Inhaled Nitric Oxide Delivery Systems for Mechanically Ventilated and Nonintubated Patients: A Review

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Nitric oxide (NO) is a biologically active molecule approved for the treatment of pulmonary hypertension in newborn patients. Commercially available NO delivery systems use pressurized cylinders as the source of NO and a sensor to control the concentrations of NO and nitrogen dioxide (NO_2) delivered. Cylinder-based delivery systems are safe and widely used around the world, but they are bulky, expensive, and reliant on a robust supply chain. In the past few years, novel NO generators and delivery systems have been developed to overcome these limitations. Electric NO generators produce NO from ambient air using high-voltage electrical discharge to ionize air, which leads to the formation of NO, NO_2, and ozone (O_3). A scavenging system is incorporated to reduce the concentration of the toxic byproducts generated in this type of system. NO can also be generated by the reduction of NO_2 by ascorbic acid or released from liquid solutions or solid nanoparticles. The development of easy-to-use, safe, and portable NO delivery systems may enable the delivery of NO in the out-patient setting or at home. Furthermore, non–cylinder-based NO generators reduce the cost of NO production and storage and may therefore make NO delivery feasible in low-resource settings. Here we review commercially available systems that can generate and administer inhalable NO. *Key words: nitric oxide; nitrogen dioxide; respiratory therapy; inhalation.* [Respir Care 2021;66(6):1021–1028. © 2021 Daedalus Enterprises]

Introduction

Nitric oxide (NO) gas is a molecule that plays a pivotal role in many physiological processes. In the cardiovascular system, NO is released by the endothelial cell and acts as a vascular smooth muscle relaxant to induce systemic and pulmonary vasodilation.[1] Because NO is rapidly inactivated by the reaction with oxyhemoglobin, inhaled NO acts as a pure pulmonary vasodilator with negligible systemic hemodynamic effects.[2] In 1999, the U.S. Food and Drug Administration (FDA) approved gaseous inhaled NO for the treatment of "term and near-term (> 34 weeks) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hypertension where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation."[3,4] In addition to its pulmonary vasodilator effect, NO displays a mild bronchodilator effect[5] as well as antithrombotic[6-7] and anti-inflammatory effects.[8]

In adult patients with ARDS, NO reduces pulmonary artery pressure and improves ventilation-perfusion matching.[9-11] Moreover, NO reduces pulmonary vascular resistance in patients with COPD[12,13] and chronic pulmonary hypertension.[14,15]

NO is normally produced by healthy endothelium and, in the presence of intravascular hemolysis, is rapidly inactivated by its reaction with free hemoglobin,[16] inducing systemic and pulmonary vasoconstriction. The potent pulmonary vasodilator effect of inhaled NO has been demonstrated to treat the systemic and pulmonary vasoconstriction induced by free hemoglobin.[17] Cardiopulmonary bypass induces intravascular hemolysis, releasing hemoglobin and subsequently scavenging endogenous NO, leading to intrarenal vasoconstriction and causing post-cardiac surgery kidney injury. A recent randomized controlled trial demonstrated that the perioperative administration of NO reduces the incidence of acute kidney injury in cardiac surgery subjects.[18] Furthermore, NO displays a broad antimicrobial effect against bacteria,[19] fungi,[20] and viruses,[21] especially if administered at high concentrations (> 100 parts per million [ppm]).[22-25] The antimicrobial properties of high-concentration NO are now being tested in several clinical trials (ClinicalTrials.gov registration NCT02498535, NCT04685720, NCT04606407, NCT04305457).

The increased spectrum of pathologies treated with NO gas has urged innovators and industries to find solutions to produce versatile, inexpensive, and readily available NO delivery systems for in-hospital, out-patient, and home use. While offering novel solutions, companies are also focusing on the safety of delivery systems. The main safety issue of high-dose inhaled NO delivery is the generation of nitrogen dioxide (NO_2). When NO and oxygen (O_2) mix in a gaseous environment, NO_2 is formed spontaneously by the following reaction: $NO + NO + O_2 \rightarrow NO_2 + NO_2$. The speed of NO_2 production has a first-order dependence on O_2 concentration and a second-order dependence on NO concentration with a time constant of $1.19 \pm 0.11 \times 10^{-11}/\text{PPM} \times \text{s}$.[26]

Inhaled NO reacts with water and generates nitric acid (pH 1.0), causing chemical lesions to the bronchial mucosa. Therefore, to avoid pulmonary injury, the American Conference of Governmental Industrial Hygienists established a limit of 3 ppm NO_2 in an 8-h time weighted average and 5 ppm as a short-term exposure limit.[27] More conservatively, the National Institute for Occupational Safety and Health recommends a short-term exposure limit of 1 ppm.[27] It is therefore imperative that any NO delivery system reduces the delivered NO_2 levels below the safety thresholds. In this review we will describe the systems currently developed to administer inhaled NO to patients (Table 1).

Nitric Oxide Generators and Delivery Systems

Cylinder-Based Systems

Cylinder-based systems represent the vast majority of commercially available NO delivery systems. (Table 2). Pressurized cylinders contain various concentrations of NO buffered with an inert gas (ie, not containing oxygen) such as nitrogen (N_2) to avoid the generation of NO_2 within the cylinder.

The N_2/NO pressurized cylinder is connected to a flow-regulated injector that delivers a set NO flow and therefore the target NO concentration into the inspiratory arm of a respiratory circuit. A central processing unit continuously measures the gas flow delivered to the patient (ie, from a ventilator) and regulates the NO flow from the pressurized cylinder in real time. The targeted NO concentration is obtained by maintaining the ratio between the NO flow from the pressurized cylinder and the total gas flow delivered to the patient. The NO administration can be continuous throughout the respiratory cycle or synchronized with ventilation. All commercially available NO delivery systems are able to continuously measure NO, NO_2, and O_2 concentrations through an electrochemical sensor cell.[28] The schema of a cylinder-based NO delivery system is summarized in Figure 1.

Specific cylinder-based delivery systems that should be highlighted are the INODD (Novoteris, Garden Grove, California) and the INOpulse (Bellerophon Therapeutics, Warren, New Jersey). The INODD is, up to now, the only commercially available cylinder-based system designed to deliver high-dose NO (160 ppm) and is currently employed in a trial testing the safety and efficacy of 160 ppm NO on multi-resistant bacterial lung infection and in subjects with coronavirus disease-2019 (COVID-19) (NCT03331445).

The INOpulse is a portable system designed to deliver NO in an ambulatory/home environment using a 0.16-L mini-cylinder as the NO source. The INOpulse delivers a set pulsed volume of NO at the beginning of each breath via a special nasal cannula connected to the device. Consequently, the INOpulse delivers a constant dose of NO over time (expressed in μg/kg/h)[12] that is independent of minute ventilation and inspiratory flow. In spontaneously breathing patients, the administration of a pulsed NO dose has 2 potential advantages. First, a pulsed NO dose can be

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Dr Gianni has disclosed no conflicts of interest. Dr Carroll has disclosed a relationship with Unitaid. Dr Kacmarek had disclosed relationships with Medtronic, Orange Med, Medtronic, and Venner Medical. Dr Berra has disclosed relationships with the NIH, iNO Therapeutics, Praxair, Masimo, and the Mercatus Center of George Mason University.

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DOI: 10.4187/respcare.08856

Table 1. Summary of the Available Nitric Oxide Delivery Systems

Manufacturer	Product	Market Release
Cylinder-based systems		
Mallinckrodt	INOMax DSIR	North America, Europe, Australia
Praxair	NOxBOXi	North America, Europe
EKU	NO-A	Europe, Asia
Air Liquide Healthcare	SoKINOX	North America, Europe, Australia
Ingeniería y Técnicas Clínicas	NOXTEC	Europe
Novoteris	INODD	Research use only (NCT02498535)
International Biomedical	Aeronox	North America, Europe
Zysense	NOx plus	North America, Europe, South America
Bellerophon	INOpulse	Research use only (NCT03267108, NCT04421508, NCT03727451, NCT02652429)
Electric NO systems		
Third Pole Therapeutics	eNOX-200	Under development
Beyond Air	LungFit	Research use only (NCT04685720, NCT04397692, NCT04606407)
Chemical-based NO systems		
Vero Biotech	Genosyl Ds	United States of America
Nu-Med plus	Clinical Unit	Under FDA approval
NO-releasing solutions		
SaNOtize	NORS	Research use only (NCT04163978, NCT04443868, NCT04337918)
Nanoparticle NO technology		
NMB Therapeutics	NanoNOx	Under development
Vast Therapeutics	TBD	Under development

Table 2. Technical Characteristics of Cylinder-Based NO Delivery Systems

System	What Is Set	NO Delivery	Concentration Range, ppm	NO Injector	Flow Sensor
INOMax (Mallinkrodt)	NO concentration	Synchronized, continuous	0–80	Reusable	Reusable
NOxBOXi (Praxair)	NO concentration	Synchronized, continuous	0–80	Single use	Single use
SoKINOX (Air Liquide)	NO concentration	Synchronized, continuous	0–80	Single use	Single use
AeroNOx 2.0 (International Biomedical)	NO flow	Continuous	Depends on NO/N_2 cylinder concentration and set flow	Single use	Single use
NO-A (EKU)	NO concentration	Synchronized, continuous	0–100	Single use	Single use
NOXTEC 1000 (ITC)	NO concentration	Continuous	0–100	Single use	Single use
NOx plus (Zysense)	NO flow	Continuous	Depends on NO/N_2 cylinder concentration and set flow	Reusable	Reusable

The operator can set the desired NO concentration or the NO flow necessary to achieve it. The NO delivery can be continuous (ie, the system maintains a stable NO concentration throughout the respiratory cycle) or synchronized with ventilation (ie, the system delivers an NO flow proportional to the inspiratory flow delivered by the ventilator). All the delivery systems use an electrochemical cell sensor to measure NO and NO_2 concentration.
NO = nitric oxide
NO_2 = nitrogen dioxide
ppm = parts per million

precisely delivered using a nasal cannula without the need for a snug, tight-fitting mask, improving patient comfort in an ambulatory or home setting. Second, the brief pulse of NO minimizes the amount of drug dispensed and reduces the environmental exhaust.[29]

Clinical trials are ongoing to test the efficacy of pulsed inhaled NO for the treatment of pulmonary hypertension associated with interstitial lung disease (NCT02734953), COPD,[12] and sarcoidosis (NCT03727451). A phase 3 clinical trial (NCT02725372) testing the effect of pulsed inhaled NO in subjects with symptomatic pulmonary arterial hypertension was stopped for futility. Furthermore, there are 2 phase 3 clinical trials under way to test whether the administration of NO through INOpulse can reduce mortality and acute respiratory failure in spontaneously breathing subjects with COVID-19 (NCT04421508 and NCT04388683).

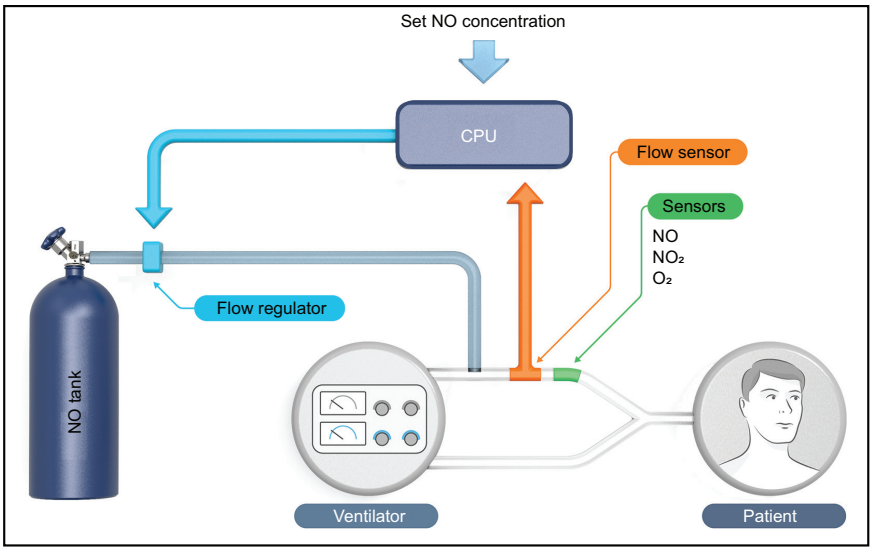

Fig. 1. Schema of a cylinder-based NO delivery system. NO = nitric oxide; NO_2 = nitrogen dioxide; CPU = central processing unit.

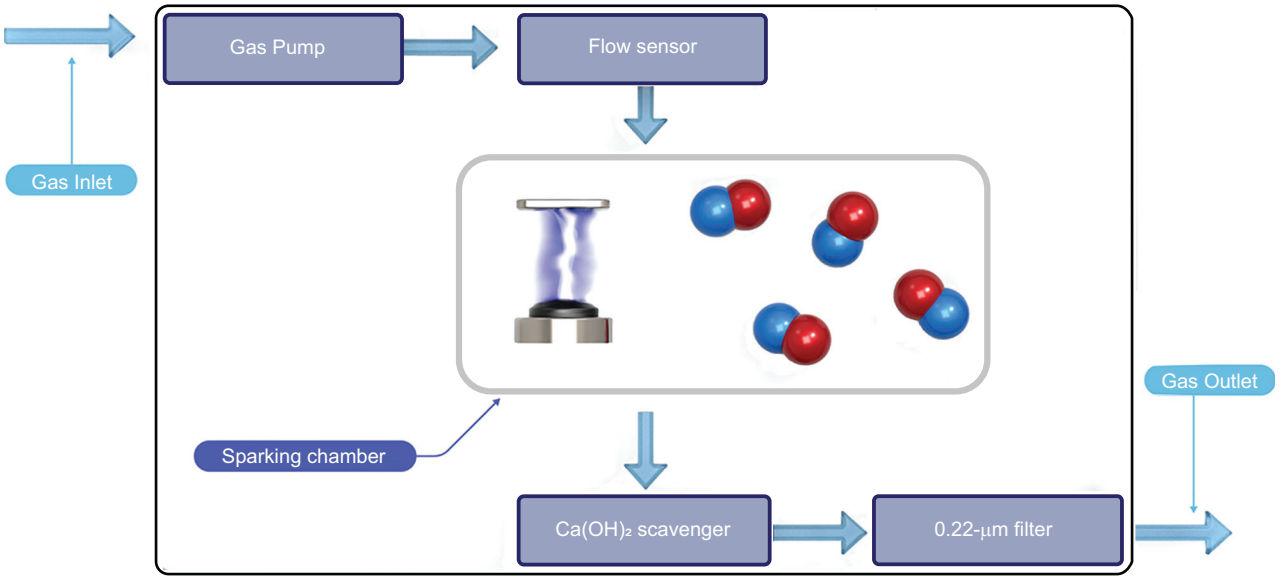

Fig. 2. Schema of a nitric oxide electric generator. $Ca(OH)_2$ = calcium hydroxide.

Systems that utilize cylinder-based delivery are the more widely available NO delivery systems, with > 450,000 patients treated worldwide.[30] They are safe and reliable and can deliver a wide range of NO concentrations by simply changing the flow from the NO cylinder. There are 2 main disadvantages of cylinder-based delivery systems: (1) pressurized gas cylinders require an extensive supply chain, inventory at the hospital, and trained personnel to connect cylinders to ventilators or noninvasive respiratory systems; (2) cylinder-based NO therapy is expensive for institutions themselves,[31] with the average charge for providing NO therapy for 5 d to a newborn patient with persistent pulmonary hypertension being ~ $14,000.[30] These factors greatly reduce the feasibility of NO gas in several settings, such as referral hospitals, out-patient clinics, in the home, and in low-resource settings.

Electricity-Generated NO

Electricity-generated NO systems use high-voltage electrical discharges to generate NO from air or another gas mixture (Fig. 2). The application of an electric potential between 2 electrodes ionizes air (or other gas mixture), causing an electron flow from the cathode to the anode.

The frequent electron collisions due to the high current raise the temperature between the 2 electrodes up to 10,000 Kelvin, causing the dissociation of N_2 and O_2 into a plasma state and the subsequent generation of NO, NO_2, and ozone (O_3). Air flows used are in the range of 0.9–4.5 L/min. The NO and NO_2 production increase with frequency of the sparking pulse, increase with greater atmospheric pressure, and decrease with increasing air flow.[32,33]

Because NO electric generators produce both NO and NO_2, it is imperative to minimize the NO_2/NO ratio to achieve more efficient and safer NO production. One of the main determinants of the NO_2/NO ratio is the temperature of the plasma: the higher the plasma temperature, the lower the NO_2/NO ratio. The plasma temperature is dependent on the capacitance of the pulse-forming capacitor (increasing with the increase in capacitance, up to 20nF), and on the current running through the sparker (plasma temperature increases from 900 to 1130 K as the current increases from 600 to 1,400 ampere).[34] The NO_2/NO ratio is also dependent on the inter-electrode gap: increasing the inter-electrode gap from 1.0 to 7.5 mm decreases the NO_2/NO ratio from 0.12 to 0.09. Namihira et al[35] measured how NO production is affected by variations of $O_2 + N_2$ gas mixture using an NO electric generator with the following settings: 1.5 L/min of total gas flow and pulse frequency of 3 Hz. The highest NO production was observed with an F_{IO_2} of 0.26. The NO_2/NO ratio was also found to be dependent on the electrode material, ranging from 0.13 for tungsten to 0.05 for iridium, across an array of metals (ie, tungsten > carbon > nickel > iridium).

To administer pure NO, it is critical to remove the byproducts such as NO_2, ozone (O_3), and brass particles emitted by the electrode during arc discharges. Because NO_2 is continuously produced whenever oxygen and NO are mixed, a system that removes NO_2 from the outlet of the NO generator is always necessary, and 2 mechanisms can be used: the catalytic conversion of NO_2 into NO, and the selective adsorption of NO_2.

There are 4 primary methods to convert NO_2 into NO. One method is to use a molybdenum wire[33,36] or a molybdenum powder filter that can convert NO_2 into NO when heated to $\sim$ 870 Kelvin through the reaction $3NO_2 + Mo \rightarrow 3NO + MoO_3$, Namihira et al[33] reported a decrease of NO_2 concentration from 138 ppm to 48 ppm accompanied by an increase of NO concentration from 455 ppm to 540 ppm when employing this reaction. Another method utilizes ascorbic acid loaded on silica gel pellets (see Chemical NO Generators). A third method is to have barium oxide (BaO) react with NO_2, producing NO according to the following reaction: $3NO_2 + BaO \rightarrow Ba(NO_3)_2 + NO$. The fourth method involves calcium hydroxide ($Ca(OH)_2$), which is capable of selectively reacting with NO_2 though the following reaction[37]: $Ca(OH)_2 + NO_2 + NO \rightarrow Ca(NO_2)_2 + H_2O$. Yu et al[38] reported a reduction of NO_2 levels between 70% and 90% using a scavenger containing $Ca(OH)_2$, when using both in-line and offline electric plasma generators.

An electrical discharge can also produce ozone (O_3) as a potential toxic by-product. O_3 can be detected in the output arm of the generator and ranges from 10 ppm[32] to 18 parts per billion (ppb).[38] The U.S. Environmental Protection Agency dictates that O_3 levels must be kept below 0.07 ppm (70 ppb). O_3 is removed (< 0.1 ppb) by bubbling the gas through water[32] or by using a $Ca(OH)_2$ filter.[38]

Brass and platinum nanoparticles can be generated from the etching of the electrode material during electric discharges and may be present in the unfiltered gas. A high-efficiency particulate (HEPA) air filter can remove the metal particles from the NO generated by electrical discharge.[39]

The generation of NO from air using pulsed electrical discharges is a growing field of research, and a handful of companies are building NO generators and delivery systems using this technology. The FDA approval process for these devices is ongoing. The opportunity to generate NO from air without the need for expensive and bulky gas cylinders is promising and could make NO widely available in underrepresented regions, both domestically and abroad.

Electric NO generators, when compared to cylinder-based delivery systems, have 2 potential limitations: NO production and safety. Because the NO production decreases when the air flowing through the sparker increases, the NO generated may not be sufficient to maintain the desired NO concentration (eg, in the setting of a patient receiving NO through a mechanical ventilator delivering high minute ventilation). Another limitation of the electric NO generators is the higher risk of safety issues compared to cylinder-based delivery systems. In case of a system malfunction (ie, damage to the HEPA filter or to the $Ca(OH)_2$ scavenger), the device may deliver unmeasured toxic products such as metal brass particles or ozone (O_3).

Chemical NO Generator

In 2011 Lovich et al[40] described the generation of NO from the reduction of NO_2 using ascorbic acid. The authors developed a cartridge composed of a thermoplastic structure wherein powdered ascorbic acid and silica gel are adhered to the inside of a solid tube. The pores in the silica gel provide a matrix in which ascorbic acid and NO_2 react at high efficiency. NO_2 flows through the inlet of this cartridge and is directed to the tube margins, where it permeates radially through the wall of a tube of ascorbic acid/silica gel particles imbedded in the thermoplastic structure, ultimately yielding NO. The authors reported the complete conversion of NO_2 (80 ppm) into NO with an output NO_2 concentration < 1 ppm (0.6–0.8 ppm using 1 cartridge with F_{IO_2} of 1.0).

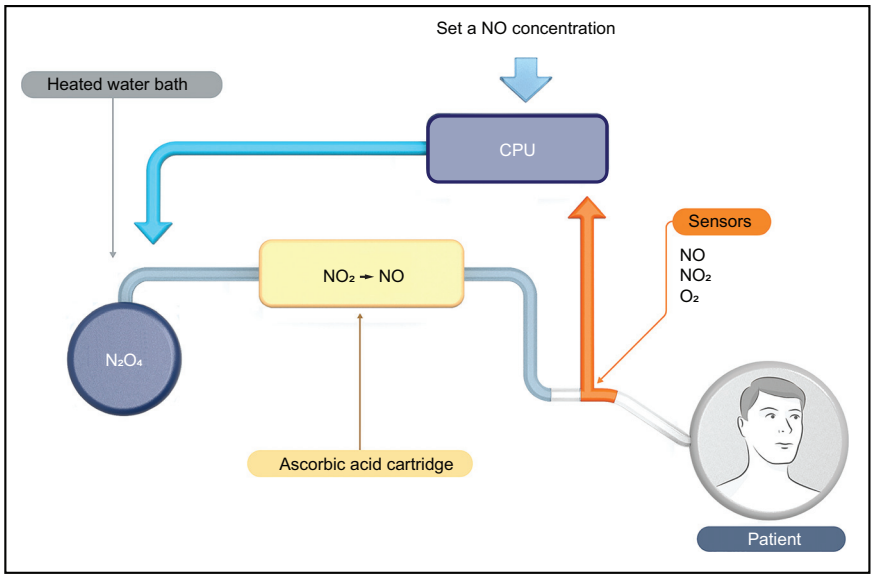

Fig. 3. Schema of the Genosyl DS NO generator and delivery system. NO = nitric oxide; NO_2 = nitrogen dioxide; N_2O_4 = dinitrogen tetroxide; CPU = central processing unit.

In a subsequent manuscript, Lovich et al[41] described an NO generator and delivery system based on this concept. NO generation follows a 2-step process: the first step is the generation of NO_2 from the vaporization of liquid dinitrogen tetroxide (N_2O_4), and the second step is the conversion of NO_2 to NO using the previously described ascorbic acid cartridge (Fig. 3).

Liquid N_2O_4 is contained in a 2-mL evaporation chamber; the walls of this chamber are stainless steel and transmits heat to the contained liquid N_2O_4 to promote the formation of gaseous NO_2. Then the NO_2 flows through a capillary tube of defined cross-sectional area and length and is mixed with air or nitrogen before entering the reactor cartridge and being converted to purified NO. Consequently, NO production is regulated only by the evaporation chamber temperature and by cross-sectional area and length of the capillary tube linking the N_2O_4 reservoir to the manifold. For a fixed resistance and for a fixed carrier stream flow, the pressure and the generated NO_2 concentration are directly proportional. The authors reported that the natural logarithm of the NO_2 concentration in the carrier stream is linearly related to the inverse of the temperature.

At the time of this writing, the only FDA-approved NO delivery system that uses this technology is the Genosyl DS (Vero Biotech, Atlanta, Georgia).[42] This system can deliver NO at a concentration of 20 ppm with NO_2 concentration < 2 ppm. The main advantage of this device is the reduced device size and weight compared to cylinder-based delivery systems. Despite being more portable, the Genosyl DS shares the same drawback of the cylinder-based delivery systems: the need for an extensive production and supply chain to allow widespread use.

NO-Releasing Solutions

NO-releasing solutions are liquid solutions that, when exposed to specific conditions, can produce and release NO. Stenzler et al[43] patented the use of a solution composed of an NO-releasing compound (such as sodium nitrite) and a water- or saline-based solution. The production and release of NO is dependent on the pH of the solution: if the pH is > 4, the production of NO is negligible; with pH values < 4, the production of NO increases in a pH-dependent manner. Consequently, the solution is kept in a "dormant" state at a pH > 4, which allows it to be easily prepared, stored, and transported without losing any appreciable amount of NO gas. Then, for administration, the solution is activated by adding citric acid monohydrate, driving the pH < 4.[44] Interestingly, the NO produced (generating NO_2 that, reacting with the aqueous solution produced nitric acid) keeps the pH of the solution < 4, auto-maintaining the NO release.

The NO production from the described NO-releasing solution is dependent on the concentration of sodium nitrite ($NaNO_2$) and on the pH of the solution. The authors described an NO production of up to 300 ppm (in the presence of a continuous air flow of 3 L/min) from 64 mL of a 60 mM $NaNO_2$ solution.

The target clinical use of this technique is the use of high-dose NO to treat topical infections (eg, bacteria, fungi, and viruses). In addition, phase 1 and phase 2 trials are ongoing to test the efficacy of intranasal administration of a NO-releasing solution for the treatment of COVID-19 (NCT04337918) and chronic bacterial sinusitis (NCT04163978).

NO-Releasing Nanoparticles

A NO-releasing nanoparticle consists of a small particle (measured in nanometers) that contains either NO or an inactive NO precursor in a stable form that, when applied to the target tissue, releases NO in a controlled manner. Friedman et al[45] reported that NO can be efficiently generated by the conversion of nitrites enclosed in a solid matrix (derived from trehalose glass or hydrogel/glass composite). The nitrites are stable in dry form, and NO is released from the solid matrix when exposed to moisture. The pattern of NO released from the nanoparticle is dependent on the composition of the matrix: a glassy matrix releases NO more rapidly than does a hydrogel/glass matrix.

Like the NO-releasing solutions described above, NO-releasing nanoparticles have been used to topically administer antimicrobial doses of NO (eg, treatment of cutaneous bacterial or mycotic infection). Furthermore, the nanoparticles, due to their small diameter, could potentially be delivered through an aerosol directly into the lung.[46] Further preclinical and clinical trials are needed to address the safety and the efficacy of this new NO delivery system.

Summary

NO gas is used worldwide for the treatment of neonates with hypoxic respiratory failure and pulmonary hypertension. The growing interest in administering high-dose NO for its antimicrobial effect[19-21] poses a major challenge to the available NO generation and delivery systems both in terms of cost and safety. In recent years, an effort has been made, both by researchers and biomedical and pharmaceutical companies, to create NO delivery systems that are more portable and easier to use. These technological advancements will allow the use of gaseous NO outside the hospital (ie, for patients with COPD or primary pulmonary hypertension). Furthermore, the newly designed NO generators, by reducing NO production and storage costs, will make NO administration feasible in low-resource settings.

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