs. Given the persistence of the antiviral response post-treatment, the prevention of COVID-19 via daily administration of nebulized chloride therapy should be examined. In brief, ultrasonic humidifiers could be adapted to produce an inexpensive, simple, and promising therapy for COVID-19.

Keywords: SARS-CoV2; COVID-19; Saline; Hypochlorous acid; Aerosols; Nebulizers; Ultrasonic humidifiers; Innate immunity; Antiviral activity.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has rapidly spawned an ongoing global pandemic. It is hypothesized that host cells evolved antiviral activity utilizing chloride anions, the most abundant anion in the oceans and in living organisms [1]. NaCl exerts antiviral activity against enveloped/non-enveloped, DNA, and RNA viruses that cause human respiratory infection [1]. Complete inhibition of SARS-CoV-2 replication was also demonstrated in a kidney cell model treated with a hypertonic saline solution [2]. Saline-based therapies had been considered as promising adjuvant and safe options for COVID-19 disease [3–8].

STATEMENT OF HYPOTHESIS AND DISCUSSION

NaCl can augment the antiviral activity of both pulmonary phagocytic and non-phagocytic cells

NaCl has no direct virucidal action against SARS-CoV-2 and other viruses [1,2], but augments innate immunity. This effect is related to both Na+ and Cl-, but is more generally attributed to Cl- [1].

Phagocyte myeloperoxidase (MPO) converts H2O2 and Cl- to hypochlorous acid (HOCl) in phagosomes [1]. HOCl, the most potent mammalian microbicide inhibits viruses, possibly via chlorination [1].

MPO is expressed at high levels in phagocytes and lower levels in many cells, including cells of the human respiratory system [1,3] and an MPO-dependent increase in intracellular hypochlorous acid enhances antiviral innate immunity in respiratory epithelial cells [1].

Thus, chloride anions enhance the antiviral innate immunity of both phagocytic and non-phagocytic cells in the human respiratory system.

Phagocytes are deployed to airway/alveolar surface liquid and actively mobilize Cl- from their surroundings [1] so a shortage of chloride could thus threaten viral inhibition by epithelial cells. Saline supply enhances mucociliary clearance [9]. NaCl treatment induces cell membrane depolarization, Na+ influx, increased cytosolic Ca2+ and a low energy state (high ADP/ATP ratio), impairing SARS-CoV-2 replication [2]. Thus, increasing saline

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Received: February 09, 2021; **Accepted:** February 24, 2021; **Published:** March 02, 2021

Citation: Le T, Nguyen PV, Wilkinson D (2021) Nebulized saline produced by ultrasonic humidifiers should be considered as an option for COVID-19. Immunome Res. 17:185.

availability may be essential to triggering and maintaining antiviral innate immunity in the human respiratory system.

Aerosolized chloride produced by nebulizers and ultrasonic humidifiers could be adjuvant therapies for COVID-19

For an inexpensive, readily available, nontoxic, therapy against COVID-19, NaCl may be ideal. Aerosol therapy with nebulizers provides a continuous chloride supply to upper and lower airways. This could alternatively be achieved with humidifiers. Humidifiers are easy-to-operate and widely used to increase the relative humidity in living spaces. Ultrasonic humidifiers have a similar operating principle to nebulizers [10], enabling them to aerosolize 85-90% of dissolved minerals to expel a mixture of micron- and submicron-particles at concentrations linearly correlated to the mineral content of consumed water [11]. Such aerosols access the entire respiratory tract to supply chloride anions to airway/alveolar surface liquid, triggering and maintaining innate immune defenses. Interestingly, the total droplet concentration of mineral-containing water remains stable during humidifier operation as a steady-state condition is approached [11], providing a constant supply of chloride aerosols from input saline solution. This is important for viral prevention, treatment of groups in enclosed spaces and for long-term treatment. Ultrasonic humidifiers can be adapted for use in the same manner as nebulizers or to generate a steady-state chloride mist in enclosed living areas.

The administered dose of NaCl is crucial. Hypertonic saline is often recommended for treating respiratory infection [12] but might lead to side effects, e.g. irritation, cytotoxicity, and ciliostasis. Patients experienced oropharyngeal irritation when they inhaled nebulized 12% hypertonic saline, making this the upper limit of tolerability [9]. Usually concentrations range from 3% to 7%, and volumes from 2 ml to 7 ml per dose, 2-3 doses per day [13]. Hypertonic saline can be administered without bronchospasm, coughing, or aggrevated wheezing [14] but may result in ciliostasis, i.e. the loss of cilia beating. Irreversible ciliostasis may occur in nasal epithelia within minutes of administration of a 14.4% saline solution [15] while ciliostasis was partially reversible at concentrations below 11% [16]. Interestingly, treatment of respiratory epithelial cells with 2% NaCl for 30 min resulted in completely reversible ciliostasis [16]. Whether 2% NaCl is the upper limit for safe inhaled therapy needs to be addressed in future studies. Treatment with up to 285 mM NaCl (1.7%) produced no evidence of cytotoxicity in monkey kidney cells while only 260 nM NaCl (1.5%) completely inhibited replication of SARS-CoV-2 [2]. Treatment of human A549 respiratory epithelial cells with up to 210 nM NaCl (1.2%) also showed no cytotoxicity but significant reduced the viral load [1]. Given the importance of continuous chloride supply, long-term administration should be considered but could lead to overdose if hypertonic saline were administrated. Therefore, isotonic and even hypotonic (<0.9%) saline should be considered, particularly for long term treatment (unlikely with most commercial nebulizers due to small tank volume). Isotonic and hypotonic saline solutions also show antiviral activity against SARS-CoV-2 and other viruses [1,2,13,17].

CONCLUSION

Antiviral therapies for COVID-19 must be administrated during the pre-symptomatic stage (lasting 1-3 days), or within 3 days of the onset of symptoms when innate responses play a dominant role in viral clearance. The virus might readily reaches the lower respiratory tract (LRT) so treatment should reach the LRT, which can be achieved using nebulizers and other inhaled therapies. The virucidal action of chloride-based treatment via enhancement of innate immunity was demonstrated against various viruses, including SARS-CoV-2. Hypertonic saline, often recommended for the treatment of respiratory infections, causes adverse side effects such as irritation, ciliostasis, and cytotoxicity. The upper limit for human tolerability was claimed to be a relatively high concentration (12%). Several studies conjectured that a 3%-7% nebulized saline solution is safe. However, in vitro assay indicated that the concentration must be less than 2% to exclude ciliostasis. No evidence of cytotoxicity was observed at up to 1.7% NaCl in an in vitro test with monkey kidney cells while 1.5% NaCl completely inhibited SARS-CoV-2 replication. For respiratory epithelial cells, approximately 1.2% NaCl was sufficient to completely inhibit viral shedding without cytotoxicity. A hypotonic saline solution (0.7%) also boosts viral inhibition by non-phagocytic cells. A far lower concentration may be sufficient to augment antiviral cell responses via increased MPO-dependent, HOCl production. Plasma membrane depolarization may contribute to the virucidal action of hypotonic saline against SARS-CoV-2. Given the efficacy and safety of hypotonic saline and to avoid side effects, we urge long-term administration of nebulized saline solution at concentrations well below 0.9% as a starting point for future experimental designs. Similar to nebulizers but with larger tank volumes, ultrasonic humidifiers merit adaptation for constantly supplying chloride via the creation of a steady-state chloride mist. If the addition of a small amount of NaCl to input water is sufficient to induce the antiviral innate immune response, we then have an inexpensive, simple, safe option to tackle SARS-CoV-2. Otherwise, given the cost-effectiveness of saline-based therapy, it is worth considering the use of ultrasonic humidifiers for short-term treatment with a hypertonic saline solution. Importantly, antiviral responses may persist post-treatment e.g. membrane depolarization that inhibits SARS-CoV-2 replication was maximal at 24 hours post-treatment. Daily administration of nebulized saline therapy should be examined for the purpose of COVID-19 prevention, particularly to reduce COVID-19 transmission within households or enclosed areas.

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