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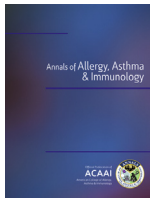

Review

Characterization of reactive nitrogen species in allergic asthma

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ABSTRACT

Objective: To investigate the molecular mechanism of reactive nitrogen species (RNS) in the pathogenesis of asthma and examine the use of fractional exhaled nitric oxide (FE_{NO}) measurements in close conjunction with standard clinical assessments of asthma.

Data Sources: Through PubMed, Google Scholar, and Medline databases, a broad medical literature review was performed in the following areas of asthma pathobiology and management: allergic asthma, RNS, nitric oxide (NO), airway inflammation, and FE_{NO}.

Study Selections: Studies were selected based on the physiologic and pathophysiologic roles of RNS in relation to allergic asthma. Current evaluations on clinical applications of FE_{NO} in asthma treatment also were selected.

Results: At the onset of an asthma attack, an enhanced production of NO strongly correlates with increase inducible NO synthase (NOS) activity, whereas endothelial NOS and neuronal NOS regulate primarily normal metabolic functions in the central and peripheral airways. During allergic inflammatory responses, NO and superoxide form peroxynitrite, which has deleterious effects in the respiratory tract. RNS directly accentuates airway inflammation and cytotoxicity through nitrosative stress. Moreover, the use of FE_{NO} to monitor eosinophilic-mediated airway inflammation is a potentially valuable assessment that supplements standard procedures to monitor the progression of asthma.

Conclusion: This review examines recent evidence implicating the molecular mechanisms of NO and NO-derived RNS in the pathobiology of asthma and suggests that monitoring FE_{NO} may markedly contribute to asthma diagnosis.

Introduction

Asthma, a chronic lung disease of airway inflammation, is normally characterized by bronchial hyper-reactivity, tissue remodeling, and reversible airflow obstruction, which leads to symptoms of recurrent coughing and dyspnea.[1] Patients are diagnosed with allergic or nonatopic asthma based on the presence or absence of clinical symptoms and allergen-specific antibodies at exposure to various environmental allergens.[2] Although similar immunopathogenic mechanisms are involved in the establishment and progression of the 2 modes of asthma,[3] key differences in the airway microenvironment exist. Increased goblet cell hyperplasia, mast cell degranulation, epithelial damage, and thickening of the subepithelial basement membrane have been observed in allergic asthma compared with its nonatopic counterpart.[4–6] Most often, allergic asthma is defined by IgE-mediated airway inflammation and an underlying airway hypersensitivity to inhaled allergens. Although many physiologic processes have been implicated in asthma pathogenesis, recent evidence supports the key roles of endogenous nitric oxide (NO) and NO-derived reactive nitrogen species (RNS) in modulating airway function and inducing asthma.[7] NO is a gaseous free radical produced by highly specialized cells of the respiratory epithelium and vascular endothelium in response to inflammatory cytokines. More specifically, NO plays a critical role in the systemic vasodilation of the airway smooth muscle.[8] The correlation between high exhaled NO concentrations and eosinophilic-mediated airway inflammation in patients with asthma has been well documented in the past decade. Thus, the use of clinical breath testing to measure the fraction of exhaled NO (FE_{NO}) has become an increasingly valuable diagnostic tool for asthma.[9,10]

Since its discovery as an endothelium-derived relaxing factor,[11,12] NO has proved to be a versatile intracellular messenger regulating a wide range of biological processes in multiple organ systems. Previous studies have shown that some NO metabolic byproducts exhibit cytotoxic effects in the central and peripheral

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bronchioles and alveoli. For instance, the rapid reaction between superoxide ($O_2^{\bullet-}$) and NO produces a highly unstable RNS, peroxynitrite ($ONOO^-$), which has been involved in cellular damage and airway hyper-responsiveness.[13] Highly bioactive NO and NO-derived RNS can inflame the airways and subsequently damage the DNA, enzymes, and lipids of the targeted cell.[14] This deleterious activity can be attributed to the oxidative and nitrosative stresses elicited by allergens. As a result, excessive NO-adduct molecules including $ONOO^-$, nitrogen dioxide (NO_2), dinitrogen trioxide (N_2O_3), and higher oxides of nitrogen can induce post-translational modifications.[8,14,15]

This review describes the biosynthetic pathway, physiologic and pathophysiologic roles of RNS in patients with allergic asthma. In addition, the medical applications of FE_{NO} in assessing asthma are discussed.

Biosynthesis of NO in Asthma

Tissue-specific isoforms of NO synthase (NOS) catalyze the conversion of amino acid L-arginine to L-citrulline and NO. These NOS isoenzymes differ in their expression, regulation, and pathophysiologic function.[9,16] More importantly, the increased levels of NO in the exhaled breath of patients with asthma can be explained by NOS overexpression. Constitutive NOS isoenzymes are composed of endothelial NOS (eNOS/NOS3) and neuronal NOS (nNOS/NOS1), which release small to moderate amounts of NO required for the normal metabolic functions of vascular tone and autonomic neurotransmission, respectively.[17,18] Endothelial NOS is constitutively expressed in the endothelial cells of the pulmonary and systemic circulations and the alveolar endothelium of the respiratory tract.[9] For vascular injuries, eNOS regulates regional blood pressure, platelet aggregation, leukocyte adhesion, and smooth muscle cell proliferation.[17] Moreover, traces of NO from eNOS activity have been isolated in the respiratory epithelium and found to play a role in ciliary movement and mucus ejection.[19,20] There also has been recent evidence linking certain eNOS gene polymorphisms to increased serum IgE levels in patients with allergic asthma.[21] Functionally, nNOS mediates neuronal bronchodilation in the skeletal muscles and cholinergic airway nerves.[9] At the onset of allergic asthma, increased arginase activity attenuates the nNOS-catalyzed synthesis of NO and induces neural bronchoconstriction.[22] Thus, eNOS and nNOS genotypes appear to be actively involved in the pathophysiology of allergic asthma.

However, severe airway inflammation in patients with allergic asthma is due primarily to NO generated from the inducible NOS (iNOS/NOS2) isoform, which is prevalent in immune cells such as macrophages, phagocytes, and various smooth muscle cells. Unlike constitutive NOS activity, iNOS expression does not depend on intracellular Ca^{2+} and calmodulin concentrations. Inducible NOS produces large amounts of proinflammatory NO after induction by numerous cytokines and bacterial products, including interferon-γ, tumor necrosis factor-α, interleukin-1β, and lipopolysaccharide.[23] Particularly in patients with asthma, the exogenous stimuli originate from allergens and environmental pollutants, initiating transcriptional activation of iNOS in the respiratory tract. Abnormally increased iNOS mRNA/protein and high NO output in patients with asthma are likely due to a continuous transcriptional regulation of the iNOS gene by Stat1 protein activation.[24] Numerous studies have confirmed that elevated levels of exhaled NO and iNOS activity correlate with increasing asthma exacerbations and eosinophilic airway inflammation.[24–26] In addition, it has been shown that an increase in exhaled NO leads to worsening baseline forced expiratory volume in 1 second (FEV_1) values and that subsequent treatment with inhaled corticosteroids can significantly improve FEV_1 and decrease FE_{NO} levels in patients with asthma.[16] Thus, FE_{NO} concentration is a good surrogate marker for steroid responsiveness in the central and peripheral airway sites of patients with asthma, which is discussed further in the following sections.[27,28] After administering a selective inhibitor of iNOS, FE_{NO} levels dramatically decreased in healthy subjects and patients with asthma.[29] Therefore, high NO output in patients with asthma is due mainly to amplified transcriptional activation of the iNOS gene. In addition, the use of selective iNOS inhibitors might treat asthma-related inflammatory responses by modulating NO bioactivity in the lungs.[24,30]

NO Signaling and Bioactivity in the Airway

Endogenous NO is a crucial physiologic regulator of airway function and serves as a bronchodilator through relaxation of the smooth muscle myosin.[31] In response to agonist-receptor binding, NO is synthesized by surface endothelial cells in the airway. As a gas, it can diffuse through the lipid membrane of the target cells and bind to the Fe(II) heme group of guanylyl cyclase, which initiates an intracellular cyclic guanosine monophosphate (cGMP)-mediated downstream signaling for vasodilation.[32] More specifically, cGMP induces an intracellular response for vasodilation by coupling with cGMP-dependent protein kinase to decrease intracellular calcium ions. Activated cGMP-dependent protein kinase also can induce Ca^{2+} desensitization of the actin cytoskeleton organization and relax vascular smooth muscle by inhibiting RhoA-dependent pathways.[33]

The biological function of NO ultimately depends on its concentration and interaction with other bioreactive molecules and proteins.[34] High concentrations of NO, released by iNOS, respond as an immune defense molecule by killing tumor cells and preventing viral replication.[35,36] Yet, the simultaneous release of reactive oxygen species (ROS) and high concentrations of NO in the presence of the L-arginine cofactor can severely damage airway cells and tissues, because NO reacts with the $O_2^{\bullet-}$ anion to form a potent reactive nitrogen intermediate, $ONOO^-$, leading to severe cellular damage.[37] Moreover, a comprehensive study by Nguyen et al[38] showed that NO can directly impair DNA and cause mutations in mammalian cells through its reaction with O_2 to form N_2O_3. Thus, the cytotoxicity and mutagenicity associated with acute inflammatory responses in patients with asthma likely stems from a variety of nitrogen oxide and oxygen radicals.

RNS Formation, Nitrosative Stress, and Airway Damage

Because of the complex chemistry and short half-lives of RNS, the exact metabolic fate and biosynthetic pathways by which RNS stimulate an asthmatic response remain uncertain. Over the past few decades, however, RNS have been increasingly accredited as mediators of airway damage and nitrosative stress. Endogenous RNS are highly reactive toward biomolecules, cells, and tissues of the airway, targeting amino acid residues (tyrosine, thiols, and amines), heme groups of metalloproteins, and unsaturated lipids.[14] More specifically, apoptosis and necrosis in patients with asthma often occur when the respiratory epithelial cells are exposed to excessive $ONOO^-$, which triggers a signal transduction cascade involving mitogen-activated protein kinases.[39] Compared with healthy controls, patients with asthma have higher levels of NO and total nitrites and nitrates in exhaled breath condensate.[40] During nitrosative stress, NO rapidly degrades into nitrite (NO_2^-), which can further oxidize to nitrate (NO_3^-), as illustrated in Figure 1. Under the acidic conditions of an acute asthma attack, endogenous NO_2^- can convert to NO and thereby account for an increased NO concentration in the exhaled breath of patients with chronic asthma.[41] Further, $ONOO^-$ decays into $NO^-{}_3^-$ or causes tyrosine nitration after an oxidative burst of $O_2^{\bullet-}$ by leukocytes in the NO-abundant respiratory tract.[8] Exogenously administered $ONOO^-$ stimulates airway hyper-responsiveness, respiratory