Topical Review

Nitric oxide and the respiratory system in health and disease

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Introduction

Oxides of nitrogen including nitric oxide (NO) are gases present in atmospheric air, and have been considered as air pollutants derived predominantly from automobile exhaust and domestic gas cookers. Indeed at one stage NO was considered as a potential toxic lethal contaminant of nitrous oxide cylinders used in anaesthesia (1). However, following the independent identification of the endothelium-derived relaxing factor (EDRF) by Palmar et al. (2) and Ignarro et al. (3) in 1987 as nitric oxide, and the subsequent recognition of the L-arginine:NO pathway (4,5), NO was named molecule of the year by Science in 1992. Since then a considerable body of research has been conducted to characterize the regulatory role of NO in various organ systems in animals and humans, in both health and disease, and it is now appreciated that NO has a potential involvement in a number of aspects of respiratory physiology and pathology. The intention of this review is to integrate the recent advances in this rapidly growing field.

Nitric Oxide Metabolism

SYNTHESIS AND FUNCTION

NO is synthesized from the semi-essential amino acid L-arginine (Fig. 1) in a reaction that involves five-electron oxidation of its guanidino nitrogen and is catalysed by a family of enzymes called nitric oxide synthases (NOSs) (6). This process requires several co-factors including molecular oxygen (O2), calcium (Ca2+), reduced nicotinamide adenine dinucleotide (NADPH), flavine mononucleotide (FMN), flavine adenine dinucleotide (FAD), and tetrahydrobiopterine (BH4) (7).

There are now at least three isoforms of NOS which are cloned and sequenced (8-10), two being constitutive and one inducible (Table 1). The constitutive isoforms (cNOS) comprise the endothelial (eNOS) or type III NOS normally present in endothelial cells, and the neuronal isoform (nNOS) or type I NOS, present in neuronal cells of the brain and the peripheral nerves. The constitutive isoforms are calcium dependent and induce transient production of picomolar concentrations of NO in response to various physiological stimuli, while the inducible isoform (iNOS), or type II NOS, is upregulated in various cells including macrophages, endothelial cells and pulmonary epithelial cells in response to stimulation by endotoxins or cytokines, such as interferon-γ (INF-γ), interleukin-1β (IL-1β), and tumour necrosis factor α (TNF-α) (11). This isoform generates relatively large (nanomolar) concentrations of NO and for a more sustained period of time than its constitutive counterparts. It is generally thought that iNOS is calcium independent. This has, however, been questioned by a recent report by Cho et al. (12). These workers identified that iNOS contains calmodulin which is tightly bound and only requires very low levels of calcium for activation and thus gives the impression that it is calcium independent. In contrast cNOS binds calmodulin loosely and requires much higher cytosolic levels of calcium to produce closer

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association with calmodulin, a step necessary for NO production. Further support for the dependence of iNOS on Ca\(^{2+}\) is the identification that calcium chelation attenuates the activity of iNOS in human hepatocytes (9).

NO being a small lipophilic readily diffusible gas is an ideal intra- and intercellular messenger. Once it is formed it diffuses into the effector cell and binds to the haem iron complex of soluble guanylate cyclase (sGC), forming nitrosyl-haem that activates guanylate cyclase resulting in increased amounts of cyclic guanosine monophosphate (cGMP) which leads to activation of the cGMP-dependent protein kinase, the mediator of smooth muscle relaxation (13). Other functions of NO which are relevant to the respiratory system include its inhibitory effect on smooth muscle proliferation (14), inhibition of platelet aggregation and adhesion (15) and being a neurotransmitter of bronchodilator nerves in human airways (16), together with its cGMP-independent actions which include cytotoxic effects due to inhibition of mitochondrial Fe-S enzymes, and cyclo-oxygenase activation in macrophages (17,18).

**MODULATION OF NO PRODUCTION**

**NO Inhibitors**

Interference at any step in the L-arginine:NO pathway can lead to inhibition of NO production (Table 2). Binding or depletion of the essential co-factors will lead to suppression of NO synthesis (1). The guanidino-substituted L-arginine analogues are potent inhibitors of NO production, as are the guanidines, especially aminoguanidine. Calcium chelators, such as EDTA, and glucocorticoids are also effective in this regard. Transforming growth factor β (TGF-β), macrophage-deactivating factor (MDF) and interleukins (IL-1, IL-2) are known to stimulate NO production. The presence of NO donors results in increased NO synthesis, which is also promoted by inhibition of NOS induction, and by NO adducts such as S-nitrosothiols, which include S-nitroso-albumin and S-nitroso-N-acetylcycteine. ACE inhibitors are known to inhibit NO synthesis, and nitric oxide donors have been shown to prevent the immunosuppression associated with transplantation. The modulation of NO production by drugs and other agents has important implications in the treatment of various diseases. **

**TABLE 1. Nitric oxide synthases (NOS); types and salient features**

<table>
<thead>
<tr>
<th>Enzyme type</th>
<th>Isoform</th>
<th>Gene location</th>
<th>Molecular weight (kDa)</th>
<th>Calcium dependent</th>
<th>Cellular site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I neuronal (nNOS)</td>
<td>Constitutive</td>
<td>Chromosome 14</td>
<td>150</td>
<td>Yes</td>
<td>Cytosolic</td>
</tr>
<tr>
<td>Type II endothelial (eNOS)</td>
<td>Constitutive</td>
<td>Chromosome 7</td>
<td>135</td>
<td>Yes</td>
<td>Membrane bound</td>
</tr>
<tr>
<td>Type II inducible (iNOS)</td>
<td>Inducible</td>
<td>Chromosome 17</td>
<td>130</td>
<td>?</td>
<td>Cytosolic</td>
</tr>
</tbody>
</table>

nNOS, neuronal nitric oxide synthase; eNOS, endothelial nitric oxide synthase; iNOS, inducible nitric oxide synthase.

**TABLE 2. Nitric oxide (NO) modulators with their mode of action**

<table>
<thead>
<tr>
<th>Co-factor binding-depletion</th>
<th>Inhibitors of NOS induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium chelators</td>
<td>Glucocorticoids</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>Cytokines</td>
</tr>
<tr>
<td>Sodium nitroprusside</td>
<td>TGF-β, MDF, IL-4, IL-10</td>
</tr>
<tr>
<td>Nitroglycerine</td>
<td>ACE inhibitors</td>
</tr>
<tr>
<td>Nitrosated thiols</td>
<td>IL-2</td>
</tr>
</tbody>
</table>

analogues are an important group of NOS competitive inhibitors commonly used in research. Several analogues has been identified such as Nω-monomethyl-L-arginine (L-NMMA), Nω-nitro-L-arginine methyl ester (L-NAME), and Nε-iminooethyl-L-ornithine (ε-NIO). All have the same mode of action but vary in potency (19), with ε-NIO being five times more potent than others, while L-NMMA and L-NAME are active orally (20).

Guanidines are another group of compounds including aminoguanidine and N,N-diaminoguanidine which have been shown to inhibit NOS. The mechanism whereby these compounds exert their inhibitory effect is unknown (21), but it is of interest that aminoguanidine appears to be a relatively specific inhibitor of iNOS (22).

Glucocorticoids as well as cytokines, such as members of the transforming growth factor β family (TGF-β1, -β2, and -β3) and macrophage-deactivating factor (MDF), inhibit the induction but not the activity of iNOS, although neither has any effect on eNOS activity or expression (23,24).

**NO Promoters**

Although nitrates have been used clinically as vasodilators for more than a century, it is only recently that their mode of action has been realized. It is now appreciated that NO is their active intermediate, being generated through the non-enzymatic pathway of nitrate metabolism (25). By contrast sodium nitroprusside (SNP) releases NO spontaneously (26), although a recent report suggested that SNP-induced smooth muscle relaxation is probably not mediated by cGMP or NO (27). Nitrosylated thiols form a new group of compounds known as NO adducts, acting as carrier molecules for NO (28) which is released under physiological conditions to mediate smooth muscle relaxation. Part of the therapeutic effect of angiotensin-converting enzyme inhibitors could be linked to their ability to potentiate the duration of action of bradykinin, which is a known agonist for NO release (29).

**FATE AND TOXICITY OF NO**

Although endogenous NO comes from a single pathway, the L-arginine: NO pathway, its fate is more complicated and follows several routes (Fig. 2). NO is a free radical and its nitrogen atom can exist in multiple oxidative states, depending on the conditions of the surrounding microenvironment and on the concentration of NO in that medium (30,31). The relevance of all these pathways in vivo...
is uncertain as studies investigating NO metabolites in vitro usually use much higher NO concentrations than those present under physiological conditions. However, notwithstanding these limitations some broad lines can be drawn regarding the fate and toxicity of NO.

Firstly, in oxygenated biological systems, whether aqueous or at air–aqueous interfaces as in the airways, NO reacts with oxygen (O2) producing nitrogen dioxide (NO2) and other oxidants as intermediates, while nitrite is probably the major end-product. The rate of NOx production depends on the concentration of NO and oxygen in the airways, so under physiological conditions this reaction is relatively slow in the gas phase and yields negligible amounts of NO2, which is unlikely to affect the biological activity of NO (30,31). However, if for any reason significant amounts of intermediates are produced, NO2 can lead to lipid peroxidation and the other intermediates dinitrogen trioxide (N2O3) and dinitrogen tetroxide (N2O4) can act as nitrosylating species (32). Nitrite will remain stable for a few hours in aqueous solution, but is rapidly converted in the blood to nitrate, which is excreted by the kidneys. Nitrates and methaemoglobin form the major end-products of the metabolic pathway for endogenously produced NO (33).

Secondly, the lung is a rich source of superoxide anion (O2−) which interacts rapidly with NO to produce peroxynitrite anion (ONOO−) and peroxynitrous acid (OONOH), faster than the rate of O2− scavenging by superoxide dismutase. It was initially considered that this reaction was potentially beneficial, in protecting the lungs from 02• scavenging by superoxide dismutase (34), but recent reports suggested that ONOO− mediates extensive nitrotyrosine formation in lung tissue of paediatric patients with acute lung injury (35) and induces airway hyperresponsiveness with epithelial damage and possible eosinophilic activation in guinea-pigs (36).

Thirdly, NO has a high affinity for iron-containing compounds such as haem, non-haem metalloproteins and certain enzymes (32,37,38). It is through this property that NO mediates its biological activity by binding to sGC (13) and is also inactivated in the blood through its interaction with haemoglobin. This form of interaction can also be detrimental, however, as when NO binds to iron-containing enzymes such as aconitase and complex I and II of the respiratory chain and to ribonucleotide reductase; these enzymes become nitrosylated with resultant cytotoxic effects, as is evident in activated macrophages (39).

Finally, through its oxidation to higher oxides of nitrogen, NO can mediate nitrosylation of various compounds such as glutathione, cysteine and albumin. The resultant S-nitroso compounds can act as carrier molecules for NO or ameliorate the toxicity of the other oxides of nitrogen by its combination with O2− (28,32,34). In the presence of high concentrations of NO, as in cigarette smoke (40), however, nitrosamine formation and deamination may occur and this has carcinogenic and genotoxic potential (41).

MEASUREMENT OF NO

There are two principal approaches to the measurement of NO, direct and indirect methods (42). Direct methods depend on trapping of NO by oxyhaemoglobin to form nitrosylhaemoglobin adduct which is detected by electron paramagnetic resonance, or trapping of NO by reduced haemoglobin to form methaemoglobin which is detected by spectrophotometry or the reaction of NO with ozone to form partially excited NOx and the detection of light emission (chemiluminescence) which accompanies conversion of excited-state to ground-state NO2 by a photomultiplier tube. Microelectrode techniques, using amperometric means, are another form of direct method with extreme low detection thresholds and may be useful in single-cell preparations (42). However, for clinical measures of NO in exhaled air, chemiluminescence is commonly used and is very sensitive with a detection threshold of 20 pmol (1 ppb), and its specificity has been confirmed (43), although the ideal technique for its measurement is still controversial.

The indirect methods use measurement of cGMP and nitrite levels as indicators of NO activity or oxidation respectively, while measurement of citrulline, a byproduct of the L-arginine:NO pathway, has also been used (42). All these methods, however, lack specificity.

Nitric Oxide and the Respiratory System

Following the discovery of the L-arginine:NO pathway it has been proposed that NO is a mediator of biological functions in almost all body systems, and the respiratory system is no different in this respect, with considerations concerning the involvement of NO in normal physiology as well as abnormal pathophysiology.

PRODUCTION SITE

Although the presence of NO in exhaled air of humans and animals has been confirmed (44–46), the exact source of its production is not yet clear. While some workers (47–52) believe that most of the orally exhaled NO in healthy subjects is derived from the upper airways, particularly the nose or paranasal sinuses (52), others believe that the lower airways are the major source of orally exhaled NO at rest (53,54) and the contribution of the lower airways is more evident during exercise (54). However, all workers agree that healthy subjects have significantly higher levels of NO in nasally exhaled air than orally exhaled samples, and direct measurement of NO in nasal airways still gives much higher results.

The question thus arises as to why the upper airways produce such a high concentration of NO and which NOS isoform is responsible. One suggested explanation is that this relates to bacterial colonization of the nose. Consistently with this, it has been shown in long-term ventilated patients that NO synthesis is reduced by antibiotics (47). However, antibiotic therapy in healthy volunteers has no effect on exhaled NO (48,51), and the presence of high nasal NO in neonates even within the first hour after birth (55) makes a bacterial contribution to nasal NO unlikely. Nasal biopsy studies have helped to clarify the situation. There is evidence that eNOS is expressed in vascular endothelium, surface epithelium and submucosal glands of human nasal
Nitric oxide is a potent relaxant of porcine airway smooth muscle in vitro (70), and NOS inhibitors enhance the increase in pulmonary resistance induced by intravenous histamine in guinea-pigs in vivo (71), indicative of the potential for NO to regulate human airway tone. Consistent with the relevance of airway epithelium to NO synthesis and release, NOS inhibitors have been shown to increase the basal tone in guinea-pig tracheal tubes with intact epithelium, but not in preparations denuded of epithelium. Furthermore, bradykinin mediates relaxation in guinea-pig tracheal tubes with intact epithelial lining but causes contraction in the presence of NOS inhibitors or in epithelially denuded preparations (72), suggesting that NO released from epithelial cells can modify the constrictor response to bradykinin. This protective effect of NO might be relevant to the normal surface of the lung.
to asthma, a disease in which there is epithelial disruption and potentially a loss of this anti-bronchoconstrictor protective mechanism.

The airway effects of NO depend not only on the dose administered but also on the state of smooth muscle tension. Although low levels of inhaled NO (5 ppm) produced a significant reduction of the increased pulmonary resistance induced by intravenous methacholine in guinea-pigs (73), much higher concentrations of inhaled NO (300 ppm) were required to produce a 10% decrease in basal airway resistance. Similarly, inhaled NO (100 ppm) has been shown to reduce significantly the increase in canine airway resistance induced by aerosolized acetylcholine or hypocapnia but to have no effect on basal airway resistance (74). These protective effects of NO appear to relate to a central rather than a peripheral airway effect, as inhalation of 80 ppm NO has been shown to protect rabbits effectively from the bronchoconstrictor effect of nebulized methacholine (75) but not to ameliorate the induced drop in lung compliance.

Consistent with these animal findings, in humans the bronchoconstrictor effect of inhaled bradykinin is greatly inhibited by the formation of NO in the airways of asthmatic patients (76), and inhaled NO (40–80 ppm) in healthy volunteers (77–80), and in patients with chronic obstructive pulmonary disease (COPD) (78,80), has no effect on basal tone but has a small and inconsistent effect on resting airway calibre in asthmatic patients and in healthy subjects with hyperreactive airways (78,79,81), in whom there is likely to be enhanced basal tone. This effect is much less marked than that produced by β2-adrenoceptor agonists and again relates to modulation of central rather than peripheral airway tone (81).

This central effect of NO can be linked to the airway distribution of the inhibitory non-adrenergic non-cholinergic nerves which are part of the respiratory system innervation, as there is evidence of NOS immunoreactivity localized to airway neurones in both animals and humans (82–84). These neurones are more prominent in proximal than distal airways (84). Nitric oxide is considered to be the prime neurotransmitter in such (nitrergic) nerves and is the mediator of neurally mediated bronchodilatation (16). It has been considered that as diseases like asthma are characterized by generalized airway narrowing, there might be a defect in the neural nitrergic responses. Studies in vivo on resected airway tissue have, however, revealed that the nitrergic responses are similar in tissue from both asthmatics and normal human donors (85). Interestingly the nitrergic response to stimulation is markedly reduced in airway tissue from cystic fibrosis patients, even though the overall NOS activity is generally higher in the presence of inflammatory lung diseases (86).

**NO AND PULMONARY VASCULAR TONE**

Vascular endothelial cells produce several vasodilators and of these NO and prostacyclin are potentially the most important. Although prostacyclin is a powerful vasodilator with beneficial effects in primary pulmonary hypertension (PPH) (87), it is unlikely that it has a major role in maintaining the low resistance of pulmonary circulation under physiological conditions (88). This physiological role is regulated, at least in part, by endothelially derived NO.

Pulmonary arterial and venous endothelial cells are capable of producing NO (89), and in vitro studies have demonstrated that inhibition of the endothelium-dependent NO production by NOS inhibitors or by endothelial removal enhances the response to vasoconstrictor stimuli (90,91). In isolated perfused lungs of rabbits (92) and humans (93), L-NMMA and methylene blue (a guanylate cyclase inhibitor) increases pulmonary vascular resistance, and injection of L-NMMA into the pulmonary artery of normal humans has been shown to cause a significant increase in pulmonary vascular resistance (94). Furthermore, in healthy conscious adults local infusion of L-NMMA into segmental pulmonary arteries resulted in a dose-dependent decrease in local flow velocity, while acetylcholine infusions resulted in a dose-dependent increase in flow velocity (95). All these observations suggest that NO is considered the endogenous nitrovasodilator which regulates the normoxic pulmonary vascular tone under basal conditions, and there is evidence that endothelial generation of NO is stimulated by physiological stimuli such as pulsatile blood flow and shear stress (2).

Acute hypoxia induces pulmonary vasoconstriction, and this pressor response is counteracted by NO release and potentiated by NOS inhibitors (93,96). Nitric oxide inhalation prevents and reverses the hypoxic pulmonary vasoconstrictor response in lambs (97) and humans (98). Cremona et al. (99) reported low levels of exhaled NO in patients with PPH, while Reily et al. (100) in a recent report showed that patients with PPH produce normal levels of NO in exhaled air at rest, but during exercise the levels failed to rise as in normal subjects. These observations are probably related to reduced NO activity, as there is evidence of marked reduction in eNOS expression in pulmonary arteries of patients with PPH (101), and the degree of expression is inversely correlated with the total pulmonary resistance. Chronic inhibition of NO production in utero has been shown to produce persistent pulmonary hypertension in new born lambs (102). It is thus evident that NO plays a significant role in the regulation of pulmonary vascular tone.

Chronic hypoxia is an important cause of pulmonary hypertension, and there is evidence that hypoxia markedly reduces NO activity with a decrease in NO release (44). Pulmonary arterial rings from patients with COPD have marked impairment of endothelial-dependent relaxation in response to acetylcholine, with preserved and rather augmented response to SNP (103), suggesting that COPD patients have impaired synthesis or release of NO. Of interest therefore is the report that there is only weak eNOS immunoreactivity in the pulmonary arteries of patients with secondary pulmonary hypertension due to COPD or other causes (101). As this disease is characterized by medial thickening and intimal proliferation, and as NO has an inhibitory effect on vascular mitogenesis and cellular proliferation (14), it seems likely that the NO deficiency state in patients with chronic pulmonary hypertension contributes not only to the rise in pulmonary vascular resistance but also to the structural vascular changes seen in such patients.
NO AND PULMONARY GAS EXCHANGE

The main function of the respiratory tract is gas exchange, whereby oxygen and carbon dioxide are transported across the alveolar-capillary membranes in opposite directions, to maintain normal arterial blood gases. For optimal gas exchange ventilation-perfusion (V/Q) matching is a prerequisite. As NO has both vasodilator and bronchodilator actions it is probable that the local generation of this gas has a role in gas exchange through modulation of ventilation and perfusion.

Some investigators (47,104) believe that autoinhalation of NO produced in the upper airways might be implicated in ventilation-perfusion matching, as there is evidence that aspirated nasal air, in intubated patients, when added to the inspiratory limb of the ventilator produces significant fall in pulmonary vascular resistance (PVR) and an increase in PaO₂ (104), and low doses of inhaled NO (10-200 ppb) similar to those produced in normal human upper airways (105) produce a significant reduction in PVR and improve arterial oxygenation in patients with acute respiratory failure (106,107). However, the effect of inhaled NO on oxygen saturation in healthy volunteers is controversial: while some investigators (77,108) demonstrated a significant drop in oxygen saturation, which is attributed to alteration in perfusion with resultant disturbances in V/Q ratios, other investigators using similar doses of inhaled NO have failed to find any significant change in arterial oxygen saturation (80,98).

In animal models of adult respiratory distress syndrome (ARDS) (109,110) and smoke inhalation injury (111), inhaled NO improves V/Q matching with significant increases in PaO₂. In humans inhaled NO reverses acute hypoxic pulmonary hypertension without impairment of gas exchange (98), and in adults with ARDS (107,112) as well as in neonates with severe hypoxemia not due to extrapulmonary shunting (113), inhaled NO improves oxygenation through a significant reduction in shunt fraction and improvement in V/Q ratios. The same improvement has been demonstrated to occur in children and neonates with acute respiratory failure and pulmonary hypertension (114,115). Recently, inhaled NO has been found to improve arterial oxygenation in high altitude pulmonary oedema (116), probably through redistribution of blood to non-oedematous regions, thereby improving V/Q ratios.

Smokers are known to have lower levels of exhaled NO (117,118), and this level is inversely proportional to the amount smoked (117). It is thus possible that such an NO deficiency state in smokers could contribute to the V/Q mismatching seen in many smokers. However, COPD patients where shunting is trivial but V/Q mismatching is the main cause of hypoxemia (119), studies have given conflicting reports as to the value of inhaled NO. Administration of NO has been found to produce a significant reduction in arterial oxygenation through worsening of V/Q ratios (108,120), no change (121) and even improvement (122) in oxygenation and gas exchange with the use of the same dose of inhaled NO (40 ppm). In the absence of consistent findings it is thus unlikely that NO will be of therapeutic value in such patients.

NO AND RESPIRATORY DEFENCE MECHANISMS

For a long time it has been known that resistance to infection and cancer can be enhanced in a non-specific way by bacterial products (123) and that this resistance can be attributed to changes in macrophage behaviour, triggered by bacteria of their toxins (124) and inhibited by red blood cells or haemoglobin (125). These changes in macrophage activity are not due to an alteration in phagoctosis and may be either cytostatic or cytotoxic in nature (37). There is evidence indicating that NO synthesized by iNOS in activated macrophages plays an important role in host defence against bacteria, viruses, fungi and parasitides as well as its role against tumour cells (39). Furthermore, there is also evidence that NO inhibits neutrophil adhesion to vessel walls (126) and modulates chemotaxis (127). Such diverse activities of NO make it indispensable for the immune system, and indeed mice lacking the iNOS gene have increased susceptibility to infections (128). It is thus possible that the relative NO deficiency state in smokers (117,118) might contribute to their increased susceptibility to infections and tumour growth. In ARDS patients, inhaled NO has been found to reduce the activity status of neutrophils in addition to reducing IL-8 and IL-6 release (129). These effects of NO will tend to ameliorate lung inflammation in ARDS.

The respiratory system is unique in being directly exposed to the surrounding environment and acts as an immunological filter, so it is probable that the high NO concentration in upper airways (45,47-53) reflects a role for this gas in mucosal defence mechanisms and that the very high levels identified in sinuses by direct puncture sampling are involved in maintaining the sterility of this environment (52).

There is also evidence that the L-arginine:NO pathway plays a major role in the control of mycobacterial infections. Virulent strains of Mycobacterium avium induce NO production by human monocyte-derived macrophages (130), and it has been demonstrated that killing of mycobacteria by human macrophages is mediated by the L-arginine:NO pathway (131). Animal studies have confirmed that an NO-dependent mechanism is important for the expression of immunosuppressive activity by macrophages against M. avium (132) and that the macrophage L-arginine dependent cytotoxic pathway effectively kills virulent strains of mycobacteria (133). In support of this, NOS inhibitors have deleterious effects on tuberculous infection in mice and the severity of mycobacterial infection has been shown to be increased in mice lacking the iNOS gene (134). Ethanol has been found to attenuate the increase in iNOS mRNA and reactive nitrogen metabolites in bronchoalveolar lavage fluid induced by instillation of mycobacteria into rat lungs (135), suggesting that ethanol has an inhibitory effect on alveolar macrophage NO production. In humans alcohol ingestion has been reported to result in a significant reduction of exhaled NO in asthmatics but not in normal subjects (136), probably reflecting a preferential action of alcohol on iNOS which is expressed in asthmatic airways.
A further important respiratory defence mechanism is the mucociliary escalator, and again there is evidence of a regulatory role for NO. Nitric oxide upregulates ciliary motility in response to stimulation (60) and probably mediates the increase in ciliary beat frequency induced by $\beta_2$-agonists (137). The reduced NO production in cigarette smokers (117,118), with its possible effect on ciliary motility, provides an additional possible explanation for the increased incidence of respiratory infections.

However, NO overproduction is not always beneficial, as data from animal studies indicate that overproduction of NO in acute lung injury is detrimental, particularly in association with the production of superoxide anion seen in such states, as the resultant peroxynitrite formation may lead to oxidative tissue injury (35) and might contribute to epithelial damage and airway hyperresponsiveness (36). As such, there is evidence implicating NO in the pathogenesis of pertussis (138), paraquat lung injury (139), immune-complex-mediated lung injury (140) and the hypotensive state of septic shock syndrome (141). Also, there is evidence that NO may be an important autoregulatory molecule preventing the over-expansion of Th1 and CD8$^+$ T cells (142), but at the same time its overproduction in asthmatics may tip the balance in favour of Th2 cells. This effect may have a role in perpetuating and amplifying allergic inflammation through the release of Th2 cytokines, such as IL-4 and IL-5 with their stimulatory effect on IgE synthesis and enhancement of eosinophilic function. Also, it is possible that NO overproduction in uncontrolled asthmatics may play a central role in epithelial damage seen in such patients as it does in pertussis (143). Thus the circumstances and magnitude of NO production determine its contribution to health or disease.

NO AS A MARKER OF PULMONARY INFLAMMATION

The low levels of exhaled NO seen in normal subjects (44-51) are probably produced by constitutive isoforms of NOS, while in inflammatory disorders of the respiratory tract there is evidence of increased NO production following iNOS induction.

Asthmatic patients have significantly higher levels of exhaled NO (45,46,117,144, 145) together with increased expression of iNOS in bronchial epithelium (65,146) and both NO levels (46,144,145) and iNOS expression (146) are reduced by corticosteroid therapy. Acute asthma attacks are associated with higher levels of exhaled NO (147), and such levels start to fall in 48 h after initiation of corticosteroid therapy, coinciding with the improvement in lung function. In stable asthmatics inhaled steroids reduce exhaled NO levels (46,144,148) as well as methacholine responsiveness before any observed changes in FEV$\textsubscript{1}$ (144). Thus exhaled NO may be a very sensitive index of corticosteroid activity within the airway as changing the dose of inhaled steroids leads to changes in exhaled NO well before any changes in lung function, symptom scores or $\beta_2$-agonist use (148). Furthermore, allergen-induced late asthmatic reactions are associated with increased levels of exhaled NO (149), while early asthmatic reactions (149) as well as acute changes in asthmatic airway calibre induced by methacholine and salbutamol have no effect on exhaled NO levels (150). All these findings further support to the suggestion that exhaled NO is a marker of airway inflammation rather than an endogenous modulator of airway tone. At present, however, the cost of equipment needed prohibits the routine use of exhaled NO levels in daily practice within the community.

Other inflammatory disorders of the respiratory tract are also associated with higher production rates of NO; symptomatic seasonal and perennial rhinitis patients have increased levels of exhaled NO (151-153), and NO metabolites are increased in nasal lavage fluid from patients with house dust mite allergy (154). Increased levels of exhaled NO are also reported with viral respiratory tract infections (45,155), although this is not a consistent finding (156). In bronchiectasis the level of exhaled NO has been found to correlate with the extent of the disease, as judged by CT scan scores, and bronchiectatic patients not on inhaled steroids are reported to have significantly higher levels of exhaled NO (157), while children with cystic fibrosis have normal levels of orially exhaled NO (158,159) whether or not they are on inhaled steroids (158) but have significantly lower levels of nasal NO.

Rejection and graft versus host disease are important causes of morbidity and mortality in transplant patients, and there is evidence from animal models of transplantation indicating that NO production is increased during rejection and graft versus host disease (160,161) and that urinary nitrite levels rise dramatically during cardiac allograft rejection in rats (161) before any other sign of rejection. As immunosuppressives attenuate urinary nitrite levels, NO or its metabolites may form the basis for a non-invasive test to detect and monitor rejection in transplant patients.

DIAGNOSTIC AND THERAPEUTIC POTENTIALS OF NO

Although oral L-arginine increases exhaled NO levels in asthmatics (162), and inhaled NOS inhibitors reduce it (145), there is no evidence that any of these has an effect on airway function as measured by FEV$\textsubscript{1}$. Direct inhalation of NO in asthmatics has a small and inconsistent effect on airway function (78,79,81) which is far less significant than that of $\beta_2$-agonists. The increased NO levels in asthmatics are a reflection of the underlying inflammatory process and may contribute to the pathogenesis of asthma through enhancement of vascular permeability and plasma exudation. Consistent with this, inhibition of endogenous NO production reduces neurogenic plasma exudation in guinea-pig airways (163), and pre-treatment with L-NNAME suppresses microvascular leakage in sensitized guinea-pig airways in a dose-dependent manner (164). Furthermore, NOS inhibitors attenuate bradykinin- and histamine-induced albumin extravasation in human nasal airways (165). In contrast to these reports, however, Gaboury et al. (166) demonstrated that an exogenous NO donor reduces...
microvascular permeability induced by direct activation of perivascular mast cells. However, as mentioned earlier, measurement of exhaled NO levels in asthmatics could have a role in monitoring disease activity and response to treatment, and the balance of evidence suggests that iNOS inhibition may have beneficial effects on asthmatic airways (167).

In ARDS patients (106,107,112,168), inhaled NO does improve oxygenation and haemodynamic variables in most patients, with a possible beneficial effect on lung inflammation through attenuation of the polymorphonuclear oxidative burst and adhesion as well as cytokine release in the lungs (129). However, so far there is no evidence that inhaled NO reduces mortality in ARDS patients (168), although it might have an impact on morbidity related to oxygen toxicity and barotrauma. Similar responses to inhaled NO have also been reported in neonates and children with acute respiratory failure and pulmonary hypertension due to various causes (113–115,169), and there is evidence that NO inhalation could obviate the need for extracorporeal membrane oxygenation (ECMO) in such patients (169).

In COPD patients (120–122), the hypoxic pulmonary vasoconstrictor response limits the disturbance in VQ matching, and through release of this response inhaled NO probably worsens oxygenation (120); however, as COPD patients have reduced NOS activity (101) with impaired NO release, it is possible that exhaled NO in COPD patients might predict those who reached the stage of chronic respiratory failure requiring long-term oxygen therapy.

In PPH inhaled NO has proven to be a selective and specific pulmonary vasodilator (170,171) that is superior to infused prostacyclin and can predict accurately and safely the vasodilator response to calcium channel blockers (172). There is a report of one patient with end-stage PPH who was maintained on inhaled NO via a transtracheal catheter for 68 days until she had a heart-lung transplant (173), and her explanted lung showed no evidence of NO toxicity; also, a recent report suggested that pulsed delivery of inhaled NO through nasal prongs might become an option in the long-term treatment of ambulatory patients with PPH (174).

Post-cardiac-surgery pulmonary hypertension is still an important cause of morbidity and mortality, and there is good evidence supporting a therapeutic role for inhaled NO in such patients (175,176), as well as in patients with postoperative graft dysfunction after lung transplantation (177). Also, the response to inhaled NO could help in discriminating those patients who need heart alone or heart–lung transplant (178).

Finally, studies on pulmonary toxicity of inhaled NO are scarce, but the few studies in animals have not revealed evidence of toxicity when NO is inhaled for up to 6 months (178,179). There is, however, an early human report of inhalation of high concentrations of NO (≥1.5%) causing severe methaemoglobinaemia with pulmonary oedema and death (1). So, as with any other drug, the inhaled NO dose has to be tailored to the patient’s needs and the minimum effective dose used, with close monitoring of methaemoglobin and nitrogen dioxide levels (31).

Conclusions

It is thus apparent that NO has the potential to be involved in many processes within the respiratory tract and lungs and to contribute both to the maintenance of normal homeostasis and to the pathogenesis of the disease, dependent on the nature and extent of its synthesis. With further understanding it is hoped that selective modulation or enhancement, when appropriate, may lead to novel therapeutic approaches to lung disease and that the measurement of NO in exhaled air will provide a valuable marker for monitoring airway inflammation in conditions such as asthma and rhinitis and assist in the management of transplanted patients and possibly also those with COPD.

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References


149. Kharitonov SA, O'Connor BJ, Evans DJ, Barnes PJ. Allergen-induced late asthmatic reactions are associated with elevation of exhaled NO. Am J Respir Crit Care Med 1995; 151: A128.


160. Winlaw DS, Schyvens CG, Smythe GA et al. Urinary nitrate excretion is a noninvasive indicator of acute


167. Barnes PJ. NO or no NO in asthma? Thorax 1996; 51: 218–220.


