Received: 14 April 2020 | Revised: 22 April 2020 | Accepted: 23 April 2020

DOI: 10.1111/bph.15085

LETTER TO THE EDITOR

Inhaled NO and COVID-19

NO is a unique signalling molecule in the mammalian species. NO is produced by a variety of cell types to elicit distinct physiological actions. In the vascular system, NO is produced by the endothelium, a single layer of cells forming the inner lining of all blood vessels. Endothelium-derived NO has several different functions, one of which is vascular smooth muscle relaxation, resulting in vasodilation and a consequent decrease in blood pressure and increase in local blood flow. In the erectile tissue, NO is released as a neurotransmitter from the nerves innervating the corpus cavernosum during sexual stimulation and causes profound smooth muscle relaxation and increased blood flow to the erectile tissue. This results in engorgement with blood and consequent penile erection.

The uniqueness of NO as a signalling molecule derives, at least in part, by the fact that it is a gaseous molecule in its native state. However, despite being a gas, NO, like oxygen (O_2), elicits its pharmacological effects as a solute in aqueous solution. Another unique characteristic of NO is its fleeting action because of its highly unstable chemical nature and reactivity. Unlike many other signalling molecules, NO elicits its wide array of physiological effects by distinct mechanisms. For example, vascular and nonvascular smooth muscle relaxation and inhibition of platelet function are mediated by intracellular cyclic GMP (cyclic 3′,5′-guanosine monophosphate). NO elicits many cyclic GMP-independent effects as well. For example, NO is a reactive free radical that can covalently modify protein function. One good example is protein S-nitrosation, which can result in both regulatory and aberrant effects. By this and a variety of other mechanisms, NO also reacts with other molecules, such as ROS, in invading cells such as bacteria, parasites, and viruses to kill them or inhibit their replication or spread.

The first pharmacological action of NO, demonstrated several years before its production in mammals was actually discovered, was vascular and nonvascular smooth muscle relaxation. One of many examples of the latter is the smooth muscle enveloping the sinusoidal cavities within the corpus cavernosum. Another important example is the airway smooth muscle in the trachea and bronchioles of the lungs. In an in vitro preparation of neuronally intact, guinea pig isolated tracheal smooth muscle, electric field stimulation caused NO-mediated relaxation (Tucker, Brave, Charalambous, Hobbs, & Gibson, 1990). Indeed, inhalation of NO gas causes bronchodilation and increased delivery of air into the lungs. However, perhaps more significant than the bronchodilator effect of inhaled NO is its vasodilator effect. In fact, advantage was taken of the vasodilator action of NO in the lungs by Warren Zapol, MD, from the Massachusetts General Hospital in Boston, who discovered that inhalation of very small amounts of NO gas by newborn babies with life-threatening, persistent pulmonary hypertension (PPHN) results in a dramatic and permanent reversal of pulmonary vasoconstriction. Inhaled NO (INO) literally turned blue babies into pink babies. Without INO, most babies would have died while others would have required highly invasive procedures (extracorporeal membrane oxygenation; ECMO) to oxygenate their lungs and may not have survived. In stark contrast to PPHN, the use of INO in adults with ARDS (acute respiratory distress syndrome) has met with little success. The reason for this is unknown.

Regarding its antiviral action, NO has been shown to increase the survival rate of mammalian cells infected with SARS-CoV (Severe Acute Respiratory Syndrome caused by coronavirus). In an in vitro study, NO donors (i.e., *S*-nitroso-*N*-acetylpenicillamine) greatly increased the survival rate of SARS-CoV-infected eukaryotic cells, suggesting direct antiviral effects of NO (Keyaerts et al., 2004). In this study, NO significantly inhibited the replication cycle of SARS-CoV in a concentration-dependent manner. NO also inhibited viral protein and RNA synthesis. Furthermore, NO generated by inducible NO synthase inhibited the SARS-CoV replication cycle. SARS-CoV shares most of the genome of SARS-CoV-2, which is responsible for COVID-19 infection, indicating potential effectiveness of inhaled NO therapy in COVID-19 patients.

In 2004, during the SARS-CoV outbreak in China, the administration of inhaled NO reversed pulmonary hypertension, improved severe hypoxia, and shortened the length of ventilatory support as compared to matched control patients with SARS-CoV not receiving treatment (Chen et al., 2004). The mechanism of action was thought to be pulmonary vasodilation and consequent improved oxygenation in the blood of the lungs, thereby killing the virus, which does not do well in a high oxygen environment. In addition, however, I would offer the opinion that the NO also interacts directly with the virus to kill it and/or inhibit its replication, as shown in a prior study (Keyaerts et al., 2004). Moreover, the failure of INO in treating ARDS patients may suggest that INO is more effective as an antiviral agent than simply as a vasodilator in the pulmonary circulation. Alternatively, the distinct aetiologies of ARDS and COVID-19 may explain the difference in responsiveness to INO.

Although studies have not yet been reported with COVID-19, NO has been shown to have an antiviral effect on several DNA and RNA virus families (Colasanti, Persichini, Venturini, & Ascenzi, 1999). The NO-mediated S-nitrosation of viral molecules might be an intriguing general mechanism for the control of the virus life cycle. In this regard, it is conceivable that NO could nitrosylate cysteine-containing enzymes and proteins, including nucleocapsid proteins and glycoproteins, present in the coronavirus.

In view of the knowledge gained by treating SARS-CoV-infected patients with INO, it follows that INO might be effective in patients with the current SARS-CoV-2 (COVID-19) infection. Indeed, a clinical trial of inhaled NO in patients with moderate to severe COVID-19 with pneumonia and under assisted ventilatory support recently received IRB (Institutional Review Board) approval at the Massachusetts General Hospital. Warren Zapol is director of this project. This trial has now been expanded to include at least two additional hospitals in the United States. In the successful treatment of persistent pulmonary hypertension in newborns, the amount of NO inhaled is generally 1 ppm (part per million). In the clinical trial using COVID-19 patients, the amount of NO will be approximately 100-fold higher, about 100 ppm. This is a safe dose of INO, which could prove to be effective in killing the virus and allowing recovery of the patient. The effective use of INO would also lessen the need for oxygen, ventilators, and beds in the ICU.

One practice to consider during this coronavirus pandemic is to breathe or inhale through the NOSE and exhale through the mouth. Swedish investigators at the Karolinska Institute in Stockholm have shown that the cells and tissues in the nasal sinusoids, but not the mouth, constantly and continuously produce NO, which is a gas, and can be easily detected in the exhaled breath. The physiological significance of this may be that nasally derived NO, when inhaled through the nose, improves oxygen delivery into the lungs by causing bronchodilation. This physiological action of inhaled NO is recognized by competitive athletes, especially runners. Moreover, when inhaling through the nose, nasal NO is inhaled into the lungs where it stands a chance of meeting up with the coronavirus particles and killing them or inhibiting their replication. Inhaling through the mouth may not accomplish this. By the same token, exhaling through the nose is highly wasteful in that the NO would be expelled away from the lungs, where it is needed most.

Louis J. Ignarro

Department of Pharmacology, UCLA School of Medicine, Beverly Hills, California, USA

Correspondence

Louis J. Ignarro, Department of Pharmacology, UCLA School of Medicine, 264 El Camino Drive, Beverly Hills, CA 90212, USA.
Email: lignarro@gmail.com

REFERENCES

Chen, L., Liu, P., Gao, H., Sun, B., Chao, D., Wang, F., … Wang, C. G. (2004). Inhalation of nitric oxide in the treatment of severe acute respiratory syndrome: A rescue trial in Beijing. *Clinical Infectious Diseases*, *39*(10), 1531–1535. Epub 2004 Oct 22

Colasanti, M., Persichini, T., Venturini, G., & Ascenzi, P. (1999). S-nitrosylation of viral proteins: Molecular basis for antiviral effect of nitric oxide. *IUBMB Life*, *48*(1), 25–31.

Keyaerts, E., Vijgen, L., Chen, L., Maes, P., Hedenstierna, G., & Van Ranst, M. (2004 Jul). Inhibition of SARS-coronavirus infection in vitro by *S*-nitroso-*N*-acetylpenicillamine, a nitric oxide donor compound. *International Journal of Infectious Diseases*, *8*(4), 223–226.

Tucker, J., Brave, S., Charalambous, L., Hobbs, A., & Gibson, A. (1990). L-N^G-nitro arginine inhibits non-adrenergic, non-cholinergic relaxations of guinea-pig isolated tracheal smooth muscle. *British Journal of Pharmacology*, *100*, 663–664.