Review Article

Inhaled nitric oxide: clinical applications, indications, and toxicology

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Purpose: Although the analogy of nitric oxide (NO) to Endothelium-derived Relaxing Factor remains controversial, medical use of exogenous NO gas by inhalation has grown exponentially. This review presents the mechanisms of action of inhaled NO in pulmonary hypertension, hypoxæmia, inflammation and œdema, as well as its therapeutic and diagnostic indications with emphasis on acute respiratory distress syndrome (ARDS) and toxicology.

Source: Two medical databases (Current Contents, Medline) were searched for citations containing the above-mentionned key words to December 1996. Moreover, many presentations in congresses such as 4th International Meeting of Biology of Nitric Oxide, 52nd and 53rd Annual Meeting of Canadian Anæsthetists' Society or 10th Annual Meeting of European Association of Cardiothoracic Anæsthesiologists were used.

Principal findings: Inhaled NO is now recognized as an invaluable tool in neonatal and paediatric critical care, and for heart/lung surgery. Other clinical applications in adults, such as chronic obstructive pulmonary disease and ARDS, require a cautious approach. The inhaled NO therapy is fairly inexpensive, but it would seem that it is not indicated for everybody with regards to the paradigm of its efficiency and potential toxicity. The recent discovery of its anti-inflammatory and extrapulmonary effects open new horizons for future applications.

Conclusion: Clinical use of inhaled NO was mostly reported in case series, properly designed clinical trials must now be performed to establish its real therapeutic role. These trials would permit adequate selection of the cardiopulmonary disorders, and subsequently the patients that would maximally benefit from inhaled NO therapy.

Objectif : Même si la relation entre le monoxyde d'azote (NO) et l'EDRF (endothelium-derived relaxing factor) n'est pas établie de façon absolue, l'utilisation médicale du NO exogène à l'état gazeux a cru de façon exponentielle. Ce survol de la littérature rappelle les mécanismes d'action du NO inhalé dans l'hypertension pulmonaire, l'hypoxémie, l'inflammation et l'oedème et jette un regard sur ses indications diagnostiques et thérapeutiques principalement en rapport avec le syndrome de détresse respiratoire de l'adulte (SDRA) et la toxicologie du NO.

Sources : Deux bases de données (*Current Contents, Medline*) incluant décembre 1996, ont été consultées en faisant appel aux mots-clés mentionnés plus haut. En outre, on a révisé les travaux présentés à des congrès comme le *4th International Meeting of Biology of Nitrous Oxide*, les 52e et 53e congrès annuels de *La Société canadienne des anesthésistes* et le 10e congrès annuel de l'*Association européenne des anesthésiologistes cardiothoraciques*.

Principales constatations : L'inhalation de NO est maintenant reconnue comme faisant partie de l'arsenal thérapeutique néonatalogique, pédiatrique et chirurgical cardiopulmonaire. Chez les adultes, les autres indications comme la maladie pulmonaire obstructive chronique et le SDRA sont moins évidentes. L'inhalation thérapeutique de NO ne coûte pas cher mais ne constitue pas une panacée, compte tenu de son efficacité et de sa toxicité potentielle. La découverte récente de ses effets anti-inflammatoires et extrapulmonaires ouvre toutefois la porte à de nouvelles applications.

Conclusion : En clinique, l'inhalation de NO a surtout fait l'objet d'observations anecdotiques mais des essais cliniques validés doivent être menés pour établir sa valeur thérapeutique réelle. Ces essais devraient permettre d'établir ses indications cardiopulmonaires et ainsi procurer aux patients les avantages maximaux de la thérapie par inhalation au NO.

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SEVENTEEN years ago, Furchgott and Zawadzki [1] demonstrated that the relaxation induced by acetylcholine requires the presence of endothelial cells, and that this effect was mediated by a humoral factor later known as endothelium-derived relaxing factor (EDRF). The subsequent discovery that the formation of nitric oxide (NO), or a closely related molecule synthesized from the guanido group of L-arginine, by endothelium accounts for the biological activity of EDRF, and has stimulated intensive research about NO biology. The gaseous physical state of NO, its role in the evolution of living systems, its ubiquitous distribution,[2] the new concept of its autocrine signal transduction by the L-arginine-NO pathway, and its participation in fundamental biological functions, led to a revision of scientists' understanding of biochemistry, physiology, neuroscience and immunology. Because of this, Science magazine awarded NO with the title of molecule of the year in 1992.

The potent vasodilators sodium nitroprusside and organic nitrate esters, such as nitroglycerin, are metabolized to NO, their active moiety. Nitric oxide gas can be administered as inhaled NO (inhNO) *via* the gas mixture of the patient's breath. Inhaled NO reaches pulmonary vascular smooth muscle by diffusion from ventilated *alveoli*, hence causing vascular relaxation of the adjacent vessels.[3] As inhNO in blood stream is rapidly and very specifically inactivated by hæmoglobin, its vasodilatory effect seems to be restricted to the pulmonary vasculature and no clear systemic vasodilatation can be seen.[3,4] This relative selectivity generated the enthusiastic concept of a safe and effective therapy.

We will present the clinical applications of inhNO in various pulmonary pathologies with emphasis on inflammation, followed by the therapeutic and diagnostic indications of inhNO with emphasis on ARDS. Finally, we will discuss the potential dangers associated with the use of inhNO.

Biochemistry

The mediator EDRF/NO is formed from L-arginine by the activity of NO synthase (NOS):

$$\text{L-arginine} + O_2 + \text{NADPH} \rightarrow \text{NO} + \text{L-citrulline} + \text{NADP} \quad (1).$$

At least three NOS isoforms have been identified. Constitutive forms of NOS (cNOS) include endothelial (eNOS; NOS III), which mediates endothelium-dependent vasodilator responses, and neuronal (nNOS; NOS I) form. There is also an inducible form (iNOS; NOS II) stimulated by cytokines and bacterial endotoxins, which expression is inhibited by corticosteroids.

After its endothelial production, EDRF/NO relaxes adjacent smooth muscle cells by binding to iron (Fe) in the hæm of soluble guanylate cyclase, thereby activating the enzyme to generate cyclic guanosine 3′,5′-monophosphate (cGMP). This intracellular messenger elicits smooth muscle relaxation through numerous actions, particularly by stimulating the phosphorylation of poorly defined substrates by cGMP-dependent protein kinase(s) or as a consequence of cGMP-mediated activation or inhibition of phosphodiesterase(s) (PDE) which usually inactivate(s) cGMP and/or cyclic adenosine 3′,5′-monophosphate (cAMP).[5] These in turn lead to a decrease in the intracellular free Ca^{2+} (sarcoplasmic reticulum uptake, complexation with phosphorylated transporters, interference with receptor-operated Ca^{2+} channels, inhibitory effects on phosphatidylinositol 4,5-diphosphate - PiP_2 - hydrolysis), and in the sensitivity of myosin light chain kinase for Ca^{2+}.[6]

In water, ultrafiltrate, and plasma, NO is oxidized to NO_2- which is stable for several hours. In whole blood, however, NO_2^- is rapidly converted to NO_3^-. Intermediates produced during the formation of these anions could indeed nitrosylate thiols, although the yield would be very low. Nitric oxide reaching the blood stream can be metabolised *via* three pathways:[7] 1) interaction with dissolved O_2 in blood to form NO_2^- 2) reaction with oxyhæmoglobin to form methæmoglobin, which is in turn reduced back to hæmoglobin and NO_3^- mainly by the NADPH-methæmoglobin reductase and $OONO^-$ pathways, and 3) combination with deoxyhæmoglobin to form rather stable nitrosohæmoglobin or with carrier molecules to form *S*-nitrosothiols or other packaged forms. (see *Extrapulmonary effects of inhaled nitric oxide* section).

Clinical applications of inhaled nitric oxide

The three NOS isoforms have been identified in human airways.[8] Established sources of endogenous NO in the lung include the arterial and venous endothelial cells, epithelial cells, inflammatory cells (macrophage, neutrophil, mast cell), fibroblasts, smooth muscle cells, and nonadrenergic-noncholinergic (nitrergic) nerves. High levels of NO are continuously produced in the human upper airways and inhaled at each inspiration.[9] Pulmonary-produced EDRF/NO maintains the low pulmonary artery pressure (PAP) at rest and during exercise, controls the pulmonary blood flow distribution, opposes hypoxic pulmonary vasoconstriction (HPV)[3] and the pulmonary response to endogenous or exogenous vasoconstrictors. Nitric oxide released by nitrergic nerves might control the bronchomotor tone[9] and NO released by the bronchial epithelium might decrease the submucosal œdema formation, an effect mediated by cGMP. Nitric oxide plays a major role in inflammatory processes, infectious pneumonia, and nonspecific defense of the respiratory tract.

Pulmonary hypertension

Pulmonary hypertension is generally characterized by increased pulmonary vascular resistance (PVR), increased thickening of pulmonary artery walls, and right-sided heart failure. The primary goal in pulmonary hypertension is to improve right ventricular output without increasing its work, impairing tissue oxygenation delivery, compromising the hæmodynamic function or integrity of the systemic circulation. Therefore, the pulmonary *versus* systemic selectivity of vasodilators is of critical importance.

The decrease in the expression of eNOS observed in pulmonary hypertension contributes to the pulmonary vasoconstriction and the excessive growth of the *tunica media*.[10] In many respects, inhNO can be considered as a replacement therapy for the loss of endogenous NO production in patients with pulmonary hypertension. Inhaled NO has been used to reverse the pulmonary hypertension induced by hypoxia,[3] chronic obstructive pulmonary disease (COPD),[11] interstitial pulmonary fibrosis,[12] acute respiratory distress syndrome (ARDS),[13–15] persistent pulmonary hypertension of the newborn (PPHN),[16] primary pulmonary hypertension (PPH)[4] and cardiac surgery.[17]

The vascular reactivity to inhNO in clinical pulmonary hypertension varies widely, possibly because chronic pulmonary hypertension leads to various degrees of vascular remodeling and medial hypertrophy in the musculature of small pulmonary arteries. It was observed that the degree of acute pulmonary hypertension predicts the degree of responsiveness to inhNO.[17,18]

It has been shown that zaprinast[19] and dipyridamole[20] (PDE-V inhibitors) potentiate and prolong the pulmonary vasodilating action of inhNO, without changing its pulmonary vascular selectivity. Phosphodiesterase-V is a specific cGMP binding PDE found in highest levels in the lung, smooth muscle and platelet. By decreasing the catabolism of cGMP these drugs increase the level of cGMP after its synthesis has been increased by inhNO. The same authors proposed that PDE inhibition increases the response rate to inhNO or allows the use of much lower concentrations of inhNO (Fi_{NO}).[19,20]

Hypoxæmia

The pulmonary circulation is normally tightly controlled such that there is a matching of perfusion to ventilation through regulation of HPV. Attenuation of HPV results in areas of low ventilation/perfusion ratio ($\dot{V}/\dot{Q}$ mismatching) and right-to-left shunting of blood through pulmonary routes. This is the major cause of impaired gas exchange and hypoxæmia in ARDS. Other ætiologies of hypoxæmia involve $\dot{V}/\dot{Q}$ imbalance (high and low $\dot{V}/\dot{Q}$) with weak intrapulmonary shunt, such as in COPD, or right-to-left shunting through extrapulmonary routes, such as in some cardiac congenital disorders.

Inhaled NO is qualified as "microselective" in that it dilates only the vessels directly adjacent to the alveolar units being ventilated. Therefore, in patients with intrapulmonary shunt, inhNO can increase oxygenation by improving $\dot{V}/\dot{Q}$ matching with redistribution of blood flow from unventilated shunted areas to ventilated but underperfused areas, the so-called "steal phenomenon." This is a major advantage compared to intravenous vasodilators which tend to worsen $\dot{V}/\dot{Q}$ mistmatching by nonselectively dilating the pulmonary vasculature. Also, alveolar dead space[21] has been shown to decrease with inhNO. Some authors explained the improved oxygenation by the fact that inhNO produces

local bronchodilation,[21] decreases vascular permeability and the pressure-driven œdema formation, and exerts a platelet anti-aggregating effect. Paradoxically, inhNO worsened the gas exchanges in some patients with COPD,[11] where hypoxæmia is due to broad $\dot{V}/\dot{Q}$ heterogeneity, and may reverse HPV.

In patients suffering from ARDS, PAP might be high enough to increase right atrial pressure and induce a right-to-left shunt through a patent *foramen ovale* present in approximately 27% of the population. Inhaled NO, by reducing pulmonary and right atrial pressures while reducing or suppressing the anatomical shunt, may have a major effect on oxygenation,[12] where partial pressure of arterial O_2 (PaO_2) may be increased twenty six-fold.[22]

The effect of inhNO on oxygenation can be enhanced by vasoconstrictors, such as almitrine,[23] $PGF_{2\alpha}$, which are thought to enhance HPV, or by a PDE-V inhibitor.[20] The effect of inhNO can also be improved if more *alveoli* are recruited and if an optimal lung volume is achieved such as with positive end-expiratory pressure (PEEP)[21] and high frequency oscillating ventilation. Finally a combination of gravitational (prone position) and inhNO therapy resulted in an enhancement of the beneficial effects of both therapies.[24]

Inflammation and pulmonary œdema

Most forms of protective inflammation are exaggerated out of proportion to stimulus, because humoral homeostatic amplification systems (complement and kinin systems, coagulation cascade) recruit additionnal components of immune system (polymorphonuclear neutrophils - PMNs -, lymphocytes, and monocytes/macrophages) and platelets, initiating the production of pro-inflammatory mediators including cytokines (tumor necrosis factor - TNF- α- and interleukins - ILs -), lipid mediators (prostaglandins - PGs -, TxA_2, platelet-activating factor - PAF -, leukotrienes, etc), reactive O_2 species (ROS: superoxide anion - $O_2^{\cdot-}$ -, hydrogen peroxide - H_2O_2 -, hydroxyl radical - $OH^{\cdot}$ -, etc), and NO/cGMP pathway. Moreover, the expression of cells (leucocyte, platelet, endothelium) adhesion molecules (integrins, selectins, etc) and proteolytic enzymes (proteases, collagenase, elastase, gelatinase) contributes to epithelial and endothelial injury (Figure 1). Nitric oxide owes its ambiguous place in inflammation to the various active isozymes of NOS.

Anti-inflammatory activity of NO: The cNOS produce picomolar NO concentrations. Their dysfunction during inflammation might induce vascular (hypertension) and respiratory (hypoxæmia) disorders. It is well known that EDRF/NO exerts a tonic suppressive action on platelet and leucocyte activation, leucocyte adhesion,

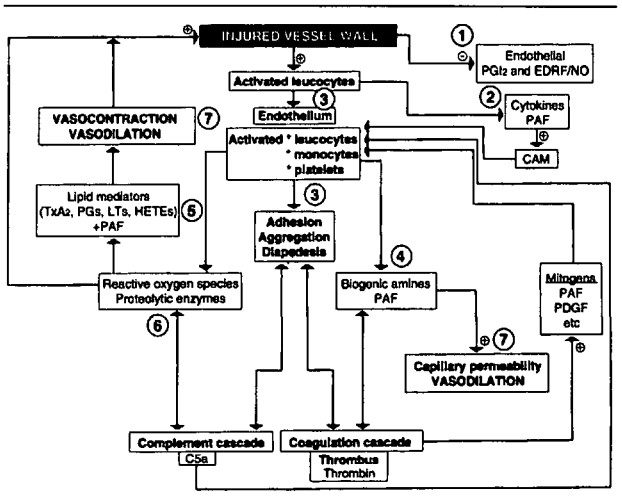

FIGURE 1 Inflammation process in the blood vessel.

The injury of the vessel wall stimulates macrophages, leucocytes and platelets, resulting in their activation, endothelial adhesion, aggregation, and diapedesis. Two main mechanisms are implicated in the inflammation process: the complement and coagulation cascades. Circled numbers indicate the potential sites of action of inhaled nitric oxide on:

1. the rehabilitation of tonic inhibitory influences; **2**. cytokines; **3**. inflammatory cells; **4**. biogenic amines; **5**. lipid mediators; **6**. reactive O_2 species; **7**. hypertension and ventilation/perfusion mismatchings.

PGI_2: prostacyclin, EDRF/NO: endothelium-derived relaxing factor/nitric oxide, PAF: platelet activating factor, CAM: cell adhesion molecules, PDGF: platelet-derived growth factor, TxA_2: thromboxane-A_2, PGs: prostaglandins, LTs: leukotrienes, HETEs: hydroxyeicosatetraenoics, C5a: C5a anaphylatoxin.

platelet aggregation and adhesion to endothelium, and mast cell degranulation. It inhibits expression of $ß_2$-integrin CD11b/CD18, and recent *in vitro* data suggest a predominant role of EDRF/NO in the reduction of endothelial cells adhesiveness to inflammatory cells.[25] The action of NO on *in vitro* pro-inflammatory cytokines production is controversial. Increased TNF-α production by monocytes and PMNs has been described by some investigators,[26] whereas others have reported decreased production of IL-1, IL-6, IFN-γ and TNF-α by monocytes and lymphocytes.[26,27] Racke *et al.*[28] have shown that EDRF/NO suppresses the phospholipase A_2-lipoxygenase pathway in rat alveolar macrophages. It attenuates the PiP_2 hydrolysis and consequent action of biogenic amines such as histamine, or eicosanoids such as TxA_2, and it presents a blocking and inactivating effect on protein kinase C activity. Through all these ways, EDRF/NO attenuates the expression of the acute phase response caracterised by cell adhesion molecules, cytokines, C-reactive protein, complement, and metal binding proteins.

Experimental evidences support that NO diminishes formation or reactivity of ROS through many potential mechanisms, e.g., by direct interaction with ROS such as $O_2^{\cdot-}$ producing peroxynitrite ($ONOO^-$). Thus, it was concluded by Maulik *et al.*[29] that NO is far more anti-oxidant than pro-oxidant.

Superoxide dismutase (SOD) is the classical endogenous mechanism of $O_2^{\cdot-}$ catabolism, and in physiological conditions, the enzyme's concentration is approximatively 10^6-fold greater than those of $O_2^{\cdot-}$ and NO, making $ONOO^-$ formation unlikely.[30] During inflammation, $ONOO^-$ formation may be favored owing to the relatively slow enzymatic dismutation of $O_2^{\cdot-}$ by SOD, as well as the relatively longer half-life (up to seconds) of NO. The reactivity and decomposition pathways of $ONOO^-$ are influenced by the chemical environment, the type of target molecules, and are strongly pH-dependent with regard to its *cis* (very stable diffusion form) and *trans* isomeric configurations (Figure 2).[30] Simply removing ROS from cells and tissues during inflammation is an important detoxification mechanism. Moreover, NO may compete with SOD for $O_2^{\cdot-}$, thereby removing $O_2^{\cdot-}$ and preserving SOD, further supporting its anti-oxidant role.

Inhibition of EDRF/NO synthesis with N^{ω}-nitro-L-arginine methyl ester (L-*N*AME) facilitates the contribution of H_2O_2 to the leucocyte-endothelial cell adhesion and consequent microvascular permeability in rat mesenteric postcapillary venules.[31] Cyclic GMP and PDE-II inhibitor reduce the porcine pulmonary artery endothelial hyperpermeability.[32] In previous studies, the same authors determined the endothelial PDE isozyme spectrum and found in swine that these cells lack PDE-V, the "classical" cGMP-degrading PDE in human. However, PDE-II was identified in swine as the major endothelial cGMP-degrading pathway. These studies outline the importance of NO and the second messenger system, cytosolic guanylate cyclase-cGMP, as a primary homeostatic regulator of microvascular permeability.

Pro-inflammatory activity of NO: Nitric oxide is released in high nanomolar concentrations by activated macrophages and PMNs for its cytostatic and cytotoxic effect mainly by alterating Fe intracellular homeostasis.[2] It has also been shown that iNOS is present in airway epithelium of asthmatic subjects and that lung tissue contains significant amount of Ca^{2+}-independent NOS activity in inflammatory diseases such as cystic fibrosis and obliterative bronchiolitis.[33] Estrada *et al.*[34] showed that cytokines-induced NO production might injure endothelial cells. The deleterious effect of excessive NO production in sepsis patients may not be limited to uncontrolled vasodilation and/or vasoplegia. Elusive "myocardial depressant factor(s)" of sepsis may be cytokines (particularly IL-1, TNF-α) which act to depress myocardial performance *via* NO/cGMP-dependent mechanisms.[35]

$ONOO^-$ with:

$R\text{-}SH \rightarrow R\text{-}S_{OX}$ (2)

$H^+ \rightarrow$ "$OH^{\cdot}$ ..... $NO_2^{\cdot}$" (3)

SOD, Metals $\rightarrow$ $NO_2^+ + OH^-$ (4)

$OH^- \rightarrow NO_3^-$ (5)

Adapted from *Beckman*.[30]

FIGURE 2 Summary of the peroxynitrite reactivity and decomposition pathways.

In the *cis* form and at high physiological pH, peroxynitrite ($ONOO^-$) is very stable (diffusion form), and should preferentially react (2) with sulfhydril (-SH) group and ascorbate, which are present in most biological systems. Oxidation of critical SH groups is responsible for the inhibition of mitochondrial and cytosolic aconitase (necessary for the Krebs cycle) and other critical enzymes in the mitochondrial respiratory chain and disruption of the zinc-thiolate center at the active site of enzymes. In the *trans* form, a slight acidic pH will favor reaction (3) supported mainly by *in vitro* experiments, whereas at neutral pH, nitration reaction (4) need to be catalyzed by superoxide dismutase (SOD) or transitional metals (Fe, Cu). For instance, tyrosine nitration may lead to dysfunction of nitrated proteins such as SOD, cytoskeletal actin, and neuronal tyrosine hydroxylase. At slight alcaline pH (8), $ONOO^-$ will isomerise directly (5) in nitrate (NO_3^-). Moreovever, in the presence of plasma, proteins, glucose, or gluthatione, $ONOO^-$ can form intermediates that act as NO donors.

Moreover, $ONOO^-$ mediated tissue injury has been implicated as being important in the pathogenesis of ARDS. Peroxynitrite is able to inhibit mitochondrial respiration, or α_1-antiprotease which protects the lung from PMNs-dependent proteases, to damage surfactant components, and to alter amiloride sensitive-Na^+ channels which play an important role in the control of pulmonary œdema.[30] It might also induce lipid peroxidation as well as protein and DNA dysfunction with tyrosine nitration.[36] Nitric oxide could also react with H_2O_2 generating a highly ROS, the singlet O_2.[37]

Sites of inhNO action on inflammation: The 7th site of action noted in Figure 1 indicates the anti-hypertensive and oxygenative properties of inhNO. Moreover, inhNO might have a predominant effect on postcapillary sphinc-

ters reducing the resistance more on the venous than on the arterial side of the pulmonary circulation,[38] and the pressure-driven œdema. Inhaled NO attenuates the increase in pulmonary vascular permeability resulting from infusion of xanthine/xanthine oxidase, and glucose/glucose oxidase, respectively $O_2^{\cdot-}$, and H_2O_2 generating systems (**#6** action). After 4 days of NO inhalation (18 parts-per-million - ppm -), PMNs from bronchoalveolar lavage in ARDS patients showed a reduction in spontaneous H_2O_2 production[39] (possibly explained by NO scavenging of $O_2^{\cdot-}$: **#6** action). Indeed, Garat *et al.*[40] observed that 10 ppm inhNO during prolonged exposure to 100% O_2 decreased pulmonary œdema, decreased lung endothelium permeability, and increased alveolar liquid clearance. Inhaled NO reverses the cardiovascular and respiratory disorders of eicosanoid (**#5** action)[41] or amine (**#4** action) infusion. It reduces the pulmonary platelet and PMNs sequestration,[42] probably by inhibition of aggregation[43] and/or adhesion of these cells (**#3** action). Chollet-Martin *et al.*,[39] also showed that inhNO decreases $ß_2$-integrin CD11b/CD18 expression (**#3** action) and the high levels of cytokines (IL-6 and IL-8) decreased in bronchoalveolar lavage fluid supernatants after NO inhalation (**#2** action). Finally, the potential beneficial interrelations of these different actions, and the critical timing of inhNO initiation in a rat model of acute lung injury[44] outline the importance to re-establish as soon as possible the tonic inhibition of EDRF/NO suppressed by inflammation (**#1** action).

EXTRAPULMONARY EFFECTS OF INHALED NITRIC OXIDE

The fundamental question of how EDRF/NO exerts its biological activity, particularly its transport to molecular targets, remains unknown.[7] Metal nitrosylation with hæm and nonhæm proteins as well as the reactions of NO with nucleophil groups (e.g., sulfhydryl, amine) of amino acids, peptides, and proteins lead to the potential interactions and formation of many nitrosyl-hæm adducts, *S*-nonprotein-nitrosothiols, *S*-nitrosylated proteins,[2] nitrosylated Fe-sulfur clusters, nitrosamines, and others. Thiols present the greater prevalence and reactivity over other biological nucleophils, and sulfhydryl radicals are found abundantly in proteins. Particularly, the *S*-nitroso albumins[45] and *S*-nitrosohæmoglobins[7] might constitute a reservoir which could protect EDRF/NO from its inactivation in the blood stream, and have EDRF-like properties distant (spacially and temporally) from its site of production.[45]

CARDIOVASCULAR EFFECTS OF INHALED NITRIC OXIDE

In a previous study,[46] we have already suggested that inhNO has a direct cardiac effect identical to that of endothelium-independent nitrovasodilators, such as depressed myocardial contractility, improved ventricular relaxation and diastolic distensibility.[47] Frostell *et al.*[3] observed a slow decaying effect on PAP from previous NO inhalation for which they proposed a mechanism of storage or binding of NO to proteins or the formation of thiols in lung tissue. Shah *et al.*[48] reported a small but significant drop in systemic arterial pressure only at the highest Fi_{NO} (80 ppm).

PLATELET EFFECTS OF INHALED NITRIC OXIDE

It is well known that platelets flowing through the lungs treated with inhNO will show increased cGMP level,[43] and this may prolong the bleeding time. Recent data have shown that inhNO decreased platelet aggregation in patients suffering from ARDS,[43] but had no effect on platelet aggregability nor bleeding time in healthy subjects.[49]

RENAL EFFECTS OF INHALED NITRIC OXIDE

We have also established that 40 ppm inhNO increases renal blood flow, glomerular filtration rate and diuresis in pigs, independently of its effect on pulmonary and systemic hæmodynamics.[50] In view of the rapid renal response to application and discontinuation of inhNO, we postulate that inhNO may be accompanied by non-selective, extrapulmonary effects due to local delivery of NO on peripheral territories including the renal bed.

Therapeutic indications of inhaled nitric oxide

Pediatric patients

PERSISTENT PULMONARY HYPERTENSION OF THE NEWBORN

In neonates with PPHN, inhNO is very efficient to reduce PAP and PVR, improve the oxygenation, and avoid the treatment with extracorporeal membrane oxygenation (ECMO).[16] The increase in flow through the lung can in turn increase EDRF/NO production by the pulmonary endothelial cells, through stimuli such as PaO_2 (O_2 is a cosubstrate of NOS) and shear stress. The persistent fœtal circulation is probably due to immature EDRF/NO response or impaired endogenous EDRF/NO activity.[51] Several prospective clinical trials are underway to determine if inhNO is really the ideal therapeutic agent for this pathology, without any toxicity and adverse reactions.

CONGENITAL DIAPHRAGMATIC HERNIA

Similar to PPHN, congenital diaphragmatic hernia is a disease in which potentially reversible pulmonary hypertension may produce hypoxæmia. Various reports mention the usefulness of inhNO improving oxygenation before proceeding with repair of the diaphragmatic hernia.[52]

CONGENITAL HEART DISEASE

Of the various congenital heart diseases, many are associated with progressive pulmonary hypertension, reversing the left-to-right shunt (due to a defect at the atrial, ventricular, or aortopulmonary level) to a right-to-left or bidirectional shunt (Eisenmenger's syndrome) leading to clinical cyanosis and the secondary manifestations of chronic hypoxæmia. In these conditions, reports indicate that inhNO is efficient in reducing PAP and improving oxygenation.[53] Other applications of inhNO involve perioperative pediatric surgery, and are adressed in the ***Heart/lung surgery*** section.

Heart/lung surgery

The pathogenesis of organ injury following extracorporeal circulation involves many inflammatory cascades and cellular components of the immune system. Pulmonary hypertension is a common feature following congenital heart defect repair in children, valvular or coronary artery bypass surgery in adults,[17] or heart and/or lung transplantation.

One therapeutic approach is to target the PMNs with mechanical removal of circulating PMNs or modulation of the adherence of PMNs to endothelium. Another approach is to recreate the inhibitory influences which were attenuated during the inflammatory process (see ***Inflammation*** section).

Several publications[17,54] have shown that inhNO is efficient in reducing pulmonary hypertension in these situations and most of the pediatric cardiac surgery teams, which have the opportunity, are using inhNO as first medication. Inhaled NO is the medication of choice for treatment of pulmonary hypertension and hypoxæmia following cardiopulmonary bypass or the use of a ventricular assist device,[55] for mitral valve replacement,[17] coronary artery bypass graft,[17] heart or lung transplantation, and pulmonary embolism.[56]

Primary pulmonary hypertension

Primary pulmonary hypertension was the first pulmonary hypertensive syndrome reported to respond to inhNO. Sitbon *et al.*[57] concluded that inhNO could thus now be regarded as the gold standard screening agent in patients with PPH evaluated for vasodilator therapy, although its long-term use and safety in these patients remains to be evaluated. Other issues such as dose-response data, its effects on oxygenation and its role as a bridge to lung transplantation also need to be adequately assessed.

Chronic obstructive pulmonary disease and pulmonary fibrosis

The efficacy of inhNO to decrease the pulmonary hypertension observed in COPD[11] and pulmonary fibrosis[12] is well recognized. However, the effect of inhNO on gas exchange in such patients has sometimes been disappointing despite concomitant and consistent reduction in pulmonary hypertension.[11] As mentionned in the ***Oxygenation*** section, this defect is due to the reversion of HPV by inhNO. Other authors reported an improvement in arterial oxygenation.[58] Once again appropriate studies are necessary to determine the real adequacy of inhNO with such patients as a long-term therapeutic strategy.

Bronchospastic disease

Inhaled NO is a powerful bronchodilator comparable, and additive, to $ß_2$-agonists in animal models of induced bronchospasm.[59] In healthy men, inhNO (80 ppm) had a modest bronchodilatory effect compared to ß-sympathomimetic drugs, where it only slightly increased conductance.[60] The same dose had no effect on airway tone of healthy volunteers and COPD patients, and only weak bronchodilatory effect in asthmatic patients.[61] There is a relative dose/effect relationship with increased doses showing successively stronger effects on oxygenation, pulmonary hypertension, and bronchomotor tone.

Acute respiratory distress syndrome

Initially viewed solely as a surfactant abnormality, identical to that seen in neonatal respiratory distress syndrome,[62] ARDS is now considered as an inflammatory process, the magnitude of which transcends the pulmonary lesion and includes involvement of the microvasculature in multiple organ systems.[63] Therefore, in patients suffering from ARDS, the pattern of inflammation, as previously described, is prominent, and the endothelial dysfunction seems most often to preceed the epithelial injury.

Almost three decades after the first description of ARDS by Ashbaugh *et al.*,[62] the survival rate is not markedly improved despite complex and expensive curative technologies.[64] The intrinsic heterogeneity of study populations, the potential confounding influence of variables other than primary damage, and the highly variable time-course to complete resolution or death, all hamper the ongoing evaluation of new therapies and underline the need for larger sample size. Ventilatory support remains the cornerstone of ARDS clinical management. The benefits of PEEP were first noted by Ashbaugh *et al.*[62] Several other ventilatory approaches have been tested including inverse-ratio, pressure-controlled and high-frequency jet ventilation, permissive hypercapnia, ventilation in prone position, ECMO, or intravascular oxygenators. The main goal of these strategies is to increase systemic oxygenation and at the

same time reduce the potential O_2 toxicity, barotrauma and/or volotrauma. However, no randomized controlled trials have found clear benefit of these therapies. The other experimental approaches to ARDS aim at limiting the pulmonary damage and the inflammatory process. Using exogenous surfactant, perfluorocarbon, almitrine, pentoxifylline, PGE_1, antagonists of PAF, IL-1, TNF-α or leukotriene B_4 receptors have shown some encouraging results. But are they efficacious on human ARDS, without side effects and complications, and are they affordable?

As previously demonstrated, inhNO's local effects on pulmonary hypertension and consequently on right ventricular dysfunction, oxygenation, inflammation or pulmonary œdema, argue for its use in ARDS. Moreover, the extrapulmonary effects of inhNO (i.e., against platelet activation,[43] cardiac positive lusitropic,[65,66] and renal protective[50] effects) could also be helpful on the systemic disorders observed with ARDS.

However, at this time, it is impossible to corroborate the prognosis of the disease and the efficiency of the inhNO treatment on the pulmonary function. Wenstone and Wilkes[67] mentionned that it seems unlikely that new therapies directed solely at treatment of the lung will reduce the mortality significantly from what is essentially an inflammatory systemic disease. Owing to the absence of appropriate studies, several unresolved questions persist: 1) Incidence of inhNO on ARDS mortality? 2) Methodological criteria for NO inhalation on the choice of time and patients to treat, NO dose-response, concomitant treatments? 3) Potential toxicity of inhNO? (see **Toxicology** section).

We undertook an analysis, to our knowledge, of all available data pertaining to the treatment of ARDS with inhNO in order to obtain a larger sample size and a better idea of the heterogeneity between studies (Table).

1) McIntyre *et al.*[68] noted that "In the studies done by Rossaint[13] and Gerlach,[14] the majority of patients had ARDS as the result of direct lung injury. Two-thirds of the patients also had ECMO. Thus, it is unclear if these results may be extended to patients with ARDS due to systemic ætiologies not treated with ECMO." If the noteworthy work of Murray *et al.*[69] helped establish a universal definition of ARDS, the critical remark of McIntyre *et al.*[68] questions the heterogeneity of populations and treatments between the actual studies on ARDS treated with inhNO. With our analysis, the global survival rate of ARDS patients with inhNO (n=256) is 54.7%, which is not different from previous studies not using inhNO.[62] Moreover, when we distinguish the pulmonary from systemic ætiology of ARDS, we find respective survival rates of 69.4% (n=98) and 40.2% (n=97).

Since the first enthusiastic report of Rossaint *et al.*,[13] and despite the recommendations of the American-European ARDS consensus,[63] no study has been appropriately designed to address the clinical outcome of ARDS patients treated with inhNO. Small sample sizes, heterogeneous populations and treatments, as well as selection bias (only 5/28 studies[13,65,70–72] were clearly randomized), plagued previous studies on the effect of inhNO in ARDS patients. Moreover, none of them used a control group without inhNO monitored in parallel except Chollet-Martin *et al.*[39] whose main objective was not the survival rate, Blaise *et al.*[72] who realized a pilot randomized controlled clinical trial, and Rossaint *et al.*[73] whose study was retrospective. Several of these studies carried out a cross-over design with PGI_2[13,65,71] or almitrine[74,75] to study the efficiency of inhNO on hypoxæmia. This design, however, cannot address important clinical outcomes such as survival or weaning and is very limited by the fact that ARDS condition is not stable in time. The lack of consistency in previous studies could result from the high variety of ARDS ætiologies, the large Fi_{NO} (10 parts-per-billion - ppb -[14] to 128 ppm[76]) and age (1[73] to 81[70,77] years) ranges. Most of the authors used a unique Fi_{NO} dose, which does not optimize treatment. Finally, several studies used concomitant treatments such as ECMO,[13,14,65,73,78] permissive hypercapnia,[70,77,79,80] or vasoactive drugs,[18,66,74,76,79,81,82] which make it difficult to compare outcomes. Two other multicenter randomized prospective clinical trials are under way to determine the exact role of inhNO in ARDS morbidity and mortality.

2) Despite the improvement in PAP and oxygenation, it was not possible to find an improvement in the survival rate induced with inhNO in patients sufferring from moderate to severe lung injury. It is possible that inhNO has been used at a late stage of the disease,[15] and that early treatment could be more efficient on mortality, as suggested by experimental report.[44] The effects of inhNO appear within 10 seconds after initiating treatment,[70] and reverse as rapidly after stopping treatment. Tachyphylaxis has not been demonstrated, but dependence on inhNO for maintaining arterial oxygenation and pulmonary hæmodynamic stability occurs. In our own experience[72] of sepsis-induced ARDS, we have observed that inhNO initially improved PAP and oxygenation. This effect was sustained in time but no secondary improvement in pulmonary function occured after the initial effects, and the mortality rate was the same in the group of patients treated with inhNO (n=15) and in the control group (n=15).

A first predictive indication seems to come from the ARDS ætiology (direct *versus* indirect lung injury). During the past two years, it became evident that several authors tried to define a subgroup of patients to

TABLE Available data on treatment of ARDS with inhNO.

Reference	*n=*	*Age-range* (Years)	*LIS-range*	*iPAP$_M$* (mmHg)	*fPAP$_M$* (mmHg)	*iPaO$_2$/FiO$_2$* (mmHg)	*fPaO$_2$/FiO$_2$* (mmHg)	*Fi$_{NO}$* (ppm)	*% NR*	*Survival rate* (%)
Rossaint[13]	10	17–46	3.20 – 4.00	37 ± 3	30 ± 2	152 ± 15	199 ± 23	18 and 36	–	80
Gerlach[14]	12†	9–53	3.00 – 4.00	41.7 ± 4.2	28.9 ± 1.4	75.3 ± 8.2	–	0.001–100	–	–
Gerlach[78]	3	13–35	3.00 – 3.75	–	–	58 ± 13.1	75.4¶¶	0.06–0.23	0	100
Wysocki[80]	4	–	2.9 ± 0.2‡‡	34 ± 2	28 ± 7	82 ± 37	126 ± 79	10–20	0	50
Ricou[83]	5	–	> 2.50	35 ± 3.5	31.8 ± 3.4	–	–	25–45	80	–
Bigatello[18]	13	22–71	3.00–4.00	34 ± 1.9	30 ± 1.9	126 ± 9.6	149 ± 10.1	0–40	54	31
Germann[24]	6	–	> 2.50	33.3 ± 2.1	26.9 ± 2.5	117.2 ± 11.8	148.1 ± 13.8	10	–	–
Puybasset[70]	6	20–81	2.50–3.50	33 ± 3	–	–	–	0.1–5	0	83
Wysocki[74]	17	19–71	2.75–3.66	30 ± 1.2	26 ± 1.2	88 ± 7.3	98 ± 9	5–10	59	–
Young[76]	14	20–75	–	37.6 ± 5.3	35.9 ± 5.9	94 ± 11.2	–	8–128	39	–
Puybasset[77]	11‡	20–81	2.50–3.75	31 ± 6	28 ± 4	184 ± 67	270 ± 87	2	–	–
Mira[81]	6	–	3.00–3.50	43	36	79	118	15	100	17
Monchi[84]	19	–	–	–	–	–	–	15	47	–
Roupie[85]	17	–	> 2.50	–	–	–	–	10	65	–
Steltzer[86]	28	–	> 2.50	–	–	–	–	–	64	43
Samama[43]	6	25–64	2.00–3.70	26 ± 8	21 ± 8	151 ± 70	244 ± 83	3	–	100
Rossaint[65]	10§	11–30	2.50–4.00	33 ± 2	28 ± 1	135 ± 18	195 ± 27	18 and 36	–	70
Fierobe[66]	13	18–45	2.50–4.00	36.1 ± 1.2	31.3 ± 1.7	103 ± 13	142 ± 17.5	5	23	69
McIntyre[68]	14	18–80	–	41.1 ± 1.8	34.3 ± 1.3	69.5 ± 3.9	100.8 ± 9.5	20 and 40	29	50
Blaise[72]	15¶	20–72	2.50–3.75	30.2 ± 1.2	26.9 ± 1.3§§	119.4 ± 13.6	189.8 ± 13.4§§	8.5 ± 2.6§§	33.3	40
Rossaint[73]	26‖	1–62	2.50–4.00	35 ± 2	31 ± 2	98 ± 8	132 ± 12	0.01–25	46	69
Lu[75]	6**	25–69	2.30–3.00	31 ± 8.2	–	99 ± 8.7	158.4¶¶	0.15–45	–	67
Levy[79]	20	20–80	3.60 ± 0.2‡‡	31 ± 3	30 ± 2	78 ± 10	130 ± 25	5–10	5	70
Levy[82]	30	–	3.45‡‡	29 ± 3	28 ± 2	81 ± 8	–	5	7	–
Lowson* 1/2[15]	10	36–67	2.75–4.00	38 ± 3.5	–	136 ± 16.8	171.4¶¶	5–20	10	50
Lowson* 2/2[15]	8	22–72	2.75–4.00	36.4 ± 2.5	–	93.8 ± 7.3	114.4¶¶	0.1–10	13	38
Chollet-Martin[39]	9††	16–60	2.00–4.00	–	–	114 ± 9.2	182 ± 11.5	18	11	–
Walmrath[71]	16	22–72	2.60–3.30	34.8 ± 2.2	33 ± 1.8	115 ± 11.7	144 ± 14.5	2–40	–	56
Krafft[87]	25	19–74	2.50–4.00	–	–	93 ± 7.6	–	18 and 36	60	44
TOTAL (28)	379	1–81	2.00–4.00	26–43 34.3 ± 0.9	21–36 29.4 ± 0.8	58–184 106 ± 6.7	75.4–270 155 ± 9.9	0.01–128	115/301 38.2%	140/256 54.7%

Abbreviations:
n=sample size; LIS-range: lung injury score of Murray *et al.*[69]; iPAP$_M$: initial mean pulmonary arterial pressure; fPAP$_M$: final mean pulmonary arterial pressure after inhNO treatment; iPaO$_2$/FiO$_2$: initial hypoxia score; fPaO$_2$/FiO$_2$: final hypoxia score after inhNO treatment; Fi$_{NO}$: inspired fraction of NO; %NR: percentage of nonresponders to inhNO.

Footnotes:
* The study of Lowson *et al.*[15] was divided in 2 parts;
† The 3 patients of Gerlach *et al.*[78] were included in this study;
‡ The authors have excluded 7 ARDS patients of sepsis origin, 3 of which were nonresponders to inhNO treatment;
§ Five of these patients were previously presented[13];
¶ The authors used a control group treated with usual care comprising of 15 patients;
‖ The ARDS patients were matched with 57 control patients;
** Five nonresponders to inhNO treatment were excluded;
†† The authors used a control group comprising of 5 patients;
‡‡ Only mean values were available;
§§ These results were obtained after determination of the initial optimal Fi$_{NO}$ during the inclusion-day;
¶¶ The values were calculated from indicated percentage increases.

treat. The consequence was the appearance of nonresponders to inhNO. First described by Ricou and Suter,[83] several authors proposed different criteria to define nonresponders.[15,68,71–74,76,81–87] A level of improvement of 20% in hypoxia score (PaO$_2$/FiO$_2$) is found in the majority of the literature, but we also agree with Lowson *et al.*[15] that all patients presenting an increase in PaO$_2$/FiO$_2$ due solely to inhNO must be considered as a responder. By using each author's criteria, we found that the survival rate in responders was 59.2% (n=71), significantly higher than that of nonresponders (23.2%; n=43).

Possible reasons about nonresponsiveness to inhNO include the patient's age,[85] ætiology (sepsis is mainly incriminated),[72] pathophysiology and severity of the insult,[73,82] particularly the $\dot{V}/\dot{Q}$ mismatching (as in COPD)[68,73] or an impaired cardiac reserve.[87] Other hypotheses include a failure in the target enzyme (i.e., the soluble guanylate cyclase of pulmonary vascular smooth muscle)[81,82] and an inappropriate concentration of exogenous NO.[73]

To avoid this latter situation, we recommend the establishment of an optimal dose against oxygenation and/or pulmonary hypertension. In our clinical trial,[72] the minimum Fi_{NO} which had a maximal effect on PaO_2 was determined daily for every patient. This optimal Fi_{NO} varied from 500 ppb to 40 ppm, according to the protocol, and the mean dose used for the total inhNO treatment duration (8.1 ± 1.3 days) was 5.6 ± 1.8 ppm (mean ± SE).

Inhaled NO could reversibly inhibit the NOS present in the airways and pulmonary circulation and decrease the endogenous pulmonary NO produced.[18,78] This down-regulation of pulmonary NOS could explain the rebound pulmonary hypertension often seen when inhNO treatment is suddenly interrupted.[88] Daily optimal Fi_{NO} determination, through regular reverse dose-response assessments allowed patients to be gradually weaned from inhNO and limits this risk.

It is interesting to note that Levy *et al.*[79] found a mortality rate of 30% (n=20) when using therapeutic optimization (inhNO, vasoactive drugs, permissive hypercapnia, PEEP, pressure-controlled with inverse ratio ventilation, tracheal gas insufflation, prone position, pleural drainage, continuous hæmofiltration, transfusion, treatment of infection and corticosteroids) in ARDS patients. The use of concomitant treatment to inhNO seems to be one other way of the future.

Diagnostic indications of nitric oxide

Diagnosis of pulmonary hypertension reversibility

BEFORE CARDIAC TRANSPLANTATION

Inhaled NO (80 ppm) was used in 11 patients to test the reversibility of pulmonary hypertension, and establish the indications for heart or heart/lung transplantation.[89] Inhaling low levels of NO may provide an important and safe means for evaluating the pulmonary vasodilatory capacity of patients with congenital heart disease without producing systemic vasodilation. In addition, studies have suggested that inhNO can be successfully used for graft dysfunction following lung transplantion, even in life-threatening situation.

However, several cases of pulmonary œdema have been observed in patient candidates for heart transplantation in whom inhNO was used as a screening for the reversibility of pulmonary hypertension.[90] This adverse effect will be discussed in the **Toxicology**, ***Potential toxicity pertaining specifically to inhaled nitric oxide*** section.

AFTER NEONATAL CARDIAC OPERATION

The use of a short challenge with inhNO was reported after operation in the neonate with congenital heart disease and proximal pulmonary artery hypertension or excessive cyanosis. The results of such a challenge may be used advantageously to direct therapy in the patient with pulmonary vasoconstriction who shows response to inhNO or in the patient who is nonresponder to inhNO with indication of further investigation of surgically remediable obstruction to pulmonary blood flow.

Determination of the diffusion capacity at the alveolocapillary membrane

Carbon monoxide (CO) is used to measure the pulmonary diffusing capacity (D_L) because of its very high affinity for hæmoglobin, and because of its uptake almost independent of capillary blood flow. Various factors favor the replacement of CO by NO. The velocity constant of combination of NO with hæmoglobin is almost 280 times faster than that of CO and about 5 times faster than that of O_2. Nitric oxide is close to twice as soluble in water than CO (at 37.5°C). Nitric oxide has 1 500 times more affinity for hæmoglobin than CO, and a D_L value four-fold greater.[91] Nitric oxide is therefore more independent of capillary blood flow than CO, and better suited to measure D_L.

Exhaled nitric oxide

Breathholding from 5 to 60 seconds increased peak exhaled NO from 6.1 ± 2.2 ppb to 143 ± 48 ppb respectively, but end-expiratory NO levels only increased from 3.9 ± 0.7 ppb to 11 ± 2.9 ppb.[92] Smokers are known to have lower levels of exhaled NO, while intubated patients showed no exhaled NO, or extremely low levels in their exhaled air.[9] It is now known that asthmatic and bronchiectatic patients exhale greater peak levels of NO when not treated with inhaled corticosteroids, as opposed to those under treatment and normal subjects. Furthermore, symptoms of upper respiratory tract infections markedly increased the concentration of exhaled NO (315 ± 57 ppb).[93] These studies emphasize the fact that chronic inflammation in the respiratory tract may be cytokine-mediated, and that exhaled NO could be used to assess disease severity and thereafter to judge the efficacy of appropriate treatment. Exhaled NO in septic shock might be used as a marker of endogenous NO production and correlated to the plasma level of NO_x^-.

Gerlach *et al.*,[9] found that exhaled NO was mostly produced in the upper airways, and Lundberg *et al.*[94] confirmed that NO originates mainly from the epithelium of the paranasal sinuses in concentrations ranging from 890 ppb to 23 300 ppb. Inhaled endogenous NO may enhance the pulmonary O_2 uptake or contribute to the successful circulatory adaptation necessary at birth by controlling pulmonary and airway blood flow and airway diameter. Localized responses, including secretory immunoglobulin A, mucus, and the rapid flow in the respiratory tract are important first-line host-defense mechanisms that protect our internal and external environments. Inhaled endogenous NO was shown to be implicated in all these phenomena, particularly controlling the epithelial ciliar motility or killing bacteria.

Toxicology

Acute toxicity of nitric oxide and nitrogen dioxide

The inhalation toxicology of NO and NO_2 are difficult to ascertain separately because of the oxidation of NO to NO_2 (and other NO_x) in the presence of O_2. Nitric oxide and NO_2 have been responsible for a death which occured in 1967 during anæsthesia. A nitrous oxide (N_2O) cylinder had been contaminated by a large amount of NO and NO_2. Nitric oxide and NO_2 are also responsible for the death occurring as a result of silo filler's disease, when farmers go inside poorly ventilated silos and inhale large amounts of NO and NO_2. In these situations, a pulmonary œdema with increased permeability was induced by the oxidative stress due to NO, NO_2, resulting in an acid pneumonitis. It is expected that human exposure to 25 ppm NO_2 for 60 minutes can lead to respiratory irritation and chest pain, whereas 50 ppm can cause pulmonary œdema.[95]

Health and safety standards

Threshold limit values have been set by the U.S.A. Occupational Safety and Health Administration (OSHA) for permissible exposure limit for workers. The OSHA has set permissible exposure limits for NO not to exceed 25 ppm for an 8-hour time-weighted average period, and for NO_2 not to exceed 5 ppm during any part of the working day. On the other hand, the U.S.A. National Institute for Occupational Safety and Health (NIOSH) has set the recommended exposure limit for NO_2 not to exceed 1 ppm for a 15 minutes exposure, and a maximum inhaled NO_2 level of 5 ppm. The U.K. standard set by the Health and Safety Executive is 3 ppm NO_2, and in France 2 ppm for NO_xs. Although there is consensus for NO exposure limits, NO_2 is still controversial.

Toxicity pertaining specifically to nitrogen dioxide

Nitrogen dioxide is an oxidant gas that contaminates ambiant air in many urban and industrial sites, and indoor air in homes with combustion appliances. The primary site of damage after NO_2 inhalation is the terminal bronchioles and the proximal *alveoli*. Like ozone (O_3), NO_2 causes oxidative damage that results in the generation of free radicals that may oxidize amino acids and initiate lipid peroxidation in pulmonary cell membrane. The lung injury is characterized by increased extravascular lung water, extravasated erythrocytes, type II pneumocytes hyperplasia and accumulation of fibrin, polymorphonuclear cells and alveolar macrophages in the *alveoli*. Nitrogen dioxide may adversely affect the efficiency of lung defense such as the mucocilliary clearance, the alveolar macrophage, and the immune system. However, the combined effect of pulmonary inflammation, high FiO_2, high ventilatory pressure and NO_2 on long term pulmonary function is not known.

Potential toxicity pertaining specifically to inhaled nitric oxide

Nitric oxide is 5 times less oxidant than NO_2 and 25 to 50 times less oxidant than O_3. Concentrations of inhNO as high as tens of ppm have been given to patients for weeks without apparent toxic pulmonary effects, and 80 ppm inhNO in healthy volunteers or in patients with COPD did not affect airway conductance.[61]

The safety levels of the above mentionned administrative authorities are based on earlier studies on NO and NO_2 toxicity. Interpretation of those studies in the context of long-term administration of inhNO is complicated by several factors. In our opinion, standards must be revised and adapted, taking into account that patients may inhale the gas 24 hours a day for long periods of time, and our better knowledge of inhNO dose-efficiency and indications (dose-response relationship for bronchodilation, vasodilation, and oxygenation).

It was suggested that the potential toxicity of inhNO comes not from NO itself but from its reaction product with $O_2^{\cdot-}$, i.e., $OONO^-$ (Figure 2).[96] Direct intratracheal administration of inhNO could be noxious since it might induce local mucosal lesions on the epithelial lining of airways, and decrease the surface activity of alveolar surfactant dependently of the relative concentrations of individual species ($O_2^{\cdot-}$, NO, $ONOO^-$) responsible of its pro-oxidant activity. Inhaled NO may exert a negative feed-back on the endogenous pulmonary vasodilation which creates dependency of treated patients to the inhNO therapy.

The other potential dangers of inhNO come from its extrapulmonary effects. The platelet effects of inhNO

increase the bleeding time only in presence of coagulopathy.[43] Cardiac activity may have adverse effects (pulmonary œdema) in patients with severe heart failure.[90] The cause of pulmonary œdema development was unclear and a mechanical origin (increased pulmonary wedge and left ventricular filling pressure), associated with a poor left ventricular function, and/or an alteration of the permeability induced by inhNO was proposed. However, a theoretic analysis[97] has established that, in this situation, an increase in pulmonary venous pressure was compatible with a drop in PVR. We have encountered the same problem with two young women with PPH who developed pulmonary œdema (Blaise G, Francœur M, Troncy E, *unpublished data*, 1996). Also, inhNO caused a severe systemic hypotension in a cardiac neonate defect by reversing the left-to-right shunt.[22]

In most clinical situations, methæmoglobin levels remain low during inhNO therapy and no bleeding complications occured. The disease and the conditions of use of inhNO therapy[98] (high concentrations, long duration of treatment, concomitant intravenous nitrovasodilators, etc) may influence the rate of formation of methæmoglobin. Indeed, Dyar *et al.*[99] observed a linear increase of methæmoglobin with 512 ppm inhNO (in sheep), and found that it increased to 11% after only 20 minutes of inhalation, confirming that increase in methæmoglobinemia is both time and dose-dependent. Moreover, some patients may have a partial (such as native Americans) or complete (immature system in neonates) deficiency in methæmoglobin reductase. With careful monitoring and appropriate management, it appears that inhNO is safe for these patients. An increased methæmoglobinemia is usually easily treated by reducing the Fi_{NO}; only rarely does a treatment with methylene blue or *N*-acetyl-L-cysteine have to be used. In one case,[98] methæmoglobinemia increased up to 14% (with 80 ppm) and was treated with 500 mg vitamin C and a blood transfusion where it returned to 8%. The dose-dependent methæmoglobinemia could be increased with a deficiency in glucose 6-phosphate dehydrogenase (G6-PD). Individuals with this deficiency, most frequently of African or Mediterranean extraction, are asymptomatic until oxidative stress such as H_2O_2 depletes the glutathione reductive pathway in the erythrocytes, inducing methæmoglobinemia formation.

Conclusion

In 1994, Frostell [100] wrote "It is a sincere hope that the promising development of inhaled nitric oxide as therapy ... is not jeopardized by our own over enthusiastic and ill-advised use outside randomized trials." In 1997, the question of the real therapeutic role of inhNO remains. With the discovery of extrapulmonary effects of inhNO, its useful range of action will transcend the pulmonary area and the classical view of inhNO as a selective pulmonary vasodilator. The importance of indicating inhNO in neonatal and pediatric critical care is becoming more evident every day. With regards to its potential deleterious effects, the parameters of inhNO use need to be assessed, particularly in adults. Inhaled NO should be used in a selective and controlled manner in intensive care units if we wish to avoid situations where disappointments surpass expectations. During and after cardiac and/or pulmonary surgery, inhNO is a powerful therapeutic tool as a selective pulmonary anti-hypertensive and oxygenative agent. Its diagnostic indications of the pulmonary vascular reactivity and/or gas diffusion capacity status of the patient provide a new path which should be enthusiastically explored. Moreover, exhaled NO may be useful as marker for asthma or inflammation evolution. In other pathologies such as acute lung injury, it seems more appropriate to classify inhNO as an adjuvant therapy than as a miracle drug, and its potential preventive role in development of acute lung injury is really promising but also needs to be fully elucidated.

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