The potential role of inhaled nitric oxide for postexposure chemoprophylaxis of COVID-19

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Abstract

Background: Several vaccines have been fast-tracked in an attempt to decrease the morbidity and mortality of COVID-19. However, post-exposure prophylaxis has been overlooked in battling COVID-19.

Main text: Inhaled nitric oxide is a potential tool in post-exposure prophylaxis of COVID-19. It decreases cytosolic calcium levels, which impairs the action of Furin. SARS-CoV-2 uses Furin to replicate in the respiratory tract.

Short conclusion: Inhaled nitric oxide could decrease the viral load in the upper respiratory tract, abort clinically symptomatic infection, and prevent subsequent complications. Nitric oxide might be a tool for post-exposure chemoprophylaxis in at-risk groups, especially medical personnel.

Background

SaNOtize (Canada, Vancouver-based/NCT04443868 biotech firm) recently created a self-administered nitric oxide nasal spray (NONS) that could potentially reduce coronavirus disease 2019 (COVID-19) viral load in infected patients. After completing early-stage clinical trials in Canada and the United Kingdom (UK), SaNOtize, Ashford and St. Peter’s Hospitals, the National Hospital System (NHS) foundation, and a few pathology services in the UK announced the results of phase II trials. The results indicate that NONS can be a powerful and safe antiviral treatment. It could prevent COVID-19 transmission, shorten its duration, and reduce the severity of its symptoms.

Some reports have discussed the use of nitric oxide against COVID-19. Lotz et al., for example, highlighted its potential to improve acute respiratory distress syndrome in COVID-19. However, SaNOtize’s clinical trial results suggest that it has a much earlier antiviral role against COVID-19. We will discuss the exact mechanism behind this in this report [1].

Main text

Protease is critical to determine the viral load of COVID-19

Furin is a member of the PCSK (pro-protein convertase subtilizing/Kexin) family. Furin is a type 1 membrane-bound protease utilized by multiple pathogens including human immune deficiency virus (HIV), Ebola virus, Marburg virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), and even some bacterial toxins. Pathogenicity can increase several folds once they react with Furin and other pro-protein convertases. After Furin is cleaved, latent precursor proteins are activated. Hence, Furin-dependent infections may respond to therapeutics targeting host cell Furin [2].

The spike protein of SARS-CoV-2 is the cleavage site of Furin. It plays an essential role in the pathogenesis, host range, and infectivity of the virus. Furin requires a polybasic instead of a monobasic cleavage site. Hence, cleavage occurs at the junction of the two polybasic, spike protein subunits (S1 and S2). High virulence and
Table 1: Review of in vivo and in vitro studies of the antiviral effect of nitric oxide

| Reference number in text | Virus                        | Type of nitric oxide therapy | Study model                              | Main outcome                                                                 |
|--------------------------|-----------------------------|------------------------------|------------------------------------------|-----------------------------------------------------------------------------|
| [4, 5]                   | SARS-CoV                    | NO donor, SNAP               | In vitro                                 | Inhibited SARS CoV replication cycle in a concentration-dependent manner (1) |
|                          |                             |                              | SNAP                                     | SNAP and SNP inhibited the SARS CoV viral cytopathic effect (2)           |
| [7, 8]                   | SARS-CoV-2                  | inhaled NO                   | Multicenter randomized controlled trial  | Ongoing, antiviral effect of high concentrations of inhaled NO administered during early phases of COVID-19 on spontaneous breathing patients, effect on disease progression (3) |
|                          |                             |                              | Single-center, randomized (1:1) controlled, parallel-arm clinical trial | Ongoing, testing inhaled Nitric Oxide in mechanically ventilated patients with severe acute respiratory syndrome in COVID-19 (SARS-CoV-2) (4) |
| [9]                      | Coxsackievirus              | NO donors SNAP, iNO          | In vitro                                 | NO inhibits CVB3 replication by inhibiting protease activity and interrupting the viral life cycle (6) |
|                          |                             |                              | SNAP                                     | NO inhibits CVB3 replication in part by inhibiting viral RNA and protein synthesis (7) |
|                          |                             |                              | SNAP, PFC, GTN, ISDN                     | In vitro NO showed inhibition of the 2A proteinase activity CVB3-infected mice showed significantly reduced signs of myocarditis after treatment with GTN or ISDN (8) |
| [10, 11]                 | Influenza                   | Gaseous nitric oxide (gNO)   | In vitro                                 | Viral NA inhibition by gNO was shown and may be responsible for this antiviral effect (9) |
|                          |                             |                              | SNAP                                     | inhibition of influenza virus viral RNA synthesis (10)                     |
|                          | Japanese encephalitis virus (JEV) | SNAP                           | In vitro                                 | NO was found to profoundly inhibit viral RNA synthesis, viral protein accumulation, and virus release from infected cells (11) |
|                          |                             |                              | MDF to produce NO (inducible NO)         | MDF stimulated macrophages inhibited virus replication with high levels of NO production. MDF treatment increased the survival rate of JEV infected mice (12) |
| [22]                     | Rhinovirus                  | Nitric oxide donor (NONOate) | In vitro                                 | (NONOate) inhibited both rhinovirus replication and cytokine production in a dose-dependent fashion without reducing levels of cytokine mRNA (13) |
| [14]                     | Reovirus                    | iNO                          | In vitro                                 | Cytostatic effects antiviral effects e.g. reduction in DNA synthesis, protein synthesis & mitochondrial metabolism (14) |
| [15]                     | Dengue virus (DENV)         | SNAP                          | In vitro                                 | NO showed an inhibitory effect on viral RNA synthesis. The activity of the viral replicase was suppressed significantly (15) |
| [16]                     | Herpes simplex virus type 1 (HSV 1) | NO generated from (GSNO)     | In vivo, in vitro (Murine model)          | Nitric oxide had inhibitory effects on HSV1 protein and DNA synthesis as well as on cell replication (16) |
| [17]                     | Porcine circovirus type 2 (PCV2) | NO generated from (GSNO)     | In vivo, in vitro (Murine model)          | NO strongly inhibited PCV2 replication in vitro. NO reduced the progression of PCV2 infection in mice (17) |
| [18]                     | Crimean Congo hemorrhagic fever virus (CCHFV) | SNAP                           | In vitro                                 | NO reduced virion progeny yield with a reduction in expression of viral proteins; the nucleocapsid protein and the glycoprotein, and vRNA (18) |
| [19]                     | Respiratory Syncytial Virus (RSV) | INO, SNAP                     | In vitro                                 | NO has significant direct antiviral activity against RSV, which is more potent with continuous, endogenous NO production than exogenous NO (19) |
| [13]                     | Human papillomaviruses (HPVs) | NVN1000, Topical NO-releasing polymer | In vitro                                 | NO abrogated HPV-18 progeny virus production. Reduced HPV-18 E6 and E7 oncoproteins. Impaired S-phase progression and induced DNA damage in infected cultures (20) |
| [20]                     | Vesicular stomatitis virus (VSV) | NO, SNAP                      | In vitro                                 | anti-VSV effects of NO in form of significant inhibition of productive VSV infection (21) |
| [21]                     | Molluscum                   | Topical acidified             | A double-blind, group-                  | 75% cure rate in the active treatment group |

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circovirus type 2, GSNO, CVB3, inducible NO, chemotactic factor, NONOate, tick-borne hemorrhagic fever [27].

The intensity of fever spikes and the occurrence of thrombocytopenia syndrome, categorized by manifestations of tick-borne hemorrhagic fever [27].

In 2019 that calcium channel blockers (CCB) decrease calcium channel blockers can be a promising approach against Furin-activated organisms. Additionally, Li et al. stated calcium channels can be a promising approach against Furin-activated organisms. Furin directly. Molloy et al. noted that the intracellular cations—nitrite, nitric oxide liberating cream) topical SB206 (NO releasing topical gel) studies which used NO as an antiviral agent) [4–24].

Nitric oxide is an inhibitor of viral proteases and subsequently of viral replication

Previous studies have noted that the antiviral role of nitric oxide is due to its inhibition of viral protease activity. It also prohibits viral replication. In a study, several viruses demonstrated the mechanism behind this phenomenon. These included coxsackievirus, picornaviruses, hantavirus, herpesvirus, rhinovirus, Japanese encephalitis, vaccinia, retrovirus, and many more (Table 1 exposes the clinical and laboratory trials which used NO as an antiviral agent) [4–24].

Nitric oxide inhibits viral protease activity by decreasing intracellular cations

Furin is a cellular protease enzyme expressed from the FURIN gene in humans. Furin shows an intriguing interplay between intracellular ions, especially cations. Potassium ions are the most common intracellular ions in our bodies, followed by magnesium—which can activate Furin directly. Molloy et al. noted that the intracellular calcium level noticeably influences the activity of Furin. Thus, Furin is a calcium-dependent enzyme [25].

Yamada and colleagues further supported the relationship between Furin and calcium levels. Inhibiting Furin prevented further neuronal damage caused by calcium influx after hypoxic injury [26]. Hence, impeding calcium channels can be a promising approach against Furin-activated organisms. Additionally, Li et al. stated in 2019 that calcium channel blockers (CCB) decrease the intensity of fever spikes and the occurrence of thrombocytopenia syndrome, categorized by manifestations of tick-borne hemorrhagic fever [27].

Nitric oxide encourages calcium efflux from cells, leading to decreased intracellular calcium levels. Van Hove et al. demonstrated this and proved that nitric oxide stimulates smooth muscle cells (SMCs) to relax directly or indirectly by decreasing the elevated calcium level [28]. As such, nitric oxide could inhibit Furin’s action by decreasing cytosolic levels of calcium.

**Inhaled nitric oxide as post-exposure prophylaxis**

Argyropoulos et al. concluded that a diagnostic viral load has no prognostic value [29]. While in a more recent report, Silva et al. found the saliva viral loads to be significantly higher in patients with chronic respiratory conditions, cardiovascular conditions, kidney disease, and diseases that compromise the immune system [30]. Patients with four or more risk factors had much higher saliva viral loads than patients with fewer risk factors, as did male patients. However, there was no relation between nose and throat viral loads and risk factors. Saliva viral loads were also higher in patients with worse clinical outcomes. As such, early interruption of viral replication in the upper respiratory tract might abort the development of significant symptoms and complications. This rationale might have led to the current inclusion criteria of SaNOtize’s ongoing clinical trial, which involves administration of the intranasal medication within the first 48 h of a diagnosis. SaNOtize could potentially be administered to medical personnel as post-exposure chemoprophylaxis.

**Conclusion**

Early reports of the role of nitric oxide in the treatment of COVID-19 suggested its use for the treatment of established acute respiratory distress syndrome. However, nitric oxide seems to have a much earlier and more efficient prophylactic role. It inhibits Furin, a protease needed for canonical viral replication of SARS-CoV-2, by decreasing cytosolic calcium levels. This action can prevent the exponential increase of viral load in the upper respiratory tract leading to the abortion of...
clinically symptomatic infection and subsequent complications. Nitric oxide could be a tool for post-exposure chemoprophylaxis in the at-risk groups, especially medical personnel.

Figure 1 summarizes the antiviral effect of nitric oxide and its possible uses in the context of COVID-19.

Abbreviations
CCB: Calcium channel blocker; COVID-19: Coronavirus 2019; NHS: National Hospital System; NO: Nitric oxide; NONS: Nitric oxide nasal spray; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; UK: United Kingdom

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