Nitric Oxide to Fight Viral Infections

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Coronavirus disease 2019 (COVID-19) is an infectious disease caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) that has quickly and deeply affected the world, with over 60 million confirmed cases. There has been a great effort worldwide to contain the virus and to search for an effective treatment for patients who become critically ill with COVID-19. A promising therapeutic compound currently undergoing clinical trials for COVID-19 is nitric oxide (NO), which is a free radical that has been previously reported to inhibit the replication of several DNA and RNA viruses, including coronaviruses. Although NO has potent antiviral activity, it has a complex role in the immunological host responses to viral infections, i.e., it can be essential for pathogen control or detrimental for the host, depending on its concentration and the type of virus. In this Essay, the antiviral role of NO against SARS-CoV, SARS-CoV-2, and other human viruses is highlighted, current development of NO-based therapies used in the clinic is summarized, existing challenges are discussed and possible further developments of NO to fight viral infections are suggested.

1. Introduction

Infections have plagued mankind for centuries and they remain among the leading causes of death and disability worldwide.[11] They are caused by pathogenic microorganisms, such as viruses, bacteria, parasites, or fungi. Several factors contribute to the emergence/re-emergence of infectious diseases, including microbial diversity and evolution, social factors, and environmental conditions.[1–3] An example of the interaction among these factors is zoonotic diseases, which arise at the interface of human, animal, and environmental health. According to Keusch et al., at least 65% of recent major human infectious disease outbreaks have originated from zoonotic viruses,[11] including: acquired immune deficiency syndrome (AIDS), severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), Ebola, swine influenza, avian influenza, dengue fever, and Zika fever. Coronavirus disease 2019 (COVID-19), a respiratory disease caused by the zoonotic severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), is the latest example of infectious disease with over 60 million infections and a million deaths globally to date.

Nitric oxide (NO) is a signaling molecule produced in mammalian cells by NO synthase and it is involved in a wide range of physiological processes, including inflammatory response,[4] bronchodilation (relaxation of the smooth muscles in the lungs to open airways),[5,6] vasodilation (widening of blood vessels to increase blood flow),[7–9] modulation of intraocular pressure,[10,11] regulation of neuronal function and signal transmission.[12] It has been well established that NO has a role in the pathogenesis of many human viral infections[13] as well as direct or indirect antiviral activity.[14] Direct antiviral activity means that NO can directly inactivate viral particles or inhibit their replication, while indirect activity means a modulation of the host immune response that usually produces an inflammatory response.[15] NO can also react with other molecules to produce several reactive oxygen/nitrogen species, including peroxynitrite (ONOO–), dinitrogen trioxide (N2O3), and nitrogen dioxide (NO2). These reactive oxygen/nitrogen species themselves may have a direct antiviral effect on the pathogen, but they could also induce oxidative stress that may cause severe cytotoxic effects (Figure 1).[15–17] Interestingly, whether the overall effect of NO is positive (antiviral) or negative (pathogenic) seems to be virus-dependent (discussed later in this Essay).

NO activity is also concentration-dependent. During viral infection, intracellular NO production increases.[18,19] While local and controlled production of NO may be beneficial—aiding in the control of infections—generalized activation and overproduction may contribute to tissue damage and cell death—contributing to the pathogenesis.[16–23] Therefore, NO production in the host must be tightly controlled to exert antiviral rather than harmful effects.[24,25] NO is produced endogenously in a number of cells and tissues by the enzymatic action of NO synthase (NOS), which catalyzes the oxidation of l-arginine to NO and l-citrulline.[15,26] In mammals, there are three isofoms of NOS: neuronal NOS (nNOS or NOS-1), inducible NOS (iNOS or NOS-2), and endothelial NOS (eNOS or NOS-3).[15,26] eNOS and nNOS produce...
NO only for short periods of time (seconds to minutes) following enzyme activation. In contrast, iNOS produces 10–100 times more NO for comparatively long periods of time (hours to days) and, during an infection, its expression is localized in specific tissues and cells. It is the iNOS that has been mainly associated with the host response to viral infections. Because of the high levels of NO that iNOS can produce, regulation of this enzyme is particularly important. Mediators that regulate iNOS expression are proinflammatory cytokines, such as interferon-γ (IFN-γ), interleukin 12 (IL-12), and tumor necrosis factor-alpha (TNF-α). The mechanism of this regulation is cell-dependent and has been demonstrated in several human cells, including hepatocytes, lung epithelial cells, alveolar macrophages, and monocytes. When the iNOS gene is strongly upregulated, the high amount of NO generated might lead to tissue damage. However, defining what concentration of the radical leads to negative effects is not trivial, because of the difficulties involved in the direct quantification of NO in humans only indirectly, relying on the quantification of NO metabolites (nitrites and nitrates) in plasma and urine as a surrogate for NO production. Another method to infer localized NO production is the detection of iNOS, performed directly on tissue samples via immunohistochemical staining or indirectly via quantification of iNOS mRNA using polymerase chain reaction (PCR). Measurement of exhaled NO is also performed, as the radical is considered a biomarker of airway inflammation. Reported concentrations of NO in the airways of healthy subjects varies between 0.1 and 41 × 10⁻⁹ m, depending if the gas is measured in the lower or upper respiratory tract, respectively.

In this Essay, we highlight the key findings on the production of NO during viral infections and the role of NO in the treatment of SARS-CoV, SARS-CoV-2, and other human viruses. We provide an overview of the current NO-based therapies used for the treatment of respiratory viral infections and discuss existing challenges.

2. NO Production During Viral Infections

A list of experimental studies on the production of NO in response to viral infections is reported in Table 1. Herein, we aim to illustrate the conflicting role of NO (antiviral or pathogenic effects) in viral infections, and the crucial difference between respiratory and non-respiratory viruses. For a more exhaustive overview of the role of NO on specific diseases, readers are referred to the reviews of Chaturvedi et al. (dengue), Akaike...
Table 1. In vitro and in vivo studies on the effect of nitric oxide on respiratory and non-respiratory infections caused by DNA and RNA viruses. ss: single-stranded, ds: double-stranded. In preparing this table, priority was given to the selection of in vivo studies in humans.

| Family            | Viral nucleic acid | Envelope | Virus (common name)                                                                 | Studies |
|-------------------|-------------------|----------|-----------------------------------------------------------------------------------|---------|
| Respiratory       |                   |          |                                                                                   |         |
| Coronaviridae     | Positive-sense ssRNA | Yes      | Severe acute respiratory syndrome coronavirus (SARS-CoV)                           | In vitro [17,46,47] |
|                   |                   |          | Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2)                      | In vitro [48] |
| Picornaviridae    | Positive-sense ssRNA | No       | Human rhinovirus                                                                  | In vivo (humans) [49] |
| Orthomyxoviridae  | Negative-sense ssRNA | Yes      | Influenza A subtype H1N1 (swine flu)                                              | In vitro [51] |
|                   |                   |          | Influenza A subtype H3N2                                                           | In vivo (humans) [52] |
|                   |                   |          | Influenza A subtype H2N2                                                           | In vitro [53] |
| Non-respiratory   |                   |          |                                                                                   |         |
| Flaviviridae      | Positive-sense ssRNA | Yes      | Dengue virus                                                                      | In vitro [54,55] |
|                   |                   |          | Hepatitis C                                                                       | In vivo (humans) [56-58] |
| Retroviridae      | Positive-sense ssRNA | Yes      | Human immunodeficiency virus (HIV)                                                | In vitro [54,55] |
|                   |                   |          |                                                                                    | In vivo (humans) [56,67] |
| Herpesviridae     | dsDNA             | Yes      | Herpes simplex virus                                                              | In vitro [48] |
|                   |                   |          |                                                                                    | In vivo (mice) [60,61,62] |

et al. [42] (influenza), Torre et al. [43] (HIV), Proud et al. [44] (common cold), and Iwakiri et al. [45] (hepatitis).

2.1. Respiratory Viruses

Human rhinovirus (HRV) is the predominant cause of the common cold, one of the most frequently experienced acute respiratory illnesses in humans. [27] In response to HRV infections, Sanders et al. found elevated iNOS expression in the nasal cells obtained from infected patients. [50] This virally induced increase in iNOS expression was associated with higher levels of exhaled NO (≈550% higher compared to the baseline) from both the nasal and lower airways. Interestingly, the increase in exhaled nasal NO levels was inversely related to the symptoms, suggesting that NO may play an important role in the innate immune response to rhinovirus infections, contributing to the resolution of the cold. [27,50] Elevated NO levels were also observed in patients infected with influenza A, a respiratory virus responsible for many of the seasonal outbreaks. [27] Murphy et al. infected 14 healthy volunteers with influenza A H1N1 and monitored the levels of exhaled NO from the nose and the mouth. [27] Only oral NO levels increased, peaking at day 8 post infection (15.4 ppb). This increase was not associated with patient symptoms, which peaked at day 3 postinfection. This suggested that elevated NO levels may not be directly responsible for the respiratory tract symptoms of infection with influenza A. From this study NO seems not to have harmful effects on the hosts in response to influenza; however, the concentration of exhaled NO measured was much lower compared with HRV infections. Unlike what was observed in humans, in a mouse model infected with influenza A H2N2, Akaike et al. found that NO is crucial for the pathogenesis of pneumonia but not as an antiviral effector mechanism. [51] Although studies in mouse models have indicated that the release of NO after infection with influenza viruses contributes to the pathogenesis of pneumonia, it may be argued that the production of NO in the respiratory tract may also provide a first-line defense mechanism against influenza viruses. [24]

2.2. Non-respiratory Viruses

In opposition to respiratory viruses, NO production during non-respiratory infections is mostly associated with pathogenic effects. Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV) that primarily affects the liver. Mihm et al. analyzed liver tissue from chronically HCV-infected patients, where the iNOS expression was found to be positively correlated to the level of viral RNA. [62] A positive correlation was also found between iNOS activity and tissue damage, suggesting a role of NO in the pathogenesis of human chronic viral hepatitis. [62,63] Valero et al. first reported the presence of increased NO levels (determined by assaying for nitrite and nitrate) in the serum of humans with dengue fever, a disease caused by the dengue virus. [56] Since NO induces blood vessels to relax and dilate, which lowers blood pressure, the authors hypothesized that increased NO in classical dengue could be important in the evolution of the disease into its severe hemorrhagic form. [56] NO also has a role in the pathogenesis of pneumonia from herpes simplex virus 1 (HSV-1). Adler et al. infected mice with HSV-1 via intranasal inoculation, resulting in the development of pneumonia. [21] Some mice were treated with an inhibitor of nitric oxide synthase activity (L-NMMA), while a control group was not. L-NMMA treatment significantly improved survival and pulmonary compliance of HSV-1 infected mice, despite the presence of a 17-fold higher pulmonary viral titer. Therefore, instead of worsening the disease by diminishing the antiviral effect of NO, iNOS inhibition was beneficial. It reduced the proinflammatory effects of NO, which...
resulted to be more important for the pathogenesis of HSV-1 pneumonia than the antiviral effects of NO.[20,21]

3. Antiviral Activity of NO

Antiviral effects of NO on viral infections in vitro and in vivo were first documented in the early 1990s on ectromelia, vaccinia, vesicular stomatitis, and herpes simplex-1 viruses,[68,69,72-74] later on for HIV,[75] influenza,[24] and—most importantly in light of the current pandemic—SARS-CoV[17,46,47] and SARS-CoV-2/COVID-19.[48]

In itself, this notion is worth highlighting; current understanding in the field is that broad spectrum antivirals are highly warranted but at present are hardly available. From the early days, it was clear that NO exhibits a rather broad spectrum of activity and limits infectivity of diverse viruses, RNA and DNA containing, enveloped and naked. These observations are rather unique among antiviral drugs on the market today.[76]

Target proteins for the antiviral activity of NO include proteases, reverse transcriptases, ribonucleotide reductases, as well as the zinc-fingers and related domains.[77] Mechanistically, for proteins in which cysteine (Cys) is directly involved in catalysis (e.g., cysteinyl proteases), the mechanism of activity lies in nitrosylation of the amino acid involved in the catalytic process. For other proteins, Cys is found in the overall microenvironment and a seemingly small change via S-nitrosylation exerts a dramatic effect on catalysis. Interestingly, even for the HIV aspartyl protease, NO has a strong inhibitory effect on the protease activity, through nitrosylation of Cys (two per subunit in this homodimeric protein), which appears to be essential for the catalytic activity of the protease.[75] Furthermore, HIV reverse transcriptase Cys38 also appears to be important for the overall microenvironment (the finger domain) and nitrosylation decreases the catalytic activity of this enzyme.[78]

One study we deem worth highlighting is that from Saura et al., in which antiviral activity of NO on Coxackie virus was deciphered with confidence.[79] Coxackie is a non-enveloped ssRNA-virus, which has a cysteinyl protease, indispensable for virus proliferation. The antiviral effect of NO against Coxackie was shown to proceed via Cys nitrosylation, and changing cysteine for serine (converting the cysteinyl protease into serine protease) rendered the protease NO-resistant.

Another study that stands out considered that NO can exert antiviral activity on the virion and/or interfere with intracellular viral replication.[80] On an example of the hantavirus (Bunyaviridae family, RNA-virus), this study showed that S-nitroso-N-acetylenicillamine (SNAP) and cytokine-induced NO had strong antiviral effects, whereas incubation of viral stocks in the presence of SNAP had a limited effect on infectivity—thus revealing that NO-mediated effects are intracellular rather than occurring on the virion. Interestingly, viral titers in iNOS −/− mice were higher than in control mice, suggesting that NO inhibits virus replication in vivo.

Specifically for SARS-CoV, to our knowledge, the first report of NO as an inhibitor of viral infectivity was that from Keyaerts et al. in 2004 (using SNAP as a donor, in vitro).[47] Later, Åkerström et al. showed that the mechanism of action is at least two-pronged.[17,46] One mechanism is the decreased palmitoylation of the spike (S) protein, a step pivotal for the protein anchoring to the lipid bilayer. The other mechanism is the nitrosylation of viral proteins. Interestingly, nitrosylation documented in this work was detected using the affinity isolation step and capture of nitrotyrosine, and this work confirms tyrosine nitrosylation for the S protein. Nitrosylation of the cysteinyl protease was also experimentally shown, through the analysis of proteolytic degradation of the viral polyopeptide, whereby the content of the nucleocapsid N protein was drastically decreased in the presence of SNAP and high molar mass (nonprocessed) polyopeptide content was increased. In the case of SARS-CoV-2, Akaberi et al. showed that SARS-CoV-2 3CL cysteine protease was a possible target of nitrosylation, which led to inhibition of the protease activity and viral replication.[48]

While all the studies discussed above present antiviral effects of NO generated by donor compounds, one and only study (to our knowledge) presents antiviral effects of gaseous NO. Specifically, Regev-Shoshani et al. investigated three strains of the influenza virus in vitro and showed that gaseous NO provided highly efficacious antiviral effects.[51] NO doses were matched to those approved in the clinic, further highlighting the translational potential of NO gas for antiviral treatment. Remarkably, this study was all but neglected and all studies on antiviral effects of NO that appear in press continue to rely on NO donors.

4. NO-Based Therapies to Fight Viral Infections

Response to non-respiratory viral infections in humans is often accompanied by an overproduction of endogenous NO, which mostly contributes to pathogenesis. In these cases, the treatment often includes iNOS inhibitor drugs such as L-NMMA.[21] In stark contrast, the production of endogenous NO seems to have a positive (antiviral) effect against human respiratory viruses. It might be hypothesized that for these diseases, when the endogenous levels of NO are not sufficient to achieve antiviral effects, the administration of additional NO may have a positive therapeutic effect.

There are various strategies to administer additional NO to the body; some of these are early stage technologies, while others have made it to the clinics.[126] NO can be introduced via catalytic generation using enzymes or enzymes mimics with NO donors in viral infections employed SNAP, a synthetic S-nitrosothiol that has been shown to inhibit in vitro the replication of influenza A, dengue, herpes, HIV, SARS-CoV, and SARS-CoV-2 viruses.[24,46,48,54,65,68] Such a recurring use of SNAP might be justified by its higher stability compared to primary alkyl S-nitrosothiols (RSNOs).[81] In most in vitro studies, the donor is added into the cell culture and the biological effects (e.g., inhibition of viral replication) are monitored. However, the authors usually do not quantify the concentration of the NO generated,
and therefore the correlation between the therapeutic level of the donor and virus inhibition is not clear. Another important class of synthetic NO donors is diazeniumdiolates (NONOates), for which the rate of decomposition is not influenced by reducing agents or biological tissues.\textsuperscript{[86]} 3-(2-hydroxy-2-nitrosomethylhydrazino)-1-propanamine (PAPA NONOate) has been shown to inhibit both rhinovirus replication and cytokine production in a dose-dependent fashion.\textsuperscript{[49]} The diethylenetriamine-NO adduct (DETA/NO) has been used in aerosol form for the treatment of acute respiratory distress syndrome in phase I clinical trial.\textsuperscript{[87]} However, defining pharmacokinetics of NO in vivo using donor compounds is a highly challenging task, as it requires controlling blood and body residence time of the donor compounds and to factor in the kinetics of NO release in a complex environment such as blood. Furthermore, even the quantification of NO concentration in blood in real time is a stand-alone challenge. Finally, NO donors are most often administered systemically, which means that an add-on challenge is found in drug targeting to the diseased tissues.

A facile, powerful alternative is found in direct pulmonary administration of gaseous NO, via inhalation. This is particularly important in the context of the main subject of this discussion, namely the treatment of COVID-19. Inhalation of gaseous NO has proven successful for the treatment of circulatory and respiratory conditions including pulmonary infections, pulmonary arterial hypertension, recurrent pulmonary infections associated with cystic fibrosis, and tuberculosis.\textsuperscript{[88,89]} Traditionally, NO is delivered from gas cylinders connected to a delivery system that monitors in real time the concentration of NO, nitrogen dioxide, and oxygen. Companies that commercialize this technology include Mallinckrodt Pharmaceuticals (INOmax, FDA approved), Praxair Technologies (Noxivent, FDA approved), and Novoteris (Thiolanox). More recently, portable devices that can generate NO on demand—even outside the hospital setting—have been developed.\textsuperscript{[90]} Currently, Genosyl by VERO Biotech is the only FDA approved NO-generating machine, but companies such as Third Pole Therapeutics, Beyond Air, and Bellerophon Therapeutics are also developing similar devices.

The therapeutic range of inhaled NO depends on the disease; for persistent pulmonary hypertension of the newborn, it is recommended a continuous dose of 20 ppm ($832 \times 10^{-9}$ m; 1 ppm of NO corresponds to a concentration of $41.59 \times 10^{-9}$ m) for 14 days, with a therapeutic range of 5–80 ppm. Viral infections such as bronchiolitis are usually treated with higher doses of NO (160 ppm, $6654 \times 10^{-9}$ m) for shorter periods (e.g., 30 min, five times a day), while other infections might be treated with even higher concentrations (up to 250 ppm, $10397 \times 10^{-9}$ m).\textsuperscript{[91,92]} Taken together, these data illustrate the most important aspect of inhaled NO, namely that—unlike the systemic use of NO donors—concentration of gaseous NO is literally dialed to the desired level, which opens up broad opportunities for efficient, personalized treatments. Another highly advantageous aspect associated with the delivery of gaseous NO is that for the treatment of respiratory conditions such as COVID-19, the drug is delivered directly to the diseased tissue.

We note that the therapeutic effects of inhaled NO, as for every medicinal agent, have to be carefully balanced against the possible side effects. One potential complication of inhaled NO, especially at high doses (above 80 ppm), is methemoglobinemia, which is a condition characterized by an abnormal level of methemoglobin (i.e., oxidized hemoglobin) in the blood. NO oxidizes heme iron (of hemoglobin), changing its iron oxidation state from Fe$^{2+}$ (ferrous) to the Fe$^{3+}$ (ferric) state. The oxidized hemoglobin has a decreased ability to bind oxygen and this leads to an overall reduced ability of the red blood cells to deliver oxygen to the tissues. Other side effects may be due to the products of NO oxidation such as NO$_2$ gas. In our Essay, we focus on the effects of NO against diverse viral pathogens and leave the detailed discussion of side effects to specialized reviews on the subject,\textsuperscript{[93,94]} as well as to the ongoing clinical trials that evaluate the efficacy of inhaled NO as a treatment for COVID-19.

Clinical studies conducted during the SARS outbreak reported that inhalation of 30 ppm NO for at least 3 days helped in preventing disease progression.\textsuperscript{[95]} In light of these results, NO is currently trialled for patients with COVID-19, with seventeen active clinical studies as of October 2020.\textsuperscript{[96]} The treatment is based on inhalation of gaseous NO in fourteen studies, with some trials focusing on the continuous delivery of low doses of NO, while others using up to 300 ppm of the radical. The remaining three studies are based on the NO donor sodium nitrite, which is administered as a nasal spray (called “nitric oxide releasing solution”, NORS). These strategies may be important to mitigate the symptoms of COVID-19, especially in view of the current shortage of ventilators.\textsuperscript{[97]} The details of each clinical trial are reported in Table 2.

5. Conclusions

Although NO’s role in viral infections is still not completely understood, the production of the radical seems to have a positive antiviral effect on respiratory viruses, while mostly leading to pathogenesis in non-respiratory infections. This difference is further confirmed by the fact that NO-based therapies (e.g., inhaled NO) have demonstrated success in clinical settings for the treatment of respiratory viruses. Interestingly, the concentration of inhaled NO is in the range of nm, orders of magnitude higher than the endogenous levels (µm–nm). The difference between the concentration of NO produced endogenously by the body and externally administered by NO-based therapies is an important factor to consider, taking into account that the bioavailability and reactivity of NO dissolved in the tissue might be different from the gaseous one. In light of the above, to develop NO therapies with better outcomes and less side effects, it is important to characterize what is the amount of inhaled NO that locally dissolves in the tissues, and how its final concentration compares to the endogenous levels. Yet, this kind of measurement is particularly challenging in vivo, and more interdisciplinary research in NO-detection technologies is required.

Taken together, existing evidence of activity of NO against viruses, FDA approval for inhaled NO as prior evidence of safety, and therapeutic efficacy of inhaled NO against pulmonary hypertension and thrombocytopenia strongly suggest that inhaled NO is a unique, ready available agent for the treatment of respiratory infections, including COVID-19.
Table 2. Active clinical studies (October 2020) that use nitric oxide for the treatment or prevention of COVID-19. Results collected from the databases ClinicalTrials.gov and ClinicalTrialsRegister.eu. No results were found on ChiCTR.org.cn and ISRCTN registry. ARDS: acute respiratory distress syndrome, iNO: inhaled NO, NORS: NO releasing solution.

| Identification code             | Status                  | Drug | Administration | Aims                                                                 | Expected outcomes                                                                 |
|---------------------------------|-------------------------|------|----------------|----------------------------------------------------------------------|-----------------------------------------------------------------------------------|
| EudraCT 2020-001490-68          | Ongoing                 | NO   | Inhalation     | Measure the difference in ventilator treatment time in patients with ARDS. | A decrease in the duration of continuous ventilator treatment of the group treated with iNO compared to the control group. |
| EudraCT 2020-002394-94          | Ongoing                 | NO   | Inhalation     | Clinical evaluation of iNO for patients with COVID-19 using clinical and imaging (high resolution computed tomography) endpoints. | Improvement of functional pulmonary imaging parameters, including imaged airway volume, imaged airway resistance, and blood vessel volume. |
| EudraCT 2020-001329-30          | Ongoing                 | NO   | Inhalation     | Assess the rate of change in oxygenation in mechanically ventilated patients with ARDS. | Increase in oxygenation of the group treated with iNO compared to the control group. |
| NCT04388683                    | Recruiting              | NO   | Inhalation     | Investigation of iNO to prevent systemic deoxygenation and inflammation, with escalation to higher levels of oxygen and ventilatory support or death. | Prevention of disease progression (time frame: 28 d). Treatment will be given for up to 14 d unless the patient deteriorates and requires escalation. |
| NCT04338828                    | Recruiting              | NO   | Inhalation     | Determine whether iNO improves short term respiratory status and prevents future hospitalization in patients diagnosed with COVID-19 specifically in the emergency department. | Decreased likelihood to return to the emergency department with worsening symptoms. |
| NCT04421508                    | Recruiting              | NO   | Inhalation     | Assess the efficacy of pulsed iNO versus placebo in subjects with mild or moderate COVID-19 who are hospitalized and require supplemental oxygen without assisted ventilation. | Decreased proportion of subjects who died or had respiratory failure (time frame: day 28). |
| NCT04397692                    | Recruiting              | NO   | Inhalation     | Evaluate the efficacy of 80 ppm NO given four times a day in addition to the standard of care of patients. | Increased time to deterioration of respiratory symptoms (time frame: 14 d), determined by escalation to either noninvasive ventilation, high-flow nasal cannula, or intubation. |
| NCT04306393                    | Recruiting              | NO   | Inhalation     | Determine whether iNO improves oxygenation in patients with ARDS. | Improvement of arterial oxygenation at 48 h from enrolment. |
| NCT04312243                    | Recruiting              | NO   | Inhalation     | Assess whether intermittent delivery of iNO gas in air at a high dose before and after the work shift may protect healthcare workers from SARS-CoV-2 infection. | Decreased percentage of subjects with COVID-19 diagnosis in the treatment group. |
| NCT03331445                    | Recruiting              | NO   | Inhalation     | Assess the efficacy of NO in acting as an antiviral agent, resulting in the reduction of incidence of oxygen therapy. | Reduction in the incidence of mechanical ventilation during the NO treatment. |
| NCT04383002                    | Recruiting              | NO   | Inhalation     | Test whether high dose iNO is safe and can reverse virus burden and respiratory failure in patients on mechanical ventilation. | Negative COVID-19 PCR at the completion of treatment (day 7) from tracheal aspirate. |
| NCT04456088                    | Not yet recruiting      | NO   | Inhalation     | Evaluate the efficacy of 150 ppm NO given in addition to the standard of care of patients with COVID-19. | Decreased time to deterioration (up to 14 d) for patients treated with NO. |
| NCT04476992                    | Active, not recruiting  | NO   | Inhalation     | Compare the intermittent versus continuous inhalation of NO in spontaneous breathing patients. | High concentration iNO with an adjunct of continuous low dose administration between the high concentration treatments can be safely administered in hypoxemic COVID-19 patients compared to the high dose treatment alone. |

(Continued)
Table 2. Continued.

| Identification code | Status                   | Drug   | Administration          | Aims                                                                 | Expected outcomes                      |
|---------------------|--------------------------|--------|-------------------------|----------------------------------------------------------------------|----------------------------------------|
| NCT04305457         | Active, not recruiting   | NO     | Inhalation              | Test whether iNO therapy prevents disease progression in spontaneous breathing patients with mild to moderate COVID-19. | Reduction in the incidence of patients requiring intubation and mechanical ventilation. |
| NCT04337918         | Recruiting               | NORS   | Gargle + nasal spray + nasopharyngeal flush | Evaluate the efficacy of NORS treatment for the prevention of SARS-CoV-2 infections in healthcare workers. | Decreased proportion of subjects with positive COVID-19 in NORS versus control group by 21 d. |
| NCT04460183         | Recruiting               | NORS   | Inhalation using a nebulizer | Evaluate the efficacy of NORS in reducing the rate of progression to a more severe level of COVID-19. | Increased proportion of participants with safe levels of oxygen saturation by day 14 versus standard of care alone. |
| NCT04443868         | Not yet recruiting       | NORS   | Nasal spray + nasal irrigation | Evaluate the efficacy of NORS to treat and prevent the exacerbation of mild COVID-19 infections. | Reduction of clinical symptoms as compared to saline placebo. |

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

coronavirus, COVID-19, nitric oxide, viral infections, virus

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