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Themed Section: Nitric Oxide 20 Years from the 1998 Nobel Prize

REVIEW ARTICLE

Inhaled nitric oxide

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Nitric oxide (NO) is a gas that induces relaxation of smooth muscle cells in the vasculature. Because NO reacts with oxyhaemoglobin with high affinity, the gas is rapidly scavenged by oxyhaemoglobin in red blood cells and the vasodilating effects of inhaled NO are limited to ventilated regions in the lung. NO therefore has the unique ability to induce pulmonary vasodilatation specifically in the portions of the lung with adequate ventilation, thereby improving oxygenation of blood and decreasing intrapulmonary right to left shunting. Inhaled NO is used to treat a spectrum of cardiopulmonary conditions, including pulmonary hypertension in children and adults. However, the widespread use of inhaled NO is limited by logistical and financial barriers. We have designed, developed and tested a simple and economic NO generation device, which uses pulsed electrical discharges in air to produce therapeutic levels of NO that can be used for inhalation therapy.

LINKED ARTICLES

This article is part of a themed section on Nitric Oxide 20 Years from the 1998 Nobel Prize. To view the other articles in this section visit http://onlinelibrary.wiley.com/doi/10.1111/bph.v176.2/issuetoc

Abbreviations

AKI, acute kidney injury; ARDS, acute respiratory distress syndrome; CPB, cardiopulmonary bypass; HBOCs, Hb-based oxygen carriers; HEPA, high-efficiency particulate absorption; RBCs, red blood cells; SCD, sickle cell disease; STEMI, ST-elevation myocardial infarction

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Introduction

NO is a gas that is produced in the body by a family of three **NOS**. The enzymes use oxygen and L-arginine to produce NO and L-citrulline. NO stimulates **soluble guanylate cyclase (sGC)** to synthesize **cGMP**, which activates **cGMP-dependent PKG**, leading to vascular relaxation. **PDEs** catabolize cGMP, thereby limiting its activity (Ichinose and Zapol, 2017b). In the presence of oxygenated haemoglobin (Hb), NO is rapidly metabolized to form nitrate and methaemoglobin. In erythrocytes, methaemoglobin reductase converts methaemoglobin to ferrous-Hb.

Drugs that generate NO, such as **nitroglycerin** and **sodium nitroprusside (SNP),** have long been used to reduce blood pressure (BP) and treat angina pectoris. NO-donor compounds can also dilate the pulmonary vasculature, but their efficacy is limited by systemic hypotension. In patients with lung injury, NO-donor compounds given systemically may induce vasodilatation in regions of the lung that are poorly ventilated, thereby increasing ventilation-perfusion mismatching and leading to systemic arterial hypoxaemia. Frostell and colleagues reasoned that NO administered *via* inhalation would relax the pulmonary vasculature but, upon reaching the bloodstream, would be rapidly scavenged by Hb thereby preventing systemic vasodilatation (Frostell *et al*., 1993). These investigators observed that inhalation of NO produced a dose-dependent decrease in pulmonary artery pressure and pulmonary vascular resistance in awake sheep with pulmonary hypertension. No effect of inhaled NO was observed in sheep with normal pulmonary vascular tone (Frostell *et al*., 1991). The pulmonary vasodilator effects of breathing NO were readily reversible upon discontinuation of breathing the gas. Breathing NO did not alter systemic arterial BP. The selective dilation of the pulmonary vasculature induced by NO has been observed in a wide range of species, including man. Pilot studies in critically ill newborns with acute pulmonary hypertension showed that inhaled NO improved oxygenation without causing systemic hypotension (Kinsella *et al*., 1992; Roberts *et al*., 1992). Subsequent randomized, placebo-controlled studies confirmed these results and led to the approval of inhaled NO to treat hypoxic newborns by the US Food and Drug Administration in 1999, by the European Medicine Evaluation Agency and European Commission in 2001 and by the Ministry of Health, Labour and Welfare of Japan in 2008 (NINOS group, 1997; Kinsella *et al*., 1997; Roberts *et al*., 1997; Clark *et al*., 2000).

A number of studies now indicate that inhaled NO has an important role in treating pulmonary hypertension of paediatric and adult patients with respiratory and cardiac failure (Kinsella *et al*., 1992; Roberts *et al*., 1992; Krasuski *et al*., 2000; Cockrill *et al*., 2001; Ozturk *et al*., 2016). Inhaled NO can also be used during cardiac catheterization to determine the vasodilatory capacity of the pulmonary vascular bed in patients with pulmonary hypertension. A data meta-analysis was conducted on 1240 preterm infants who received either placebo (nitrogen gas) or NO >5 p.p.m. for a minimum of 7 days (Askie *et al*., 2018). This study suggested that inhaled NO prevented bronchopulmonary dysplasia (BPD) in preterm African American infants and was presented as an example of a racially customized therapy for infants with BPD. Recent studies suggest inhaled NO may prevent ischaemia-reperfusion injury and reduce haemolysis-induced vasoconstriction and renal failure after prolonged cardiopulmonary bypass (Lei *et al*., 2018). This review article will focus on recent developments in the use of NO inhalation therapy to treat patients with acute respiratory distress syndrome (ARDS) and the vascular consequences of haemolytic diseases. In addition, advances in the development and testing of an inexpensive, lightweight portable NO generating device from air will be described. The use of inhaled NO in other clinical areas including paediatric and adult pulmonary hypertension and chronic obstructive pulmonary disease have been reviewed elsewhere (Ichinose and Zapol, 2017a).

Inhaled NO in acute respiratory distress syndrome

ARDS is characterized by pulmonary hypertension and increased intrapulmonary shunting of blood through hypoventilated regions. Pulmonary hypertension contributes to pulmonary oedema and can cause right ventricular dysfunction and heart failure. The use of inhaled NO for the treatment of ARDS is one of the most widely studied pharmacological interventions over the past three decades. In 10 patients with severe ARDS, inhalation of NO from 5–20 p.p.m. for 3 to 53 days reduced pulmonary arterial pressure, decreased intrapulmonary shunting and improved arterial oxygenation without producing systemic vasodilatation (Rossaint *et al*., 1993). Benzing and colleagues demonstrated that inhalation of 40 p.p.m. NO vasodilated pulmonary vasculature and thereby lowered pulmonary capillary pressure in patients with acute lung injury (Benzing and Geiger, 1994). However, subsequent clinical trials reported disappointing results, in that inhalation of NO did not improve the survival rate in patients with ARDS (Troncy *et al*., 1998; Gerlach *et al*., 2003; Taylor *et al*., 2004). These randomized, controlled trials were unfortunately performed in the 1990s, before low-volume ventilation was shown to be beneficial in patients with ARDS. With the widespread adoption of the low tidal volume ventilation strategy, Bronicki *et al*. enrolled 55 paediatric patients with ARDS in a prospective, randomized placebo-controlled trial of inhaled NO that showed a significant reduction in duration of mechanical ventilation and a significantly greater survival without the need for using extracorporeal membrane oxygenation (Bronicki *et al*., 2015). In 161 children with ARDS, Dowell and coworkers demonstrated that inhalation of NO, for at least 1 h, within 3 days of ARDS onset was associated with a decrease in the average number of days that patients required ventilator support (Dowell *et al*., 2017). In an open-label prospective crossover pilot study, breathing 20 p.p.m. of NO significantly improved oxygenation in 15 adult patients with ARDS (Albert *et al*., 2017). Accumulating evidence suggests that NO inhalation therapy is beneficial in patients with ARDS. Large randomized trials are needed to determine whether inhaled NO improves survival of adult patients with ARDS.

Inhaled NO in haemolysis

Endothelial cells produce NO, which acts as a potent dilator of vascular smooth muscle cells. NO depletion can lead to vasoconstriction, impaired tissue perfusion and inflammation. During haemolysis, plasma NO is consumed by circulating plasma free oxyhaemoglobin, which is transformed into methaemoglobin by the dioxygenatoin reaction. Breathing NO converts circulating cell-free oxyhaemoglobin to methaemoglobin, thereby reducing the ability of oxyhaemoglobin to scavenge intrinsic NO. Minneci and coworkers used water-induced haemolysis in dogs to investigate the effect of free Hb on vascular tone and renal function. By scavenging endothelium-derived NO, free Hb in plasma induced vasoconstriction and decreased creatinine clearance (Minneci *et al*., 2005). Dogs that were treated with inhalation of 80 p.p.m. NO had decreased haemolysis-induced hypertension and renal dysfunction. Based in part on these observations, it has been proposed and in many cases proven that NO inhalation attenuates the vasoconstriction that is associated with clinical haemolysis, including haemolysis induced by, or associated with, prolonged cardiopulmonary bypass, sickle cell anaemia, malaria and blood transfusion.

Cardiac surgery

Pulmonary hypertension is a recognized risk factor for mortality in cardiac surgery. In a murine model of cardiac arrest, Kida and coworkers showed that inhaled NO exerts protective effects and improves outcomes after cardiac arrest and cardiopulmonary resuscitation with or without therapeutic hypothermia (Kida *et al*., 2014). In 33 paediatric patients who underwent a palliative surgical procedure to treat univentricular heart, Latus and colleagues found that inhalation of NO increased pulmonary and systemic blood flow, demonstrating beneficial effects on cardiac output and tissue perfusion (Latus *et al*., 2016). Elmi-Sarabi and colleagues conducted a meta-analysis of 10 studies including 434 patients, to compare the efficacy of inhaled aerosolized vasodilators (including NO) in the treatment of pulmonary hypertension during cardiac surgery (Elmi-Sarabi *et al*., 2017). The authors concluded that inhaled NO improved right ventricular performance when compared to i.v. administered agents. Janssens and coworkers conducted a multicentre, double-blind, randomized controlled trial of inhalation of NO in 250 patients with ST-elevation myocardial infarction (STEMI) (Janssens *et al*., 2018). Inhalation of NO at 80 p.p.m. for 4 h in these patients after cardiac catheterization was safe, and there was a tendency towards decreased rates of adverse events at 4 months ($P = 0.10$) and 1 year ($P = 0.06$) in patients who received NO. The results suggest that further studies of the potential benefits of inhaled NO in patients with STEMI are needed.

The most common complication associated with prolonged cardiopulmonary bypass (CPB) is acute kidney injury (AKI), which markedly increases postoperative mortality (Karkouti *et al*., 2009; Wrobel *et al*., 2015). Prolonged CPB causes haemolysis with high levels of circulating plasma Hb that scavenges NO *via* the dioxygenatoin reaction, depleting endogenous NO and causing vasoconstriction, proximal renal tubular injury and AKI. Lei and colleagues conducted a single centre, prospective, randomized, double-blind controlled trial involving 217 patients with normal kidney function, who underwent elective multiple valve replacement surgery that required prolonged CPB (Lei *et al*., 2018). The incidence of AKI in patients treated with NO decreased from 63 to 50% ($P < 0.05$). Treatment with inhaled 80 p.p.m. NO during (*via* oxygenator) and for 24 h after the operation was safe, with blood methaemoglobin levels remaining below 10%. Based on these observations, inhalation of NO may prove to be beneficial for patients undergoing prolonged cardiac surgery.

Sickle cell disease and cerebral malaria

Sickle cell disease (SCD) is an autosomal-recessive disorder caused by mutations in the β-globin gene. Mutant Hb S polymerizes in erythrocytes, altering the shape of red blood cells and causing occlusion of small blood vessels. Patients with SCD experience episodes of severe pain (vaso-occlusive crisis), with subsequent damage to major organs, and premature death. There has been considerable interest in the possible contribution of NO depletion to the pathogenesis of SCD and a potential role for inhaled NO as a treatment for SCD. Case reports (Atz and Wessel, 1997; Sullivan *et al*., 1999; Head *et al*., 2010) and a single-institution, placebo-controlled study (Weiner *et al*., 2003) suggested beneficial effects of NO inhalation in patients with SCD. However, Gladwin and colleagues performed an 11 centre, double-blind, randomized, placebo-controlled clinical trial involving 150 SCD patients and found that breathing 80 p.p.m. NO for up to 72 h, with pulsatile inspiratory delivery of NO through nasal prongs, did not decrease the duration of painful crisis (Gladwin *et al*., 2011). Maitre and colleagues conducted a prospective, double-blind, randomized, placebo-controlled clinical trial in 100 adult SCD patients with acute chest syndrome, which is characterized by fever and/or respiratory symptoms accompanied by new abnormalities on their chest radiograph. Inhalation of 80 p.p.m. NO for 3 days did not reduce the rate of mortality in this group of patients (Maitre *et al*., 2015). Future trials should target more severely ill SCD patients with hypoxaemia and/or acute pulmonary hypertension and investigate whether inhalation of NO provides benefit to this subgroup of patients.

Cerebral malaria is the most severe neurological complication of infection with *Plasmodium falciparum*, with a mortality rate of approximately 20%. Survivors of cerebral malaria often experience long-term cognitive and neurological deficits (Idro *et al*., 2007; Serghides *et al*., 2011; Postels *et al*., 2012). In a murine model of cerebral malaria, treatment with 40 p.p.m. NO improved survival by inactivating NO-scavenging by free Hb in the plasma (Gramaglia *et al*., 2006). Serghides and colleagues demonstrated that mice treated with NO during infection had reduced systemic inflammation and endothelial cell activation, decreased intercellular adhesion molecule 1 (ICAM-1) expression, preserved integrity of the blood–brain barrier and decreased parasite accumulation in the brain (Serghides *et al*., 2011). In addition, inhaled NO co-administered with artesunate (a medication currently used to treat malaria), starting 5.5 days after infection, improved the murine survival rate compared to treatment with artesunate therapy alone (Serghides *et al*., 2011). Hawkes and colleagues performed a

randomized, blinded, placebo-controlled trial in which standard artesunate treatment was supplemented with either 80 p.p.m. NO in air (delivered by nonrebreathing mask) or air alone. In 180 children with severe malaria, inhaled NO did not significantly decrease angiopoietin-2 levels, an endothelial biomarker of malarial severity. There was also no observed effect of NO treatment on clinical outcomes, including mortality (Hawkes *et al.*, 2015). Mwanga-Amumpaire and colleagues performed a randomized open-label, phase II, controlled trial of breathing 80 p.p.m. NO in air or air alone in 92 children with cerebral malaria in Uganda (Hawkes *et al.*, 2011; Mwanga-Amumpaire *et al.*, 2015). Although this trial did not demonstrate a reduction in mortality or neurological impairment in children treated with NO, breathing NO for 48 h was safe and was associated with an increase in methaemoglobin and plasma nitrate levels (Mwanga-Amumpaire *et al.*, 2015). In the future, it may be valuable to focus NO inhalation therapy on cerebral malaria patients with laboratory evidence of NO scavenging and pulmonary or systemic vasoconstriction.

Blood transfusion

For many decades, Hb-based oxygen carriers (HBOCs) have been investigated as substitutes for the use of red blood cells in blood transfusions. One of the major obstacles hindering the successful clinical development of HBOCs is systemic vasoconstriction. Yu and colleagues demonstrated in mice that HBOCs cause systemic vasoconstriction by scavenging NO produced by endothelial NOS (also known as NOS3; Yu *et al.*, 2008). Administration of inhaled NO before an i.v. infusion of HBOCs prevented systemic vasoconstriction without causing methaemoglobinaemia in mice and sheep (Yu *et al.*, 2008, 2009a). Inhalation of NO, at a concentration as low as 5 p.p.m., prevented HBOC-induced pulmonary vasoconstriction in healthy awake lambs. Yu and coworkers studied the effects of HBOC infusion on mice with endothelial dysfunction caused by diabetes mellitus or by consuming a high-fat diet for 4–6 weeks. The endothelial damage induced by these conditions resulted in decreased NO bioavailability and increased susceptibility to HBOC-induced vasoconstriction (Yu *et al.*, 2010). Inhaled NO can prevent the systemic vasoconstriction induced by infusion of HBOCs in these mice with endothelial dysfunction. In a case report, Marrazzo and colleagues described an 87-year-old patient with acute life-threatening anaemia (Hb level at 40 $g{\cdot}L^{-1}$) (Marrazzo *et al.*, 2018). This patient had a history of an anti-Jk3 alloantibody and could only receive packed red blood cells lacking the Jk3 antigen, which is an extremely rare phenotype in most populations. Therefore, two units of HBOC were administered for compassionate use. The administration of inhaled NO combined with HBOC infusion improved cardiac output, arterial oxygen content, lactate clearance and reduced vasopressor requirement in this patient. In the future, treatment with inhaled NO may prove to be a novel strategy that permits the use of HBOC transfusion without causing systemic and pulmonary hypertension.

During *ex vivo* storage, red blood cells (RBCs) undergo numerous biochemical, structural and functional alterations, which are collectively termed the 'storage lesion'. Transfusion of blood that has been stored for more than 2 weeks was associated with an increased rate of infection and multiorgan failure, longer hospitalizations and increased mortality (Koch *et al.*, 2008; Weinberg *et al.*, 2008; Zimrin and Hess, 2009). In diabetic mice, inhalation of 80 p.p.m. NO prevented systemic vasoconstriction and hypertension associated with transfusion of RBCs stored for 14 days (Yu *et al.*, 2012). Lei and colleagues used a murine model of haemorrhagic shock, in the setting of hyperlipidaemia-induced endothelial dysfunction and decreased NO bioavailability, to investigate the effects of NO on stored-blood induced injury. Inhalation of NO during transfusion of 14-day-old blood decreased tissue injury, inflammation and mortality (Lei *et al.*, 2012). Baron and colleagues developed a model of autologous blood transfusion in lambs and found that inhaled NO attenuated the pulmonary hypertension induced by transfusion of red blood cells stored for 40 days (Baron *et al.*, 2012). Furthermore, in a sheep model of haemorrhagic shock, inhalation of NO attenuated the pulmonary hypertension and inflammation associated with transfusion of 40-day-old red blood cells (Baron *et al.*, 2013). Berra and coworkers demonstrated in human volunteers that inhalation of NO prevented pulmonary hypertension associated with the transfusion of autologous leukoreduced blood stored for 40 days (Berra *et al.*, 2014). The results of these studies suggest that inhaled NO should be considered as an adjunctive therapy for blood transfusion, especially in critically ill patients with pulmonary hypertension, endothelial dysfunction and acute lung injury.

Generating NO from air using pulsed electrical discharges

NO inhalation therapy requires gas cylinders and a cylinder distribution network, a complex delivery device to regulate NO and oxygen (O_2) concentrations, and trained respiratory therapists. For many hospitals, inhaled NO is the most expensive drug used in neonatal medicine (Subhedar and Dewhurst, 2007). Because of the complexities and expense of delivering NO, this treatment is not available in many parts of the world and is not practical for outpatient use. Several approaches have been used to produce NO for biomedical purposes, including chemical methods and various electrical systems (Namihira *et al.*, 2000, 2002; Stoffels *et al.*, 2006; Kuhn *et al.*, 2010). However, these reactions produce large amounts of toxic byproducts, such as nitrogen dioxide (NO_2) and ozone, and therefore require complex purification systems (Samaranayake *et al.*, 1999; Hu *et al.*, 2007). Lovich and colleagues proposed that NO might be produced by catalytic conversion of liquid NO_2/N_2O_4. However, this approach would require a large amount of highly toxic NO_2 as the starting material (Lovich *et al.*, 2014). Ren and coworkers developed a NO releasing system that used a copper (II)-tri(2-pyridylmethyl) amine complex to mediate electrochemical reduction of nitrite. Unfortunately, the system only produced very low levels of NO (Ren *et al.*, 2014, 2015). Subsequent modifications, based on the electrochemical reduction of nitrite using copper (II)-ligand as a mediator, increased the yield of NO, from 400 p.p.b. to 500 p.p.m. (Qin *et al.*, 2017). However, the instability of the mediators and the short lifespan of the copper-complex covered electrodes (currently less than

48 h) suggest that this approach requires further investigation and improvement.

Recently, our group designed, developed and tested a lightweight, portable and economical NO generator system that uses pulsed electrical discharges (Figures 1 and 2) (Yu *et al.*, 2015). The NO generator produces NO in a therapeutic range (5–80 p.p.m.) at gas flow rates of 0.5 to 5 $L \cdot min^{-1}$. Iridium electrodes were found to be superior to stainless steel, nickel, carbon and tungsten electrodes in that they produced the least amount of NO_2 during NO production. The small amount of potentially toxic gases and metals that were produced in the electrically generated plasma were removed by a small (12 g) in-line, calcium hydroxide ($Ca(OH)_2$) scavenger and a high-efficiency particulate absorption (HEPA) filter. In lambs with acute pulmonary arterial hypertension, breathing electrically generated NO reduced pulmonary arterial pressure, as effectively as NO diluted from a conventional cylinder (Yu *et al.*, 2015). To save energy, reduce the consumption of the scavenger and preserve the electrodes, we improved the NO generator by triggering NO production only during inspiration. The newly developed NO generator can be installed in series with the ventilator or can be used to inject NO into the airway *via* a transtracheal catheter.

Prolonged use of the NO generator resulted in erosion of the surface of the electrodes, potentially introducing contaminating metal particles into the gas stream (Yu *et al.*, 2016). We used quadrupole mass spectroscopy to show that a single HEPA filter was sufficient to remove all metal particles from the effluent gas. Mice breathing electrically generated NO, 50 p.p.m. in air for 28 days, did not develop pulmonary inflammation or structural changes, and no trace metals were detected in the lungs of these mice (Yu *et al.*, 2016).

Berra and colleagues tested the NO generator on six healthy volunteers and six patients with chronic pulmonary hypertension (Berra *et al.*, 2016). Each subject received 25 p.p.m. of NO for 10 min, and no adverse effects were detected. In six patients with chronic pulmonary hypertension, the acute pulmonary vasodilator haemodynamic effects of electrically generated NO were similar to those seen using NO obtained from commercially available cylinders (Berra *et al.*, 2016).

To develop a lighter NO generator for potential portable, outpatient use, we designed a miniaturized version of the prototypic NO generator, designated the 'mini-NO generator' (Yu *et al.*, 2018). The mini-NO generator weighs approximately 14 g and consists of two iridium electrodes within a ceramic insulator surrounded by a 3 mm aluminium housing. Two HEPA filters are used to contain the 0.8 g $Ca(OH)_2$ scavenger and to remove potential metal particles released from the electrodes during NO generation (Figure 3). When placed adjacent to the endotracheal tube of anaesthetized rabbits with acute pulmonary hypertension, the mini-NO generator induced selective vasodilatation of the pulmonary vasculature. We showed that a small amount of $Ca(OH)_2$ scavenger (0.8 g) was sufficient to remove potentially toxic gases.

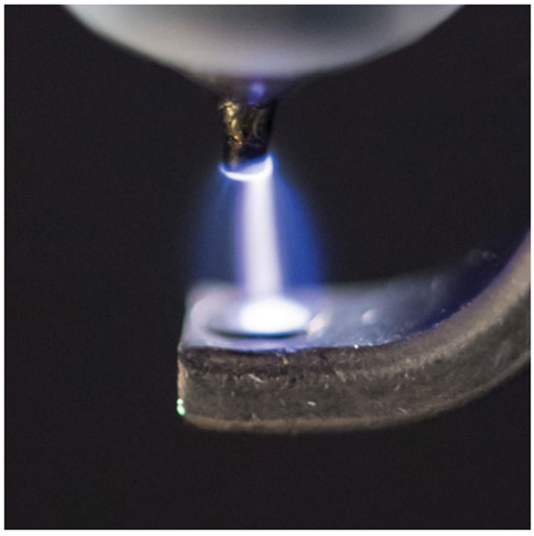

Figure 1

Producing NO by pulsed electrical discharge from air (from Yu *et al.*, 2015; used with permission).

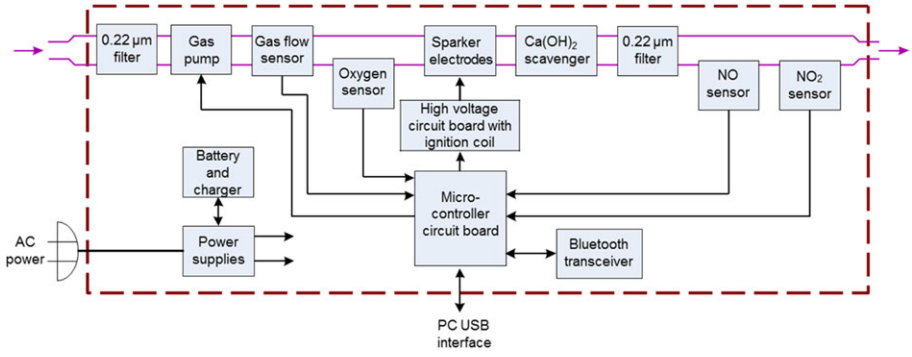

Figure 2

Detailed internal components of the NO generator. Purple arrows indicate gas entering and leaving the device. Black arrows indicate the sensors and pump that are connected to or controlled by the circuit board. Air or an O_2/N_2 mixture is pumped and filtered through a 0.22 µm high-efficiency particulate absorption (HEPA) air filter. The gas flow rate is measured with a meter. Sensors for O_2, NO and NO_2 indicate the concentration of each gas. The electrodes are powered by a microcontroller circuit. The $Ca(OH)_2$ scavenger and a 0.22 µm filter remove potential toxic gases (NO_2 and O_3) and metal particles before delivery of the gas. (From Yu *et al.*, 2015; used with permission). O_3, ozone.

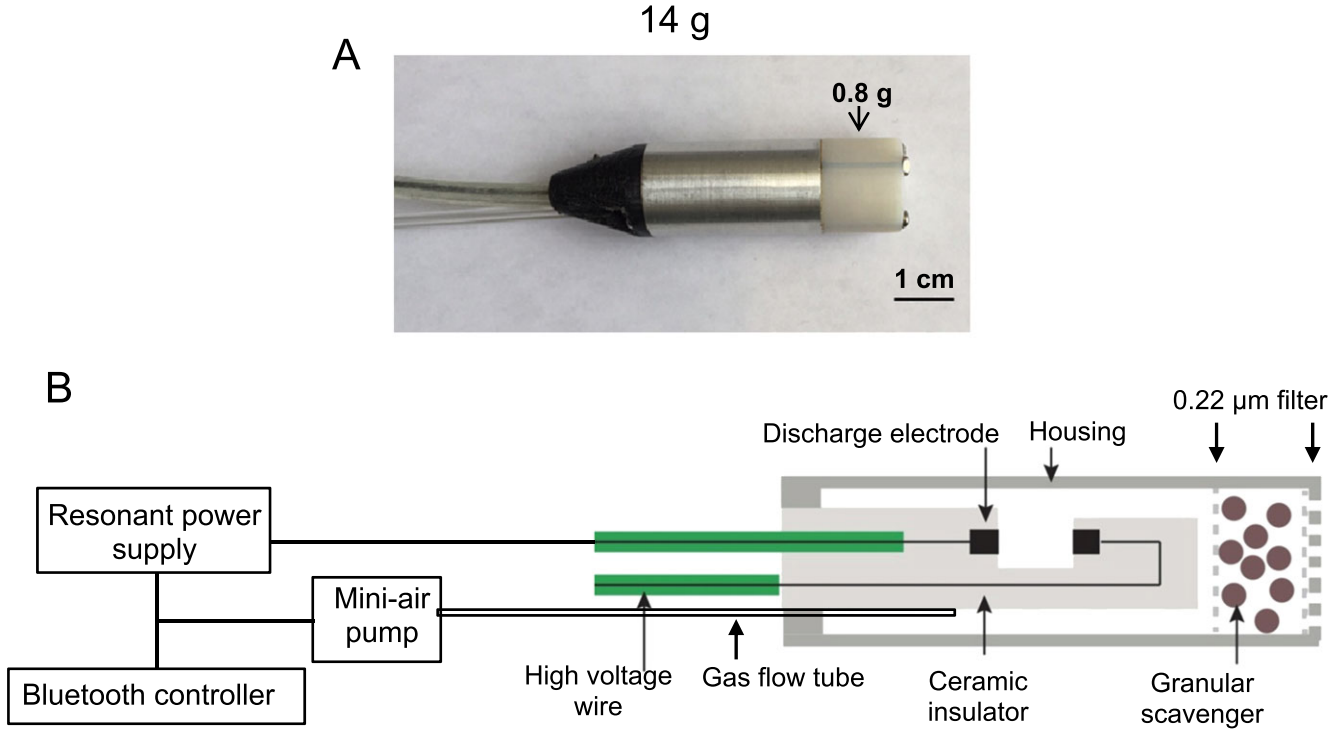

Figure 3

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^{-1} airflow) to facilitate NO delivery, a mini-air pump, resonant power supply and Bluetooth controller. The device is surrounded by a ceramic insulator. (From Yu *et al.*, 2018; used with permission).

Table 1

Summary of potential therapeutic applications of inhaled NO therapy

Cardiopulmonary diseases	Acute respiratory distress syndrome (ARDS) (Rossaint *et al.*, 1993; Benzing and Geiger, 1994; Troncy *et al.*, 1998; Gerlach *et al.*, 2003; Taylor *et al.*, 2004; Bronicki *et al.*, 2015; Albert *et al.*, 2017; Dowell *et al.*, 2017)
	Chronic obstructive pulmonary disease (COPD) (Barbera *et al.*, 1996; Yoshida *et al.*, 1997; Vonbank *et al.*, 2003; Hajian *et al.*, 2016)
	Bronchopulmonary dysplasia (BPD) (Schreiber *et al.*, 2003; Ballard *et al.*, 2006; Kinsella *et al.*, 2006; Askie *et al.*, 2018)
	Interstitial lung disease (ILD) (Blanco *et al.*, 2011)
	Cardiac or lung transplantation (Stobierska-Dzierzek *et al.*, 2001; Fojon *et al.*, 2005; Moreno *et al.*, 2009; Tavare and Tsakok, 2011)
	Myocardial ischemia/reperfusion injury (I/R) (Hataishi *et al.*, 2006; Janssens *et al.*, 2018)
	Cardiac arrest and cardiopulmonary resuscitation (Minamishima *et al.*, 2011; Kida *et al.*, 2014; Derwall *et al.*, 2015)
	Univentricular heart surgery (Latus *et al.*, 2016)
	Pulmonary hypertension during and after cardiac surgery (Elmi-Sarabi *et al.*, 2017)
	ST-elevation myocardial infarction (Janssens *et al.*, 2018)
	Elective multiple valve replacement surgery-prolonged CPB (Lei *et al.*, 2018)
Haemolytic diseases	Sickle cell disease (Atz and Wessel, 1997; Sullivan *et al.*, 1999; Weiner *et al.*, 2003; Head *et al.*, 2010; Gladwin *et al.*, 2011; Maitre *et al.*, 2015)
	Cerebral malaria (Gramaglia *et al.*, 2006; Serghides *et al.*, 2011; Hawkes *et al.*, 2015; Mwanga-Amumpaire *et al.*, 2015)
	Stored blood transfusion (Yu *et al.*, 2008, 2009b, 2010, 2012; Baron *et al.*, 2012, 2013; Lei *et al.*, 2012, 2018; Berra *et al.*, 2014; Marrazzo *et al.*, 2018)

Scanning electron microscopy and energy-disperse X-ray spectroscopy measurements demonstrated that a single HEPA filter was sufficient to remove all trace metal particles produced during prolonged NO generation (Yu *et al.*, 2018). An airflow of 70 mL·min^{-1} was sufficient to maintain the housing of the mini-NO device at an acceptable low temperature during prolonged NO generation.

In summary, the electric plasma NO generator produces therapeutic levels of NO from air, with scavenging and filtration systems that effectively eliminate toxic gas and metallic impurities from the effluent gas. The device provides safe, efficient and economical NO generation, which will expand the applications of NO therapy to hospitalized and ambulatory patients around the world. Table 1 lists conditions that may benefit from treatment with inhaled NO.

Conclusions

Inhaled NO is the first drug to produce selective pulmonary vasodilatation without reducing systemic arterial pressure. Inhaled NO is a life-saving therapy in children and adults with a variety of diseases. By 2018, an estimated half a million of Americans with various causes of pulmonary hypertension had received NO inhalation therapy. With the recent breakthrough invention and testing of an electric NO generator, a simple, lightweight and economic device to produce NO from air by pulsed electrical discharge, inhaled NO will be affordable and available to patients in developing countries. Furthermore, this portable NO generator is likely to expand the indications for inhaled NO therapy, especially for patients in the ambulatory setting.

The newly developed NO generator can produce therapeutic levels of NO gas, which can be delivered through a face mask, nasal cannulas, an endotracheal tube or a ventilator. Because the device is lightweight and inexpensive, we anticipate that the device will have a wide range of applications including (i) treatment of hospitalized patients with cardiac and/or pulmonary diseases, to replace heavy and expensive tanks; (ii) treatment of outpatients with chronic respiratory illnesses, either in the ambulatory setting or at home; (iii) facilitation of research and preclinical studies; (iv) treatment of patients who are facing extreme conditions, such as those with respiratory failure who are injured on the battle field or while fighting fires; and (v) treatment of pulmonary arterial hypertension, for example, in hypoxic mountain climbers (Scherrer *et al.*, 1996).

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Harding *et al.*, 2018), and are permanently archived in the Concise Guide to PHARMACOLOGY 2017/18 (Alexander *et al.*, 2017).

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Conflict of interest

W.M.Z. and B.Y. have filed patents at MGH on the electric generation of NO. W.M.Z. is on the scientific advisory board of Third Pole Inc., which has licensed patents on NO generators from MGH. Other authors declare no conflicts of interest.

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