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Nitric Oxide 104-105 (2020) 29–35

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Analytical Methods

Ideation and assessment of a nitric oxide delivery system for spontaneously breathing subjects

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ARTICLE INFO

Keywords:
Nitric oxide
Nitrogen dioxide
Delivery system

ABSTRACT

Background: There is an increasing interest in safely delivering high dose of inhaled nitric oxide (NO) as an antimicrobial and antiviral therapeutics for spontaneously breathing patients. A novel NO delivery system is described.

Methods: We developed a gas delivery system that utilizes standard respiratory circuit connectors, a reservoir bag, and a scavenging chamber containing calcium hydroxide. The performance of the system was tested using a mechanical lung, assessing the NO concentration delivered at varying inspiratory flows. Safety was assessed in vitro and in vivo by measuring nitrogen dioxide (NO_2) levels in the delivered NO gas. Lastly, we measured the inspired and expired NO and NO_2 of this system in 5 healthy subjects during a 15-min administration of high dose NO (160 parts-per-million, ppm) using our delivery system.

Results: The system demonstrated stable delivery of prescribed NO levels at various inspiratory flow rates (0–50 L/min). The reservoir bag and a high flow of entering air minimized the oscillation of NO concentrations during inspiration on average 4.6 ppm for each 10 L/min increment in lung inspiratory flow.

The calcium hydroxide scavenger reduced the inhaled NO_2 concentration on average 0.9 ppm (95% CI -1.58, −0.22; $p = .01$). We performed 49 NO administrations of 160 ppm in 5 subjects. The average concentration of inspired NO was 164.8±10.74 ppm, with inspired NO_2 levels of 0.7±0.13 ppm. The subjects did not experience any adverse events; transcutaneous methemoglobin concentrations increased from 1.05±0.58 to 2.26±0.47%.

Conclusions: The system we developed to administer high-dose NO for inhalation is easy to build, reliable, was well tolerated in healthy subjects.

1. Introduction

Nitric oxide (NO) is a therapeutic gas approved by the US Food and Drug Administration (FDA) in 1999 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" [1]. In addition to its pulmonary vasodilator effect, NO produces broad antimicrobial activity on bacteria [2] and viruses such as SARS CoV [3,4], the virus responsible for the SARS epidemic in 2003. Based on the established anti-viral effects, several clinical trials are now testing the efficacy of NO inhalation on patients infected by SARS CoV-2, in the midst of the ongoing pandemic [5–7].

In spontaneously breathing patients, NO gas is traditionally delivered through a mechanical ventilator or through a high flow nasal cannula (HFNC) system [8]. Nitric oxide is blended with medical air and oxygen in the ventilator and may be delivered to the patient through a snug fitting facemask [8]. Despite this approach's ability to administer a high concentration of NO gas (>100 parts per million [ppm]), widespread adoption is challenged by the lack of a safe delivery system. In addition, in the phase of the SARS-CoV-2 pandemic, delivering NO via HFNC or ventilator-driven respiratory systems potentially aerosolizes droplets with virus, which raises further concerns for safety.

Two major patient safety aspects of administering high-dose NO are the generation of nitrogen dioxide (NO_2) and methemoglobin (MetHb). Nitrogen dioxide is formed by the reaction between NO and oxygen, and

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https://doi.org/10.1016/j.niox.2020.08.004
Received 9 June 2020; Received in revised form 12 August 2020; Accepted 16 August 2020
Available online 21 August 2020

when combined with water in the airways, NO_2 forms nitric acid, leading to a caustic burn of the airways. When inhaling NO, methemoglobin is generated by oxidation of the iron contained in circulating hemoglobin. Methemoglobin cannot bind oxygen, so levels must be closely monitored in all subjects receiving NO (particularly high doses). Methemoglobin levels of 10%, or less, are well tolerated in a healthy subject [9]. After cessation of NO treatment, intracellular methemoglobin reductase rapidly reduces RBC MetHb levels.

An in-hospital system that is simple, inexpensive, and capable of delivering a constant and predictable concentration of NO over time, while minimizing NO_2, without generating aerosolized particles is needed to allow use of high-dose NO outside the intensive care unit (ICU).

In this study, we designed and developed a breathing system capable of delivery high concentrations of NO. We evaluated performance and safety of the device both in vitro and in healthy adults by accurately sampling and measuring NO and NO_2 concentrations in the inhaled and exhaled breath.

2. Materials and METHODS

2.1. System design

The system incorporates standard respiratory circuit connectors (Fig. 1). The distal portion of the inspiratory limb begins with a one-way valve (Hudson RCI, Wayne, PA, USA) and two gas inlet connectors (Hudson RCI, Wayne, PA, USA), which inject medical air and NO gas, respectively. A T-connector joins a 3 L bag to the inspiratory limb. The bag serves as an NO reservoir to stabilize the NO concentration throughout the inspiratory phase. A scavenger (internal diameter = 60 mm, internal length = 53 mm, volume = 150 mL) containing 100 g of calcium hydroxide (Spherasorb™, Intersurgical Ltd, Berkshire, UK) absorbs the NO_2 generated in the gas mixture [10]. A flexible connector was inserted to accommodate patient movement and positioning. Two gas inlet connectors act as oxygen inlet and NO/NO_2 sampling line, respectively. A second inspiratory one-way valve was placed after the set reservoir/scavenger to avoid additional gas mixing due to expired backflow. This system was created for the treatment of subjects with coronavirus disease 2019 (COVID-19), and a high-efficiency particulate air (HEPA) filter was connected between the Y-piece and the patient interface (full face mask or mouthpiece) to remove any aerosolized virus.

Active humidification was not added, and relative humidity ratio was not tested, as the device, here described, has been built for delivering intermittent, short periods of high dose nitric oxide [11].

2.2. Experimental assessments

The NO delivery system performance was tested using a bench testing lung (Dual Adult Test Lung, Michigan Instruments, Michigan, USA) (Fig. 2) and a mechanical ventilator to simulate an inspiratory effort (Hamilton G5, Hamilton Medical AG, Bonaduz, Switzerland). The ventilator was connected to the right lung, which acts as the "diaphragm" to lift the left lung by a coupling clip. An inspiratory sinusoid flow waveform was produced by the ventilator during a volume-controlled ventilation mode. The iNO system was connected to the left lung (the "breathing" lung), which was set with a compliance of 0.05 L/cmH_2O. No airway resistor was added. A digital flowmeter (Mallinckrodt Puritan-Bennett PTS 2000) was used to measure the air, O_2, and NO gas flow rates. The inspired oxygen fraction was assessed with an oxygen analyzer (MiniOX® 1, Ohio Medical Corporation®, 1111 Lakeside Drive, Gurnee, IL 60031 USA).

The NO concentration was measured by an NO analyzer (Sievers 280i Nitric Oxide Analyzer, GE Analytical Instruments, Boulder, CO) connected to the inspiratory limb via a sampling line proximal to the Y-connector. NO_2 levels were simultaneously evaluated by the Cavity Attenuated Phase Shift (CAPS) NO_2 monitor (Aerodyne Research Inc, Billerica, MA) using the same sampling port and line. NO and NO_2 concentrations were measured during the inspiratory phase (Fig. 3). Additionally, we measured NO and NO_2 concentration using an electrochemical gas sensor from a commercially available NO delivery system (iNOmax DSIR® Plus, Mallinckrodt Pharmaceuticals, Bedminster, NJ, USA).

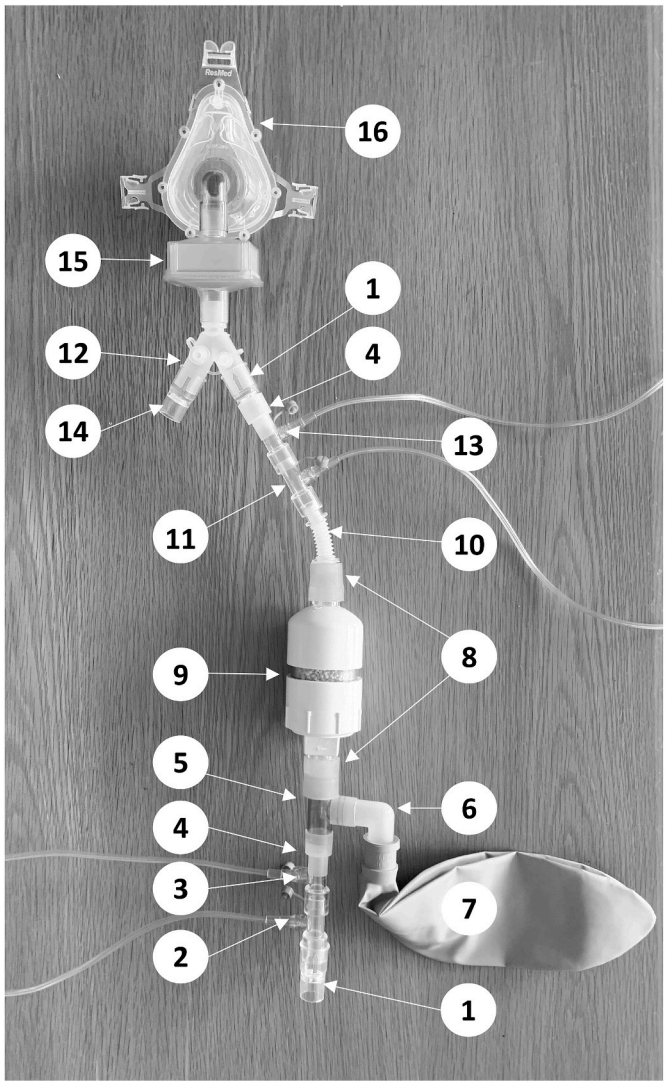

Fig. 1. System for delivering inhaled nitric oxide (NO) during spontaneous breathing. 1. Inspiratory one-way valve; 2. Gas connector for medical air; 3. Gas connector for NO; 4. Two-step adapter; 5. T Adaptor; 6. Elbow Adaptor; 7. Reservoir bag (3 L); 8. Silicone adapter; 9. NO_2 Scavenger; 10. Flex Connector; 11. Gas connector for O_2; 12. Y-piece; 13. Gas sample port for NO and NO_2 analyzers; 14. Expiratory one-way valve; 15. HEPA filter; 16. Snug fitting full face mask.

The NO tank was provided at either 857 ppm (150 A, Airgas, Radnor Township, Pennsylvania, content = 4089 L at STP) or 800 ppm (*Noxivent*, size AQ aluminum cylinders Praxair Shimersville Road Bethlehem Pennsylvania, content = 2239 L at STP). The duration of a 2239 L tank at STP ranged between 3.1 and 37.3 h when NO was delivered at 50 ppm or 250 ppm, respectively. One should note, however, that the delivery system we described here is independent from the tank of NO employed. By introducing a standard gas connector, an operator could use our delivery system with any desirable NO source.

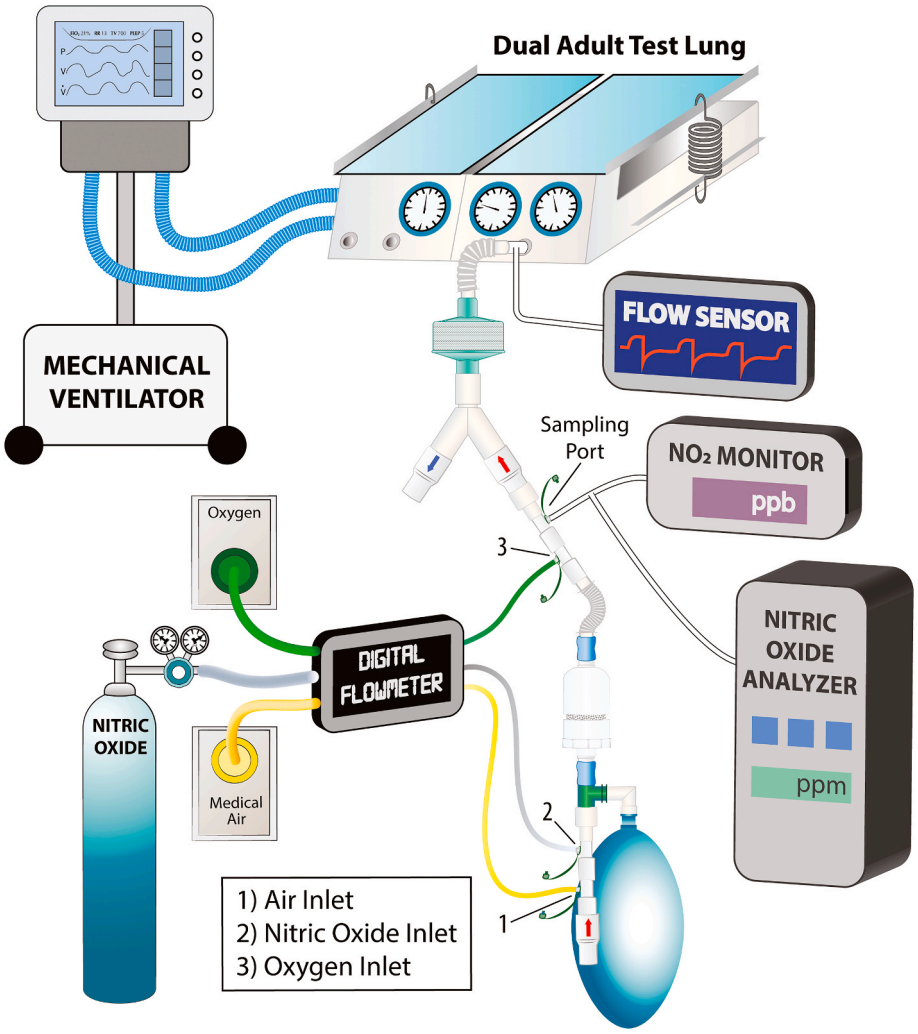

Fig. 2. Experimental setup. The mechanical ventilator connected to the right lung acts as a diaphragm of the left lung. The left lung is connected with the tested delivery system. During the experiment we continuously monitor the inspiratory/expiratory flow after the Y and FiO_2, NO and NO_2 concentration on the inspiratory limb of the circuit.

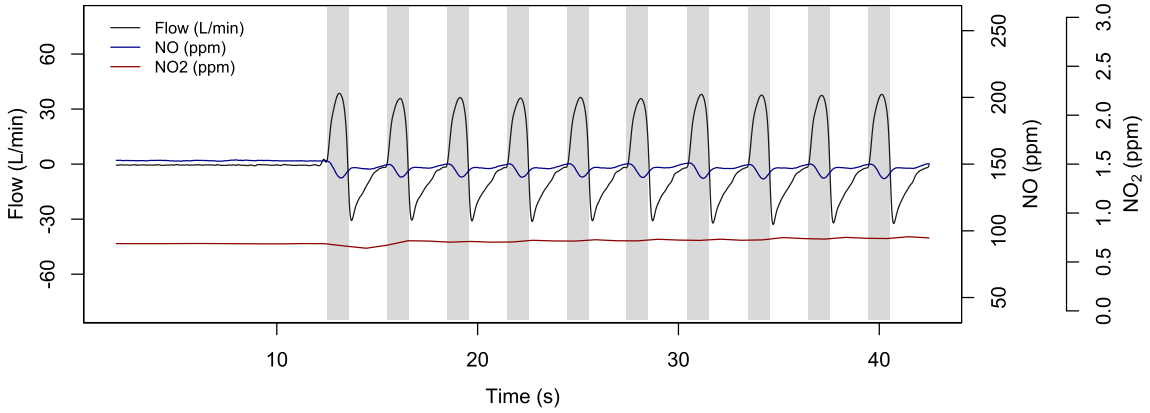

Fig. 3. NO and NO_2 signals synchronized with the airway flow during a bench test with the artificial lung. In all the subsequent analyses, we used the average concentration during the inspiratory time (grey/shadowed area) for the NO and NO_2 concentration.

2.3. Performance tests

2.3.1. Reservoir effect

To reduce the fluctuations of delivered NO, we examined the efficacy of adding a reservoir bag to stabilize the concentration of the inspired NO. The experimental setup involved a respiratory rate (RR) of 15 breaths/min; tidal volume (V_T) of 0.25, 0.5, 0.75 and 1 L; an inspiratory time of 1 s, and a sinusoidal flow wave. During this test the calcium hydroxide scavenger was incorporated into the system. The average inspiratory flow required to archive the set V_T was used as an

independent variable in the analysis. Nitric oxide concentration was measured over 2 min during the inspiratory phase of each set of tidal volume, with and without the reservoir bag. We used three NO concentrations: 50, 150 and 250 ppm. FiO_2 was set at 0.21.

Similarly, we evaluated the inspired concentration of NO_2, using the same experimental settings, with or without the 3 L reservoir bag.

2.3.2. Air flow effect

We examined the effect of various levels of air flow on NO concentration during ventilation. We measured the inspiratory NO concentrations at 5, 10, and 15 L/min of air flow. At every level of air flow, before starting ventilation, we set the NO gas flow to achieve a static concentration of 180 ppm NO in the inspiratory limb of the circuit.

Ventilator settings included a respiratory rate of 20 bpm, a tidal volume of 0.5 L, an inspiratory time of 1 s and sinusoidal flow wave.

2.3.3. NO and NO2 assessment in vitro

We tested the performance of the system to find the NO and oxygen flow required to obtain the desired concentration of inspired NO. Mechanical ventilation was set with a tidal volume 0.5 L, a respiratory rate of 20 bpm, an inspiratory time of 1 s and a sinusoidal flow wave. We tested 3 different target NO concentrations: 50, 150, and 250 ppm at different FiO_2: 0.21, 0.30, and 0.40. NO_2 levels were also measured.

2.4. Safety assessment

2.4.1. Scavenger effect

To assess the efficacy of the calcium hydroxide scavenger in reducing the inspiratory levels of NO_2, we used the same mechanical ventilator settings as above, a range of target NO concentrations (50, 150, and 250 ppm), two different levels of FiO2 (0.21 and 0.40) and measured NO_2 levels in the inspiratory limb with and without the scavenger.

2.4.2. NO2 assessment with healthy subjects

We administered high-dose NO with our system to healthy adult subjects as part of a randomized controlled trial [7] conducted in our center (NCT04312243). Each administration lasted for 15 min. FiO_2, NO and NO_2 concentrations were monitored in the inspiratory limb of the circuit. Peripheral oxygen saturation (SpO_2) and methemoglobin (MetHb) were continuously and non-invasively monitored with a pulse co-oximeter (Masimo rainbow SET, Irvine, CA 92618) [12,13].

2.4.3. Exhaled NO2 measurement

Additionally, we evaluated the exhaled concentration of NO_2 in one healthy subject. We administered 150 ppm NO using the previously described system. To monitor the expiratory NO_2 concentration we placed the sampling line between the mouthpiece and the HEPA filter (Fig. 1). Since the continuous gas flow from the inspiratory limb of the circuit can interfere with the measure, a 3-way stopcock (2100 series, Hans Rudolph INC. Shawnee, KS, USA) was positioned before the Y: its closure at the beginning of exhalation stopped the washout effect of fresh gas coming from the inhalation arm of the circuit, allowing a sampling of exhaled gas only.

2.5. Statistical analysis

We used linear mixed models to analyze the effect of the reservoir and the scavenger on the system's performance. Statistical significance was assumed at a two-tailed P value $< .05$. The statistical analyses were performed in R (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria). Variables were expressed as mean and standard deviation (SD).

3. Results

3.1. System inspiratory and expiratory resistance

The total inspiratory resistance, from the inspiratory one-way valve to the HEPA filter, was on average 8.6 $cmH_2O/L/s$. The total expiratory resistance, measured from the HEPA filter to expiratory one-way valve, was on average 7.2 $cmH_2O/L/s$.

3.2. Reservoir effect

For NO concentrations of 50, 150, and 250 ppm, the reservoir bag decreased the inspiratory NO fluctuations, respectively, by 0.79 ppm (95% CI -1.7, 0.2; p = .09), 5.2 ppm (95% CI -7.6, −2.6; $p = .004$), and 7.8 ppm (95% CI -14.3, −1.3; $p = .02$) for each 10 L/min increment in the average lung inspiratory flow (Fig. 4).

The accumulation of NO and O_2 in the reservoir bag produced an increase of inspiratory NO_2 on average of 0.29 ppm (95% CI 0.14, 0.43; $p < .001$) (Fig. 5). The NO_2 levels were kept below 2 ppm in all NO concentrations with the reservoir bag. These data suggest that adding a reservoir bag (3 L) efficiently reduces the fluctuations of delivered NO and keeps NO_2 concentrations below the recommended level [14].

3.3. Air flow effect

At a low air flow of 5 L/min, we measured a marked reduction of NO concentration in the inspiratory limb of the circuit: from 180 ppm to 96 ppm with 51 ppm of variation during the inspiratory phase. At 15 L/min, the concentration during ventilation was stable and decreasing of only about 8 ppm from 180 ppm to 169 ppm (Fig. 6). These findings indicate that maintaining air flow at 15 L/min reduces the effect of ventilation on the variation of the NO concentration in the respiratory limb.

3.4. Scavenger effect

The scavenger positioned in the inspiratory limb of the circuit reduced the inhaled NO_2 concentration to an average of 0.9 ppm (95% CI -1.58, −0.22; $p = .01$) (Fig. 7). At 150 ppm of inhaled NO, the NO_2 concentration was maintained below 1.2 ppm with FiO_2 from 0.21 to 0.40. Our data suggest that the scavenger can efficiently reduce NO_2 in the circuit for NO delivery.

3.5. NO flow for different NO and FiO_2 target

The delivered NO concentrations varied depending on the flows of NO and O_2. Three concentrations of NO (50, 145, and 245 ppm) targeted for delivery were tested. First, to obtain a concentration of 50 ppm NO (48.5 ppm measured with the chemiluminescence method, 44 ppm using the electrochemical gas phase sensor), the flows of NO ranged from 1.0 to 1.4 L/min for FiO_2 levels of 0.21 and 0.41, respectively, using the 800 ppm NO/N_2 tank. Using the 857 ppm tank, NO flows were set from 0.9 to 1.2 L/min for FiO_2 levels of 0.21 and 0.41, respectively. Second, to obtain an NO concentration of 145 ppm (143 ppm measured with the chemiluminescence method, 130 ppm using the electrochemical gas phase sensor), the required NO flow was at 3.5 and 5.8 L/min of O_2 for FiO_2 levels of 0.21 and 0.41, respectively, using the 800 ppm tank. Using the 857 ppm tank, NO flows was at 3.1 L/min, and 4.5 L/min for FiO_2 levels of 0.21 and 0.41, respectively. Finally, to obtain an NO concentration of 245 ppm, the required NO flow was at 7.0 and 11.9 L/min for FiO_2 levels of 0.21 and 0.41, respectively, using the 800 ppm tank. Using the 857 ppm tank, NO flow was at 6.2 and 10.1 L/min for FiO_2 levels of 0.21 and 0.41, respectively.

NO_2 concentration was measured by the most accurate Cavity Attenuated Phase Shift (CAPS) NO_2 monitor and by a hospital commonly used electrochemical gas phase sensors. In-vitro studies were performed by using 800 ppm of NO/N_2 tanks. Same studies, then, were repeated

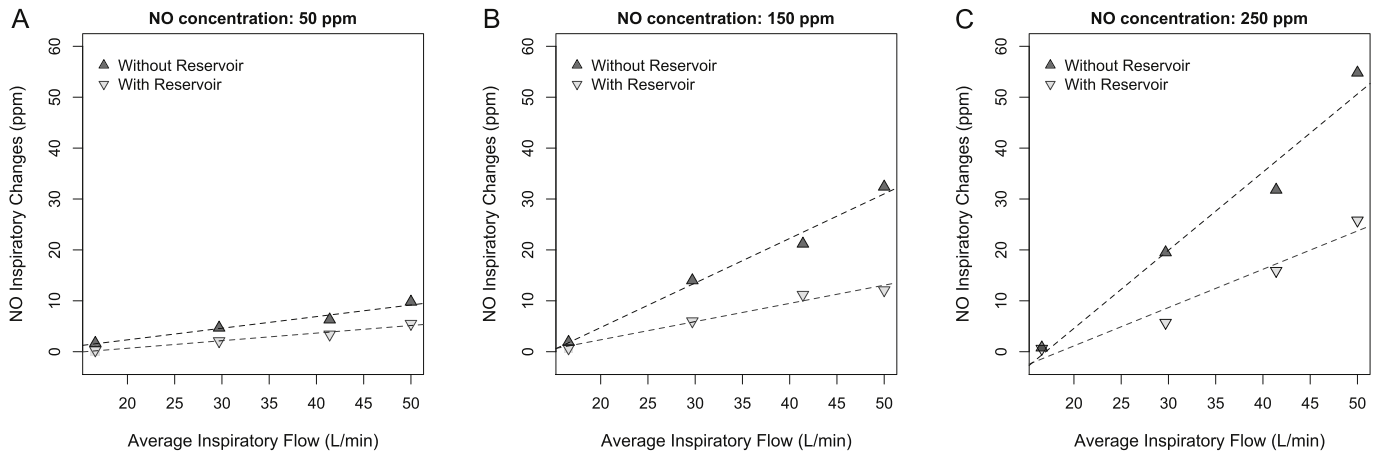

Fig. 4. Changes in inspiratory NO (ppm) during incremental inspiratory flow, with and without the reservoir bag. Nitric oxide concentrations were 50 ppm (Panel A), 150 ppm (Panel B) and 250 ppm (Panel C).

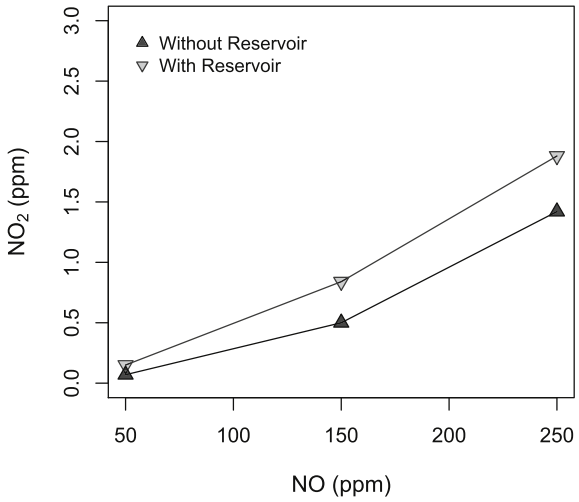

Fig. 5. Inspiratory NO_2 concentrations (ppm) at increasing NO concentrations (ppm) with and without the reservoir bag. FiO_2 was 0.21 in each step.

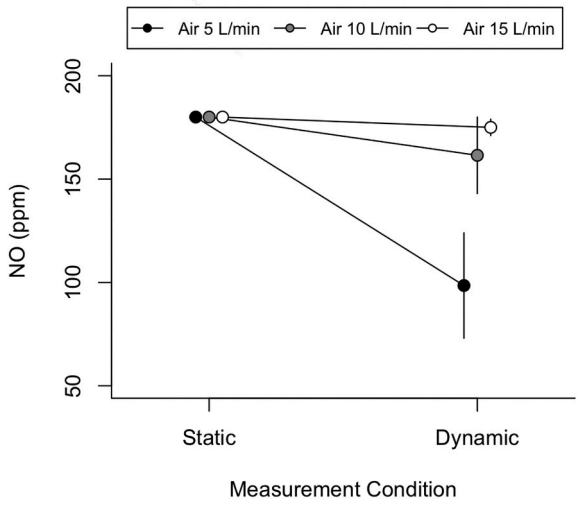

Fig. 6. Nitric oxide concentration (ppm) in static and dynamic conditions (during mechanical ventilation) at different air flows: 5 L/min (black dots), 10 L/min (grey dots) and 15 L/min (white dots). The dots represent the average inspiratory concentration, and error bars represent the intra-tidal swing of NO concentration during the inspiration phase.

with 857 ppm NO/N_2 tanks. When NO tank was set to deliver 50 ppm of NO, NO_2 concentration in the inspiratory limb of the circuit by CAPS monitoring was 0.13 at FiO_2 of 0.21, 0.18 at FiO_2 0.3 and 0.18 at FiO_2 0.41. When NO flow was increased to reach 250 ppm of NO, NO_2 was 1.57 at FiO_2 0.21, 2.35 at FiO_2 0.3 and 2.61 at FiO_2 0.41.

By electrochemical methodology, at 50 ppm of NO, NO_2 concentration measured 0.1 at FiO_2 0.21, 0.3 at FiO_2 0.3 and 0.3 at FiO_2 0.41. At 250 ppm of NO, NO_2 concentration measured 2.37 at FiO_2 0.21, 3.17 at FiO_2 0.3 and 3.61 at FiO_2 0.41.

Similar NO_2 values were found when 857 ppm NO/N_2 tanks were used (see Table 1). These data provide a reference for adjusting desired NO delivery in our designed system.

3.6. Clinical NO and NO_2 assessment with healthy subjects

We administered NO to 5 adult health care subjects: 2 males and 3 females. Median age was 32. The subjects had no history of cardiovascular or lung disease. The total number of NO administrations was 48. The average concentration of inspired NO was 164.8 ± 10.74 ppm with NO_2 levels of 0.7 ± 0.13 ppm; these levels remained stable throughout the administration (Fig. 8). We administer oxygen to keep FiO2 0.21 (see Table 1). During 15 min of administration of gaseous NO, methemoglobin levels increased from a baseline value of 1.05 ± 0.58% to 2.26 ± 0.47%. The subjects did not experience any discomfort during the procedure. No adverse events were reported. Despite the small number of administrations, these results indicate that breathing high concentration of NO for short period of time using our newly developed NO breathing system is feasible and well tolerated without adverse events.

3.7. Exhaled NO_2 measurement

The average inspired NO and NO_2 concentration were 153 ppm and 0.51 ppm, respectively, using an FiO_2 of 0.205. At the end of exhalation NO_2 concentration decreased to 0.03 ppm (see Fig. 9). Exhaled FiO_2 was 0.195.

4. Discussion

We built a device to reliably administer high concentration of NO in spontaneously breathing subjects without the need for a mechanical ventilator. The use of a 3-Liter reservoir and high medical air flow allow this system to maintain a stable concentration of NO throughout a wide range of tidal volumes (from 0.25 to 1 L) independently form the source of gaseous nitric oxide. This system administers a concentration of NO

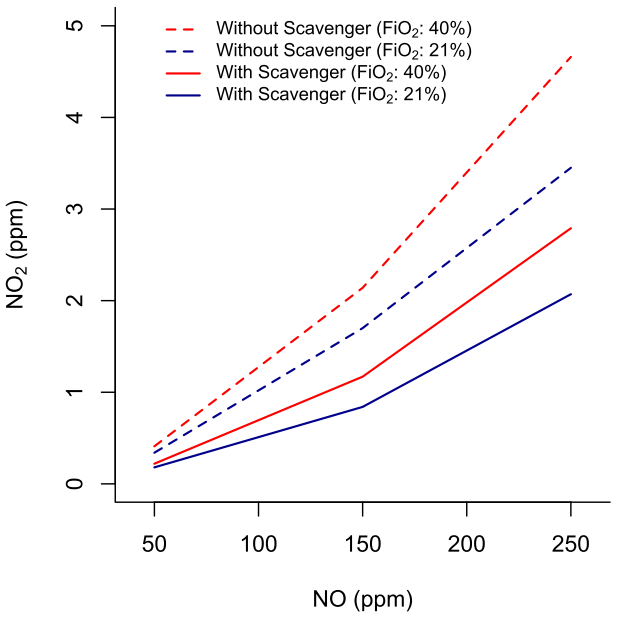

Fig. 7. NO_2 concentration with increasing NO concentrations (50 ppm, 150 ppm, 200 ppm) at 2 levels of FiO_2 (0.21 and 0.4) with (continuous line) or without (dotted line) the calcium hydroxide scavenger.

Table 1
Nitric oxide and oxygen flow (in liters/minute) were set to obtain a desired NO inspiratory concentration (50, 150, and 250 ppm) at a desired FiO_2. We set medical air flow at 15 Liters/min in every setting. We used two different tanks with different NO concentrations (800 and 857 ppm).

NO/N_2 tank concentration = 800 ppm				
	NO (ppm)	NO_2 (ppm)	NO flow (L/min)	O_2 flow (L/min)
$FiO_2 = 0.21$	49	0.13	1	0.36
	145.5	0.72	3.46	1.11
	249.5	1.57	6.99	2.10
$FiO_2 = 0.30$	48.5	0.18	1.2	3.50
	140	0.97	4.25	4.82
	250	2.35	9.10	7.30
$FiO_2 = 0.41$	48	0.18	1.40	7.12
	145	1.17	5.08	10.20
	241.5	2.61	11.91	17.40
NO/N_2 tank concentration = 857 ppm				
	NO (ppm)	NO_2 (ppm)	NO flow (L/min)	O_2 flow (L/min)
$FiO_2 = 0.21$	47.50	0.24	0.92	0.58
	143.50	0.77	3.07	1.16
	237.50	1.98	6.18	2.37
$FiO_2 = 0.30$	49	0.26	1.05	3.46
	143	1.24	3.84	5.40
	253	2.79	7.66	7.22
$FiO_2 = 0.42$	40	0.29	1.22	8.48
	142	1.40	4.48	10.74
	236	3.11	10.05	15.70

up to 250 ppm at a range of FiO_2 from 0.21 to 0.4. Using this system, NO_2 levels are maintained at the prescribed low levels [14].

Over the last few years, there has been increased interest in administering high-dose NO to spontaneously breathing patients outside the ICU setting and, possibly outside the hospital environment. Previous in vitro and in vivo evidence support the use of high-dose of NO as an antimicrobial [2,15]. Over the past two years, at Massachusetts General Hospital (Boston, MA), we have treated a teenage patient with 46-intermittent inhalation of 160 ppm NO for antibiotic resistant *Burkholderia multivorans* lung infection in the setting of cystic fibrosis. No adverse events or delivery system failures were observed, respiratory symptoms of the patient improved and the antibiotic pattern of the *Burkholderia multivorans* changed to allow common antibiotic coverage (i.e., Bactrim and Levofloxacin) [16]. Additionally, inhaled NO therapy has also shown to be a potent anti-inflammatory agent, reducing lung thrombosis after lung transplant [17].

In the setting of the current COVID-19 pandemic, we are examining whether the administration of high-dose NO in spontaneously breathing COVID-19 patients leads to a reduced rate of hospital admission (NCT04338828) and respiratory failure requiring intubation and mechanical ventilation (NCT04305457). Furthermore, we recently published a case series of 6 COVID 19 positive pregnant patients that received high dose (160–200 ppm) nitric oxide using our delivery system [18].

To avoid overwhelming COVID-19 systemic inflammation, we designed a way to deliver NO treatment early. Therapy with NO inhalation starts in the emergency department or upon hospital admission to the general care wards. One could envision treatments with NO gas with a similar prototype of NO delivery system for home use [19].

The system we designed and evaluated in this study allows for the administration of NO outside the ICU setting without a mechanical ventilator. If evidence continues to mount that high-dose inhaled NO is an effective anti-inflammatory, anti-thrombotic and anti-microbial agent, the system described can be used to treat or prophylactically treat many patients without the need for a dedicated mechanical ventilator. It has potential applications on the front lines, in an emergency room or rural clinic setting, or in low-resource settings, in the face of a pandemic due to a susceptible respiratory pathogen. The system is reproducible, inexpensive, reliable, and easy to build and maintain.

There are two important limitations of this system. First, this system does not continuously measure NO and NO_2 during administration without the use of external gas analyzers. However, once calibrated, using consistent gas flows and concentrations, and fresh calcium hydroxide, the NO and NO_2 levels showed to remain consistent in repeated laboratory tests. Second, to set medical air, NO, and oxygen flows, we used a high precision digital flowmeter. These flowmeters are not in widespread clinical use. The flowmeters commonly used in the clinical setting cannot reach this level of resolution, which may introduce some unexpected variability in the predicted NO and NO_2 levels.

In conclusion, we built an NO delivery system that provides an alternative to a ventilator-based system [8] to give high dose NO to spontaneously breathing patients. Despite some limitations, this system can efficient to allow administering high concentrations of NO via a comfortable fitting mask.

Funding information

S.G, C.C.A.M., G.L, R.P, R.C. have nothing to declare.

LB receives salary support from K23 HL128882/NHLBI NIH as principal investigator for his work on hemolysis and nitric oxide. LB receives technologies and devices from iNO Therapeutics LLC, Praxair Inc, Masimo Corp. LB receives a grant from iNO Therapeutics LLC. This study was supported by the Reginald Jenney Endowment at Harvard Medical School to LB, by grants from NHLBI B-BIC/NCAI (#U54HL119145) to W.M.Z, an NHLBI grant (#R21HL130956) to B.Y. This study was also supported by laboratory funds of the Anesthesia Center for Critical Care Research of the Department of Anesthesia, Critical Care and Pain Medicine at Massachusetts General Hospital (MGH). W.M.Z. and B.Y. received patents at MGH on the electric generation of nitric oxide (NO). W.M.Z. is on the scientific advisory board of Third Pole Inc, which has licensed patents on electric NO generation from MGH.

CRediT authorship contribution statement

Stefano Gianni: Formal analysis, Writing - review & editing, Writing - original draft. **Caio C.A. Morais:** Writing - original draft, Formal analysis, Writing - review & editing. **Grant Larson:** Writing - original

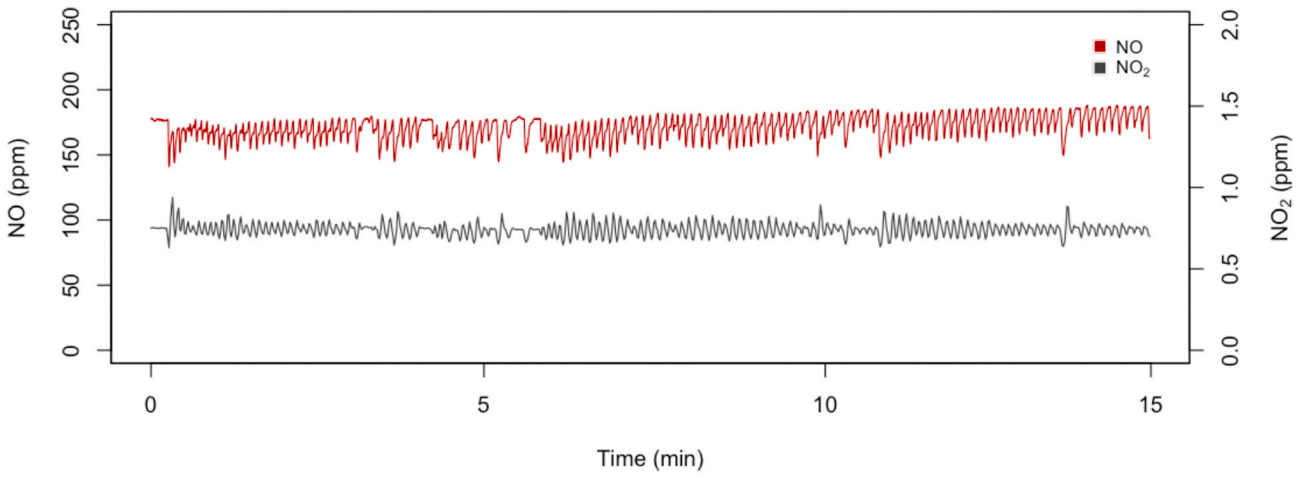

Fig. 8. NO and NO_2 concentrations during 15 min of NO gas inhalation using a mechanical lung. Target NO dose = 160 ppm.

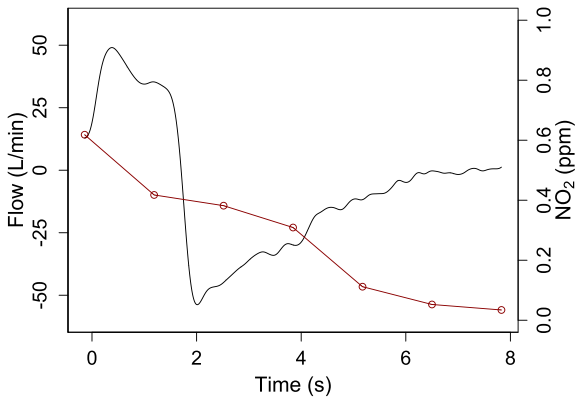

Fig. 9. Exhaled nitrogen dioxide concentration (red line) during a single breath in a healthy subject. The black line represents flow.

draft, Writing - review & editing. **Riccardo Pinciroli:** Writing - original draft. **Ryan Carroll:** Writing - original draft, Writing - review & editing. **Binglan Yu:** Writing - original draft, Writing - review & editing. **Warren M. Zapol:** Writing - original draft, Writing - review & editing. **Lorenzo Berra:** Writing - original draft, Writing - review & editing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.niox.2020.08.004.

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