

Review

Bench-to-bedside review: Inhaled nitric oxide therapy in adults

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Abstract

Nitric oxide (NO) is an endogenous mediator of vascular tone and host defence. Inhaled nitric oxide (iNO) results in preferential pulmonary vasodilatation and lowers pulmonary vascular resistance. The route of administration delivers NO selectively to ventilated lung units so that its effect augments that of hypoxic pulmonary vasoconstriction and improves oxygenation. This 'Bench-to-bedside' review focuses on the mechanisms of action of iNO and its clinical applications, with emphasis on acute lung injury and the acute respiratory distress syndrome. Developments in our understanding of the cellular and molecular actions of NO may help to explain the hitherto disappointing results of randomised controlled trials of iNO.

Introduction

Nitric oxide (NO) is an important determinant of local blood flow and is formed by the action of NO synthase (NOS) on the semi-essential amino acid L-arginine in the presence of molecular oxygen. Inhaled NO (iNO) results in preferential pulmonary vasodilatation and lowers pulmonary vascular resistance (PVR), augments hypoxic pulmonary vasoconstriction (HPV), and improves oxygenation. These effects are harnessed in the therapeutic applications of iNO to patients with acute lung injury (ALI)/acute respiratory distress syndrome (ARDS), to those with acute right ventricular failure (RVF) complicating cardiac surgery or acute pulmonary embolism, or in acute sickle chest crisis. Despite dramatic physiological improvements that are often seen during the therapeutic use of iNO, there remains a lack of evidence concerning any beneficial effect on outcomes. This 'Bench-to-bedside' review focuses on the mechanisms of action of iNO and its clinical applications, with particular attention to ALI and ARDS. Alterations in endogenous NO production and the use of exogenous intravenous NO donors in acute inflammatory conditions are beyond the scope of this review.

Administration of inhaled nitric oxide to adults

The licensed indication of iNO is restricted to persistent pulmonary hypertension in neonates, yet most iNO is administered for unlicensed indications. Pharmaceutical iNO is available at a very high cost, and in light of this and concerns over potential adverse effects of iNO, international guidelines have been developed. An advisory board under the auspices of the European Society of Intensive Care Medicine and the European Association of Cardiothoracic Anaesthesiologists published its recommendations in 2005 [1]. Although this valuable project was sponsored by the manufacturer of iNO (INO Therapeutics, now part of Ikaria Holdings, Clinton, NJ, USA), the board stated that the sponsor had no authorship or editorial control over the content of the meetings or any subsequent publication.

iNO is administered most commonly to invasively ventilated patients, although other routes are possible. To minimise the admixture of high concentrations of oxygen with NO (risk of nitrogen dioxide [NO_2] formation), the NO/nitrogen mixture is introduced into the inspiratory limb of the ventilator tubing as near to the patient as possible. It is obligatory to monitor the NO and NO_2 concentrations, and although concentrations of iNO administered clinically should not cause methemoglobinemia, guidelines recommend that methemoglobin levels be measured regularly. iNO administration reduces endogenous NO production, and therefore rapid withdrawal of iNO can cause a significant rebound pulmonary hypertension, but in clinical practice, this can be avoided by gradual withdrawal [2].

There is marked variation in response to iNO between patients [2] and in the same patient at different times. After prolonged use, there is a leftward shift in the dose-response curve such that, without regular titration against a therapeutic

ALI = acute lung injury; ARDS = acute respiratory distress syndrome; Hb = haemoglobin; HPV = hypoxic pulmonary vasoconstriction; iNO = inhaled nitric oxide; iNOS = inducible nitric oxide synthase; NO = nitric oxide; NO_2 = nitrogen dioxide; NOS = nitric oxide synthase; $\text{PaO}_2/\text{FiO}_2$ = arterial partial pressure of oxygen/fraction of inspired oxygen; PVR = pulmonary vascular resistance; RCT = randomised controlled trial; RNS = reactive nitrogen species; RV = right ventricle; RVF = right ventricular failure; SCD = sickle cell disease; SMC = smooth muscle cell.

goal, there is a risk of excessive iNO administration, associated with toxicity and loss of the therapeutic effect [3]. A survey of 54 intensive care units in the UK revealed that the most common usage was in treating ARDS, followed by pulmonary hypertension [4], in keeping with results of a European survey [5]. By contrast, a survey of therapeutic iNO usage in adult patients from a single US centre (2000 to 2003) demonstrated that the most common application was in the treatment of RVF in patients after cardiac surgery and then, in decreasing order, orthotopic heart transplantation, ventricular assist device placement, medical patients (mostly with refractory hypoxaemia), orthotopic lung transplantation, and for hypoxaemia in other surgery [6].

Inhaled nitric oxide in acute lung injury and acute respiratory distress syndrome

ALI and its extreme manifestation, ARDS, are characterised by hypoxaemia despite high inspired oxygen ($\text{PaO}_2/\text{FiO}_2$ [arterial partial pressure of oxygen/fraction of inspired oxygen] ratios of less than 300 mm Hg [40 kPa] and less than 200 mm Hg [27 kPa], respectively) in the context of a known cause, evidence of pulmonary oedema, and the absence of left atrial hypertension suggestive of a cardiogenic mechanism [7]. Pathologically, there is alveolar inflammation and injury leading to increased pulmonary capillary permeability and resultant accumulation of alveolar fluid rich in protein and inflammatory cells. This is manifest clinically as hypoxaemia, ventilation-perfusion mismatch, physiological shunting, atelectasis, and reduced compliance.

Since 1993, when the first investigation on the effects of iNO on adult patients with ARDS was published [8], there have been several randomised controlled trials (RCTs) examining the role of iNO in ALI/ARDS (Table 1). The first systematic review and meta-analysis [9] scrutinised five RCTs and found no beneficial effect on mortality or ventilator-free days, but given wide confidence intervals, the authors concluded that the effects were uncertain. More recently, a meta-analysis considered 12 RCTs that included a total of 1,237 patients [10] and came to conclusions that were more definitive: no benefit was seen on mortality but there was improved oxygenation at 24 hours (13% improvement in $\text{PaO}_2/\text{FiO}_2$ ratio) at the cost of increased risk of renal dysfunction (relative risk 1.50, 95% confidence interval 1.11 to 2.02). Indeed, the authors highlight a trend to increased mortality in patients receiving iNO and suggest that it not be used in ALI/ARDS. However, findings from meta-analyses of many small underpowered RCTs have significant limitations and should be viewed as hypothesis-generating, not authoritative. Further elucidation of why iNO may fail to improve patient outcomes stems from understanding recent advances in our knowledge of the biology of iNO, particularly those actions that occur outside the pulmonary vasculature.

The biological action of inhaled nitric oxide

NO is a naturally occurring colourless and odourless gas. In biological solutions, it is highly diffusible in water, with a half-

life of seconds. NO was regarded mainly as an environmental pollutant prior to its identification as an endothelium-derived relaxing factor and an important determinant of local blood flow [11]. NO has an unpaired electron and, as such, reacts very rapidly with other free radicals, certain amino acids, and transition metal ions. In biological solutions, it is stabilised by forming complexes.

The canonical source of endogenous NO is the action of NOS on the semi-essential amino acid L-arginine in the presence of molecular oxygen. Neuronal NOS was the first isoform to be identified, followed by inducible NOS (iNOS or NOS2), and finally endothelial NOS (eNOS or NOS3). iNOS is calcium-independent and generates higher concentrations of NO [12] than the other isoforms do. Its activity is implicated in the pathogenesis of the vasoplegia that characterises septic shock.

Exogenous NO is administered by controlled inhalation or through intravenous administration of NO donors such as sodium nitroprusside or glyceryl trinitrate. Traditionally, iNO was thought to work exclusively in the lung, and thus be free from remote or non-pulmonary effects, through immediate inactivation by circulating haemoglobin (Hb). However, appreciation of the remote effects of iNO has highlighted the importance of the actions of NO on circulating targets (Figure 1).

First, proteins including Hb and albumin contain reduced sulphur (thiol) groups that react reversibly with NO. Previously, NO was considered to react with oxyhaemoglobin to form methemoglobin and nitrate or heme iron nitrosyl Hb and thereby lose all vasodilating properties. However, a stable derivative that retains vasodilatory properties is formed by a reaction resulting in nitrosylation of a conserved cysteine residue of the β subunit of Hb: S-nitrosylated-Hb (SNO-Hb). This reaction is favoured in the presence of oxyhaemoglobin, whereas binding of NO to the heme iron predominates in the deoxygenated state [13]. As such, circulating erythrocytes may effectively store and release NO peripherally in areas of low oxygen tension, augmenting microvascular blood flow and oxygen delivery via hypoxic vasodilation of systemic vascular beds [14]. Thus, in isolation, NO can act as an autocrine or paracrine mediator but when stabilised may exert endocrine influences [15].

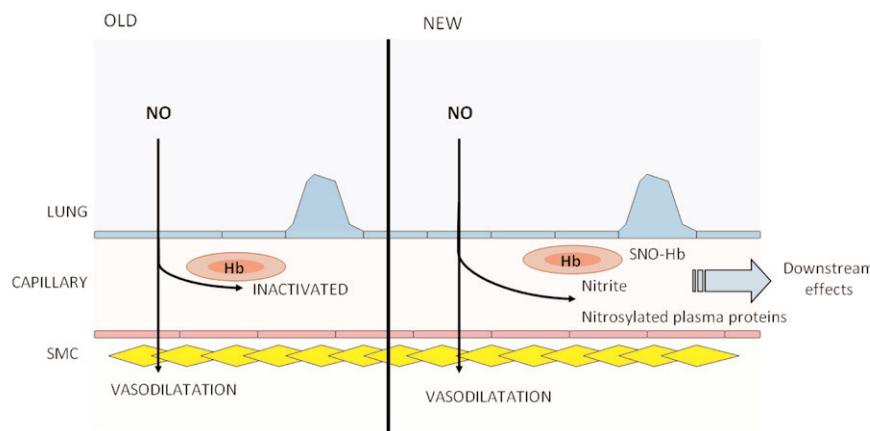
Second, in addition to *de novo* synthesis, supposedly inert anions nitrate (NO_3^-) and nitrite (NO_2^-) can be recycled to form NO. Indeed, it has been suggested that nitrite mediates extra-pulmonary effects of iNO [16]. In the absence of molecular oxygen (hypoxic environment), NOS cannot produce NO and deoxyhaemoglobin catalyses NO release from nitrite, thus potentially also providing a hypoxia-specific vasodilatory effect. Given that effects of iNO are mediated in part by S-nitrosylation of circulating proteins, therapies aiming at directly increasing S-nitrosothiols have been developed. In a small observational study, inhaled ethyl nitrite safely

Table 1
Studies of inhaled nitric oxide in adult patients with acute lung injury/acute respiratory distress syndrome

Study	Year	Number of patients/centres	Intervention		Primary outcome	Secondary outcomes
			Patient details	Inhaled nitric oxide		
Delinger, et al. [48]	1998	177/30	AEC/ARDS within 72 hours. Excluded severe sepsis or non-pulmonary organ failure	Nitrogen	1.25 to 80 ppm	Oxygenation, PAP, and 28-day survival
Michael, et al. [49]	1998	40/1	ARDS with PFR <150 mm Hg and CXR infiltrates	Usual care	5, 10, 15, and 20 ppm every 6 hours for 24 hours, then clinically adjusted	Persistence of improvements in oxygenation
Troncy, et al. [50]	1998	30/1	Lung injury score ≥2.5	Usual care	Initial titration (2.5, 5, 10, 20, 30, and 40 ppm every 10 minutes) and daily re-titration	30-day mortality and duration of MV
Lundin, et al. [51]	1999	268/43	CXR infiltrates and ARDS with PFR <165 mm Hg with MV for 18 to 96 hours	Usual care	2, 10, or 40 ppm (lowest effective dose)	Reversal of ALI 30- and 90-day survival, ICU and hospital LOSs, and organ failure
Gerlach, et al. [3]	2003	40/1	AEC/ARDS, $\text{FiO}_2 \geq 0.6$, PFR ≤150 mm Hg, PEEP ≥10 cm H ₂ O, and PAOP ≤18 mm Hg. Duration of ventilation ≥48 hours.	Usual care	10 ppm (with daily dose-response analysis)	Dose-response relationship with PaO_2 and iNO Duration of ventilation and ICU LOS
Park, et al. [52]	2003	23/1	AEC and duration of ARDS ≤2 days	Usual care	5 ppm ± recruitment manoeuvres	Oxygenation -
Taylor, et al. [53]	2004	385/46	ARDS with PFR <250. Excluded sepsis as cause of ALI and any non-pulmonary organ failure.	Nitrogen	5 ppm in 192 patients	Survival without need for MV during the first 28 days Oxygenation and PEEP and 28-day survival

AECC, American-European Consensus Conference (definitions); ALI, acute lung injury; ARDS, acute respiratory distress syndrome; CXR, chest x-ray; FiO_2 , fraction of inspired oxygen; ICU, intensive care unit; iNO, inhaled nitric oxide; LOS, length of stay; MV, mechanical ventilation; PaO_2 , arterial partial pressure of oxygen; PAOP, pulmonary artery occlusion pressure; PAP, pulmonary artery pressure; PEEP, positive end-expiratory pressure; PFR, $\text{PaO}_2/\text{FiO}_2$ (arterial partial pressure of oxygen/fraction of inspired oxygen) ratio; ppm, parts per million.

Figure 1



New paradigm of inhaled nitric oxide (NO) action. This figure illustrates the interactions between inhaled NO and the contents of the pulmonary capillaries. Previously, NO was considered to be inactivated by haemoglobin (Hb), and now it is recognised that, both through the interaction of Hb with NO and the formation of S-nitrosylated-Hb (SNO-Hb) and through the nitrosylation of plasma proteins and the formation of nitrite, the inhaled NO has effects downstream to the lungs. SMC, smooth muscle cell.

reduced PVR without systemic side effects in persistent pulmonary hypertension of the newborn [17]. In animal models, pulmonary vasodilatation was maximal in hypoxia and had prolonged duration of action after cessation of administration [18].

When inhaled with high concentrations of oxygen, gaseous NO slowly forms the toxic product NO_2 . Other potential reactions include nitration (addition of NO_2^+), nitrosation (addition of NO^+), or nitrosylation (addition of NO). Furthermore, NO may react with reactive oxygen species such as superoxide to form reactive nitrogen species (RNS) such as peroxynitrite (ONOO^-), a powerful oxidant that can decompose further to yield NO_2 and hydroxyl radicals. NO is therefore potentially cytotoxic, and covalent nitration of tyrosine in proteins by RNS has been used as a marker of oxidative stress.

Cardiovascular effects

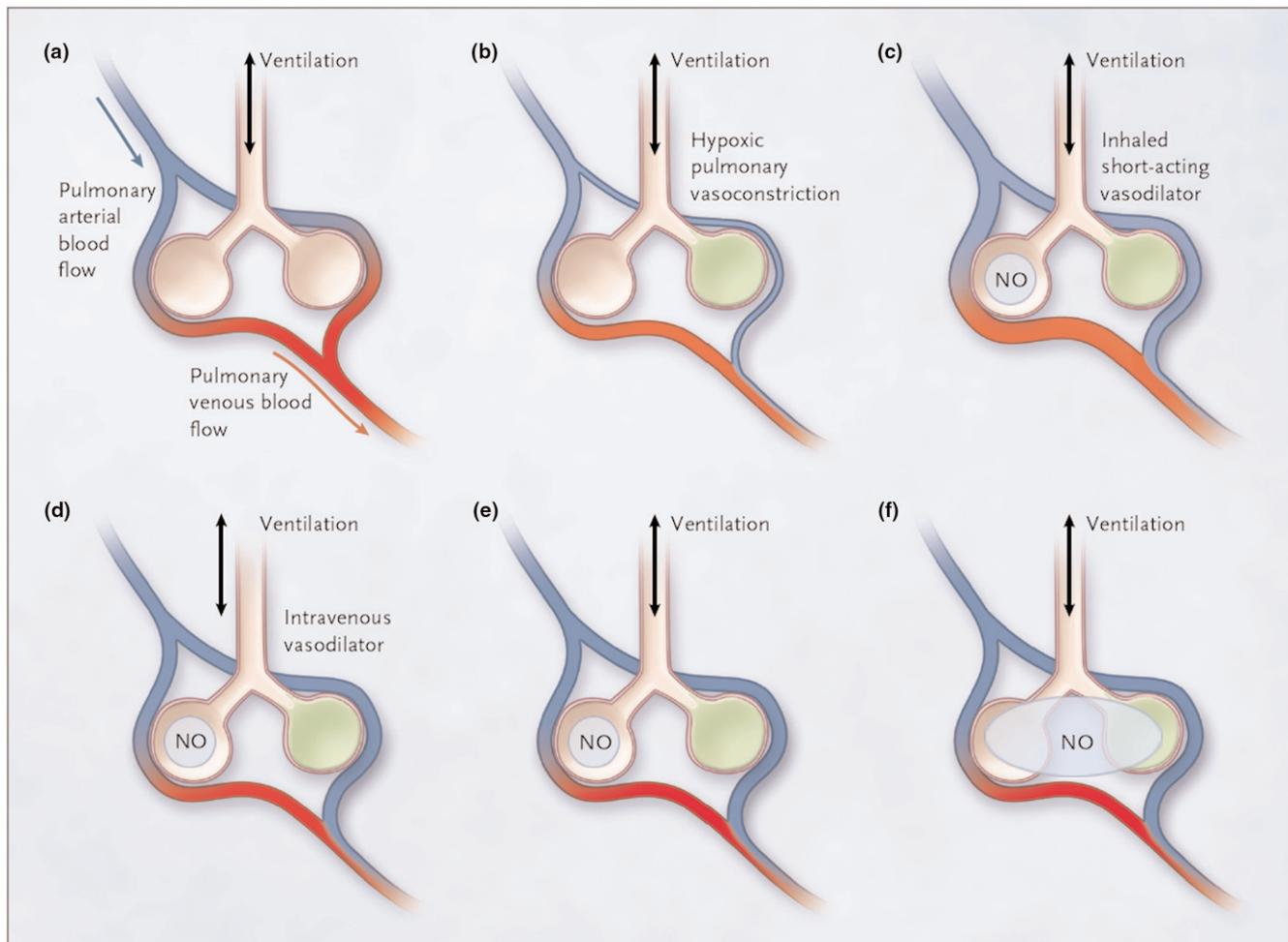
NO activates soluble guanylyl cyclase by binding to its heme group, and consequently cyclic guanosine 3'5'-monophosphate (cGMP) is formed, in turn activating its associated protein kinase. This protein kinase decreases the sensitivity of myosin to calcium-induced contraction and lowers the intracellular calcium concentration by activating calcium-sensitive potassium channels and inhibiting the release of calcium from the sarcoplasmic reticulum. These changes cause smooth muscle cells (SMCs) to relax. iNO causes relaxation of SMCs in the pulmonary vasculature with a resultant decrease in PVR. The right ventricle (RV) is exquisitely sensitive to afterload, and if RV function is impaired, it may respond favourably to the decreased afterload, improving cardiac output. iNO must be used with caution in the presence of left ventricular impairment as the decrease in PVR may permit increased right ventricular output to a greater extent than the left ventricle can accommodate and this may excessively

increase the left atrial pressure, causing or exacerbating pulmonary oedema. Similarly, pulmonary oedema can result from disproportionate vasodilatation of the pre-capillary compared with post-capillary vasculature, causing an increased transpulmonary gradient.

iNO augments the normal physiological mechanism of HPV and improves ventilation-perfusion matching and systemic oxygenation (Figure 2). In the absence of hypoxaemia being caused by ventilation-perfusion mismatching and HPV, the beneficial effects of iNO on oxygenation are severely limited. Indeed, experimental data confirm that intravenously administered vasodilators worsen oxygenation by counteracting HPV [3]. Further signs of the extent of non-pulmonary effects of iNO are increased renal blood flow and improved hepatic tissue oxygenation [14].

Non-cardiovascular effects relevant to lung injury

Neutrophils are important cellular mediators of ALI. Limiting neutrophil adherence experimentally and production of oxidative species and lytic enzymes reduce lung injury. In neonates, prolonged iNO diminished neutrophil-mediated oxidative stress [19], and in animal models, neutrophil deformability and CD18 expression were reduced [20] with resultant decreases in adhesion and migration [21]. These changes limit damage to the alveolar-capillary membrane and the accumulation of protein-rich fluid within the alveoli. Platelet activation and aggregation, microthrombosis, and intra-alveolar deposition contribute to ALI. iNO attenuates the procoagulant activity in animal models of ALI [22] and a similar effect is seen both in patients with ALI [23] and in healthy volunteers [23,24]. In patients with ALI, decreased surfactant activity in the alveoli contributes to impaired pulmonary function and is of prognostic significance [25].

Figure 2

Hypoxic pulmonary vasoconstriction (HPV). (a) Normal ventilation-perfusion (VQ) matching. (b) HPV results in VQ matching despite variations in ventilation and gas exchange between lung units. (c) Inhaled nitric oxide (NO) augmenting VQ matching by vasodilating vessels close to ventilated alveoli. (d) Intravenous vasodilation counteracting HPV leads to worse oxygenation. (e) In disease states that are associated with dysregulated pulmonary vascular tone, such as sepsis and acute lung injury, failure of HPV leads to worse oxygenation. (f) Accumulation of NO adducts leads to loss of HPV-augmenting effect. Reprinted with permission from the Massachusetts Medical Society [2]. Copyright © 2005 Massachusetts Medical Society. All rights reserved.

Although a major cause of diminished surfactant activity is the presence of alveolar exudate, iNO may have deleterious effects on the function of surfactant proteins through the alteration in their structure by reactions with RNS [26]. Finally, prolonged exposure to NO in experimental models impairs cellular respiration [27] and may contribute to cytopathic dysoxia.

The failure of iNO to improve outcome in ALI/ARDS is therefore potentially due to several factors. First, patients with ALI/ARDS do not die of refractory hypoxaemia but of multi-organ failure. The actions of NO are mainly considered to have their beneficial effects on oxygenation and are not expected to improve the outcome of multi-organ failure. Indeed, any beneficial effects of iNO on oxygenation may be

abrogated by detrimental systemic effects mediated by downstream products of iNO. Second, ALI/ARDS is a heterogeneous condition with diverse causes, potentially requiring specific interventions to affect outcome. Finally, the use of iNO without frequent dose titration risks inadvertent overdose with increased unwanted systemic effects without further cardiopulmonary benefits.

Other clinical uses of inhaled nitric oxide

Pulmonary hypertension and acute right ventricular failure

RVF may develop when there is abnormally elevated PVR and/or impaired RV perfusion. Table 2 lists common causes of acute RVF. The RV responds relatively poorly to inotropic agents but is exquisitely sensitive to afterload reduction.

Table 2**Causes of acute right ventricular failure**

Acute rise in pulmonary vascular resistance, such as due to acute pulmonary embolism or rapidly progressive pulmonary parenchymal/vascular disease

Acute right ventricular ischaemia, often due to diminished right coronary perfusion consequent upon inadequate systolic and diastolic pressures in shocked states

Acute high left atrial pressures, perhaps due to acute left ventricular failure of any cause

Decompensation of chronic pulmonary arterial hypertension

Decompensation of congenital heart defects with pulmonary arterial hypertension or left-to-right intracardiac shunts

After surgery necessitating cardiopulmonary bypass *per se*

Hypoxaemia causing hypoxic pulmonary vasoconstriction

Reducing PVR will offload a struggling ventricle with beneficial effects on cardiac output and therefore oxygen delivery. In the context of high RV afterload with low systemic pressures or when there is a limitation of flow within the right coronary artery [28], RV failure will ensue and potentially trigger a downward spiral, as diagrammatically represented in Figure 3.

iNO is commonly used when RV failure complicates cardiac surgery. Cardiopulmonary bypass *per se* causes diminished endogenous NO production [29].

There is marked variation in response to iNO between patients [30] and in the same patient at different times. After prolonged use, there is a leftward shift in the dose-response curve such that, without regular titration against a therapeutic goal, there is a risk of excessive iNO administration that is associated with toxicity and loss of the therapeutic effect [31].

Cardiac transplantation may be complicated by pulmonary hypertension and RVF that are improved with iNO [32]. Early ischaemia-reperfusion injury after lung transplantation manifests clinically as pulmonary oedema and is a cause of significant morbidity and mortality [33,34]. Although iNO is a useful therapy in this circumstance [35], it did not prevent ischaemia-reperfusion injury in clinical lung transplantation [36].

iNO has been used successfully in patients with cardiogenic shock and RVF associated with acute myocardial infarction [37,38]. Similarly, iNO was valuable in patients with acute RVF following acute pulmonary venous thromboembolism accompanied by significant haemodynamic compromise [39]. No systematic evaluation of iNO and its effect on clinical outcome has been conducted in these conditions.

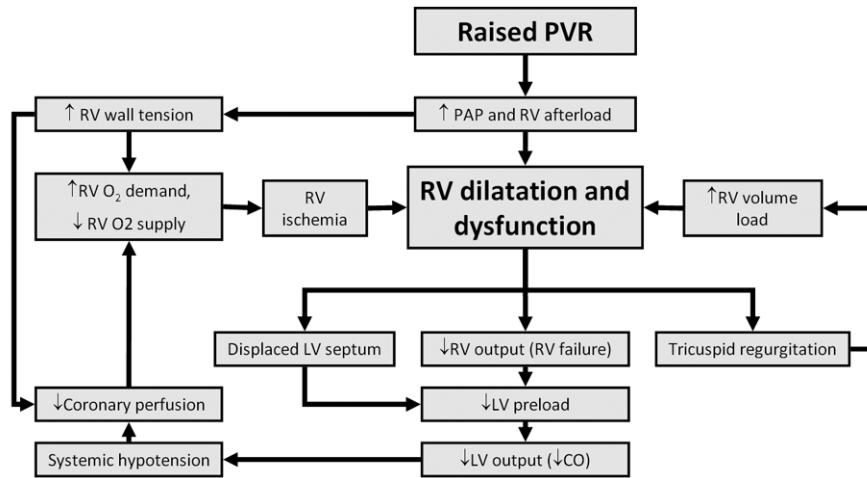
Acute chest crises of sickle cell disease

Acute chest crises are the second most common cause of hospital admission in patients with sickle cell disease (SCD)

and are responsible for 25% of all related deaths [40]. Acute chest crises are manifest by fever, respiratory symptoms or chest pain, and new pulmonary infiltrate on chest radiography. Pulmonary infection, fat emboli, and pulmonary infarction due to vaso-occlusion are the major contributory factors. Haemolysis of sickled erythrocytes releases Hb into the plasma, where it generates reactive oxygen species and reacts with NO [41]. In SCD, the scavenging systems that would usually remove circulating free Hb are saturated. Free Hb depletes NO, leading to endothelial cell dysfunction. Haemolysis also releases arginase 1 into plasma, depleting the vital substrate for NO production, arginine [42]. Furthermore, secondary pulmonary hypertension is common in adults with SCD, with estimates of prevalence ranging from 30% to 56%. Given the physiological rationale for the use of iNO and supportive data from animal studies, there have been several cases [43-45].

iNO has been used successfully in patients with cardiogenic shock and RVF due to acute myocardial infarction [46]. Similarly, iNO was valuable in patients with acute RVF due to acute pulmonary venous thromboembolism accompanied by significant haemodynamic compromise [47]. Thus far, iNO has failed to demonstrate either persistent improvements in physiology or beneficial effects on any accepted measure of outcome in clinical trials (other than its licensed indication in neonates). Therefore, iNO sits alongside interventions such as prone positioning and high-frequency oscillatory ventilation in that they improve oxygenation without demonstrated improvements in patient outcome and therefore are usually reserved for refractory hypoxaemia.

Potential problems in designing and conducting RCTs in the efficacy of iNO are numerous. Blinded trials will be difficult to conduct as the effects of iNO are immediately apparent. Recruitment will be limited as some of these indications are uncommon and rapidly life-threatening with little time for consent/assent or randomisation. Clinicians with experience of the efficacy of iNO may not have sufficient clinical

Figure 3

Pathophysiology of right ventricular failure. CO, cardiac output; LV, left ventricle; PAP, pulmonary artery pressure; PVR, pulmonary vascular resistance; RV, right ventricle.

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equipoise to recruit to placebo-controlled trials. Finally, given the expense of iNO, industry funding is likely to be necessary and this might both cast doubt on the independence of the trial results and exclude trials that may adversely influence sales.

iNO remains an important tool in the intensivist's armamentarium of rescue therapies for refractory hypoxaemia. iNO has a well-established role in managing complications of cardiac surgery and in heart/lung transplantation. There is a place for iNO in the management of ALI/ARDS, acute sickle chest crisis, acute RV failure, and acute pulmonary embolism, but it is likely to remain a rescue therapy.

Competing interests

The authors declare that they have no competing interests.

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