Assessment of nitric oxide (NO) potential to mitigate COVID-19 severity

Swati Srivastava1 · Iti Garg1 · Anju A. Hembrom1 · Bhuvnesh Kumar1

Received: 8 February 2021 / Accepted: 20 May 2021 / Published online: 3 June 2021 © Indian Virological Society 2021

Abstract Novel coronavirus disease by SARS-CoV-2 virus (also known as COVID-19) has emerged as major health concern worldwide. While, there is no specific drugs for treating this infection till date, SARS-CoV-2 had spread to most countries around the globe. Nitric oxide (NO) gas serves as an important signaling molecule having vasodilatory effects as well as anti-microbial properties. Previous studies from the 2004 SARS-CoV infection demonstrated that NO may also help to reduce respiratory tract infection by inactivating viruses and inhibiting their replication cycle and is an effective supportive measure for treating infection in patients with pulmonary complications. NO gas inhalation is being suggested as potential therapy for managing severe acute respiratory distress syndrome in COVID-19 patients. Present report summarized the therapeutic importance of NO to reverse pulmonary hypertension, restore normal endothelial activity and produce anti-thrombotic effects. In addition to this, NO also reduces viral infection by inhibiting its replication and entry into the host cell. In absence of vaccine and effective treatment strategies, we suggest that NO inhalation therapy and NO releasing foods/compounds could be considered as an alternative measure to combat COVID-19 infection.

Keywords COVID-19 · Nitric oxide (NO) · vasodilation · hypertension

Brief report

Severe acute respiratory syndrome coronavirus (SARS-CoV-2), also known as COVID-19, has emerged as a global pandemic in recent times. The virus causes flu like symptoms with high fever, cough and asthenia [24] and may progress to severe lung injury in some high-risk individuals such as the elderly people with weak immune system and individuals with other co-morbidities [6]. The infection is either asymptomatic or mild, with the most common symptoms being fever, headache, loss of smell and nasal obstruction in about 80–90 % of cases and only around 10 % of the infected patients have severe infection with dyspnoea, hypoxemia and extensive radiological involvement of the lung parenchyma. In extreme cases, this virus is likely to cause severe interstitial pneumonia, acute respiratory distress syndrome (ARDS) and subsequent multiorgan failure leading to respiratory failure and eventually death [18]. Thus, the symptoms vary from individual-to-individual ranging from asymptomatic infection to severe respiratory failure. Whereas, less than 5 % of cases present critical condition, multi-organ failure and death [9, 25]. The general symptoms of the patients remain in a state of mild upper respiratory tract disease for an extended period of 8–10 days, after which up to 42 % individuals
may develop ARDS and severe hypoxemia among which 61–81 % require urgent mechanical ventilation [7]. Currently, there are no designated drugs for COVID-19 infection treatment and even vaccine is yet to be developed. Most of the pharmacological treatment strategies being used are derived from experience gained during the SARS-CoV or MERS-CoV pandemics or from in vitro studies [5, 27]. Several potential molecules with antiviral, anti-inflammatory and immunomodulatory properties are under different stages of clinical trial for treatment of COVID-19 [7, 28].

Nitric oxide (NO) is a natural vasodilator produced by vascular endothelial cells. It acts as a signaling molecule between the cells and is also involved in wide range of processes [3]. NO is synthesized by three enzymes that catalyze the oxidation of l-arginine to NO and l-citrulline [3]; namely neuronal nitric oxide synthase (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). Whereas, nNOS and eNOS are constitutively expressed in calcium dependent manner [19], iNOS is independent of calcium ion concentration and is expressed only in activated cells [4]. Up-regulation of iNOS has been commonly seen during infections, and its anti-microbial activities have been described for several bacteria and viruses [1, 19] and serves as a critical molecule for immune response against pathogens and infections. Nitric oxide has been proven as a powerful molecule playing a critical role in a broad array of biological functions. It targets a variety of microbes such as bacteria, fungi, helminths, protozoa and viruses. The mechanism of virus inactivation by NO involves inactivation of various modifying proteins and nucleic acids involved in virus replication cycle [26]. Higher levels of basal exhaled NO have been correlated with milder symptoms of common cold in humans previously [20]. Also, NO has been demonstrated to inhibit pulmonary viral replication in pigs [11].

Due to proven inhibitory effects of NO on viral infections, it is being investigated as a candidate for therapy against COVID-19. Akerstrom and co-workers demonstrated that NO generated by iNOS inhibits the SARS-CoV replication cycle. They further reported that organic NO donor compound, S-nitroso-N-acetylpenicillamine facilitates inhibition of SARS-CoV replication in a concentration dependent manner [29]. Lower NO levels in the airways may facilitate progression of SARS-CoV-2 infection. A recent study suggests prevention of COVID-19 infection by avoiding mouth breathing, as it bypasses filtering effect by nose and decreases NO levels in airways. Rather simpler devises that help in promoting nasal breathing during sleep may also prevent infections [15].

NO is produced at 10 parts per million (ppm) in the human sinuses and diffuses into bronchi and lungs to produce vasodilatory and broncho-dilatory effects [12]. It also contributes to activation of ciliary movement [21] and secretion of mucus [17], which in turn helps in prevention of viral particles entering respiratory tract. Several clinical trials are being conducted on effectiveness of NO inhalation therapy to prevent disease progression in patients with COVID-19. A clinical trial intervention to assess the lung diffusion capacity for NO and Carbon Monoxide (CO) early after mild-to-severe COVID-19 has completed the clinical trial phase, but the details of the study has not been published yet (Table 1). Most important clinical presentation of COVID-19 is acute respiratory distress in the lungs which later propagates to other vascular networks throughout the body. It is also associated with plateletendothelial dysfunction and abnormal thrombotic clots [16]. Since intact endothelium releases NO which produces vasodilator and anti-thrombotic effects [23], the prime cause for endothelial dysfunction and thrombotic events during COVID-19 infection is NO deficiency due to suppressed eNOS in injured vessels. Thus, restoration of NO levels may contribute to vasodilation, thus releasing pulmonary hypertension and create anti-thrombotic milieu. In addition, nitric oxide interferes with the interaction between viral S-protein of coronavirus and its receptor molecule in the host, angiotensin converting enzyme-2 (ACE-2). Thus, the critical step of infection, i.e., the viral entry into the host cell is affected by the presence of NO, as it mediates S-nitrosylation of viral cysteine proteases and host serine protease, TMPRSS2 [2, 10, 22]. Thus, NO inhalation may be beneficial in mitigating severity of COVID-19 infection in many different ways as summarized in Fig. 1. The availability of nitric oxide in the human body depends on presence of NO donor compounds such as arginine, citrulline, nitroglycerin and phosphodiesterase inhibitors and consumption of NO releasing foods like green leafy vegetables, beetroot etc. [14, 13]. NO donors are a heterogeneous group of compounds which either release NO or an NO-related species. The type and extent of biological action of these compounds also depends on the form in which NO is released and amount of NO produced. The dietary inorganic nitrate releasing foods has been shown to be effective in restoring endothelial function, reducing pulmonary hypertension and inducing antimicrobial activity [8]. Thus, restoring levels of NO through dietary inorganic nitrate may prevent and even mitigate severe effects of COVID-19 infection.
| Identifier          | Brief Title                                                                 | Co-ordinating center                                                                 | Study Design | Drug                          | Dose                  | Duration | Subjects (n) | Follow-up (days) | Study Phase | Status          |
|---------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------|-------------------------------|-----------------------|----------|--------------|----------------|-------------|-----------------|
| NCT04388683         | Inhaled NO for preventing progression in COVID-19                           | Tufts Medical Center, Boston, Massachusetts, United States                           | RCT, Open label | NO gas                        | 125mcg/kg (~ 20 ppm) | 24 h     | 42           | 28             | Phase 2     | Recruiting      |
| NCT04383002         | High dose Inhaled NO for COVID-19 (ICU patients)                            | University Health Network, Toronto General Hospital, Toronto, Canada                 | RCT, Open label | NO gas                        | 160 ppm, once        | 6 h      | 20           | 7              | Phase 1     | Recruiting      |
| NCT04338828         | NO Inhalation therapy for COVID-19 infections in ED                         | Massachusetts General Hospital, Boston, Massachusetts, United States                 | RCT, Triple NO gas | 140–300 ppm                  | 20–30 min            | 260      | 28           |                | Phase 2     | Recruiting      |
| NCT04601077         | The evaluation of NO generating lozenges on outcome of newly diagnosed COVID-19 patients in African Americans | Nitric Oxide Innovations LLC                                                        | RCT, Triple NO lozenges | 30 mg, twice                  | 30 days              | 100      | 30           |                | Phase 1     | Not yet recruiting |
| NCT04610554         | Lung diffusing capacity for NO and CO early after mild-to-severe COVID-19 | IRCCS Azienda Ospedaliera Universitaria San Martino - IST Istituto Nazionale per la Ricerca sul Cancro, Genoa, Italy | –            | –                            | –                     | 74       | –            | –              | Completed   |                 |
| NCT04305457         | NO gas inhalation therapy for mild/moderate COVID-19                        | Providence HealthCare Network, Anchorage, Alaska, United States                     | RCT, Open label | NO gas                        | 140–180 ppm, twice  | 20–30 min | 70           | 28             | Phase 2     | Recruiting      |
| NCT04460183         | A Study to Assess Efficacy and Safety of RESP301 Plus Standard of Care (SOC) compared to SOC Alone in Hospitalized Participants With COVID-19 | Bradford Teaching Hospitals NHS Foundation Trust, Bradford, United Kingdom           | RCT, Open label | RESP301                       | Thrice                | 10 days  | 300          | 14             | Phase 2     | Recruiting      |
| NCT04456088         | Inhaled NO for treatment of COVID-19 caused by SARS-CoV2 (Canada trial)     | Beyond Air Inc.                                                                     | RCT, Open label | NO gas                        | 80 ppm, four times   | 40 min   | 50           | 14             | Phase 1     | Not yet recruiting |
| NCT04337918         | NO releasing solutions to prevent and treat mild/moderate COVID-19 infection | BC Diabetes, Vancouver, British Columbia, Canada                                      | RCT, Single NORS | NORS                          | five times            | 14 days  | 200          | 28             | Phase 2     | Recruiting      |
| NCT04312243         | NO prevention of COVID-19 for healthcare providers                          | Massachusetts General Hospital, Boston, Massachusetts, United States                 | RCT, Open label | NO gas                        | 160 ppm, twice       | 15 min   | 470          | 14             | Phase 2     | Recruiting      |
| Identifier         | Brief Title                                                                 | Co-ordinating center                                                                 | Study Design | Drug     | Dose                     | Duration | Subjects (n) | Follow-up (days) | Study Phase | Status               |
|-------------------|-----------------------------------------------------------------------------|--------------------------------------------------------------------------------------|--------------|----------|--------------------------|----------|--------------|-----------------|-------------|----------------------|
| NCT04443868       | NO releasing solution to treat and prevent exacerbation of mild COVID-19    | Sanotize Research and Development corp.                                              | RCT, Quadruple | NORS     | 14.4 ppm (240 mL)        | 14 days  | 50           | 28              | Phase 2     | Not yet recruiting   |
|                   | infection                                                                   |                                                                                      |              |          |                          |          |              |                 |             |                      |
| NCT04476992       | NO therapy for COVID-19 patients with oxygen supplementation                | Cardiology Research Institute, Tomsk National Research Medical Center, Russian Academy of Sciences, Tomsk, Russian Federation | RCT, Single  | NO gas-sessions and continuous | 200 ppm, twice 200 ppm + 20 ppm, twice | 30 min | 20           | 14              | Phase 1     | Phase 2, Not yet recruiting, Active |
| NCT04421508       | A study to assess pulsed inhaled NO vs. Placebo in subjects with mild or moderate COVID-19 | Banner University Medical Center, Phoenix, Arizona, United States                      | RCT, Quadruple | iNO pulse | 125 mcg/kg (~ 20 ppm)    | 24 h     | 500          | 28              | Phase 3     | Recruiting          |
| NCT04397692       | Inhaled NO for the treatment of COVID-19 caused by SARS-CoV-2 (US trial)    | Baptist Health Center for Clinical Research, Little Rock, Arkansas, United States    | RCT, Open label | NO gas   | 80 ppm, four times       | 40 min   | 20           | 14              | Recruiting   |                      |
| NCT04306393       | NO gas inhalation in SARS in COVID-19                                       | University of Alabama, Birmingham, Alabama, United States                             | RCT, Single  | NO gas   | 80 ppm 40 ppm            | 48 h     | 200          | 28              | Phase 2     | Recruiting          |
| NCT03331445       | Inhaled gaseous NO antimicrobial treatment of difficult bacterial and viral lung (COVID-19) infections | Nitric Solutions-Mobile Unit, Vancouver, British Columbia, Canada                    | RCT, Open label | NO (Thiolanox) Nitrogen gas | 160 ppm (0.5 %) 99.50 % | 24 h     | 20           | 20              | Phase 2     | Recruiting          |
Conclusions

The studies described in this report suggest that mechanisms designed to increase NO levels via gas inhalation or dietary intake may improve oxygen supply to tissues and restore normal vessel functioning. Treatment with NO inhalation therapy may reverse pulmonary hypertension and improve severe hypoxic condition in patients of COVID-19.

Funding  The authors have not received any funding for this work.

Availability of data and material:  Not applicable.

Code availability  Not applicable.

Declarations

Conflict of interest  The authors have declared that no competing interests exist.

Ethics approval  Not applicable.

Consent to participate  Not applicable.

Consent for publication  All authors have agreed for publication of this manuscript in its current form.

References

1. Adler H, Beland JL, Del-Pan NC, Kobzik L, Brewer JP, Martin TR, Rimm IJ. 1997. Suppression of herpes simplex virus type 1 (HSV-1)-induced pneumonia in mice by inhibition of inducible nitric oxide synthase (iNOS, NOS2). J Exp Med 1997;185:1533–1540. https://doi.org/10.1084/jem.185.9.1533.

2. Akerstrom S, Gunalan V, Keng CT, Tan Y-J, Mirazimi A. Dual effect of nitric oxide on SARS-CoV replication: viral RNA production and palmitoylation of the S protein are affected. Virology. 2009;395:1–9. https://doi.org/10.1016/j.virol.2009.09.007.

29. Åkerström S, Mousavi J, Klingström J, Leijon M, Lundkvist A, Mirazimi A. Nitric oxide inhibits the replication cycle of severe acute respiratory syndrome coronavirus. J Virol. 2005;79(3):1966–9. https://doi.org/10.1128/JVI.79.3.1966-1969.2005.

3. Boucher JL, Moali C, Tenu JP. Nitric oxide biosynthesis, nitric oxide synthase inhibitors and arginase competition for l-arginine utilization. Cell Mol Life Sci. 1999;55:1015–28. https://doi.org/10.1007/s000180050352.
4. Coleman JW. Nitric oxide in immunity and inflammation. Int. Immunopharmacol 2001; 1:1397–1406.

5. Dhama K, Khan S, Tiwari R, Sircar S, Bhat S, Malik YS, Singh KP, Chaicumpa W, Bonilla-Aldana DK, Rodriguez-Morales AJ. (2020). Coronavirus Disease 2019-COVID-19. Clin Microbiol Rev, 33(4), e00028-20. https://doi.org/10.1128/CMR.00028-20.

6. Garg I, Srivastava S, Rai C, Kumar V, Hembrom AA, Ghosh N, Kumari B, Bansal A, Kumar B. Coronavirus (covid-19): Prognostic risk associated with comorbidities and age. Int J Recent Sci Res. 2020;11(A):37983–6. https://doi.org/10.24327/ijrsr.2020.1104.5218. Issue, 04).

7. Gibson PG, Qin L, Puah SH. COVID-19 acute respiratory distress syndrome (ARDS): clinical features and differences from typical pre-COVID-19 ARDS. Med J Aust. 2020;213(2):54–6. https://doi.org/10.5694/mja2.50674.

8. Green SJ. Nitric oxide in mucosal immunity. Nat Med. 1995;1:515–7. https://doi.org/10.1038/nm0695-515.

9. He F, Deng Y, Li W. Coronavirus disease 2019: What we know? J Med Virol. 2020;92(7):719–25. https://doi.org/10.1002/jmv.25766.

10. Hoffman M, Kleine-Weber H, Schroder S, Kruger N, Herrler T, Erichsen S. SARS-CoV-2 Cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181:271–80. https://doi.org/10.1016/j.cell.2020.02.052.

11. Jung K, Gurnani A, Renukaradhya GJ, Saif LJ. Nitric oxide is elicited and inhibits viral replication in pigs infected with porcine respiratory coronavirus but not porcine reproductive and respiratory syndrome virus. Vet Immunol Immunopathol. 2010;136:335–9. https://doi.org/10.1016/j.vetimm.2010.03.022.

12. Lundberg JO. Nitric oxide and the paranasal sinuses. Anat Rec. 2008;291:1479–84. https://doi.org/10.1002/ar.20782.

13. Lundberg JO, Carlstrom M, Weitzberg E. Metabolic effects of dietary nitrate in health and disease. Cell Metab. 2018;28:9–22. https://doi.org/10.1016/j.cmet.2018.06.007.

14. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov. 2008;7:156–67. https://doi.org/10.1038/nrd2466.

15. Martel J, Ko YF, Young JD, Ojcius DM. Could nasal nitric oxide help to mitigate the severity of COVID-19? Microbes Infect. 2020;22(4–5):168–71. https://doi.org/10.1016/j.micinf.2020.05.002.

16. Mondal S, Quintili AL, Karamchandani K, Bose S. Thromboembolic disease in COVID-19 patients: A brief narrative review. J Intensive Care. 2020;8:70. https://doi.org/10.1186/s40560-020-00483-y.

17. Nagaki M, Shimura MN, Irokawa T, Sasaki T, Shirato K. Nitric oxide regulation of glycoconjugate secretion from feline and human airways in vitro. Respir Physiol. 1995;102:89–95. https://doi.org/10.1016/0034-5687(95)00042-C.

18. Pascarella G, Strumia A, Pliego C, et al. COVID-19 diagnosis and management: a comprehensive review. J Intern Med. 2020. https://doi.org/10.1111/joim.13091.

19. Pope M, Marsden PA, Cole E, Sloan S, Fung LS, Ning Q, Ding JW, Leibowitz JL, Phillips MJ, Levy GA. Resistance to murine hepatitis virus strain 3 is dependent on production of nitric oxide. J Virol 1998;72:7084–90. https://doi.org/10.1128/JVI.72.9.7084-7090.1998.

20. Ritz T, Trueba AF, Vogel PD, Aucsh RJ, Rosenfeld D. Exhaled nitric oxide and vascular endothelial growth factor as predictors of cold symptoms after stress. Biol Psychol. 2018;132:116–24. https://doi.org/10.1016/j.biopsycho.2017.11.006.

21. Runer T, Cervin A, Lindberg S, Uddman R. Nitric oxide is a regulator of mucociliary activity in the upper respiratory tract. Otolaryngol Head Neck Surg. 1998;119:278–87.

22. Saura M, Zaragoza C, McMillan A, Quick RA, Hohenadl C, Lowenstein JM. An antiviral mechanism of nitric oxide: inhibition of a viral protease. Immunity. 1999;10:21–8. https://doi.org/10.1016/S1074-7613(00)80003-5.

23. Toussoulis D, Kampoli A-M, Tentolouris C, Papageorgiou N, Stefanadis C. The role of nitric oxide on endothelial function. Curr Vasc Pharmacol. 2012;10:4–18. https://doi.org/10.2174/157016112798829760.

24. Wu D, Wu T, Liu Q, Yang Z. The SARS-CoV-2 outbreak: What we know. Int J Infect Dis. 2020;94:44–8. https://doi.org/10.1016/j.ijid.2020.03.004.

25. Xu Z, Shi L, Wang Y, et al. Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Resp Med. 2020;8:420–2. https://doi.org/10.1016/S2213-2600(20)30076-X.

26. Wu X, Zheng S, Dweik RA, Erzurum SC. Role of epithelial nitric oxide in airway viral infection. Free Radic Biol Med. 2006;41:19–28. https://doi.org/10.1016/j.freeradbiomed.2006.01.037.

27. Yang Y, Peng F, Wang R al. The deadly coronaviruses: The 2003 SARS pandemic and the 2020 novel coronavirus epidemic in China. J Autoimmun 2003;2020:102434; https://doi.org/10.1016/j.jaut.2020.102434.

28. Zhang T, He Y, Xu W, Ma A, Yang Y, Xu K-F. Clinical trials for the treatment of Coronavirus disease 2019 (COVID-19): a rapid response to urgent need. Sci China Life Sci. 2020;63:774–6. https://doi.org/10.1007/s11427-020-1660-2.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.