

## Nitric Oxide in Asthma

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Nitric oxide is a labile radical gas that is widely acclaimed as one of the most important molecules in biology to have been studied in the past millennium. The scientific world has witnessed an explosion of information detailing the production and function of NO in various diseases and conditions [1]. The role of NO in the pathogenesis of asthma first sparked interest following observations in the early 1990s that patients with asthma had significantly higher levels of NO in exhaled air, a finding that was since confirmed by numerous studies [2,3]. Despite the extensive basic and clinical investigations into this subject, the meaning of this finding remains unclear. We still do not know whether this high output of NO is protective, harmful or merely a surrogate marker of some other unknown mechanism in the pathogenesis of asthma. The present article reviews the available studies pertinent to NO and its role in the pathophysiology of asthma, and discusses the therapeutic options that may emerge from this knowledge.

### The synthesis of NO

Nitric oxide is a highly active small molecule that participates in many physiologic processes, among them vasodilatation, neurotransmission and host defense. It contains an unpaired electron, which accounts for its high biological activity, and does not depend on a single receptor or target to exert its effect. NO is derived from the amino acid, L-arginine, via the enzyme, NO synthase. At least three NOS isoforms, differing in activity and tissue distribution, have been identified. Two of them are constitutive enzymes, the endothelial NOS and the neural NOS, both of which produce a small amount of NO (picomole) in a tide-continuous regulation. In contrast, the third NOS isoform is not normally expressed but, once induced by diverse cytokines or endotoxins, this enzyme (the inducible NOS, iNOS) produces large amounts of NO (nanomole) for longer periods [1].

In the human and animal lung, NOS isoform expression and NO production have been demonstrated in pulmonary vascular endothelial cells, smooth muscle cells, airway epithelial cells, platelets, non-adrenergic and non-cholinergic neurons, macrophages, mast cells, and neutrophils. The constitutive NOS (eNOS and nNOS) localize mainly to endothelial and epithelial cells and to some of the airway nerves, whereas iNOS exists mainly in airway

epithelial cells and inflammatory cells. Although all the types of NOS are expressed by the airways' epithelial cells, that of iNOS usually predominates [4,5].

The level of exhaled NO is the sum of the processes of synthesis, diffusion and metabolism that occur in the airway. Most of the exhaled NO in asthma is due to the induction of iNOS, although non-enzymatic NO production, due to airway acidification, also contributes to the high exhaled NO in asthma [6]. NO is a very reactive radical with a half-life of only seconds in the bloodstream. As it is being produced, it is converted to stable end-products, such as nitrate, nitrite and peroxynitrite [1]. These stable end-products of NO can be measured in broncho-alveolar lavage or sputum, and their level was found to correlate with the level of exhaled NO [7].

### NO as a vasodilator

NO became the focus of attention following the discovery that its synthesis by vascular endothelium is responsible for the vasodilator tone that is essential for the regulation of blood pressure. The NO-dependent vasodilator tone seems to be maintained through the activation of endothelial cells by physical stimuli such as pulsatile flow and shear stress. Calcium ions enter the endothelial cells and activate the NOS or eNOS to produce NO. Once it is produced, NO is freely diffusible and is capable of entering vascular smooth muscle cells to activate soluble guanylate cyclase and produce cGMP. Increased cGMP in vascular smooth muscle cells activates a cGMP-sensitive kinase that phosphorylates a calcium-dependent potassium channel, leading to hyperpolarization and vasodilatation [1].

In some conditions, such as sepsis, certain cytokines and endotoxins can induce the calcium-independent iNOS to produce large amounts of NO and to cause profound vasodilation, hypotension and shock. Since NO is a potent vasodilator in the bronchial and pulmonary circulation, inhaled NO was used successfully in the treatment of pulmonary hypertension. It represents a promising but still experimental form of therapy for acute respiratory distress syndrome [8].

Some studies [9,10] have suggested the notion that the high output of NO in asthma can enhance the plasma exudation and edema formation by vasodilation and by increasing the bronchial blood flow. In a model of knockout mice rendered deficient for iNOS, the manifestations of allergic airways disease, including

NO = nitric oxide  
NOS = NO synthase  
eNOS = endothelial NOS  
nNOS = neural NOS

iNOS = inducible NOS

microvascular leakage, pulmonary edema and airway occlusion, were markedly less severe in the iNOS mutants than in the wild-type animals [10]. In addition, inhaled NO in chronic obstructive lung diseases had a deleterious effect, resulting in a worsened gas exchange [11]. Other studies found that NO may have an inhibitory or no effect on microvascular leakage. This contradiction can be explained by the differing effects of NO depending on the location and the amount produced by the different NOS isoforms [12,13].

### **NO as a bronchodilator**

The dilator effect of NO on vascular smooth muscles raised the possibility of there being a similar regulatory effect on the airway smooth muscles: indeed, NO was found to mediate bronchodilation and was also considered to be a promising new therapeutic modality in asthma [14]. Although the preliminary reports did show some beneficial effect of exogenous NO in asthma, the findings of larger and better controlled studies were disappointing [15,16]. The application of L-arginine, the main precursor of NO, was ineffective in treating bronchoconstriction and, in fact, even exacerbated the asthma [17,18]. While inhibition of NOS produced bronchoconstriction in asthmatic patients, the effect was not specific and no correlation was found between it and the concentration of exhaled NO [19].

When specific inhibitors to iNOS (the main source of NO in asthma) were used, there was a demonstrable reduction in exhaled NO but no reduction in bronchial hyper-reactivity [20]. There are speculations that although the main source of exhaled NO in asthma comes from the iNOS, this isoform does not participate in the regulation of the airways tone but rather the regulation is done by locally constitutive NOS, such as eNOS and nNOS [21].

nNOS is the main messenger of the inhibitory non-adrenergic non-cholinergic nerves, and dysfunction of inhibitory NANC nerves has been proposed in the pathogenesis of asthma. Recent studies have demonstrated an interaction between the NANC nervous system and airway hyper-responsiveness, and a variant of the nNOS gene was found that might be a source of genetic risk for asthma [21,22].

Thus, contemporary evidence indicates that NO in the airway, whether it is endogenously produced or exogenously administered, is only a weak bronchodilator whose role in the regulation of bronchial tone remains to be established.

### **NO as mediator of inflammation**

Numerous lines of evidence suggest that NO-dependent immunity and inflammation is a general phenomenon involving not only the reticuloendothelial system but also non-reticuloendothelial cells, such as hepatocytes, vascular muscle, and the vascular endothelium, in all of which the inducible NO synthase has been detected [1]. During inflammation, there is an increased production of various mediators such as cytokines, chemokines and leukotriens. Some of these pro-inflammatory mediators enhance the production of NO, mainly by inducing the iNOS isoform. High outputs of

NO can be found in a variety of inflammatory states, such as infections, inflammatory bowel disease, rheumatoid arthritis and asthma [23].

Even though the presence of NO in inflammation has been described in numerous studies, establishment of the role of NO in inflammation is far from settled and it seems that NO inflammation is a double-edged sword. NO has bacteriostatic, antiviral and anti-tumor properties; NO regulates upward ciliary beat frequency of epithelia cells, and increased NO production enhances host defense mechanisms [23–27]. On the other hand, excess production of NO was shown to be cytotoxic to host cells in many states and models [27].

With asthma recognized as an inflammatory disease, it has been suggested that elevated levels of NO in the exhaled breath of asthmatic patients may reflect ongoing inflammation. A high output of NO was found to be poorly correlated with the degree of bronchoconstriction but well correlated to other inflammatory markers, such as airway eosinophilia [3]. Treatment of asthmatic patients, but not healthy subjects, with inhaled glucocorticoids reduced airway hyper-responsiveness and the number of sputum eosinophils, and this reduction correlated significantly with the level of exhaled NO in a dose-dependent manner [19,20,28–30]. A similar reduction was observed in patients treated with the novel leukotrien receptor antagonist, montelukast, but the exact interaction between leukotriens and NO remains obscure [31,32]. Many studies have confirmed the fact that steroids can inhibit iNOS expression and activity [33]. In a model of murine asthma, the manifestation of disease – such as infiltration of inflammatory cells, eosinophils, loss of airway structural integrity, and airway occlusion – was markedly less severe in iNOS knockout mice than in wild-type animals, suggesting that NO is not merely a surrogate marker for asthma inflammation [10].

Although we know that NO may have a variety of effects on cells of the immune system (e.g., activation, apoptosis, migration, adhesion), we do not know whether these effects are involved in the pathogenesis of asthma. *In vitro* studies have suggested that the NO derived from airway epithelial cells may induce and amplify asthmatic inflammation by altering the balance between T helper type 1 and T helper type 2 cells, leading to an increase in the number of the latter and producing a cytokine profile that has been associated with asthma [34]. However, there is also contradictory evidence that NO plays a role in the down-regulation of adaptive immune responses and in the inhibition of the components of inflammation, including leukocyte proliferation, activation, migration and adhesion, production of leukotriens and other pro-inflammatory mediators [34–36].

### **Exhaled NO as a marker of airway diseases**

Asthma is characterized by bronchial hyper-responsiveness and inflammation, and although pulmonary function and challenge tests are effective methods for assessing bronchial hyper-responsiveness, they cannot be used for assessing airway inflammation. Sputum induction is emerging as a non-invasive method to assess and monitor airway inflammation, bronchial biopsy specimens, and bronchoalveolar lavage fluid [23,37].

NANC = non-adrenergic non-cholinergic

Furthermore, the fact that NO levels are increased in the exhaled air of patients with asthma may serve as a non-invasive means of monitoring inflammation in asthmatic airways [28–32,37,38]. Exhaled NO in the gas phase can be measured directly by chemiluminescence. Several exhaled NO chemiluminescence analyzers are now commercially available as investigative and clinical tools. However, while the measurement of exhaled NO has been shown to be reproducible and widely applicable, there are many methodologic pitfalls that should be considered. In 1997, the European Respiratory Society published a set of recommendations for measuring exhaled and nasal NO. In 1998, the American Thoracic Society and the American Lung Association convened a workshop with more than 35 investigators from the international research community to develop additional recommendations for the standardization of these procedures [38]. Although the direct measurement of exhaled NO is carried out by a non-invasive test that can be performed with ease, there is some evidence to suggest that exhaled NO may not reflect total NO production in the airways. Thus, the indirect measurement of NO in asthma by measuring the stable end-products (NO<sub>2</sub> and NO<sub>3</sub>) in induced sputum can serve as an additional useful tool [7].

High levels of exhaled NO have been found in other inflammatory lung diseases such as respiratory tract infections, fibrosing lung disease and rejection of a transplanted lung [23]. In patients with chronic obstructive pulmonary disease the exhaled NO level is considerably lower than in asthma; and most importantly, the major causative agent, tobacco smoking, dramatically reduces the level of exhaled NO and therefore masks any tendency towards a disease-related rise in exhaled NO levels [39]. Exhaled NO levels are markedly low in cystic fibrosis and primary pulmonary dyskinesia but the reason is not yet clear [23].

### Conclusion – the paradoxical nature of NO

NO is a molecule whose expression is induced by signals associated with inflammation. Thus, it is not surprising that it has been detected at high levels in people with asthma. Although the precise role of NO in the pathogenesis of asthma is still under debate, its importance in this pathology cannot be underestimated.

The NO that is formed in the airways may act as a bronchodilator and as an anti-inflammatory agent. However, in addition to these positive actions, NO may have a potential deleterious role in asthma, including increasing bronchial blood flow, exudation and edema, direct cytotoxic effect on airway epithelial cells, and diverging the phenotype of T helper lymphocyte toward the pro-allergic T helper type 2.

These paradoxical actions of NO are not restricted to asthma. NO was found to be a double-edged sword in many other pathologic conditions, and this dichotomy is probably dependent on its source, its concentration and its site of action. The dual nature of NO was investigated in a rat model of sepsis, where it was shown that the adverse renal outcome in sepsis is caused by local inhibition of the protective eNOS by high levels of NO produced by iNOS, possibly via NO autoinhibition [40]. It is likely that a similar situation exists in asthma, where the low levels of NO produced by

the constitutive NOS play a positive role in the regulation and protection of the airways in contrast to the high levels of NO produced by the inducible NOS.

Whether the modulation of NO production, activity or metabolism should be a target for therapy in asthma or if the high level of NO in asthma is merely a surrogate marker for inflammation or some other unknown mechanism have yet to be determined. We still do not know whether NO is advantageous or disadvantageous for asthma: revealing the role of NO in inflammation may be the key to this enigma.

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