REVIEW ARTICLE

DRUG THERAPY

Inhaled Nitric Oxide Therapy in Adults

Mark J.D. Griffiths, M.R.C.P., Ph.D., and Timothy W. Evans, M.D., Ph.D.

BACKGROUND AND HISTORICAL PERSPECTIVE

Nitric oxide was largely regarded as a toxic pollutant until 1987, when its biologic similarities to endothelium-derived relaxing factor were demonstrated.[1] Subsequently, nitric oxide and endothelium-derived relaxing factor were considered a single entity, modulating vascular tone through the stimulated formation of cyclic guanosine 3′,5′-monophosphate (Fig. 1).[2] Endogenous nitric oxide is formed from the semiessential amino acid L-arginine by one of three (neural, inducible, and endothelial) isoforms of nitric oxide synthase. The physiologic role of endogenous nitric oxide was first shown when an infusion of an inhibitor of all forms of nitric oxide synthase in healthy volunteers led to systemic and pulmonary pressor responses.[3] However, the role of nitric oxide in maintaining low pulmonary vascular resistance in healthy persons has since been challenged.[4] Inhaled nitric oxide had a negligible effect on pulmonary blood flow in healthy humans,[5] but when healthy persons were breathing 12 percent oxygen, it reversed the pulmonary hypertension that was induced without affecting systemic hemodynamics.[6] In 1991, inhaled nitric oxide was shown to be a selective pulmonary vasodilator in patients with pulmonary hypertension,[7] as well as in animals with pulmonary hypertension induced by drugs or hypoxia.[8] Two years later, inhaled nitric oxide emerged as a potential therapy for the acute respiratory distress syndrome (ARDS), because it decreased pulmonary vascular resistance without affecting systemic blood pressure and improved oxygenation by redistributing pulmonary blood flow toward ventilated lung units in patients with this condition.[9]

Despite such promise, the potential therapeutic role of inhaled nitric oxide in adults remains uncertain; licensed indications are restricted to pediatric practice. Furthermore, recent changes in the marketing of inhaled nitric oxide have dramatically increased its cost, which has inevitably led to a need to justify continuing its administration to adults. This review will consider the biologic actions of inhaled nitric oxide, discuss clinical indications for its administration in adults, and assess possible future developments.

CHEMICAL REACTIONS OF INHALED NITRIC OXIDE

Nitric oxide is a gas that is colorless and odorless at room temperature and is relatively insoluble in water. It is poorly reactive with most biologic molecules, but because it has an unpaired electron, it can react very rapidly with other free radicals, certain amino acids, and transition metal ions.[10] In biologic solutions, nitric oxide is stabilized by forming complexes with — for example — thiols, nitrite, and proteins that contain transition metals.[11]

Atmospheric concentrations of nitric oxide typically range between 10 and 500 parts per billion but may reach 1.5 parts per million (ppm) in heavy traffic[12] and 1000 ppm in tobacco smoke.[13] When inhaled with high concentrations of oxygen,

From the Adult Intensive Care Unit and Intensive Care Services, Royal Brompton Hospital, and Imperial College London — both in London. Address reprint requests to Dr. Evans at the Unit of Critical Care, Imperial College London, Royal Brompton Hospital, Sydney St., London SW3 6NP, United Kingdom, or at t.evans@rbh.nthames.nhs.uk.

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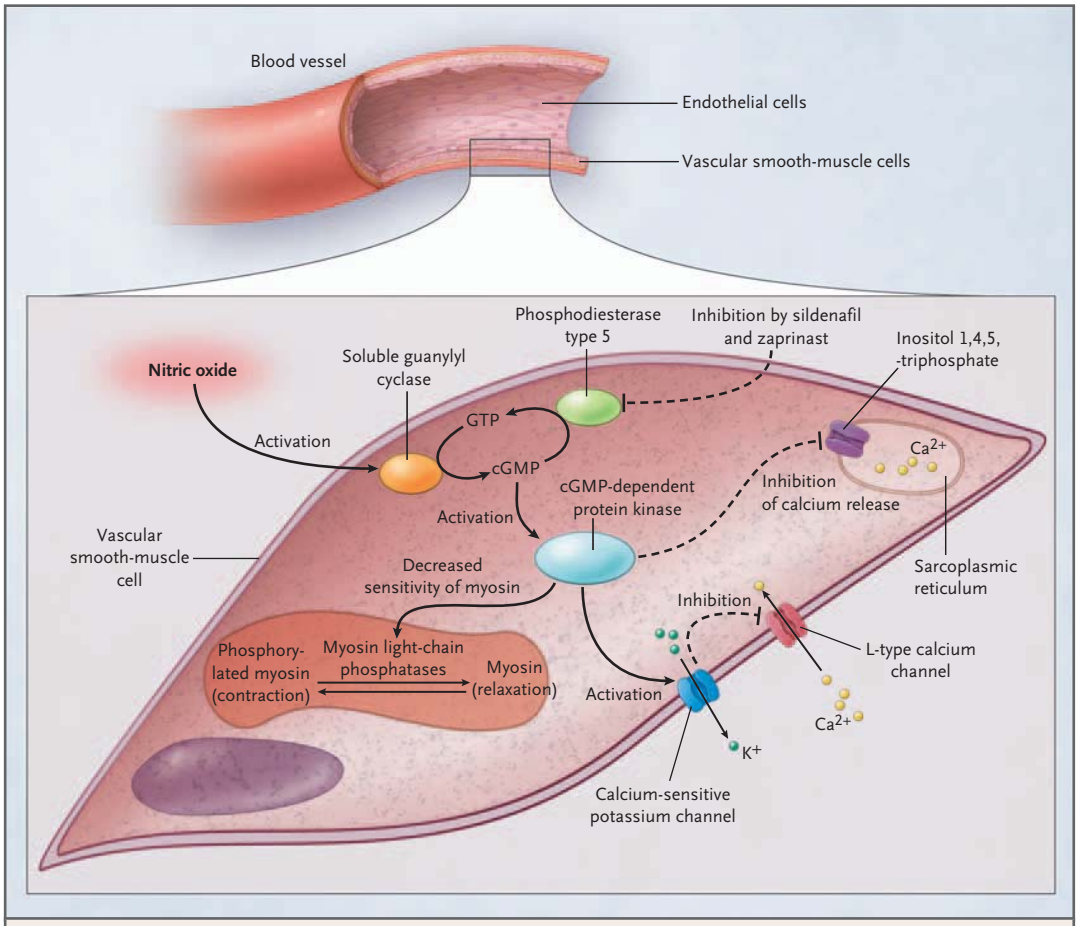

Figure 1. Regulation of the Relaxation of Vascular Smooth Muscle by Nitric Oxide.

Nitric oxide activates soluble guanylyl cyclase, leading to the activation of cyclic guanosine 3′,5′-monophosphate (cGMP)–dependent protein kinase (cGKI). In turn, cGKI 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. cGMP is degraded by phosphodiesterase type 5, which is inhibited by sildenafil and zaprinast. GTP denotes guanosine triphosphate.

gaseous nitric oxide slowly forms nitrogen dioxide.[14] Once dissolved in airway-lining fluid, nitric oxide may react with reactive oxygen species such as superoxide to form reactive nitrogen species such as peroxynitrite, a powerful oxidant that can decompose further to yield nitrogen dioxide and hydroxyl radicals (Fig. 2).[15] Therefore, nitric oxide is potentially cytotoxic, and covalent nitration of tyrosine in proteins by reactive nitrogen species has been used as a marker of oxidative stress.[16]

Nitric oxide is rapidly inactivated by hemoglobin in blood, by haptoglobin–hemoglobin complexes in plasma, and by a reaction with heme ferrous iron and ferric iron that forms nitrosylhemoglobin.[17] Nitric oxide forms methemoglobin and nitrate on reaction with oxyhemoglobin, which predominates in the pulmonary circulation. Most of the methemoglobin is reduced to ferrous hemoglobin by NADH–cytochrome *b*5 reductase in erythrocytes. In healthy subjects who have inhaled nitric oxide (80 ppm) for one hour, plasma nitrate concentrations may be four times as high as baseline levels.[18] Almost 70 percent of inhaled nitric oxide is excreted as nitrate in the urine within 48 hours.[19]

More than 100 proteins, including hemoglobin[20] and albumin,[21] contain reduced sulfur (thiol)

groups that react reversibly with nitric oxide to form *S*-nitrosothiols; these compounds are vasodilators that inhibit platelet aggregation.[22] *S*-nitrosothiols may also "store" nitric oxide within the circulation. For example, *S*-nitrosohemoglobin in red cells has been postulated to regulate microvascular flow and oxygen delivery.[23]

PHYSIOLOGIC EFFECTS OF INHALED NITRIC OXIDE ON THE CARDIOVASCULAR SYSTEM

Inhaled nitric oxide relaxes pulmonary vessels, thereby decreasing pulmonary vascular resistance, pulmonary arterial pressure, and right ventricular afterload (Table 1).[6-8] The selectivity of nitric oxide for the pulmonary circulation is the result of rapid hemoglobin-mediated inactivation of nitric oxide.[29] In the presence of biventricular cardiac failure, inhaled nitric oxide may sufficiently increase pulmonary blood flow and, hence, left atrial end-diastolic pressure to precipitate pulmonary edema.[30]

Early studies in patients with ARDS compared the effect of inhaled nitric oxide with another vasodilator (epoprostenol, or prostacyclin or prostaglandin I_2) administered intravenously.[9] The intravenously administered vasodilator worsened oxygenation owing to antagonism of hypoxic pulmonary vasoconstriction. In contrast, the advantage of inhaled nitric oxide was that only the vasculature associated with ventilated lung units was within reach of an inhaled gas diffusing across the alveolar-capillary membrane. Selective dilatation of these vessels would improve ventilation–perfusion matching (Fig. 3).

Circulating modulators of vascular tone, such as the potent vasoconstrictor endothelin-1 and endogenous nitric oxide, influence the effect of inhaled nitric oxide. Decreased responsiveness is associated with the induction of nitric oxide synthase by endotoxin both in patients with ARDS associated with septic shock[31] and in animal models (Fig. 3E).[32] Conversely, the positive effect of inhaled nitric oxide on gas exchange depends on the extent to which pulmonary vasoconstriction and ventilation–perfusion mismatching are contributing to impaired oxygenation. For example, in a study of mountaineers who were either susceptible or not susceptible to high-altitude pulmonary edema, inhaled nitric oxide decreased the pulmonary arterial pressure of susceptible subjects, but improved oxygenation only in the subjects with the greatest degree of hypoxemia (those who had pulmonary edema) by increasing the blood flow to the areas of lung that were relatively unaffected.[33]

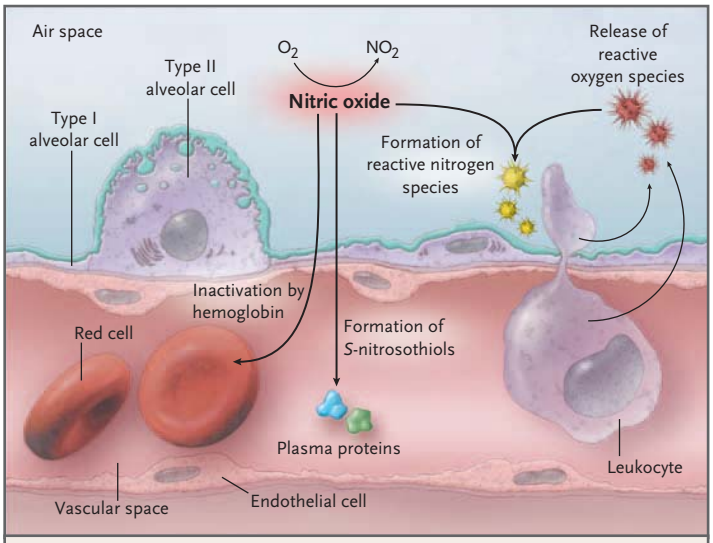

Figure 2. Biochemical Fates of Inhaled Nitric Oxide at the Alveolar-Capillary Membrane.

Small amounts of nitrogen dioxide (NO_2) may be formed if inhaled nitric oxide mixes with high concentrations of oxygen (O_2) in the air space. Depending on the milieu of the lung parenchyma, nitric oxide may react with reactive oxygen species (derived from activated leukocytes or ischemia–reperfusion injury) to form reactive nitrogen species such as peroxynitrite. In the vascular space, dissolved nitric oxide is scavenged by oxyhemoglobin (forming methemoglobin and nitrate) and to a lesser extent, plasma proteins (e.g., forming nitrosothiols, which are stable intravascular sources of nitric oxide activity).

The effects of inhaled nitric oxide also depend on vascular selectivity. For example, disproportionate arterial, as opposed to venous, dilatation would increase the pulmonary-capillary pressure and exacerbate pulmonary edema. Although many studies have not shown evidence of selectivity, others have demonstrated that 40 ppm of nitric oxide induced venodilatation with decreased pulmonary-capillary pressure[34] and reduced the risk of pulmonary edema in patients with acute lung injury.[35] Apart from changing the pulmonary-capillary pressure, nitric oxide may influence the development of edema through pulmonary vascular recruitment or by decreasing inflammation and helping maintain the integrity of the alveolar-capillary membrane. Such specific effects are difficult to identify with certainty in vivo. Because the effects of nitric oxide probably vary in different settings, apparently contradictory clinical and experimental observations have been produced.

Table 1. Comparison of Ideal Treatment Goals with Those Achieved by Inhaled Nitric Oxide in Adults with the Acute Respiratory Distress Syndrome (ARDS).

Ideal Treatment Goals	Physiological Effects of Inhaled Nitric Oxide
Improved oxygenation	20% Improvement in approximately 60% of patients for only 1 to 2 days in clinical trials, with no associated survival benefit[24,25]; may significantly improve oxygenation in very severe cases and buy time for the institution of other means of support
Decreased pulmonary vascular resistance	Selective pulmonary vasodilator of uncertain benefit in acute lung injury or ARDS characterized by mild pulmonary hypertension[26]; may have a supportive role in patients with acute right-sided heart failure, particularly in association with increased pulmonary vascular resistance and hypoxemia
Decreased pulmonary edema	May be influenced by effects on hemodynamics, inflammation, infection, and the alveolar-capillary membrane
Reduction or prevention of inflammation	Conflicting evidence of its antiinflammatory efficacy at multiple molecular and clinical levels
Cytoprotection	May contribute to the formation of cytotoxic reactive nitrogen species and reactive oxygen species, especially when administered with high concentrations of oxygen; conversely, may prevent the generation of reactive oxygen species by free iron and scavenge hydroxyl radicals[27]
Protection against infection	Direct antimicrobial effects,[28] but associated with an increased incidence of ventilator-associated pneumonia in one study[25]

Most clinical studies have provided support for the view that inhaled nitric oxide has no effect on the systemic circulation. In contrast, experimental studies have demonstrated a reduction in systemic vascular resistance[36] and restoration of mesenteric perfusion after the inhibition of nitric oxide synthase.[37] Similarly, the inhalation of nitric oxide (80 ppm) by healthy volunteers abolished the vasopressor effect of the inhibition of nitric oxide synthase in the circulation of the forearm, an effect associated with increased arterial concentrations of nitrite and *S*-nitrosylhemoglobin, but not of *S*-nitrosothiols or *S*-nitrosohemoglobin.[18] The concept of a plasma-based repository for nitric oxide activity that may be supplemented by inhaled nitric oxide has become widely accepted; probable contributors include nitrites,[38] iron nitrosyl and *N*-nitrosamine complexes,[39] and nitrated lipids.[40]

When inhaled nitric oxide is used therapeutically, its rapid withdrawal may induce rebound pulmonary hypertension and hypoxemia.[9,41] The inhalation of nitric oxide by healthy animals decreases endothelial nitric oxide synthase activity and increases plasma concentrations of endothelin-1,[42] which inactivates endothelial nitric oxide synthase by nitration.[43] In practice, rebound phenomena may be avoided by withdrawing inhaled nitric oxide gradually. Despite these concerns, in large clinical studies of patients with ARDS, the abrupt discontinuation of inhaled nitric oxide has not caused a deterioration in oxygenation.[24,25]

DIRECT CYTOTOXICITY AND EFFECTS ON INFLAMMATION

Inhaled nitric oxide 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 nitric oxide may derive from specific effects on neutrophil function — for example, by attenuation of the respiratory burst and neutrophil-derived oxidative stress.[44] Inhaled nitric oxide has decreased the accumulation of neutrophils in the pulmonary vasculature and air space in animal models of acute lung injury,[45] consistent with its known effects on the adhesion and deformability of neutrophils in vitro.[46] Furthermore, similar effects of inhaled nitric oxide outside the lung have been observed in rodent models of severe sepsis.[47] In a model in which cecal ligation and puncture were used to induce sepsis, mice lacking inducible nitric oxide synthase had fewer neutrophils sequestered in the pulmonary vasculature than normal mice, but they had greater neutrophil migration into the air spaces.[48] Subsequent experiments have confirmed that nitric oxide derived from neutrophils acts as

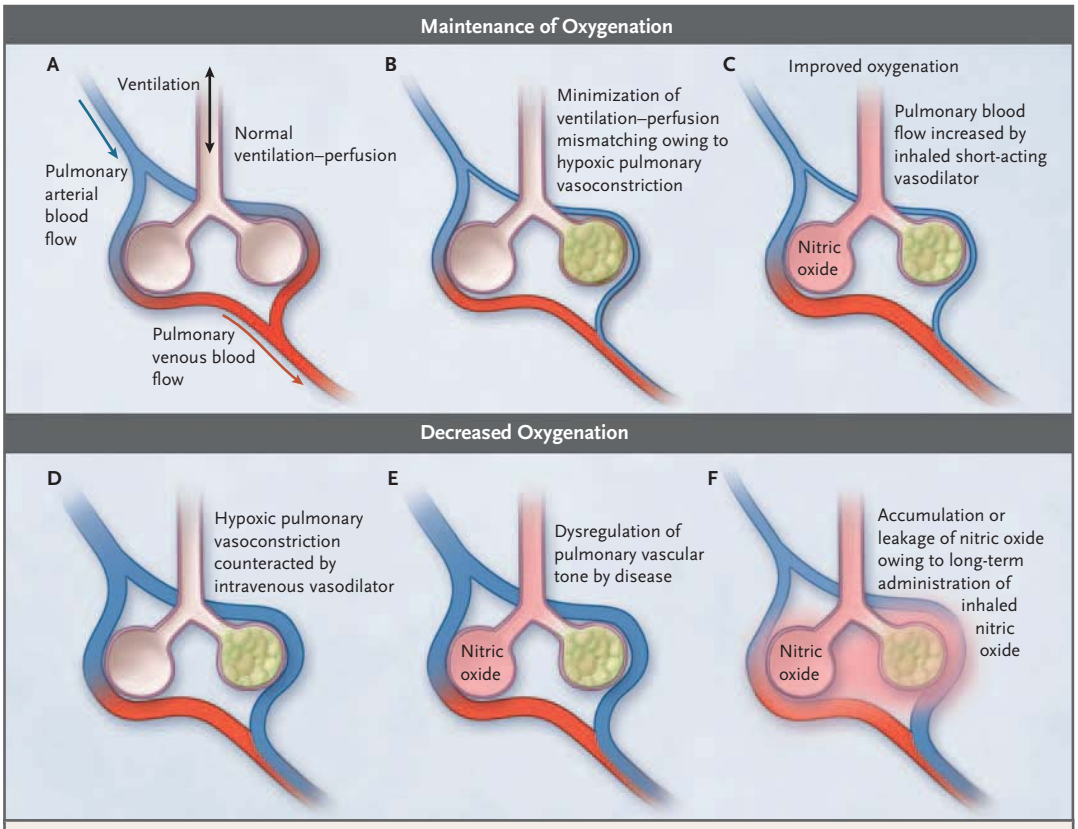

Figure 3. Mechanism of Action and Inaction of Inhaled Nitric Oxide.

Panel A shows normal ventilation–perfusion. Hypoxic pulmonary vasoconstriction (Panel B) minimizes ventilation–perfusion mismatching in the presence of abnormal ventilation. Inhaled vasodilators with a short half-life improve oxygenation by increasing blood flow to ventilated lung units (Panel C). If a vasodilator is administered intravenously (Panel D) or if diseases are associated with dysregulated pulmonary vascular tone, such as sepsis and acute lung injury (Panel E), hypoxic pulmonary vasoconstriction is counteracted, leading to worsening oxygenation. Long-term administration of inhaled nitric oxide, with the accumulation of nitric oxide or leakage between lung units associated with collateral ventilation, as may occur in chronic obstructive pulmonary disease (Panel F), may negate the beneficial effects of inhaled nitric oxide on oxygenation.

an autocrine modulating factor in infiltration of neutrophils into the lungs during sepsis.

The toxic potential of nitric oxide is well known; endogenously produced nitric oxide contributes to the control and killing of multiple pathogens[28] and malignant cells.[49] Studies involving inhibitors of nitric oxide synthase[50] and mice lacking inducible nitric oxide synthase[51] have suggested that nitric oxide–derived reactive nitrogen species contribute to epithelial damage after a variety of insults. The results of interactions between nitric oxide and reactive oxygen species are unpredictable and probably depend on the relative local concentrations of the participants in these reactions.[52] Increased concentrations of oxidative products of nitric oxide were found in the airway-lining fluid of patients with ARDS,[53] and these may be further increased by inhalation of nitric oxide.[54] In rodents, inhalation of nitric oxide (20 ppm) did not increase protein nitration unless hyperoxia was superimposed.[55] Taken together, these observations suggest an important role for oxidative damage and reactive nitrogen species in these pulmonary diseases, but the role of exogenous nitric oxide in modulating these processes is uncertain.

OTHER EFFECTS

Endogenous nitric oxide inhibits the adhesion of platelets to endothelial cells and subsequent aggregation.[2] In experimental microsphere-induced pulmonary embolism, inhaled nitric oxide attenuated increases in pulmonary arterial pressure and platelet aggregation.[56] However, in animals, healthy volunteers, and patients with pulmonary diseases, the effects of inhalation of nitric oxide on the

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