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# Our paper 20 years later: Inhaled nitric oxide for the acute respiratory distress syndrome—discovery, current understanding, and focussed targets of future applications

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**Abstract** *Introduction:* More than 20 years have passed since we reported our results of treating patients with the acute respiratory distress syndrome (ARDS) with inhaled nitric oxide (iNO). The main finding was that iNO alleviated pulmonary hypertension (PH) by selective vasodilation of pulmonary vessels in ventilated lung areas. This, in turn, improved arterial oxygenation. *Methods:* We now set out to review the time span between the discovery of NO in 1987 and today in order to identify and describe interesting areas of research and clinical practice surrounding the application of iNO. *Major Findings:* Enhancement of ventilation–perfusion matching and alleviation of PH in ARDS, treatment of PH of the newborn, and treatment of perioperative PH in congenital heart disease serve as just a few exciting examples for the successful use of iNO. Breathing NO prevents PH induced by stored blood transfusions or sickle cell disease. Exploiting the anti-inflammatory properties of NO helps to treat malaria. *Discussion:* Regarding the use of iNO in ARDS, there remains the unresolved question of whether important outcome parameters can be positively influenced. At first glance, several randomized controlled trials and meta-analyses seem to send the clear message: "There is none!" Careful analyses, however, leave sufficient room for doubt that the ideal study to produce the unequivocal proof for the inability of iNO to positively impact on important outcome parameters has, as yet, not been conducted. *Conclusion:* In summary, the discovery of and research on the many positive effects of iNO has improved care of critically ill patients worldwide. It is a noble effort to continue on this path.

**Keywords** Nitric oxide ·
Acute respiratory distress syndrome ·
Pulmonary arterial hypertension ·
Selective vasodilation ·
Arterial oxygenation

## Inhaled nitric oxide

In 1993, we published a paper in the *New England Journal of Medicine* reporting 10 patients with severe acute respiratory distress syndrome (ARDS) who inhaled 5–20 parts per million (ppm by volume) nitric oxide (NO). Breathing NO caused rapid and selective vasodilation of pulmonary vessels in ventilated lung regions leading to increased arterial oxygenation [1].

## Historical context

Pulmonary arterial hypertension (PAH) and intrapulmonary right-to-left shunting of venous blood are key and universal characteristics of ARDS. PAH contributes to lung oedema and, when PAH is severe, it can produce right heart dysfunction and cardiac failure. Reducing the elevated pulmonary artery pressure (PAP) was assumed to be beneficial. Before 1993, the drugs studied to reduce the PAP in ARDS were intravenous vasodilators, which reduced both the PAP and also the systemic blood pressure. Since patients with ARDS often have systemic vasodilation resulting from concomitant sepsis, intravenous vasodilators further decreased the systemic vascular resistance and arterial pressure, and at times produced right ventricular ischemia and consequent heart failure. Moreover, infusing an intravenous vasodilator produced global vasodilation of all the vessels. During ARDS, systemic vasodilator therapy indiscriminately vasodilated the pulmonary vasculature in both ventilated as well as non-ventilated lung regions, increasing ventilation–perfusion mismatch and leading to deterioration of pulmonary gas exchange. Therefore, in most clinical settings with acute lung injury, it was not feasible or useful to infuse intravenous vasodilators to treat PAH. Beginning in 1980, Furchgott et al. [2] reported that acetylcholine stimulates endothelial cell receptors triggering the production of a substance that diffuses into vascular smooth muscle cells and causes relaxation. He named this substance "endothelium-derived relaxing factor" (EDRF). In 1987, two independent researchers established that EDRF was the small gaseous molecule NO [3, 4]. Since NO is a byproduct of producing various gases and a common atmospheric pollutant, it was readily available to laboratory researchers. Within a short period, studies in large animals and humans demonstrated that, when NO was inhaled in low doses (5–80 ppm), it rapidly produced selective pulmonary vasodilation (Fig. 1). NO is instantly inactivated by the dioxygenation reaction when it diffuses into blood vessels containing oxyhaemoglobin. In 1991, Frostell et al. [5] reported the ability of inhaled NO to selectively induce pulmonary vasodilation in an ovine model of PAH. Pepke-Zaba et al. [6] studied the effect of inhaled NO in adults that suffered from chronic PAH. These and other promising clinical studies rapidly led us to evaluate inhaled NO as a therapy in patients with severe ARDS. Figure 2 displays selected temporal highlights surrounding the discovery of inhaled NO as a selective pulmonary vasodilator.

## Methods and major findings of our study

In our study, we compared the short-term effects of breathing two concentrations of NO (18 and 36 ppm) with an intravenous infusion of prostacyclin (4 ng/kg/min). We measured systemic and pulmonary haemodynamics, pulmonary gas exchange and ventilation–perfusion distributions (assessed by the multiple inert gas elimination technique [7, 8]). Each drug was given for 40 min to ten consecutive patients with severe ARDS. In these patients, we found breathing NO induced selective pulmonary vasodilation leading to an immediate reduction of PAP. NO inhalation did not alter either the systemic arterial pressure (SAP) or cardiac output. Surprisingly, during NO breathing, we recorded a concurrent improvement of arterial oxygenation. Analysis of ventilation–perfusion distributions revealed that this effect was caused by shifting pulmonary blood flow from collapsed and shunting lung areas to ventilated regions. In contrast, intravenous prostacyclin reduced PAP, SAP and systemic oxygenation assessed as the ratio of the partial pressure of arterial oxygen divided by the fraction of inspired oxygen ($PaO_2/FiO_2$).

In seven of ten patients, NO inhalation was continued for periods of 3–53 days. NO treatment was terminated if the $PaO_2/FiO_2$ ratio remained >250 mmHg in patients without extracorporeal membrane oxygenation (ECMO) during daily periods of NO cessation. The daily interruption of NO inhalation for 30 min revealed that breathing NO caused a reproducible decrease in PAP without influencing mean SAP and cardiac output, as well as augmenting arterial oxygenation due to a decrease of pulmonary right-to-left shunting (Fig. 3). Our study was the first to show that, in patients with ARDS, the inhalation of a gaseous vasodilator (NO) induced selective vasodilation in ventilated lung regions, a concept which was later confirmed by other researchers who studied inhaled NO or aerosolized prostacyclin [9].
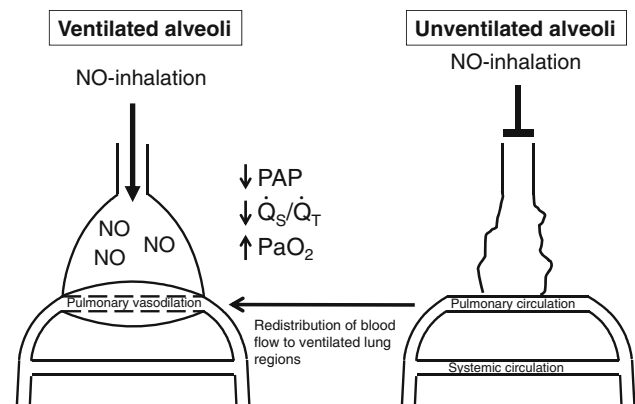


**Fig. 1** Selective vasodilation in ventilated lung areas by inhaled NO. *NO* nitric oxide, *PAP* pulmonary arterial pressure, $\dot{Q}_S/\dot{Q}_T$ intrapulmonary right-to-left shunt, $PaO_2$ partial pressure of oxygen in arterial blood
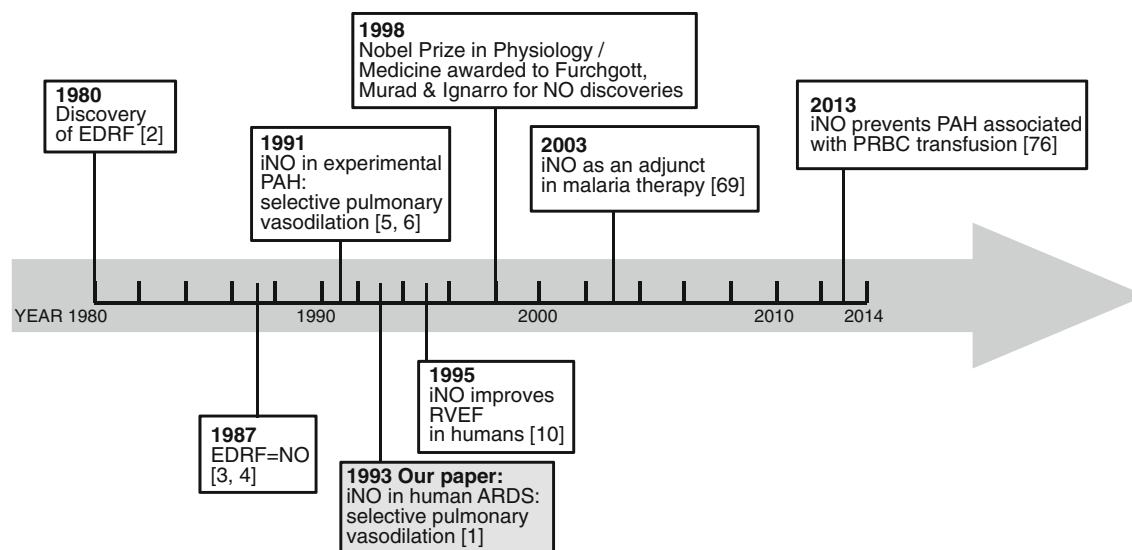
**Fig. 2** Our paper in the historical context, highlights of iNO-history, and therapeutic use of iNO in adults. *EDRF* endothelium-derived relaxing factor, *NO* nitric oxide, *iNO* inhaled nitric oxide, *ARDS* acute respiratory distress syndrome, *RVEF* right ventricular ejection fraction, *PAH* pulmonary arterial hypertension, *PRBC* packed red blood cells, *PaO₂* partial pressure of oxygen in arterial blood, *numbers in brackets* refer to the reference list

## Long-term effects of inhaled NO in ARDS

In a subsequent study, we reported that the use of a selective pulmonary vasodilator improved the ejection fraction of the right heart without producing systemic vasodilation [10]. Due to the short-term haemodynamic and gas exchange focus of our initial study of ten ARDS patients, we could not learn whether inhaled NO could improve important ARDS outcome parameters like organ failure-free days or survival. Subsequently, nine randomized controlled trials (RCT) have been performed to learn the long-term effects of breathing NO during ARDS [11–19]. These studies again demonstrated that inhaled NO reduced PAP and intrapulmonary right-to-left shunt and improved arterial oxygenation. These favourable effects, however, only appeared to last for 24–48 h. In ARDS patients, there were no significant differences of organ failure-free days, length of ICU stay or hospital mortality with inhaled NO.

A major criticism of these nine RCTs is that they were performed in patients with both severe ARDS (PaO₂/FiO₂ <100 mmHg) and moderate ARDS (PaO₂/FiO₂ >100 and <200 mmHg). A recent meta-analysis analysed all nine studies (including 1,142 patients) with respect to the effect of breathing NO upon mortality, and performed a sub-analysis of seven studies (1,070 patients total) to attempt to differentiate between the effect of NO treatment in patients with moderate and severe ARDS [20]. No beneficial effect of inhaled NO with respect to long-term outcome was discerned in either severity group. One limitation of this study is that the authors included only 329 patients in their analysis of severe ARDS and no

trend was detectable. In all of these studies patients usually received a tidal volume of over 6 ml/kg ideal body weight during mechanical ventilation. Up to now, it is unknown, but conceivable, that a protective ventilator strategy in combination with inhaled NO leads to more positive results. Experience from the use of prone position in ARDS has taught us that an improvement in outcome was pronounced if this treatment option was combined with a lung protective mechanical ventilation strategy [21].

Additive or synergistic effects of inhaled nitric oxide (iNO) and other treatment modules were reported by a number of other studies. These studies detected enhanced beneficial effects when iNO was combined with measures to enhance alveolar recruitment, such as positive end-expiratory pressure [22], high frequency ventilation [23], recruitment manoeuvres [18], prone position [24], and surfactant instillation [25, 26]. There is, however, no randomized trial assessing such combined effect on the outcome of patients with severe ARDS.

It is possible that inhaled NO might reduce the need for more invasive treatment strategies such as ECMO. This would be of interest, as Gerlach et al. [17] demonstrated, that the inhaled NO treatment group had a reduced need for invasive and costly extracorporeal membrane oxygenation (ECMO) therapy.

In summary, in the current era of protective mechanical ventilation, some clinicians employ NO breathing for a period of 24–48 h as a rescue intervention for severely hypoxemic patients to buy time and allow other therapies (ECMO, prone positioning, etc.) to improve the patients' pulmonary gas exchange. To date, however, there remain

doubts whether improved oxygenation is an adequate outcome parameter in ARDS patients [27–30]. Furthermore, iNO-response may differ dose- and time-dependently between patients [31–33]. About one-third of all ARDS patients are non-responders to iNO, i.e. their oxygenation does not improve relevantly. It is disappointing, however, that, regardless of whether patients respond or do not respond, iNO does not influence important long-term outcome parameters [14].

## Inhaled NO in neonatal hypoxaemia and persistent pulmonary hypertension

Term gestation PPH

While we were studying inhaled NO in adult ARDS, breathing NO was evaluated in term gestation neonates with persistent pulmonary hypertension (PPH) and other hypoxaemic preterm infants. PPH in the newborn may occur as a consequence of various congenital and

acquired conditions: PPH may be idiopathic or associated with premature closure of the ductus arteriosus, meconium aspiration, prematurity, or lung hypoplasia. In cases of premature closure of the ductus arteriosus and closure of the foramen ovale, right heart failure and systemic hypotension can occur. If PPH co-exists with a patent ductus arteriosus and foramen ovale, right-to-left shunting of venous blood can produce severe systemic hypoxaemia. In 1992, two independent teams of researchers described the beneficial effects of inhaled NO in PPH by reducing the PAP and right-to-left shunting of venous blood which markedly improved systemic oxygenation [34, 35]. Reduction of pulmonary hypertension occurred without decreasing the systemic blood pressure. Several years later, two prospective RCTs demonstrated breathing NO by PPHN infants reduced the need for invasive and increased survival rates (as a combined endpoint). In addition, neonates treated with NO had better lung function at 30 days [36, 37]. In 2001, these studies and others led the FDA to approve inhaled NO for treating hypoxemia in term neonates.
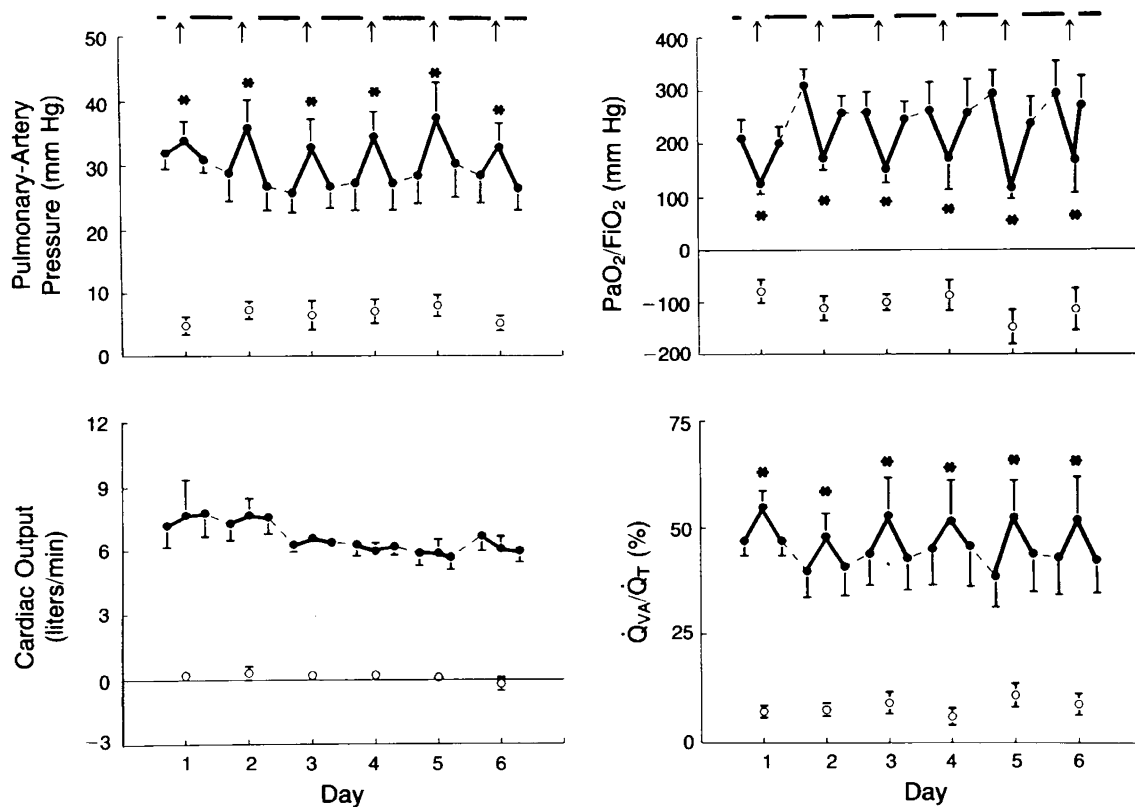


**Fig. 3** Haemodynamic function and gas exchange before, during, and after brief interruptions (*arrows*) of nitric oxide inhalation (*bars*) during the first 6 days of treatment in seven patients with ARDS. Values are means ± SE (*solid symbols*); also shown (*open symbols*) are the means ± SE of the individual differences between the values for the effect of treatment and the means of the values determined before and after interruption of nitric oxide therapy. The standard errors for the treatment effects were small, indicating that the effects of withdrawal of nitric oxide were clear and precisely estimated. Each *asterisk* denotes a significant difference from the means of the values determined before and after interruption of nitric oxide therapy (from [1], Copyright © (1993) Massachusetts Medical Society. Reprinted with permission)

**Table 1** Side effects of inhaled NO in humans

| Unwanted effect | Consequences | Recommendation for clinical practice |
| --- | --- | --- |
| Formation and inhalation of nitrogen dioxide ($NO_2$) | Alteration of alveolar permeability | Limitation of NO concentration to ≤10 ppm when long-term application is necessary |
| | Pulmonary oedema | |
| | Alveolar haemorrhage | |
| | Hypoxaemia | |
| | Alteration of surfactant activity | Monitor $NO_2$ levels and set alarm levels to 2 ppm $NO_2$ |
| | Methaemoglobinaemia | |
| | Intrapulmonary accumulation of fibrin and immune cells | |
| | Death | |
| Methaemoglobinaemia | Dyspnoea | Measurement of methaemoglobin levels continuously by transcutaneous measurement (e.g., Radical 7 of Massimo Corp) and in blood at 4 h after first NO application and then once daily |
| | Hypoxia | |
| | Cyanosis | |
| | Dysrhythmia | |
| | Loss of consciousness, coma | Stop NO breathing at methaemoglobin levels >5 % |
| | Seizures | No NO breathing for patients with methaemoglobin reductase deficiency |
| | Death | |
| Systemic vasodilation | Hypotension | Monitoring blood pressure and SVR |
| | Reduction of systemic vascular resistance (SVR) | |
| Coagulation disorders | Prolongation of bleeding time in some patients | Monitoring coagulation parameters |
| | Changes of coagulation parameters in some patients | |
| Nephrotoxicity | Progression of renal disease | Higher iNO concentrations may contribute to progression of renal disease |
| | Risk of renal failure | Monitoring of renal function |
| Rebound after iNO withdrawal | Pulmonary vasospasm | Slowly weaning from NO |
| | | Elevation of $FIO_2$ before iNO is stopped |
| | Elevation of intrapulmonary right-to-left shunt | Switch to a different pulmonary vasodilator during the weaning process |
| | Hypoxia | |
| | Haemodynamic side effects | |

Weaning the patient from breathing NO confronts the clinician with a special management problem: a "rebound phenomenon" of pulmonary vasoconstriction following acute NO withdrawal. Especially, when NO treatment is abruptly ceased, acute rebound vasoconstriction is common. Worsening of gas exchange was found in 48 % of acutely weaned patients and 26 % exhibited haemodynamic impairment [68]. Thus, NO breathing must be weaned slowly and judiciously.

In summary, NO breathing can be managed safely, given adequate attention to the well-described side effects (Table 1).

## Future directions in inhaled NO application

Malaria

Two recent laboratory studies have reported that NO may play a useful role as an adjunctive therapy to artesunate in severe or cerebral malaria due to its anti-inflammatory properties [69, 70]. Since plasma and urine nitrate levels are extremely low in severe malaria patients there may be value in replacing NO biometabolites with inhaled NO. In malaria, angiopoetin-2 release contributes to activation of the endothelium, which can be inhibited by NO. NO decreases inflammation and reduces the adhesion of parasitized erythrocytes to endothelium [69]. Thus inhaled NO could reduce inflammation and buy time for therapeutic drugs (e.g., artesunate) to eliminate the malaria parasites [70]. At present, there are two ongoing phase II RCTs in Uganda studying the safety and efficacy of breathing 80 ppm NO versus $N_2$, both gases given with artesunate, with a primary endpoint of altering angiopoietin levels [71, 72]. The latter is a quantitative biomarker of malaria severity [73, 74]. Unfortunately, recent abstracts suggest that, although breathing NO was safe to administer in severe and cerebral malaria, there was no evidence of effectiveness by altering angiopoietin levels (personal communication from Ryan Caroll and Kevin Kain).

Stored blood transfusion

Transfusion of packed red blood cells stored for over 2 weeks is an independent risk factor for organ failure and

mortality [75]. Recently, it was reported that resuscitation of lambs from haemorrhagic shock with autologous stored red blood cells induces pulmonary hypertension and inflammation. Breathing NO prevented these adverse consequences [76]. Transfusion of stored leukoreduced autologous red cells produces transient acute pulmonary hypertension in obese human volunteers with endothelial dysfunction. Breathing NO prevents the pulmonary hypertension. This is an area of active investigation.

INO in sickle cell disease

Despite initial enthusiasm for treating sickle cell crisis patients with inhaled NO, a large multicentre study showed no reduction of pain scores with NO breathing [77]. Thus, there is no reason to treat painful crises with inhaled NO. However, the pulmonary hypertension that occurs in sickle cell chest crisis does respond by vasodilation to inhaled NO [78].

## Conclusion

The study we published in 1993 was one of the first to provide evidence for selective pulmonary vasodilation produced by breathing NO in adult ARDS patients. It offered the remarkable advantage of lowering the elevated pulmonary artery pressures, improving arterial oxygenation, and protecting the right ventricle from failure without reducing systemic arterial resistance or pressure. iNO proved superior to classical intravenous vasodilators in ARDS, since breathing NO reduced and did not increase the pulmonary right-to-left shunt fraction. Researchers and clinicians working in the fields of neonatology and cardiac surgery were stimulated by this paper to explore NO therapy for hypoxemic infants with PPH, and children and adults undergoing cardiac surgery. Unfortunately, researchers studying ARDS never demonstrated an improved outcome with iNO treatment. However, breathing NO is still used to buy time to institute other therapies in severely hypoxemic ARDS. Perhaps, now that low volume ventilation has become standard therapy in ARDS, a trial of breathing NO to improve the outcome of ARDS patients should be considered.

Future research may yield synergistic beneficial effects in terms of outcome between iNO and other advanced ARDS treatment modules.

**Conflicts of interest** None.

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