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Nitric Oxide: physiology and therapeutic applications

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ABSTRACT

The biology of nitric oxide (NO) has been extensively reviewed. NO was largely regarded as an environmental pollutant until 1987, when its biological similarities to endothelium-derived relaxing factor (EDRF) were noted. Subsequently, NO and EDRF were demonstrated to be identical, modulating vascular tone through stimulated formation of cyclic cAMP. Endogenous NO is formed from L-arginine by one of three (neural, inducible, and endothelial) isoforms of NO synthase, NOS. The physiological role of endogenous NO was first shown when an infusion of an inhibitor of NOS in healthy volunteers led to systemic and pulmonary pressor responses. In 1991, inhaled NO was shown to be a selective pulmonary vasodilator in patients with pulmonary hypertension and in 1993 inhaled NO emerged as a potential therapy for the acute respiratory distress syndrome (ARDS), because it decreases pulmonary vascular resistance without affecting systemic blood pressure and improved oxygenation by redistributing pulmonary blood flow toward ventilated lung units. In patients with acute lung injury and mild pulmonary hypertension, inhaled NO has been associated with a small, short-lived decrease in pulmonary arterial pressure, which has encouraged the use of NO as a supportive treatment for acute right ventricular dysfunction complicating cardiac surgery. Currently, NO is only approved in the US for the treatment of term and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hyper-tension in conjunction with ventilatory support where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation. Endogenously produced NO contributes to the control and killing of multiple bacterial species, and while NO is not bactericidal per se, its cytotoxic effect is most likely realized by its reactive nitrogen oxides such as peroxynitrite, to produce potent cytotoxic actions against membrane lipids, nucleic acids, and proteins. In conclusion, several preclinical and clinical studies are providing evidence that the nitrate–nitrite–NO pathway critically subserves physiological hypoxic NO signalling, providing an opportunity for new nitric-oxide-based therapeutics.

Keywords: Nitric Oxide, acute respiratory distress syndrome, hypoxic respiratory failure, anti-microbial properties

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INTRODUCTION

Physiology of Nitric Oxide. The biology of nitric oxide (NO) has been extensively reviewed [1,2], and the following introduction will focus on the biological effects of inhaled NO. NO was largely regarded as an environmental pollutant until 1987, when its biological similarities to endothelium-derived relaxing factor (EDRF) were noted [1,2]. Subsequently, NO and EDRF were demonstrated to be identical, modulating vascular tone through stimulated formation of cyclic cAMP[2]. Endogenous NO is formed from L-arginine by one of three (neural, inducible, and endothelial) isoforms of NO synthase, NOS. The physiological role of endogenous NO was first shown when an infusion of an inhibitor of NOS in healthy volunteers led to systemic and pulmonary pressor responses[3]. Inhaled NO relaxes pulmonary vessels, decreasing pulmonary vascular resistance, pulmonary arterial pressure and right ventricular afterload [4-6]. The selectivity of NO for the pulmonary circulation is the result of rapid haemoglobin-mediated inactivation of NO [7].

Atmospheric concentrations of NO typically range between 10 and 500 ppb but may reach 1.5 ppm in heavy traffic[8] and 1000 ppm in tobacco smoke[9]. When inhaled with high concentrations of oxygen, gaseous NO forms nitrogen dioxide [10] and when used therapeutically, it is therefore important to limit the mixing of NO and oxygen by introducing a mixture of NO and nitrogen into the inspiratory limb of the ventilator tubing as near to the patient as possible [11]. Upon inhalation, NO is rapidly inactivated by haemoglobin in blood by haptoglobin–haemoglobin complexes in plasma, and by reaction with haeme ferrous iron and ferric iron that forms nitrosyl-haemoglobin [12]. NO forms methaemoglobin and nitrate on reaction with oxyhaemoglobin, which predominates in the pulmonary circulation. Most of the methaemoglobin is reduced to ferrous haemoglobin by NADH–cytochrome b5 reductase in erythrocytes. In healthy subjects who have inhaled 80 ppm NO for one hour, plasma nitrate concentrations may be four times as high as baseline levels [13].

In healthy volunteers, inhalation of 80 ppm NO abolishes the vasopressor effect of the inhibition of NOS in the circulation of the forearm, an effect associated with increased arterial concentrations of nitrite and S-nitrosyl-haemoglobin, but not of S-nitrosothiols or S-nitrosohaemoglobin [13]. The concept of a plasma-based repository for NO activity that may be supplemented by inhaled NO has become widely accepted; probable contributors include nitrites, iron nitrosyl and N-nitrosamine complexes, [14] and nitrated lipids [15]. Many proteins, including haemoglobin and albumin [16,17] contain thiol groups that react reversibly with NO to form S-

nitrosothiols; these compounds are vasodilators and inhibit platelet aggregation[18]. S-nitrosothiols may also 'store' NO within the circulation and S-nitrosohaemoglobin in erythrocytes has been postulated to regulate micro-vascular flow and oxygen delivery [19.]

Safety of Inhaled NO. Although inhaled NO at 500-1000 ppm or higher is rapidly fatal in the dog [20], other preclinical studies suggest that NO has minimal pulmonary toxicity when inhaled at concentrations less than 40 ppm for up to six months[21]. Up to 40 ppm of inhaled NO should therefore not cause methaemo-globinemia in adults[22]. Guidelines in the United Kingdom recommend measurement of methaemoglobin concentrations within six hours after the initiation of NO treatment and after each increase in the dose[11], while the Control of Substances Hazardous to Health Regulations suggest that environmental concentrations of NO and NO2 should not exceed 25 ppm and 2 ppm, respectively, over an eight-hour period [23].

In the presence of biventricular cardiac failure, inhaled NO may increase pulmonary blood flow left atrial end-diastolic pressure sufficiently to precipitate pulmonary oedema [24]. The effect of inhaled NO on gas exchange depends on the extent to which pulmonary vasoconstriction and ventilation–perfusion mismatching are contributing to impaired oxygenation. The effects of inhaled NO also depend on vascular selectivity. For example, disproportionate arterial, as opposed to venous, dilatation would increase the pulmonary-capillary pressure and exacerbate pulmonary oedema. Apart from changing the pulmonary capillary pressure, NO may influence the development of oedema through pulmonary vascular recruitment or by decreasing inflammation and helping maintain the integrity of the alveolar-capillary membrane. When inhaled NO is used therapeutically, its rapid withdrawal may induce rebound pulmonary hypertension and hypoxaemia [25,26]. In practice, rebound phenomena may be avoided by gradual withdrawal.

Inhaled NO may modulate the acute neutrophilic inflammation of the lung parenchyma and dysfunction of the alveolar-capillary membrane that characterizes ARDS at several levels. The protective effects of NO may derive from specific effects on neutrophil function — for example, by attenuation of the respiratory burst and neutrophil-derived oxidative stress [27].

Therapeutic Uses of NO. In 1991, inhaled NO was shown to be a selective pulmonary vasodilator in patients with pulmonary hypertension[5] and in 1993 inhaled NO emerged as a potential therapy for the acute respiratory distress syndrome (ARDS), because it decreases pulmonary vascular resistance without affecting systemic blood pressure and improved oxygenation by redistributing pulmonary blood flow toward ventilated lung units [25].

In adults with acute lung injury, inhaled NO is primarily used to improve oxygenation rather than to decrease pulmonary vascular resistance. However, results of several controlled studies have been largely negative in patients with acute respiratory failure [28]. In a French study, no decrease in the duration of mechanical ventilation or the mortality rate was reported among patients treated with inhaled NO as compared with those treated with a nitrogen placebo [29]. A US-based study reported a lack of dose response in terms of arterial partial pressure of oxygen in the concentration range from 1.25 to 40 ppm of inhaled NO [30], while a European study found no survival benefit of inhaled NO in 140 subjects with acute lung injury [31]. Another study compared the effects of continuously inhaled NO at 5 ppm with those of a placebo in patients with ARDS that was not associated with severe sepsis or multi-organ failure [32]. Despite the lower dose, the increase in oxygenation lasted only for the first day of therapy and NO treatment did not have any significant effect on any of the out-come measures. A possible contributing reason for these seemingly contradictory results is the observation that only a minority of patients with ARDS die from respiratory failure; the majority die from multi-organ failure [33].

In patients with acute lung injury and mild pulmonary hypertension, inhaled NO has been associated with a small, short-lived decrease in pulmonary arterial pressure [30,32,34], which has encouraged the use of NO as a supportive treatment for acute right ventricular dysfunction complicating cardiac surgery [35-37], although there appears to be little adequate trial data to support this practice. Inhaled NO has also been associated with marked haemodynamic improvement in patients with acute massive pulmonary embolism[38] suggesting that in these patients, reversible pulmonary vasoconstriction contributes to right ventricular dysfunction. Inhaled NO also alleviates pulmonary hypertension in patients with severe chronic obstructive pulmonary disease but exacerbates hypoxemia at rest [39]. During exercise, inhaled NO alleviates pulmonary hypertension without inducing hypoxemia[40], possibly by increasing relative ventilation and therefore increasing the delivery of NO to lung units that fill relatively quickly during inspiration, which leads to improved ventilation–perfusion matching.

Lung injury associated with ischemia and reperfusion is an important cause of morbidity and mortality after lung transplantation. Endogenous NO activity is decreased after lung transplantation, despite the increased expression of endothelial NOS [41]. Inhaled NO has been used to provide support for patients with acute lung injury after lung transplantation, and suggests potential for prophylactic use [42-45]. However, a randomized, placebo-controlled trial of inhaled NO in 84 transplant recipients demonstrated no benefit in terms of oxygenation, the time to extubation, or the 30-day mortality rate [46].

Inhaled NO causes mild bronchodilation in patients with asthma [47]. The NO–derived S-nitrosothiols, which act as bronchodilators, were present at lower concentrations in the fluid lining the airways of patients with severe asthma than of healthy subjects, suggesting that this mechanism may contribute to bronchospasm [48].

CONCLUSION

Inhaled NO acts as a selective pulmonary vasodilator that improves ventilation–perfusion matching at low doses in patients with acute respiratory failure, but trial data suggest that that benefits are short-lived in adults with acute lung injury or ARDS, and no associated improvement in mortality rates has been demonstrated and its use is therefore not recommended in these conditions. Conversely, NO may be useful as a short-term adjunct to cardiorespiratory support in patients with acute hypoxemia, life-threatening pulmonary hypertension, or both. Currently, NO is only approved in the US for the treatment of term and near-term (>34 weeks gestation) neonates with hypoxic respiratory failure associated with clinical or echocardiographic evidence of pulmonary hyper-tension in conjunction with ventilatory support where it improves oxygenation and reduces the need for extracorporeal membrane oxygenation[49]. For this indication, the dosage of 20 ppm is recommended with a duration of up to 14 days or until the oxygen desaturation has resolved.

Anti-Microbial Properties of NO. Endogenously produced NO contributes to the control and killing of multiple bacterial species, and while NO is not bactericidal per se, its cytotoxic effect is most likely realized by its reactive nitrogen oxides such as peroxynitrite [50-54], to produce potent cytotoxic actions against membrane lipids[50], nucleic acids[55,56], and proteins[52,57]. For example, Alam et al.[58] investigated the effect of NO in murine salmonellosis and showed that inducible NOS (iNOS)-deficient mice developed severe septicaemia within 6 days while wild-type mice were resistant.

The notion that NO may have antiviral properties initiated with a report from Croen in 1993[59], who studied the effect of NO on herpes simplex virus type 1 replication in vitro in murine macrophages and found that inhibition of NOS substantially reduced the antiviral effect of activated macrophages, while an NO donor inhibited viral replication in a dose-dependent manner, although no virucidal nor cytocidal effects of NO were observed. Similar studies[60] demonstrated that NO inhibited the replication of vaccinia virus and ectromelia virus as well as HSV-1 and demonstrated that mice inoculated with ectromelia an given a NOS inhibitor, the

mice developed fulminant mousepox associated with a 30% mortality, while infected control mice had a self-limited, non-fatal infection.

Others have demonstrated effects of endogenous NO on Japanese encephalitis virus [61] and Coxsackie-virus [62], who also suggest that mechanism by which NO acts is via S-nitrosylation of proteases critical for replication, although this has been challenged by others (reviewed in Akaike and Maeda [63]), proposing a less specific oxidative mechanism. Not all viral strains are equally susceptible to NO; ortho- and paramyxovirus, murine vaccinia virus, coronavirus, lymphocytic choriomeningitis virus, murine encephalomyocarditis virus, tickborn encephalitis virus are not appreciably affected by NO exposure [64-70] . It should also be noted that all available literature reports are based on in vitro or murine infection model.

Nitric Oxide in Cystic Fibrosis. The rationale for the use of NO in cystic fibrosis (CF) was based on the observation that NO formation is reduced in airways of patients with CF [71]. Reasoning that NO has bronchodilatory effects Grasemann et al.[72] suggested that a deficiency in production of NO may contribute to airway obstruction and administered nebulized L-arginine to 13 CF patients as a means to increase airway NO formation and improve pulmonary function in patients with CF. Results indicated increased exhaled nitric oxide concentrations but also a sustained improvement of FEV1 vs. baseline in comparison to control subjects administered with saline. Oxygen saturation also in-creased significantly. Conversely, the same group published data on pulmonary function of inhaled NO at 0.1, 1 and 40 ppm but found no changes in oxygen saturation or lung function at any dose and concluded that inhaled NO had no immediate effect on bronchomuscular tone in patients with cystic fibrosis [73].

In conclusion, several preclinical and clinical studies are providing evidence that the nitrate–nitrite–NO pathway critically subserves physiological hypoxic NO signalling, providing an opportunity for new nitric-oxide-based therapeutics

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