

Modulation of nitric oxide responses in asthma by extracellular antioxidants

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Nitric oxide (NO) levels are substantially elevated in the exhaled air of patients with active asthma.¹ The role of NO in the pathogenesis of asthma is a tantalizing enigma. NO has been shown to have both anti-inflammatory and proinflammatory actions in a wide range of disease states, including asthma.² Both NO and its metabolite, S-nitrosoglutathione (GSNO), are formed endogenously in the airways and can act as bronchodilators.^{3,4} NO is a nonadrenergic, noncholinergic neurotransmitter that regulates airway tone⁵ and is an inhibitor of components of the inflammatory cascade, including leukocyte activation, mobility, and adhesion.⁶⁻⁸ Along with these positive actions of NO, there are also potential deleterious actions of NO in asthmatic airways. Some of these actions may include increasing bronchial blood flow, direct cytotoxic effects on airway epithelial cells,⁹ enhancing plasma exudation into airways,¹⁰ and interfering with the balance of T_H1 and T_H2 lymphocytes through suppressing the helper T cell effect of the T_H1 subset of CD4⁺ T cells.¹¹ These paradoxical actions of NO make it challenging to interpret the role of elevated levels of exhaled NO associated with exacerbation in asthma. Three critical questions pertaining to NO remain unanswered in asthma: (1) what are the direct effects of NO, (2) which effects are mediated by NO metabolites, and (3) what factors modulate NO reactivity (Fig 1).

Under normal conditions, NO is produced in the respiratory tract by 2 constitutive forms of NO synthase (NOS), neural NOS (nNOS or type I NOS), and endothelial NOS (eNOS or type III NOS). Constitutive NOS produces relatively low NO levels found in nonadrenergic, noncholinergic neurotransmitter neurons,¹² airway epithelial cells, and endothelial cells.¹³ This source of NO production is calcium and calmodulin dependent and thought to act as a signal transduction agent modulating a variety of airway and vascular functions, including

Abbreviations used

EC-SOD:	Extracellular superoxide dismutase
GSH:	Glutathione
GSNO:	S-nitrosoglutathione
NO:	Nitric oxide
NOS:	Nitric oxide synthase
O ₂ ⁻ :	Superoxide
ONOO ⁻ :	Peroxonitrite

smooth muscle tone.¹⁴ The inducible NOS (iNOS or type II NOS) is found in a variety of cells in the respiratory tract, including macrophages, airway epithelial cells, fibroblasts, and endothelial cells.¹³ Inducible NOS is associated with larger bursts of NO and is an important component of the host defense response. Expression is induced by endotoxin and proinflammatory cytokines such as IL-1 β and TNF- α .¹⁵ It is important to note that, although NO is produced under normal conditions in airway walls, most of the exhaled NO measured in healthy individuals is thought to arise from the nasopharynx with little contribution from the lower respiratory tract. The production of exhaled NO in healthy individuals is not influenced by treatment with glucocorticoids.¹⁶ In contrast, in asthmatic patients exhaled NO is substantially increased, the primary source of that increase coming from the lower respiratory tract, and it is sensitive to glucocorticoid treatment. This suggests that the primary source of exhaled NO under conditions of asthma is an inducible form of NOS. It has been suggested that inhaled air pollutants can stimulate induction of inducible NOS by enhanced gene transcription and this might be one pathway whereby environmental air pollutants contribute to exacerbations of asthma.

Under normal conditions O₂⁻ is produced in low amounts, primarily as a byproduct of mitochondrial respiration.¹⁷ However, under conditions of inflammation the levels of extracellular O₂⁻ are dramatically elevated as part of the host defense response. The sources of extracellular O₂⁻ include macrophages, eosinophils, and neutrophils by activation of membrane-bound reduced nicotinamide adenine dinucleotide phosphate oxidases.¹⁸ Leukocytes isolated from asthmatic airways have been shown to produce higher levels of O₂⁻ on stimulation.¹⁹

Because NO reacts with O₂⁻ at close to diffusion-limited rates (the Michaelis-Menten constant for the reaction

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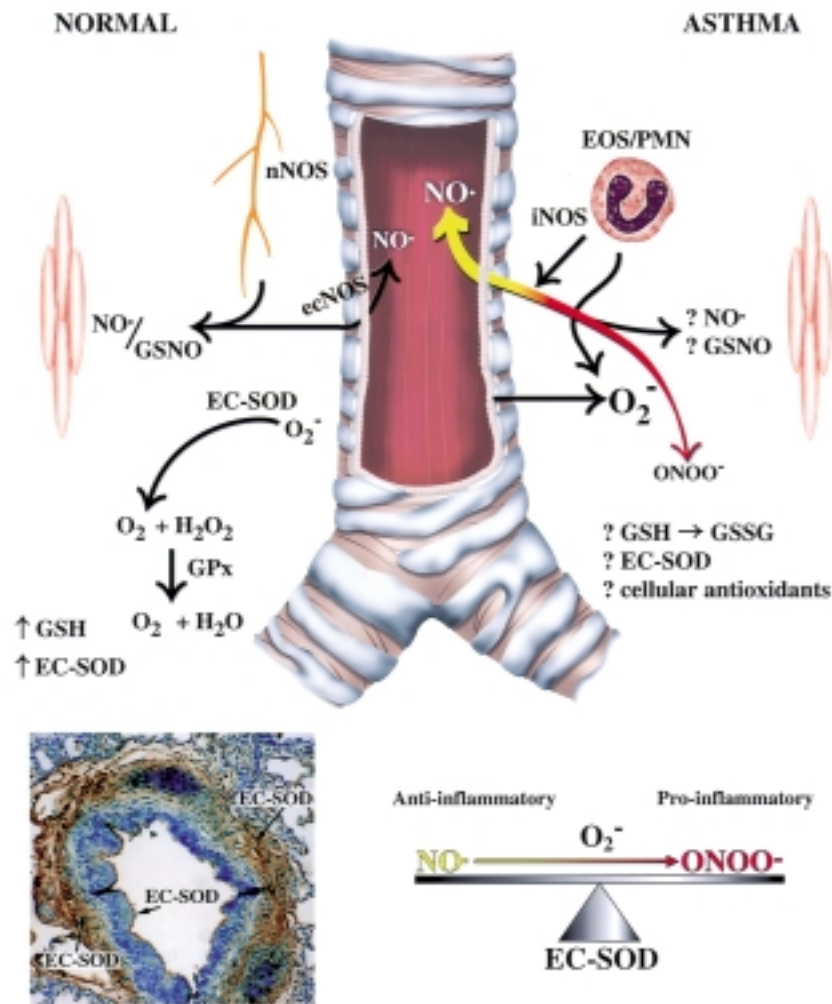


FIG 1. NO and superoxide (O_2^-) pathways in healthy and asthmatic airways. Diagram depicts reactive nitrogen-oxygen species formation in healthy and asthmatic lungs and their modulation by antioxidants such as glutathione (GSH) and extracellular superoxide dismutase (EC-SOD). Under normal conditions, neuronal and endothelial forms of NO synthase (nNOS and eNOS) generate low amounts of NO. High levels of GSH and EC-SOD in airway walls maintain O_2^- and hydrogen peroxide (H_2O_2) at extremely low levels and NO or one of its products (GSNO) can widely diffuse to function in intracellular signaling, such as mediating bronchial smooth muscle relaxation. In asthma high levels of NO are produced in lower airways with significant contribution coming from inducible NO synthase (iNOS), which can be induced in a variety of airway cells, including inflammatory cells. Inflammatory conditions also produce large amounts of O_2^- , which will preferentially react with NO to produce peroxynitrite ($ONOO^-$) and a subsequent cascade of strong oxidants. Although NO is high in exhaled air, tissue levels of NO and GSNO are not known and could even be low if O_2^- production is sufficiently high. Activity of both intracellular antioxidants and major extracellular antioxidants in airway walls of asthmatic patients is not known, and changes in these agents could largely determine the outcome of NO-mediated reactions in extracellular spaces. Predominant NO species in asthmatic airway walls (NO/GSNO or $ONOO^-$) is likely regulated by balance between O_2^- levels and its primary extracellular scavenger, EC-SOD. *Bottom left*, Immunolocalization of EC-SOD in baboon airway with use of horseradish peroxidase method. Brown stain on airway surface and in smooth muscle layer demonstrates location where EC-SOD concentrations are normally high. *Bottom right*, EC-SOD modulates extracellular O_2^- levels and therefore maintains the critical balance between NO's anti-inflammatory and proinflammatory actions.

is in excess of $10^9 \text{ M}^{-1}\text{s}^{-1}$),²⁰ then any O_2^- present in the extracellular spaces would preferentially react with NO, forming $ONOO^-$. The formation of $ONOO^-$ may change the function of NO as a signal transduction agent. $ONOO^-$ is a potent nitrating species and readily nitrates tyrosine residues of proteins.²¹ The byproduct of this reaction is the formation of nitrotyrosine residues and is

a commonly used footprint for the presence of $ONOO^-$. However, not all nitrotyrosine may be formed through the reaction with $ONOO^-$ because hypochlorous acid, formed by myeloperoxidase, can react with nitrite, producing a nitrating species.²² In this issue Kaminsky et al (p 747) have demonstrated that nitrotyrosine labeling in both the airways and lung parenchyma of asthmatic

patients is significantly increased. Thus some deleterious effects of NO may be mediated through one of its highly active metabolites, such as ONOO⁻. ONOO⁻ is capable of producing bronchoconstriction of the airways.²³ Whether increased nitrotyrosine formation is part of the pathogenesis or merely a spectator in asthma remains to be answered.

The conversion of NO to a metabolite such as GSNO may protect NO from attack from O₂⁻, enhance its half-life, and enable it to more effectively function as a signal transduction molecule. In fact, GSNO levels are decreased in the epithelial lining fluid of asthmatic patients.²⁴ In this study it was not clear whether the primary species diffusing across extracellular spaces is NO itself or one of its active metabolites. However, it is becoming increasingly clear that protection of NO from attack by O₂⁻ is a critical step that enables NO signal transduction.

Mammalian airways have unusually high levels of extracellular antioxidants. Two antioxidants that are highly expressed in the extracellular spaces of the lung are GSH and EC-SOD. GSH is found in lung extracellular fluids at levels 2-fold higher than that in serum.²⁵ GSH may serve as a substrate for GSNO formation or for glutathione peroxidase, an important enzyme that scavenges hydrogen peroxide. The combination of high levels of EC-SOD and GSH would effectively keep both O₂⁻ and hydrogen peroxide at extremely low levels in extracellular spaces in airway walls.

EC-SOD is a secreted copper- and zinc-containing enzyme with a unique tissue distribution that differs dramatically from the other 2 mammalian SOD isoforms. EC-SOD has a uniquely high expression in the lung.²⁶ EC-SOD has a heparin-binding motif at its C-terminal end that is believed to bind glycosaminoglycan in the extracellular space. EC-SOD is the major SOD enzyme in blood vessels and airways²⁷ (Fig 1). Another interesting feature of EC-SOD is that its expression is modulated by cytokines and growth factors in a manner similar to that seen with inducible NOS.²⁸ In addition to comparable regulation, both these enzymes seem to be expressed in similar lung cell types. EC-SOD has been shown to play a primary role in modulating the bioavailability of NO in an animal model of central nervous system toxicity²⁹ and to regulate the ability of NO to modulate vascular tone.³⁰ The high levels of EC-SOD expression in lung airway walls suggest a similar role for modulation of NO functions by this unique antioxidant in airway inflammation.³¹

The production of enhanced levels of exhaled NO in asthmatic patients is commonly associated with the late-phase response to allergens, which suggests that this lung NO production may be associated with exacerbation of the long-term inflammatory response. If this process is mediated by the formation ONOO⁻, it suggests that there is a significant disturbance both of the balance of NO production and the production or scavenging of O₂⁻. The same cells that induce inducible NOS in response to inflammatory stimuli also have been shown capable of

induction of high levels of O₂⁻ release and induction of EC-SOD.

In asthma we know that exhaled NO is elevated and there is activation of pathways that produce enhanced concentrations of O₂⁻, and it has now been shown that high levels of ONOO⁻ result in high levels of nitrotyrosine residues. Although it is known that there are high levels of exhaled NO in the airways of patients with asthma, it is not yet clear whether tissue levels of NO and its active metabolites such as GSNO are elevated or decreased. This would critically depend on the levels of O₂⁻, the activity of antioxidants such as EC-SOD, intracellular antioxidants, and the balance of these species in response to the higher oxidative stress in the extracellular milieu of the airway wall. The functional importance of a variety of the positive and adverse pathways stimulated by NO and its metabolites are critically dependent on the microchemistry in the airway wall and the balance of other species such as O₂⁻ and its primary extracellular scavenger, EC-SOD (Fig 1).

The high exhaled levels of NO in asthmatic patients are associated with a pro-oxidant activity in airway walls resulting in lipid peroxidation and nitration of proteins. To the extent that this pathway is mediated by a coexisting high level of production of O₂⁻, it raises the potential that therapeutic approaches designed to scavenge O₂⁻ in airway walls could inhibit a key component of proinflammatory actions cascading from NO production. If the high levels of NO being produced under conditions of asthma were preserved at high tissue levels of NO, there could be a profound impact on the progression of asthma with both smooth muscle relaxation and inhibition of inflammatory pathways.

The question of whether NO is a critical species in the pathogenesis of airway inflammation remains. Demonstration of a footprint from one of the NO reaction products (an initiator of a pro-oxidant pathway) resulting in protein nitration suggests a reasonable explanation for the apparent paradoxical reactions of NO and raises numerous questions regarding which nitrogen and oxygen species are active in the airway walls of asthmatic patients.

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