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SHORT COMMUNICATION

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

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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. In view of COVID-19 pandemic, several clinical trials are underway to examine the effects of NO inhalation on infected patients. Previously published reports on beneficial effects of endogenous NO and NO inhalation therapy were thoroughly searched to assess the potential of NO therapy for treating 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

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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 platelet-endothelial 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 sever effects of COVID-19 infection.

Table 1 Nitric Oxide (NO) therapy under various stages of clinical trial for COVID-19 treatment

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
					150 ppm, four times	40 min		14	Phase 2	
NCT04337918	NO releasing solutions to prevent and treat mild/moderate COVID-19 infection	BC Diabetes, Vancouver, British Columbia, Canada	RCT, Single	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

Table 1 continued

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 infection	Sanotize Research and Development corp.	RCT, Quadruple	NORS	14.4 ppm (240 mL)	14 days	50	28	Phase 2	Not yet recruiting
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	200 ppm, twice	30 min	20	14	Phase 1	Not yet recruiting, Active
				NO sessons and continuous	200 ppm + 20 ppm, twice	30 min		14	Phase 2	
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 gasesous NO antimicrobial treatment of dificult 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		Phase 2	Recruiting

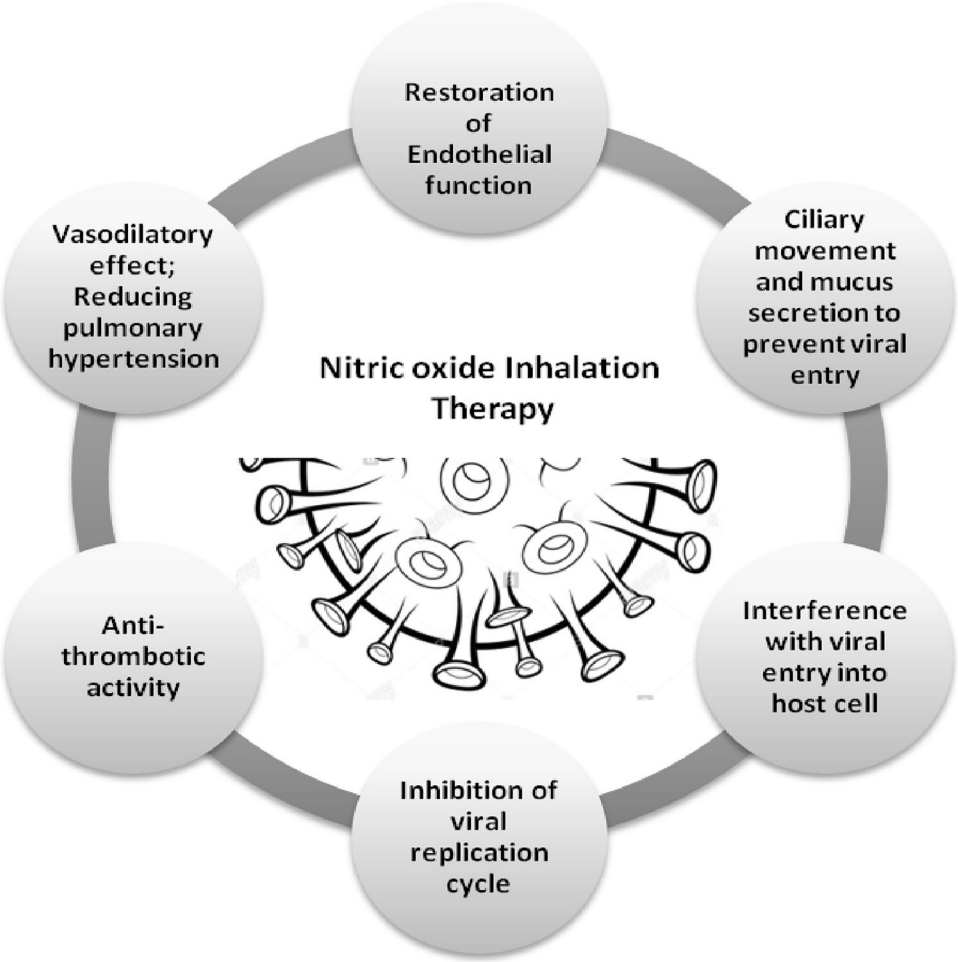

Fig. 1 Beneficial effects of NO inhalation therapy for inhibiting COVID-19 infection

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.

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Declarations

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