Nitric oxide’s physiologic effects and potential as a therapeutic agent against COVID-19

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Keywords: nitric oxide, nitric oxide synthase, pulmonary gas exchange, COVID-19, SARS-CoV-2

Abstract

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for COVID-19 pneumonia, a pandemic that precipitates huge pressures on the world’s social and economic systems. Disease severity varies among individuals. SARS-CoV-2 infection can be associated with e.g. flu-like symptoms, dyspnoea, severe interstitial pneumonia, acute respiratory distress syndrome, multiorgan dysfunction, and generalized coagulopathy.

Nitric oxide (NO), is a small signal molecule that impacts pleiotropic functions in human physiology, which can be involved in the significant effects of COVID-19 infection. NO is a neurotransmitter involved in the neural olfactory processes in the central nervous system, and some infected patients have reported anosmia as a symptom. Additionally, NO is a well-known vasodilator, important coagulation mediator, anti-microbial effector and inhibitor of SARS-CoV replication.

Exhaled NO is strongly related to the type-2 inflammatory response found in asthma, which has been suggested to be protective against SARS-CoV-2 infection. Several reports indicate that the use of inhaled NO has been an effective therapy during this pandemic since the ventilation-perfusion ratio in COVID-19 patients improved afterwards and they did not require mechanical ventilation.

The aim of this mini-review is to summarize relevant actions of NO that could be beneficial in the treatment of COVID-19.

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an RNA virus closely related to SARS-CoV. It is responsible for the disease known as COVID-19 pneumonia that was declared a pandemic by the World Health Organization (WHO) in early March 2020. Since it first emerged in December 2019 in Wuhan, China, the virus has spread rapidly around the world. As of 8 October 2020, it has been responsible for 36 306 189 confirmed cases of COVID-19 and 1 057 996 deaths in 188 countries [1]. It has also caused huge economic and social burdens worldwide. There is a great intra-individual variability in COVID-19 symptoms. Some patients remain asymptomatic, others have mild flu-like symptoms. There are others in a subset associated with the highest mortality who manifest dyspnoea, severe interstitial pneumonia, acute respiratory distress syndrome, multiorgan dysfunction, skeletal muscle fatigue and generalized coagulopathy [2–4]. The most commonly reported symptoms are fever, dry cough, fatigue, slight dyspnoea, sore throat and headache. Gastrointestinal disorders such as diarrhoea, nausea and vomiting are reported to lesser extent. Some patients have also experienced anosmia and dysgeusia. This has led to the hypothesis that SARS-CoV-2 could affect the central nervous system and that the viral neurological effects could in part be the cause of the respiratory failure [2].

2. SARS-CoV-2 infection and tobacco smoke

Initially with SARS-CoV-2 infection, the virus binds to the angiotensin converting enzyme 2 (ACE2) surface receptors of the host cell via its protein spike (S). The S protein must then be primed by host cell
proteases to allow membrane fusion and the consequent entry of the virus [5, 6]. ACE2 is expressed in several human organs; especially so in the lung alveolar epithelial cells, enterocytes of the small intestine, and arterial and venous endothelial cells where the virus can enter the body [7]. Recently, it has been demonstrated that ~85% of the cells expressing-ACE2 in the lung are the type II alveolar cells [8]. This distribution can explain the variety of symptoms caused and organs impacted by SARS-CoV-2. An allelic variability on ACE2 conformation has been found, and three more common missense changes were identified [9]. The authors think that this variability could at least partially account for the inter-individual clinical differences and likely modulate clinical severity.

A study by Smith et al showed that in the respiratory tract of mice and humans the ACE2 expression is modulated by smoke exposure in a dose-dependent manner. Moreover, they highlighted a higher ACE2 expression in subjects affected by COPD, which has been recognized as a COVID-19 risk factor [5, 10]. These observations have been confirmed by Leung et al who investigated the gene expression of ACE2 in three cohorts of individuals with and without COPD. In COPD patients and current smokers, they found that their greater levels of ACE2 mRNA were inversely related to their forced expiratory volume in 1 s [11]. Findings concerning the role of smoking in COVID-19 seem to provide controversial results. Studies from different countries revealed that the percentage of smokers affected by COVID-19 was lower than that of non-smokers, which suggests smoking has a protective effect against SARS-CoV-2 infection. On the other hand, a strong association between smoking and a poorer prognosis has been proven. Several researchers have shown that there was a higher percentage of smokers among COVID-19 patients with more severe disease [12–16]. Interestingly, the endogenous production of nitric oxide (NO) is decreased in the exhaled gas of smokers and upregulates after the cessation of smoking [17].

3. Endogenous NO production

NO is a biatomic gaseous molecule that plays a key role in a large number of physiological processes [18, 19] via the induction of cyclic guanosine monophosphate (cGMP) production [18, 20, 21]. NO generation is subsequent to consecutive redox reactions resulting in the conversion of the amino acid L-arginine to L-citrulline. The enzymes that catalyse this transformation belong to the NO synthase family (NOS) in which NOS I, NOS II, NOS III are included. Notably, are all expressed in the airways. Two of the three isoforms, NOS I and NOS III, are constitutively expressed calcium-dependent enzymes. They are also defined as neuronal (nNOS) and endothelial NOS (eNOS), respectively, since they have been originally isolated in neuronal and vascular endothelial cells. NOS II is an inducible isoenzyme (iNOS) whose expression is induced by inflammatory mediators such as interleukin 1β, interferon γ, and interleukin 13 which is the most potent inducer of iNOS in asthma [22]. The iNOS activity is independent of the intracellular calcium concentration. It yields NO that contributes to the antimicrobial activity of macrophages and represents the fraction of exhaled NO (FENO), which is considered to be an inflammatory marker [18, 23, 24].

4. NO in asthmatics with COVID-19

In vitro and ex vivo studies have shown that active smoking and secondary cigarette smoke exposure can reduce NO concentration by either inhibiting NOS activity or increasing NO degradation via reactive oxygen species (ROS) production [25, 26]. Available evidence has also shown reduced FENO values in smokers with asthma [27]. FENO is considered to be a marker of the type 2 (T2) inflammatory response [28], which characterizes allergic diseases such as asthma. Higher FINO values were measured in asthmatic patients with high blood eosinophils and atopy [29–31]. Moreover, Yamamoto et al reported that the expression of iNOS and FINO values were higher in severe asthma patients and the values correlated with their sputum eosinophil levels [32].

Kimura et al described reduced ACE2 gene expression in asthmatic children and adults with a T2-high phenotype. In the same study, the authors revealed that the ACE2 levels decreased in airway epithelial cells treated with interleukin 13, which is a key cytokine involved in the T2 response associated with increased FINO [33]. A recent study among asthmatic patients reported an association between a higher epithelial ACE2 expression found in a subset with a T2-low phenotype gene expression signature and an increased risk for severe COVID-19 [34]. Jackson et al reported reduced ACE2 expression in allergic asthmatic patients [35]. In this context, Licari et al observed that allergic children with high blood eosinophilia were less affected by COVID-19, and that there was a high frequency of eosinopenia among those who died from SARS-CoV-2 infection. Therefore, eosinopenia was considered to be a potential marker of a poor prognosis [36]. Recent enticing evidence has shown a reduced expression of ACE2 in asthmatic patients using a high dose of inhaled corticosteroids (ICS), which suggests a therapeutic regimen of ICS could be a predictor of a lower susceptibility to SARS-CoV-2 infection [37]. This could prove interesting since FINO is considered to be a biomarker of ICS responsiveness in asthma [38–41], however further studies are warranted.
Figure 1. Physiologic effects of Nitric Oxide (NO) that may have relevance in the prevention and treatment of SARS-CoV-2 infection. Further research is needed to investigate the interplay between the NO molecule and the virus responsible for COVID-19 infection.

5. NO as a signalling molecule

NO is a multifunctional signalling molecule that impacts activities that are fundamental for the human organism and could therefore play an important role in the treatment of COVID-19 symptoms (figure 1).

NO is a neurotransmitter of the inhibitory non-adrenergic non-cholinergic (NANC) system, and as such it mediates the bronchodilator NANC response [18]. As a neurotransmitter, NO is also involved in olfactory processes [18, 19]. Moreover, NO probably participates in mechanisms related to the neuronal processing of the sensory input and olfactory memory through NO/cGMP signalling and its contribution to the regenerative processes of the olfactory epithelium [42, 43].

SARS-CoV-2 infection is associated with altered coagulation parameters and a higher risk for venous thromboembolism [44–46]. This is an aspect of the infectious disease that could be improved through the action of NO due to the vasodilating and antithrombotic action of this molecule [47]. In addition, previous studies suggest that NO is able to potentiate the anti-thrombotic effect of eplerenone in a murine model of diabetes and to elongate bleeding-times in rabbits and humans after its inhalation [48, 49].

Several studies have suggested the involvement of NO in viral infection. De Gouw et al observed an increase in F\textsubscript{e}NO levels following human rhinovirus 16 (HRV-16) infection in asthmatics [50]. These findings are in keeping with subsequent evidence that showed an increase in the NO exhaled from the nasal and lower airway of humans infected with HRV-16 [51].

Moreover, a recent study in human type 2 alveolar epithelial cell line (A549) showed that when infected with the respiratory syncytial virus (RSV), an iNOS expression mediated by the transcription factor Kruppel-like factor 6 was induced. This mechanism may underline the NO-mediated apoptosis that occurs during RSV infections [52]. It should be taken into account that the iNOS gene expression can be up-regulated via interferon-γ production in response to viral infections [53]. These observations suggest that F\textsubscript{e}NO and the alveolar NO (C\textsubscript{A}NO) can be considered to be markers of epithelial damage in the proximal and distal airways, respectively, during viral infections e.g. SARS-CoV-2. Similarly, the mucin protein Krebs von den Lungen-6 [54], which is secreted by damaged or regenerating alveolar type II pneumocytes, was found to be increased in the serum levels of patients with severe COVID-19. Further studies are needed to address this issue.

On the other hand, it is well known that NO exerts an antimicrobial action and is able to inhibit viral replication [55]. In particular, NO has been shown to impair SARS-CoV replication through either a direct action on the S protein metabolism or the production of reactive nitrogen intermediates [56, 57].

Furthermore, the NO stimulatory effect on ciliary movement results in the elimination of dust and microbial (virus, bacterial, fungal) particles [58]. NO also increases the permeability of the airway epithelium and thereby enhances the ciliary motility [59]. Interestingly, Baker et al also hypothesised that asthmatics have a defective airway fluid control since cells from asthmatic donors failed to respond to NO.
or cGMP [59]. A recent study reported that patients aged <65 years who were affected by COVID-19 and had pre-existing asthma underwent a longer period of intubation than non-asthmatic COVID-19 patients [60].

The NO signalling system is involved in the physiology of most of our organs and we hypothesise that a deficiency of NO is involved in the manifestations of COVID-19. This is difficult to comprehend since NO, if not endogenously produced through enzyme activation, can be generated by the nitrate/nitrite conversion to NO [61]. DeoxyHb activates this conversion to NO and is thereby involved in the vasodilation and anti-thrombotic effects. Intriguingly, NOS 1 has a critical role in the ventilatory response to hypoxia in the brain’s respiratory centre, and the inhibition of this enzyme will attenuate this response [62].

6. NO inhalation treatment

Research has not been performed that proves whether there is a lack of NO or not, but researchers have been discussing the use of exogenous NO therapy. Hedenstierna et al hypothesised that high concentrations of inhaled NO (iNO) administered in a short burst of 1000 ppm could be protective against the COVID-19 virus [63]. The authors suggested that the beneficial effect of NO could reside in its antimicrobial action as the radical showed the capability to kill the SARS-CoV virus in a cell culture. It has been demonstrated in an in vitro model with SARS-CoV-2 infected cells, that an NO donor had an effective inhibitory effect on the replication of the virus and that the SARS-CoV-2 main protease was identified as the target [64].

In addition, during the severe acute respiratory syndrome (SARS) epidemic in 2003, iNO of 30 ppm was found to be an effective therapy by improving oxygenation in patients with significant hypoxemia. This could counteract the blood oxygenation impairment caused by SARS-CoV-2. Furthermore, the authors suggested that since smokers were under-represented among SARS and COVID-19 patients in China, a mechanism of protection against the virus could be attributed to the intake of NO in high concentration bursts during the act of smoking.

Recent evidence supports the hypothesis presented by Hedenstierna et al. In patients with severe hypoxemia, iNO has been utilized as a rescue therapy by improving ventilation-perfusion matching and by decreasing pulmonary vascular pressure. Parikh et al observed that more than half of the 39 patients with COVID-19 did not require mechanical ventilation after iNO therapy, and they suggested that iNO therapy may have a role in preventing the progression of hypoxic respiratory failure in COVID-19 patients [65]. Moreover, Zamanian et al reported that after iNO therapy, physical activity and perfusion improved for a patient with otherwise well-controlled vasoreactive pulmonary arterial hypertension but was experiencing severe breathlessness from COVID-19 [66]. Several clinical trials are being conducted with the aim to test iNO therapy among COVID-19 patients [67].

However, iNO therapy is complicated and we do not yet know what dose is best to use with COVID-19. It has been beneficial to use a low dose of NO (10 ppm) as adjunctive therapy to enhance the efficacy of antibiotics used to treat acute P. aeruginosa exacerbations in cystic fibrosis patients [68]. Pulsed iNO of 26 ppm in the first 30% of the inhalation was able to effectively increase the oxygenation and decrease the venous admixture in hypoxemic horses during anaesthesia [69]. A question that should be addressed is if the endogenous NO (in ppb) is rapidly metabolised by ROS in an extreme environment generated by the virus, what degree of danger if any exists with the inhalation of NO (in ppm). Further research is needed to answer this question and other questions regarding the use of NO in clinical practice.

7. Conclusion

We have reviewed several physiological effects exerted by NO. We have presented intriguing bases for further investigations regarding the protective influence NO might play and possible areas of use with COVID-19 infection. The different physiologic effects presented could well play beneficial roles in the treatment of a wide range of symptoms caused by this infectious disease.

Acknowledgments

The authors wish to thank Robin Quell for editing and proofreading this manuscript.

Authors’ contributions

MH, FLMR initiated the mini-review. MH, FLMR, FB, VC collected the literature and prepared the initial draft. All authors have discussed and adjusted the draft and have read and approved the final manuscript.

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