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Development of a portable mini-generator to safely produce nitric oxide for the treatment of infants with pulmonary hypertension

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Abstract

Objectives—To test the safety of a novel miniaturized device that produces nitric oxide (NO) from air by pulsed electrical discharge, and to demonstrate that the generated NO can be used to vasodilate the pulmonary vasculature in rabbits with chemically-induced pulmonary hypertension.

Study design—A miniature NO (mini-NO) generator was tested for its ability to produce therapeutic levels (20–80 parts per million (ppm)) of NO, while removing potentially toxic gases and metal particles. We studied healthy 6-month-old New Zealand rabbits weighing 3.4±0.4 kg (mean±SD, n=8). Pulmonary hypertension was induced by chemically increasing right ventricular systolic pressure to 28–30 mmHg. The mini-NO generator was placed near the endotracheal tube. Production of NO was triggered by a pediatric airway flowmeter during the first 0.5 seconds of inspiration.

Results—In rabbits with acute pulmonary hypertension, the mini-NO generator produced sufficient NO to induce pulmonary vasodilation. Potentially toxic nitrogen dioxide (NO_2) and ozone (O_3) were removed by the $Ca(OH)_2$ scavenger. Metallic particles, released from the electrodes by the electric plasma, were removed by a 0.22 μm filter. While producing 40 ppm NO,

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Conflict of interest statement:
Other authors declare no conflicts of interest.

Author contributions: W.M.Z. and B.Y. designed and conceived the study, and wrote and edited the manuscript; B.Y. M.F., and G.S. performed experimental work and collected, assembled, analyzed, and interpreted data; A.H.B. helped with developing the miniature NO generator; M.W. assisted with organizing the rabbit model of acute pulmonary hypertension; D.B.B. made major contributions revising the manuscript. All authors gave their final approval of the manuscript for submission.

the mini-NO generator was cooled by a flow of air (70 ml/min) and the external temperature of the housing did not exceed 31°C.

Conclusions—The mini-NO generator safely produced therapeutic levels of NO from air. The mini-NO generator is an effective and economical approach to producing NO for treating neonatal pulmonary hypertension and will increase the accessibility and therapeutic uses of life-saving NO therapy worldwide.

List of key words

Inhaled nitric oxide; miniaturized nitric oxide generator; pulsed electrical discharge; neonatal pulmonary hypertension; vasodilation

Introduction

The treatment of pulmonary hypertension with intravenous vasodilators, such as sildenafil, nitroglycerin, and nitroprusside, may be accompanied by systemic vasodilation, potentially leading to systemic arterial hypotension. In 1991, Frostell and colleagues used an awake sheep model of pulmonary hypertension to demonstrate that inhaled nitric oxide (NO) acts as a selective pulmonary vasodilator [1]. Breathing 5–80 ppm NO gas produced rapid and profound pulmonary vasodilation in awake 25–35 kg lambs with pulmonary hypertension induced by infusion of the potent pulmonary vasoconstrictor U46619 [1; 2]. The unique ability of inhaled NO to cause selective pulmonary vasodilation prompted a large number of pre-clinical and clinical studies [3; 4; 5; 6]. Pilot studies in critically ill near-term newborns with acute pulmonary hypertension and in infants with persistent pulmonary hypertension of the newborn (PPHN) demonstrated that inhaled NO rapidly improves oxygenation without causing systemic hypotension [7; 8]. The US Food and Drug Administration approved the use of inhaled NO for treatment of PPHN in 1999 [9]. Inhaled NO is now used to treat a spectrum of cardiopulmonary conditions, including pulmonary hypertension in children and adults [10; 11]. Recent studies suggest that inhaled NO may prevent or ameliorate other disorders, including ischemia-reperfusion injury [12; 13; 14], hemolysis-induced vasoconstriction and renal failure after cardiopulmonary bypass [15]. Wider use of this therapy is significantly limited, however, because the delivery system is cumbersome and expensive, requiring heavy NO/N_2 gas cylinders, a cylinder distribution network, a complex delivery device, gas monitoring and calibration devices, and a trained respiratory therapy staff. For many hospitals, inhaled NO is one of the most expensive drugs used in neonatal medicine [16]; the average cost of providing NO therapy for 5 days to a patient with PPHN is about $14,000. NO inhalation therapy is essentially unavailable in the Third World. Challenges of cost and delivery have also limited research into out-of-hospital conditions for which inhaled NO therapy may be of benefit. Potential ambulatory and in-home applications of inhaled NO therapy include chronic lung diseases [17] and cardiac failure with secondary pulmonary hypertension [18; 19]. Undertaking pre-clinical and clinical research and implementing future therapies in these areas require the development of a lighter, portable, inexpensive inhaled NO delivery system.

Several methods have been used to produce NO for biomedical purposes [20; 21; 22; 23; 24]. However, these methods produce large amounts of nitrogen dioxide (NO_2) and ozone

(O_3) as toxic byproducts, requiring complex purification systems [25; 26]. In a previous study, we designed, developed, and tested a simple and economic NO generation device, which uses a pulsed electrical discharge between iridium electrodes to produce NO from air [27]. After scavenging of potentially toxic gas byproducts and filtration to remove metallic particles, the electrically generated NO can provide safe and effective treatment of pulmonary hypertension [27; 28].

The objective of this study was to test a miniaturized version of the prototypic device, designated as a “mini-NO generator”, for its ability to produce therapeutic levels of NO in small tidal volumes while removing potentially toxic gases and metal particles. First, we sought to determine whether NO generated by the mini-NO generator, placed near the endotracheal tube, was capable of inducing vasodilation in the pulmonary vasculature of anesthetized rabbits with acute pulmonary hypertension. Second, we confirmed the safety and efficiency of the mini-NO generator by demonstrating that: 1) the $Ca(OH)_2$ scavenger (0.8 g) removed potentially toxic gases (NO_2 and O_3); 2) the 0.22 μm filter eliminated trace metals (nickel, iridium, or platinum) emitted by the electrodes; and 3) an airflow of 70 ml/min was sufficient to maintain the housing of the device at an acceptable low temperature.

Materials and Methods

Measurements of O_2, O_3, NO, and NO_2 levels

An oxygen analyzer (MiniOX I, Ohio Medical Corporation, Gurnee, IL) was used to measure levels of O_2. The concentration of O_3 was measured with an EC 9810 Ozone Analyzer (American Ecotech, Warren, RI). To monitor NO and NO_2 levels, an NO analyzer (Sievers 280i Nitric Oxide Analyzer, GE Analytical Instruments, Boulder, CO), and a Cavity Attenuated Phase Shift NO_2 monitor (Aerodyne Research Inc., Billerica, MA) were used, respectively.

Schematic of mini-NO generator

The mini-NO generator, which weighs 14 g, is illustrated in Figs. 1A and B. The generator consists of two iridium discharge electrodes within a ceramic insulator surrounded by a 3 mm aluminum housing, an NO_2 and O_3 scavenger consisting of 0.8 g $Ca(OH)_2$, and two 0.22 μm high-efficiency particulate air (HEPA) filters (Vital Signs Inc.). A flow of 70 ml/min of air is injected into the airway via an air-tube with internal diameter (ID) of 1.6 mm. Energy is stored and released by an autotransformer and is delivered to the spark gap (2 mm) to create plasma. The discharge of the electrodes is regulated by a microcontroller circuit. The mini-NO generator is triggered to produce sparks during the 0.5 seconds at the commencement of each inspiration. A NICO2 Respironics pediatric airway flowmeter was used to detect the beginning of each inspiration (Philips Healthcare, Thornton, CO).

Animal studies

Rabbit studies were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee (Boston, MA). We studied 8 healthy 6-month-old male and female New Zealand white rabbits (Jackson Laboratory, Bar Harbor, ME) weighing 3.4±0.4 kg

(mean±SD). General anesthesia was induced with intramuscular injection of xylazine and fentanyl, and then maintained with intravenous ketamine and fentanyl. Rabbits were paralyzed with rocuronium, and mechanically ventilated via a tracheostomy at 6 ml/kg tidal volume. The respiratory rate was 40–50 breaths per minute (bpm), with a FiO_2 of 0.5, an inspiratory time of 0.5 seconds and positive end-expiratory pressure (PEEP) of 1–2 cm H_2O. Right ventricular systolic pressure (RVSP) was monitored continuously using a 4-Fr Swan-Ganz catheter (Pediatric Double Lumen Monitoring, Edwards Lifesciences, Irvine, CA), which was placed in an external jugular vein. Pulmonary hypertension was induced by infusing the potent pulmonary vasoconstrictor U46619 (Cayman Chemical, Ann Arbor, MI) at 0.8–0.9 μg/kg/min. Mean arterial pressure and heart rate were monitored at baseline, during U46619 infusion, and before and after breathing NO. The mini-NO generator was placed immediately next to the endotracheal tube (3.5 mm internal diameter, Covidien, Mansfield, MA), so as to minimize dead space in the airway. Production of NO was triggered by the NICO2 pediatric flowmeter so as to occur for 0.5 seconds at the beginning of inspiration.

Capacity of the $Ca(OH)_2$ scavenger

To determine how often the scavenger would need to be replaced under clinical conditions, we tested the capacity of the 0.8 g $Ca(OH)_2$ scavenger to remove NO_2 and O_3. A pediatric ventilator (Inspira asv, Harvard Apparatus, Holliston, MA) was used to deliver gas with a tidal volume of 18 ml, respiratory rate at 40 bpm and airway FiO_2 of 0.5. A pediatric flowmeter (NICO2, Respironics) sensed airway flow and triggered the mini-NO generator (see schematic Figs. 1C, D), to produce NO at 40 ppm. The FiO_2 in the airway was measured at 0.47 due to dilution by air. During the test, NO was collected and measured in a 50 ml volume test lung. The levels of NO_2 were measured and recorded every hour for the first 12 hrs, and again at 24, 48, and 72 hrs.

O_3 levels were measured with or without a 0.8 g $Ca(OH)_2$ scavenger at 24 hrs after starting the mini-NO generator with an airway flow of 1 L/min. The level of O_3 was measured at different NO production rates of 20, 40, and 80 ppm and at different O_2 concentrations (21, 50, 80, and 100%) while producing a constant amount of NO (40 ppm).

Measurements of trace metals in the effluent gas produced by the mini-NO generator

The levels of trace metals were measured in the effluent gas after passing through the 0.8 g scavenger and a single HEPA filter. NO was generated at 40 ppm with an airflow rate of 0.5 L/min for 24 hrs. The effluent gas was continuously bubbled into 15 ml of 5% nitric acid, which was diluted from 70% nitric acid (Optima Grade, Fisher Scientific, Cambridge, MA) with milliQ water. All samples were collected after 24 hrs of gas delivery, and analyzed with quadrupole inductively-coupled mass spectrometry (ICP-MS) at the University of Massachusetts Mass Spectrometry Center (Amherst, MA).

Measuring the external temperature of the housing of the mini-NO generator

NO generation produces heat, and to confirm that the device would not burn a patient during NO generation, we measured the external temperature of the device. The tidal volume of the ventilator was set at 18 ml, respiratory rate at 40 bpm, airway O_2 level was 50%, and NO

concentration was 40 ppm. The temperature of the external aluminum housing of the mini-NO generator was measured with an infrared thermometer (Cole-Parmer, Vernon Hills, IL) every hour for the first 12 hrs, and again at 24, 48, and 72 hrs.

Statistical analysis

All variables were found to be normally distributed by the Shapiro-Wilk test and are expressed as mean±SD. A two-way analysis of variance with repeated measures was performed to determine the effect of breathing electrically-generated NO on the RVSP of rabbits with pulmonary hypertension (GraphPad Prism; GraphPad Software Inc.). Two-tailed analyses were performed, and a P-value < 0.05 was considered significant.

Results

Electrically-generated NO reduces right ventricular systolic pressure (RVSP) in anesthetized rabbits with pulmonary hypertension

The mini-NO generator was designed to treat babies with pulmonary hypertension. To determine whether the mini-NO generator was able to produce therapeutic levels of NO, we tested the mini-NO generator on 3.4±0.4 kg rabbits subjected to chemically-induced acute pulmonary hypertension. Anesthetized rabbits received a continuous infusion of the thromboxane analog U46619, which increased RVSP from 14±2 to 30±3 mmHg. NO was generated on inspiration and conducted into the endotracheal tube with an airflow of 70 ml/min. The RVSP was measured while rabbits breathed 50% O_2 and 20, 40, or 80 ppm NO produced by the mini-NO generator. The mini-generator produced NO during the first 0.5 seconds of inspiration and the effect of each level of NO (20, 40, and 80 ppm) was tested for 4 min. At the end of each trial, the RVSP was measured for an additional 5 min after NO production and delivery were discontinued. A 5 to 10 min interval, with the animal breathing 50% O_2 was allowed before the next concentration of NO was tested. Breathing electrically generated NO at 20, 40 or 80 ppm reduced the RVSP beginning 1 min after initiating production of NO. Each of the NO concentrations produced a similar decrease in RVSP (RVSP before vs 1, 2, 3 and 4 min after breathing NO; P<0.05 for each time point; Fig. 2A). After the mini-NO generator was turned off, RVSP returned to 30±2 mmHg within 1 min. Rabbits subjected to U46619-induced pulmonary hypertension, breathing 40 ppm NO diluted from a tank containing 500 ppm NO in N_2, had a similar decrease in RVSP. The results showed that NO produced by the mini-NO generator was as effective as NO diluted from a cylinder in terms of its ability to reduce RVSP.

Currently, the mini-NO generator uses AC power. To reduce energy consumption (and to facilitate the development of a future device that will be run on batteries), we tested whether producing an electrical discharge with either every two-breaths or every three-breaths would also reduce RVSP in rabbits with pulmonary hypertension (Fig. 2B). A Bluetooth controller was used to vary the production of NO, so as to generate NO during every breath or during every second or third breath. NO (80 ppm) generated every two- or three-breaths reduced RVSP to the same degree as NO generated on every breath (P<0.05 before NO vs after NO; 30±3 mmHg vs 26±2 mmHg). These results indicate that producing NO as infrequently as every third breath was sufficient to vasodilate the pulmonary vasculature in a rabbit model of

acute pulmonary hypertension. Furthermore, generating NO on every two- or three-breaths, will save energy and prolong battery life when batteries are used as the power source for the mini-NO generator.

To determine whether inserting air at 70 ml/min via the mini-NO generator would affect the O_2 level in the airway, we measured FiO_2 with and without introducing the 70 ml/min airflow in anesthetized rabbits. We found that, at a minute ventilation of 720 ml/min, introducing 70 ml/min airflow via the mini-generator reduced FiO_2 from 0.50 to 0.47. Because babies may require 100% O_2 at the beginning of NO therapy, we tested the effect of the 70 ml/min flow of air on the final FiO_2 when 100% O_2 was used. The final FiO_2 decreased to 0.97±0.01. Thus, the insertion of the 70 ml/min airflow into the airway ventilator does not significantly reduce FiO_2 for the ventilated gas flow that is used to treat babies.

Determining the capacity of the $Ca(OH)_2$ scavenger

To achieve the goal of decreasing the overall size of the mini-NO generator, we decreased the size of the $Ca(OH)_2$ scavenger. In the prototype NO generator, a 12 g scavenger was used to remove potentially harmful gases. In the mini-NO generator, the relatively small size and therefore the limited capacity of the scavenger might mean that the scavenger would need to be replaced frequently. To determine how long the 0.8 g scavenger would be able to remove NO_2 and O_3 during NO generation, a ventilator was used to mimic the gas delivery rate to a baby and to trigger the generation of NO on “inspiration”. The ventilator was set to deliver a tidal volume of 18 ml, respiratory rate of 40 bpm, with a FiO_2 of 0.5. The mini-NO generator provided 40 ppm NO. With these settings, NO_2 levels remained below 1 ppm (the limit set by the Occupational Safety and Health Administration (OSHA) [29]) for 48 hrs. After 72 hrs, the level of NO_2 reached 1 ppm (Fig. 3A). These results suggest that the 0.8 g $Ca(OH)_2$ scavenger is sufficient to maintain safe levels of NO_2 for at least 2 days. Because babies with pulmonary hypertension may initially require 100% O_2, we measured NO_2 levels produced by NO generation with an FiO_2 of 1.0. With the ventilator set to deliver a tidal volume of 18 ml, respiratory rate of 40 bpm and FiO_2 of 1.0, the NO_2 level was below or at 1 ppm at 24 hrs (0.81±0.05 ppm) and 48 hours (1.02±0.04 ppm).

An electrical discharge in O_2 may also produce O_3 as a potential harmful byproduct. To test the capacity of the 0.8 g scavenger to remove O_3 over time, O_3 levels were measured while the mini-NO generator was tested at varying NO (20, 40 and 80 ppm) and O_2 (21, 50, 80, and 100%) levels. With an airflow rate of 1 L/min, O_3 levels increased with increasing NO production (Fig. 3B). With NO production set at 80 ppm, the level of O_3 detected, after passage through the scavenger remained below 4 ppb for 24 hrs.

The amount of O_3 that is produced during NO production is dependent on the concentration of O_2. With varying airway O_2 levels (21, 50, 80, and 100%), O_3 levels measured after passage through the scavenger were below 1.5 ppb for each O_2 concentration tested (Fig. 3C). This level is far below the U.S. OSHA O_3 limits of 80 ppb exposure a day [30].

Assessing the capacity of the HEPA filter to remove trace metals in the effluent gas

Another potential limitation of the mini-NO generator might be that the electrically generated NO is contaminated with trace metals. Because iridium electrodes slowly erode while generating NO [31], and the alloy holders of the electrodes may contribute small amounts of nickel and platinum, we examined the effluent gas stream for trace metal particles. With an inline $Ca(OH)_2$ (0.8 g) scavenger and a single HEPA filter, the electrodes released 0.018 μg/day of nickel in a 24-hr period, which is far below OSHA's safety standards. The levels of other trace metals (e.g. iridium and platinum) were too low to be detected by ICP-MS. These results suggest that with the scavenger and a single HEPA filter were able to remove potentially harmful metals in the effluent gas when the device was run for 24 hrs.

Determining the external temperature of the housing of the mini-NO generator

A third potential limitation of the mini-NO generator is that the device might overheat and potentially burn a patient. To overcome this problem, we inserted a small air-tube into the NO generation chamber with a gas flow of 70 ml/min. We measured the temperature of the aluminum housing of the device throughout the course of NO generation. The temperature of the external housing remained constant at 31±0.5°C for more than 3 days while the device generated 40 ppm NO.

Discussion

In this study, we found that NO generated by a 14 g mini-NO generator, placed near the endotracheal tube, was capable of inducing selective vasodilation in the pulmonary vasculature of anesthetized rabbits with acute pulmonary hypertension. We confirmed the safety and efficiency of the mini-NO generator by demonstrating that: 1) the $Ca(OH)_2$ scavenger (0.8 g) removed potentially toxic gases (NO_2 and O_3); 2) the scavenger and 0.22 μm filter removed harmful metals (nickel, iridium, or platinum) emitted by the electrodes; and 3) an airflow of 70 ml/min was sufficient to maintain the housing of the device at an acceptable temperature.

The most important focus of this study was to test the mini-NO generator, which produces a therapeutic range of NO (20–80 ppm), for its ability to vasodilate the pulmonary vasculature. In rabbit studies, electrically generated NO (20–80 ppm) decreased acute pulmonary hypertension and was as effective as NO diluted from a conventional NO/N_2 tank. Breathing NO during every other breath or every third breath was as effective as NO produced for every breath in terms of lowering the chemically- induced increase in RVSP. Generating NO during every other breath or every third breath decreases electrode energy consumption, prolongs scavenger capacity and battery life, and reduces heat production. All of these characteristics improve the safety and efficiency of the mini-NO generator.

A single disposable scavenger of 0.8 g $Ca(OH)_2$ was sufficient to remove NO_2 for up to 2 days. After passage through the scavenger, the O_3 levels remained below the limit set by OSHA (80 ppb exposure per day), when measured at 24 hrs. The level of O_3 remained in an acceptable level when NO was produced at all concentrations of O_2 tested. Placement of the

device near the airway is important, because it decreases dead space and thereby decreases the production of potentially harmful NO_2 and reduces the consumption of the $Ca(OH)_2$ scavenger.

Iridium electrodes erode and release metal particles during NO generation [31]. Iridium and platinum are uncommon and inert metals, but inhaling nickel particles may cause acute and chronic lung diseases [32; 33]. After 24 hrs of NO generation, a single HEPA filter with a 0.8 g scavenger was sufficient to remove harmful metal particles. The nickel levels after the scavenger and a single filter were 0.018 μg/day, which is far below OSHA's safety standards (nickel levels: 1400 μg/day for a baby and 7200 μg/day for an adult, calculations based on OSHA's limit levels of 1.0 mg/m^3 for inhaled metallic nickel and nickel compounds in air during an 8-hour shift over a 40-hour work week [34]). Because Iridium and platinum are high boiling point, inert metals, only minimal amounts are released by the electrodes. Neither of these metals was detected in the effluent after passage through the scavenger and filter.

A novel design feature of the mini-NO generator is the addition of a small and continuous airflow (70 ml/min) into the NO generating chamber. This small amount of gas flow facilitates the delivery of newly generated NO, and cools the electrodes and chamber to avoid overheating. In fact, the temperature of the external housing remained at 31°C for 3 days while producing 40 ppm NO.

One of the current clinical limitations of NO inhalation therapy is the storage of NO in compressed gas cylinders, which are expensive and heavy. Other investigators have attempted to manufacture smaller NO/N_2 tanks but, because they require frequent replenishment, they make the usage of NO even more expensive [35]. Other methods to produce and deliver NO, such as by using liquid N_2O_4, is complicated by the production of high levels of NO_2. In this study, the novel mini-NO generator is light-weight, portable, economical, and generates medical grade NO from air. The current mini-NO generator has several advantages over our previously developed, prototypic NO generator [27; 31]. The mini-NO generator only weighs 14 g, so it can be placed adjacent to the endotracheal tube or airway. By adding a gas flowmeter, the mini-NO generator only produces NO on inspiration, which saves energy, produces less heat, and only needs a small amount of scavenger (0.8 g) to remove any potential toxic byproduct gas (e.g. O_3 or NO_2). A novel feature of the design for the mini-NO device is the introduction of small diameter tubing (ID=1.6 mm) into the generator chamber, allowing an airflow of 70 ml/min. This small airflow is used to generate and inject NO into the trachea, and cool the device.

In conclusion, the recent development of electric generation of NO from air offers the potential for delivering NO gas for inhalation for prolonged periods, including potential ambulatory and in-home applications. The device could expand the delivery of NO to hospitals and clinics around the world, because electric plasma NO generation is economical, easy to use, and safe. Future studies will focus on improving power supply efficiency by increasing NO production with even less energy consumption. In addition, it is anticipated that the size and weight of the entire portable device including power supply will be decreased to less than 500 g.

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Highlights

- The miniaturized NO (mini-NO) generator safely produced therapeutic levels of NO (20-80 ppm) from air.
- The mini-NO generator produced sufficient NO to induce pulmonary vasodilation in rabbits with acute pulmonary hypertension.
- While producing NO, the mini-NO generator was cooled by a flow of air and the housing temperature did not exceed 31°C.

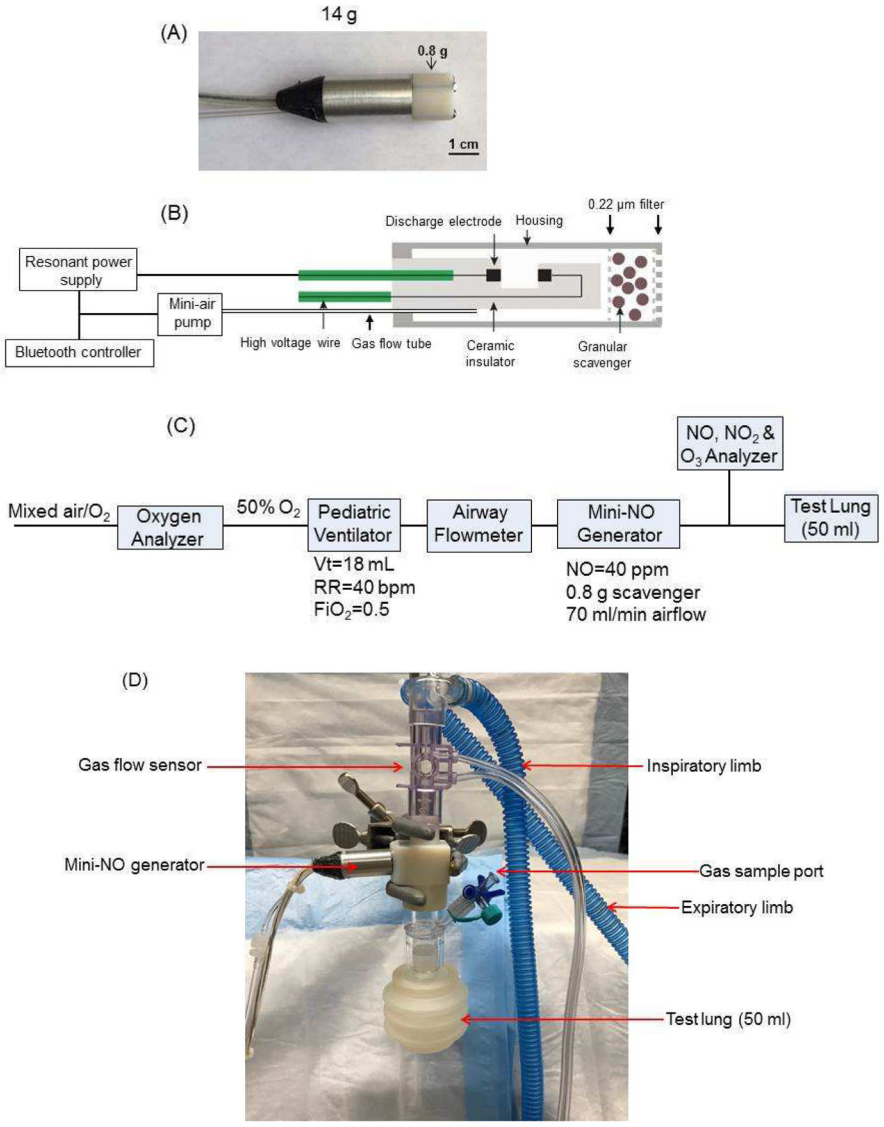

Figure 1.

Photograph **(A)** and schematic **(B)** of the mini-NO generator. The generator contains iridium discharge electrodes, a NO_2 scavenger consisting of 0.8 g of $Ca(OH)_2$, a 0.22 μm filter, a gas flow tube (e.g. 70 ml/min airflow) to facilitate NO delivery, a mini-air pump, resonant power supply, and Bluetooth controller. The device is surrounded by a ceramic insulator. **(C)** Schematic of the bench setup for measuring NO, NO_2, and O_3 levels. **(D)** Photograph of the bench setup with the mini-NO generator and a pediatric ventilator.

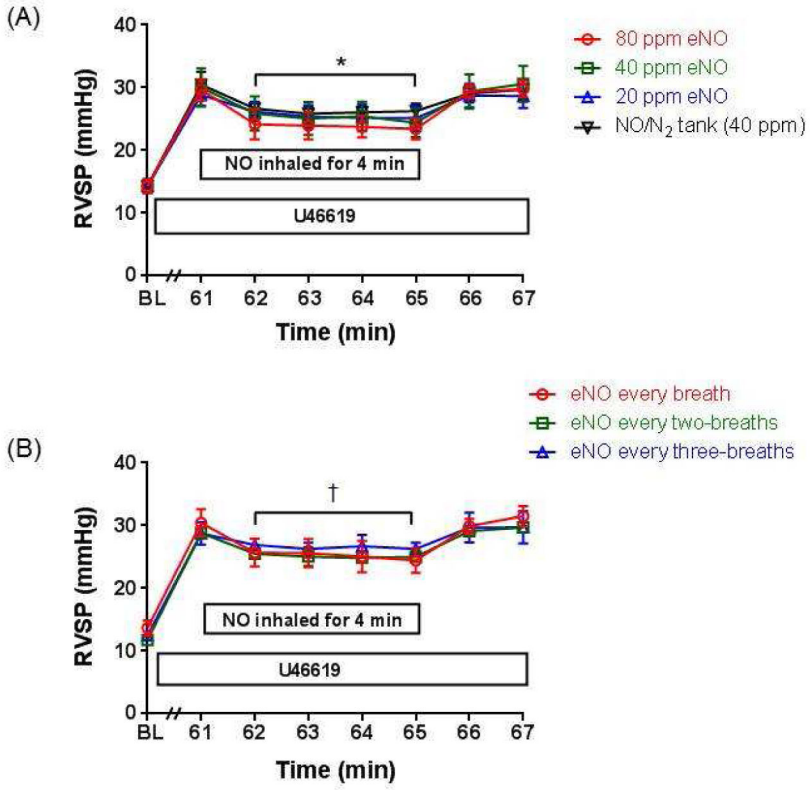

Figure 2.

Right ventricular systolic pressure (RVSP, mmHg) at baseline, during and after iv infusion of U46619, and while the anesthetized rabbits were breathing electrically generated NO. **(A)** Comparison of electrically generated NO (20, 40, 80 ppm) with NO diluted from a 500 ppm NO/N_2 tank. NO generation was triggered for 0.5 seconds during every breath. *P<0.05 for time 62 to 65 min versus 61 min (before production of NO breathing). Mean ±SD represents measurements at each NO concentration for 5 rabbits. **(B)** Comparison of triggering the production and delivery of 80 ppm NO during every breath, every other breath, and every third breath. Mean± SD represents measurements at each setting for 3 rabbits.

eNO: electrically generated NO. †P<0.05 for time 62 to 65 min versus 61 min (before production of NO breathing).

Settings: V_T= 6 ml/kg, RR=40–50 bpm, FiO_2=0.5.

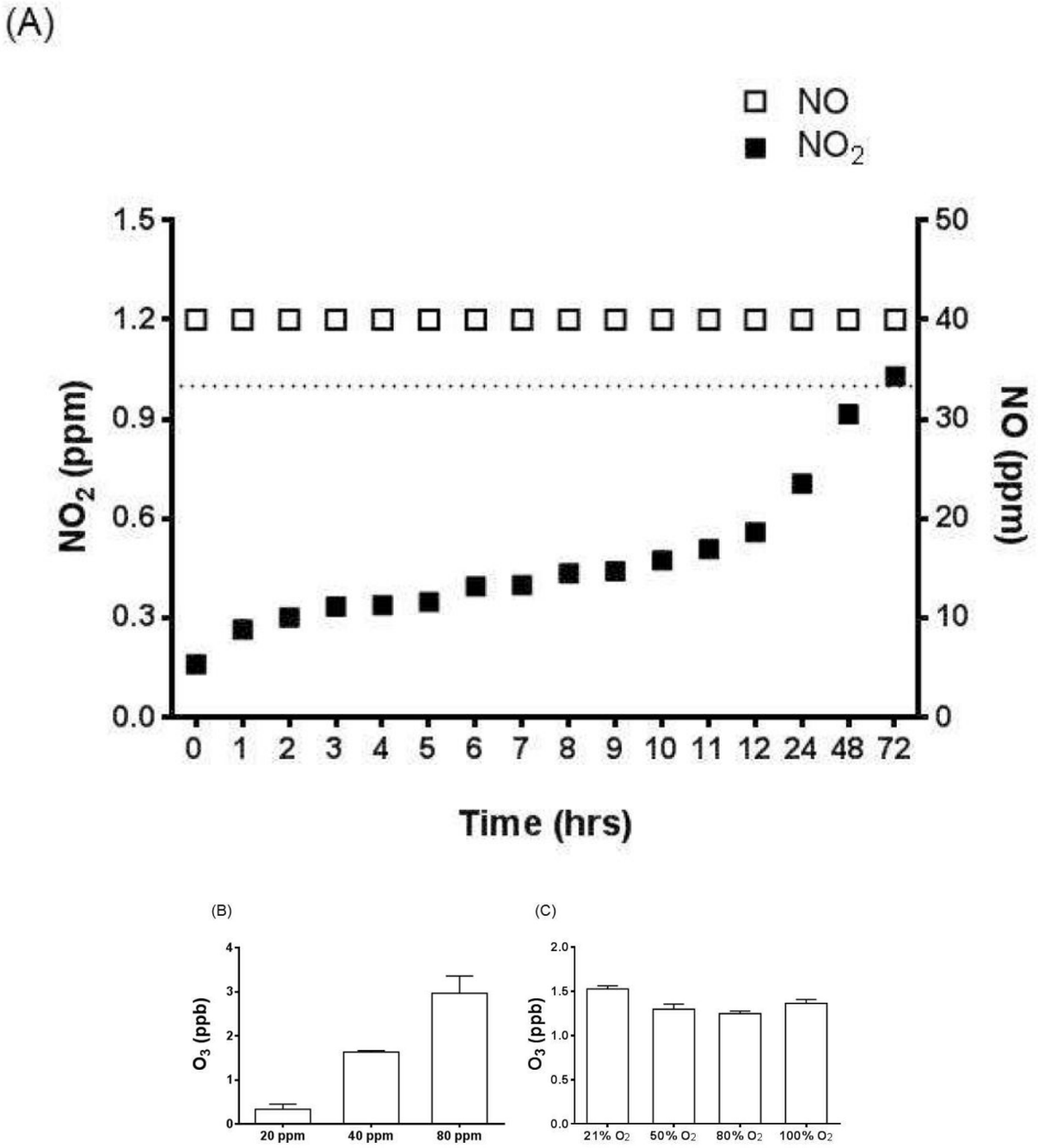

Figure 3.

(A) NO_2 levels were measured after passage through the $Ca(OH)_2$ scavenger while the mini-NO generator produced 40 ppm NO in an airflow of 70 ml/min. The pediatric ventilator was set to deliver 50% O_2, V_T=18 ml, RR=40 bpm (n=3 measurements/time point). The dotted line indicates 1 ppm of NO_2. **(B)** While the mini-NO generator produced NO at 20, 40, or 80 ppm for 24 hrs, the 0.8 g $Ca(OH)_2$ scavenger maintained the level of O_3 below the 80 ppb level recommended by OSHA over a 24 hrs period. **(C)** While the mini-NO generator produced NO at 40 ppm with FiO_2 at 21, 50, 80, and 100%, and gas flow at 1 L/min, the

scavenger maintained the level of O_3 at less than 1.5 ppb for all FiO_2 levels tested (n=3–4 measurements/time point).