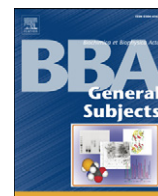




Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Review

Nitric oxide metabolism in asthma pathophysiology[☆]Sudakshina Ghosh^{a,*}, Serpil C. Erzurum^{a,b}^a Department of Pathobiology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, USA^b The Respiratory Institute, Cleveland Clinic, Cleveland, OH 44195, USA

ARTICLE INFO

Article history:

Received 11 February 2011

Received in revised form 24 May 2011

Accepted 15 June 2011

Available online xxxxx

Keywords:

Nitric oxide

Asthma

Nitritative stress

Nitration

S-nitrosylation

ABSTRACT

Background: Asthma, a chronic inflammatory disease is typically characterized by bronchoconstriction and airway hyper-reactivity.**Scope of review:** A wealth of studies applying chemistry, molecular and cell biology to animal model systems and human asthma over the last decade has revealed that asthma is associated with increased synthesis of the gaseous molecule nitric oxide (NO).**Major conclusion:** The high NO levels in the oxidative environment of the asthmatic airway lead to greater formation of reactive nitrogen species (RNS) and subsequent oxidation and nitration of proteins, which adversely affect protein functions that are biologically relevant to chronic inflammation. In contrast to the high levels of NO and nitrated products, there are lower levels of beneficial S-nitrosothiols (RSNO), which mediate bronchodilation, due to greater enzymatic catabolism of RSNO in the asthmatic airways.**General Significance:** This review discusses the rapidly accruing data linking metabolic products of NO as critical determinants in the chronic inflammation and airway reactivity of asthma. This article is part of a Special Issue entitled Biochemistry of Asthma.

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1. Introduction

Asthma is a chronic disease of airway inflammation, typically characterized by bronchial hyper-responsiveness, airflow limitation and mucus hyper-secretion, leading to the symptoms of cough, wheezing and shortness of breath [1–3]. Although many pathologic processes participate in asthma pathogenesis, a large body of evidence supports an important role for airway-derived nitric oxide (NO) in the mechanism underlying asthma. NO has effects as a bronchodilator via relaxation of airway smooth muscle [4]. NO binds to soluble Guanylate Cyclase (sGC); this leads to activation of the protein, followed by enhanced production of intracellular messenger cGMP, which is responsible for initiating downstream signaling for vasodilation [5]. Studies have established that sGC expression and activity are reduced in experimental asthma, and that this contributes to the observed airway hyperreactivity [6]. The sGC mRNA expression is transcriptionally regulated in cultured cells by inflammatory stimuli, e.g. lipopolysaccharide, IL-1 β or cytokines mixture. It may be that the high levels of inflammatory cytokines in asthmatic airways contribute to the lower level of sGC expression [6–8]. Furthermore, studies also identify an NO/cysteine interaction, in

addition to the classical heme iron-NO activation, that is important in mediating activation of sGC; formation of a putative thiol-NO adduct apparently enables maximal sGC activation. However under oxidative stress, the thiol is oxidized, which prevents NO binding and retards sGC activation [9]. Thus, the oxidative stress in the asthmatic airway may contribute to deficient sGC-mediated bronchodilation through both the loss of sGC expression as well as cysteine oxidation that consequently abrogates maximal sGC activation via NO–cysteine interactions [9]. Considerable evidence supports detrimental effects of NO in airway inflammation through formation of reactive nitrogen species (RNS) that mediate inflammation and injury [1,4]. Increased generation of RNS and reactive oxygen species (ROS) are well documented in asthma [10–12]. The asthmatic airways are infiltrated with eosinophils and neutrophils, which contain peroxidases, e.g. eosinophil-peroxidase (EPO) and myeloperoxidase (MPO) [13,14]. The peroxidases use chloride and/or bromide present in tissues to form hypohalous acids HOCl and HOBr [15–17]. Hypohalous acids are potent oxidants and chlorinate and/or brominate protein tyrosines to form 3-chlorotyrosine (Cl-Y) and 3-bromotyrosine [17] markers of MPO and EPO activity, respectively. MPO also uses the oxidation of nitrite (an oxidation product of NO) to generate the nitrogen dioxide radical [14,15,18] to nitrate protein tyrosine and form 3-nitrotyrosine [16,18–20]. The detection of 3-nitrotyrosine in the lung of asthmatic patients provides definitive evidence for numerous biochemical targets of NO that are likely to have functional consequences in the asthmatic airway [13,14,18,21]. This review will discuss the biochemical reactions of NO and its metabolic products in the pathophysiology of asthma.

[☆] This article is part of a Special Issue entitled Biochemistry of Asthma.

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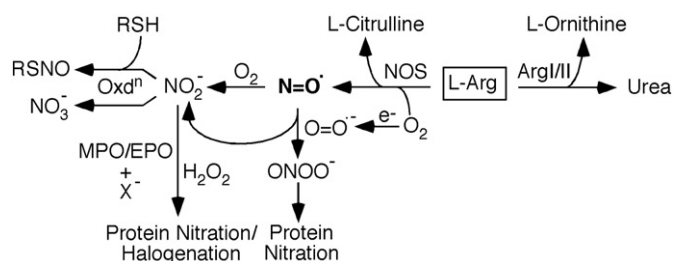


Fig. 2. Molecular consequences of nitrative stress. Nitric oxide synthase used L-Arg as substrate to produce nitric oxide (NO); L-citrulline is produced as the by-product of this reaction. Arginases I and II use L-Arg as substrate to generate urea and L-ornithine. Nitric oxide can rapidly oxidize to nitrite (NO_2^-), which can be further oxidized to nitrate (NO_3^-). Superoxide ($\text{O}_2^{\bullet-}$) reacts rapidly with nitric oxide to produce peroxynitrite (ONOO^-), which can readily nitrate proteins. In the presence of hydrogen peroxide (H_2O_2), nitrite (NO_2^-) and/or halide (X^-), myeloperoxidase/eosinophil peroxidase system can also promote protein nitration and/or halogenation. In the presence of thiols (RSH), nitrite is also involved in nitrosylation reaction to produce S-nitrosothiols (RSNO).

to NO via NOS, L-citrulline is produced. L-citrulline is converted via endogenous arginine synthetic pathways to L-arg; this may be an important source of L-arg during prolonged NO synthesis by iNOS and enable the higher levels of exhaled NO found in asthmatic individuals [78]. In addition, increased levels of exhaled NO in asthma may also result from the nonenzymatic generation of NO from nitrite in the acid airway environment typical of asthma [79,80]. Two isoforms of arginase enzymes may contribute to total arginase activity levels. Arginase I is highly expressed in the liver, where it serves a key role in the urea cycle, but is also present in cells that lack a complete urea cycle, which suggests other metabolic functions in nonhepatic tissues [81]. Arginase II is present in most tissues, including lung, and localized to the mitochondria within the cell [68,71,82,83].

Greater lung expression of both the Arginase I and II gene transcripts is present in the murine model of allergic asthma as compared with control mice [74]. The guinea pig model of allergic asthma also confirms higher arginase activity during the early response to allergen challenge [70]. In the murine model of asthma, greater L-arg metabolism through arginases linked to airway hyperreactivity [73], suggesting that L-arg levels and its utilization by specific pathways may contribute to asthma pathophysiology. Kenyon et al. have shown that treatment of mice with an arginase inhibitor increased the amount of NO produced in murine model of asthma [84]. Inhibition of arginase activity also increases protein S-nitrosylation and tyrosine nitration, and phenotypically enhances peribronchiolar and perivascular inflammation and mucus metaplasia in murine allergic asthma models [85].

Sapienza et al. showed that inhalation of L-arg amplifies the inflammatory response in the asthmatic airways [86]. Genetic studies in humans reveal that arginase I and II single-nucleotide polymorphisms are associated with an increased relative risk for asthma and atopy; the effect of polymorphisms on arginase expression or functional activity in humans is still unknown [87]. Nevertheless, it is clear that L-arg/NO metabolism is a determinant of asthmatic airway inflammation.

5. NO in oxidation chemistry: Nitrative stress

The functional role of NO in biological systems depends on its concentration and association with other biomolecules and proteins [88,89]. Furthermore it is clear that by-products of NO reactions can also have biological roles. NO reacts with oxygen or superoxide to form nitrite (NO_2^-), nitrate (NO_3^-) and reactive nitrogen species (RNS), such as peroxynitrite (Fig. 2). In comparison to healthy controls, asthmatics have higher levels of NO, NO_3^- , and nitrotyrosine in the airways. Within minutes of an allergen-induced asthmatic

response, NO_3^- increases markedly in asthmatics, while NO_2^- does not change, and NO decreases. Kinetic modeling suggests that NO reacts rapidly with $\text{O}_2^{\bullet-}$ produced by oxidative burst of leucocytes to produce ONOO^- , which may decay to NO_3^- or cause the nitration of phenolic compounds [14,90].

The half-life of peroxynitrite is 1 s at 37 °C at pH 7.4 [57]. Peroxynitrite is in equilibrium with peroxynitrous acid (ONOOH), which can covalently modify protein tyrosyl residues through nitration, leading to structural change and potential alterations in biological function [91–94]. In acid environments, ONOO^- is protonated to yield peroxynitrous acid (ONOOH) can also react with thiol residues to form S-nitrosothiols (SNO), which function in unique signal transduction pathways in the cell [95–98]. Consistent with the ability of ONOOH to participate in nitration and nitrosylation reactions, GSNO levels increase in parallel to nitrotyrosine in asthmatic airways after allergen challenge in direct proportion to formation of reactive nitrogen species [14].

NO_2^- is a substrate for hemeperoxidases such as MPO and EPO. Peroxidase-catalyzed oxidation of NO_2^- results in the formation of nitrogen dioxide radical ($\text{NO}_2^{\bullet}$) or related molecules [3,15,21,99–101]. Final products of hemeperoxidases include 3-nitrotyrosine and dityrosine (Fig. 3). In vitro studies have demonstrated that tyrosine nitration can cause either a gain or loss of protein function [102–106]. On average, proteins are composed of 4% tyrosine residues; but, chemical nitration of isolated proteins modifies only a subset of tyrosine residues, and the basis for this selectivity is not fully understood. This suggests that an innate property of the target protein or its location may predispose it toward nitration [107]. Nitration is likely not reversible and represents a pathophysiological modification, yet some reports have suggested that nitrated proteins can be repaired by a denitrase enzymic activity that is substrate dependent [108,109]. However, denitrase enzyme needs to be purified and further characterized in relevance to its repair activity. Biological nitration of protein tyrosine is associated with over 50 diseases including transplant rejection, lung infection, central nervous system and ocular inflammation, shock, cancer, neurological disorders (e.g., amyotrophic lateral sclerosis, Alzheimer's disease, Parkinson's disease, and stroke), and asthma [35,48,110–114].

Previous studies have shown that ONOO^- induces airway hyper-responsiveness in guinea pigs in vitro and in vivo [115]. Immunohistochemical studies, using antibodies to nitrotyrosine, have been used to identify modified proteins and suggest a possible involvement of

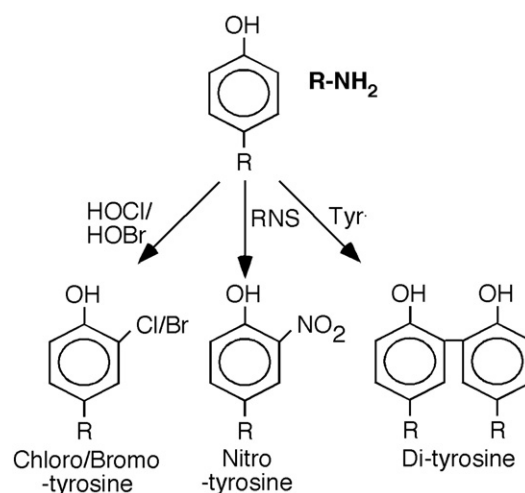


Fig. 3. Tyrosine containing amino acid oxidation and cross-linked products. Protein oxidative damage mediated by peroxidase mediated reactive brominating species (HOBr), MPO-generated reactive chlorinating species (HOCl), reactive nitrating species (RNS), and tyrosyl radical ($\text{Tyr}^{\bullet}$), may be identified by stable products formed by each pathway.

NO-derived oxidants in asthma [48,114]. Enhanced nitrotyrosine staining is present in airways of asthmatic lung [18,48,114]. Subsequent immunolocalization studies revealed that both airway epithelium and eosinophils are major cellular sources for nitrotyrosine formation in asthmatic airways [14]. Protein modification through nitration in asthma has been conclusively shown by mass spectrometry-based quantification, which demonstrates 10-fold increases in nitrotyrosine content in proteins recovered from airways of subjects with asthma compared to non-asthmatic controls [18]. Using NOS2-deficient mice in a model of allergen-induced asthmatic response, nitrotyrosine was absent in airway epithelial cells, but eosinophil recruitment and hyper-responsiveness were still present, suggesting that asthmatic response can occur independent of reactive nitrogen species production [116].

In vitro studies have shown that reactive oxygen and nitrogen species lead to oxidative and nitrative modification of tyrosine and inactivation of superoxide dismutases (SOD), MnSOD and ECSOD, while another major superoxide dismutase CuZnSOD can be inactivated by ROS and RNS through targeting of critical histidine residues and formation of histidinyl radicals [105,117,118]. As SOD scavenges superoxide, inactivation of this particular type of

enzyme may contribute to cell injury through increased free radical damage [105].

The ovalbumin sensitized and challenged mouse model of asthma has allowed for careful dissection of NO biochemical events [11]. In this model, mice were immunized with ovalbumin and two weeks later challenged with ovalbumin aerosol daily [11]. The extent and range of protein nitration was determined by two-dimensional gel electrophoresis followed by proteomic analysis. Nitrated proteins are detected within days of allergen challenge of sensitized mice (Fig. 4). Nitrotyrosine containing proteins, identified by antibody recognition, were subjected to tryptic digestion and the resulting peptides used to identify the protein by database searching (Table 2). Similar to previous reports, many of the modified proteins are key enzymes for energy production, such as pyruvate kinase, lactate dehydrogenase, malate dehydrogenase, aldolase A and glyceraldehyde phosphate dehydrogenase. This indicates that cells divert from glycolysis to other metabolic pathways for energy within cells during the inflammatory response. Dihydropyriminidase related protein, which is involved in axonal growth and has been identified as a target of protein nitration in Alzheimer's disease [119], was also identified as a target for nitration during the asthmatic response. Annexin II and III, members

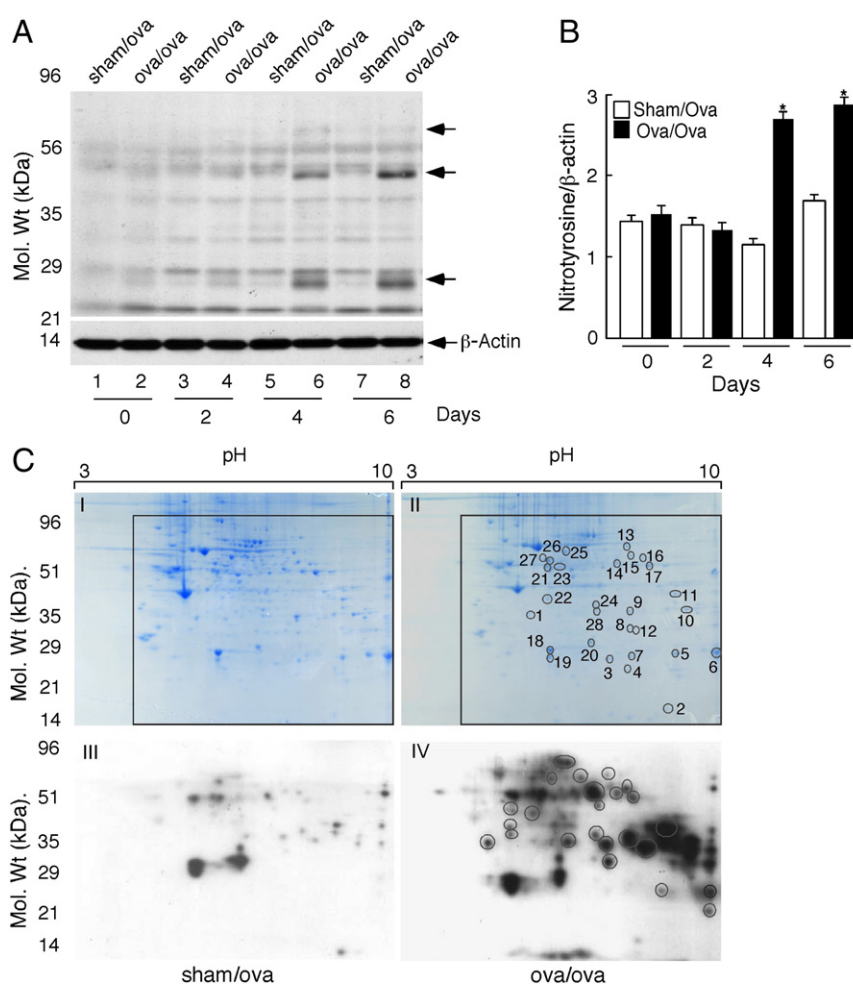


Fig. 4. Increased nitrotyrosine in ova sensitized and challenged mouse lung. [A] Western blot analysis of nitrotyrosine in tissue lysate of asthmatic mouse lung at days 0, 2, 4 and 6 (lanes 2, 4, 6 and 8) and corresponding controls at respective days (lanes 1, 3, 5 and 7) revealed more nitration in asthmatic lungs compared to controls. Lower panel is β -actin Western analyses for loading control. [B] Densitometric analysis of the Western blots shows that total nitrotyrosine band intensity compared to β -actin is significantly increased in asthmatic lung compared to control. (*) indicates $p < 0.05$. [C] 2D patterns of anti-nitrotyrosine immunopositive protein in asthmatic mouse lung compared to control at day 6. Lung tissue samples from control and ova/ova mice were subjected to proteomic analysis. Coomassie blue stained polyacrylamide gels of control [I] and ova/ova mouse lung tissue [II] are shown with the corresponding Western blots (lower panel; C and D respectively). Although the control blot [III] shows some degree of nitration, the blot [IV] representing the profile of ova/ova mouse lung shows more intense nitration. The protein spots, corresponding to the immunoreactive proteins observed in western blot [IV] on coomassie stained gel [II] were identified by tandem mass spectrometry.

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Table 2

Identification of nitrated proteins in ova sensitized and challenged mice.

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No.*	Proteins	pI	Mol wt. (kDa)	Accession no.	Coverage	Number of peptides matched	Function
1	Annexin III	5.6	36	CAA04887	62%	54	Associated with cytoplasmic granules in neutrophils and monocytes and translocates to the plasma membrane in activated cells
2	HB β chain	8.5	14	XP_489729	38%	9	Subunit of hemoglobin
	Profilin	8.5	14	CAB87382	79%	39	Regulates actin polymerization in response to extracellular signals
3	GTP binding protein	7.0	24	BAA01555	23%	20	Involved in signal transduction
4	MnSOD	8.6	24	CAA59335	24%	31	Antioxidant enzyme
5	Glutathione S-transferase μ 2	8.1	25	P10649	92%	75	Catalyze the conjugation of glutathione to numerous potentially genotoxic compounds
6	Glutathione S-transferase μ 2	7.3	25	P15626	91%	81	
7	Carbonyl reductase	9.1	26	NP_031647	95%	94	Catalyzes the NADPH dependent reduction of ketones on steroids and prostaglandins
8	GTP binding protein β chain	7.6	35	NP_034440	50%	41	Involved in signal transduction
9	Annexin II	7.6	38	AAN86740	80%	90	Ca ²⁺ -dependent phospholipid binding protein, mediates corticosteroid activity and plays a role in secretion
9	Lactate dehydrogenase	7.6	36	CAA26360	24%	19	Metabolic enzyme, converts lactate to pyruvate
10	Glyceraldehyde 3-phosphate dehydrogenase	8.4	36	AAH83149	69%	67	Enzyme in the glycolysis and gluconeogenesis pathways
	Malate dehydrogenase	8.9	36	CAA30274	48%	20	Metabolic enzyme of TCA cycle, converts malate to oxaloacetate
11	Aldolase A	8.3	39	AAA37210	65%	70	Metabolic enzyme of glycolysis pathway
12	Guanine nucleotide binding protein	7.6	35	NP_034444	29%	39	Mediates the activation of cytosolic messengers in signal transduction pathway
13	Transketolase	7.2	68	AAH55336	44%	73	Reversible link between glycolysis and the pentose phosphate pathway
14	Methylmalonate semialdehyde dehydrogenase	8.5	57	AAG44988	42%	29	Responsible for the oxidative decarboxylation of malonate- and methylmalonate semialdehydes to acetyl- and propionyl-CoA
15	Pyruvate kinase	7.1	58	NP_598428	69%	82	Metabolic enzyme, converts phosphoenolpyruvate to pyruvate
16	Catalase	7.7	59	AAA66054	58%	54	Catalyzes the conversion of toxic hydrogen peroxide into water and oxygen
17	Aldehyde dehydrogenase	7.9	54	NP_036051	52%	67	Acts in detoxifying a wide variety of organic compounds, toxins and pollutants
18	Thioether S methyl transferase	6.0	30	S52102	52%	46	Metabolic enzyme
19	Antioxidant protein 2	5.7	24	AC53277	83%	48	A member of a family of thiol-specific antioxidants
20	Carbonic anhydrase II	6.5	29	AAA37356	23%	26	Facilitates the transport of carbon dioxide
21	Selenium binding protein	5.8	53	Q91X87	56%	52	Participates in intra-golgi protein transport
22	Mouse secretory protein YM-1	5.6	44	AAB62394	45%	43	Secretory protein
23	Serum Albumin	5.7	70	NP_033784	35%	36	Transporter of many small molecules in the blood
24	Annexin I	7.0	39	NP_034860	77%	76	Carries anti-inflammatory role and regulates Ca ²⁺ -phospholipid activity
25	Dihydropyrimidinase related protein	6.0	62	1351260	43%	36	Involved in axonal growth
26	ER60 protease	5.8	57	JC2385	49%	70	Cysteine protease of the endoplasmic reticulum
27	Protein disulfide isomerase	6.0	56	AAA39906	32%	57	Resident foldase of the endoplasmic reticulum, catalyzes the formation and isomerization of disulphide bonds during protein folding
28	Aldehyde reductase	6.7	36	AA072144	37%	30	Catalyzes reversibly the oxidation of an aldose to an alditol

Nitrated proteins found on 2D gel of ova sensitized and challenged mice lung were identified by peptide mass mapping using production (MS/MS) spectra. Proteins are shown with pI, molecular weight, accession number, percentage coverage, number of peptides matched and a brief description of the function of the protein. * Corresponds to the numbered spots in Fig. 4C, II.

of the annexin superfamily of calcium and phospholipid binding proteins [120], and annexin I, an important mediator of glucocorticoid action [15] are all nitrated in the experimental model of asthma.

Notably, catalase, MnSOD, glutathione S-transferase, antioxidant protein 2 and carbonic anhydrase II are nitrated in the murine model of asthma, although these enzymes are present in different compartments of the cell. Studies identify loss of activity of catalase and SOD in asthma [121]. MnSOD is nitrated in the asthmatic airways, but the dominant oxidant modifications are related to hydroxyl radical like oxidants presumably via Fenton or Haber–Weiss chemical events [122] Nitration of MnSOD at tyr (Tyr34), which is in the active site of the enzyme, leads to loss of enzyme function, and tissue injury [123,124].

Other studies have shown that catalase and glutathione-S-transferase activity are lost by exposure to reactive nitrogen species in vitro [125,126]. Catalase activity is 50% lower in bronchoalveolar lavage of asthmatic lungs, as compared to healthy controls [11]. Catalase isolated from asthmatic airway epithelial cells has increased protein oxidation markers, including nitrotyrosine and chlorination and oxidation of sulfhydryls, linking oxidative modification to the reduced activity in vivo. Tyrosine oxidant modifications of catalase

occur in asthma: chlorination of tyrosine by peroxidase-catalyzed halogenation, and oxidative cross-linking of tyrosine as monitored by dityrosine, a product of tyrosyl radical [11]. Interestingly, catalase contains a putative chlorination site (KXHY) at Tyrosine 236, which may influence the susceptibility of the enzyme to peroxidase activity [127]. Tyrosine modification itself is not likely the complete cause of the loss of catalase activity. Other oxidative modifications, specifically oxidation of the cysteine 377 to cysteic acid, contribute to activity loss of the enzyme [11]. Altogether, the studies provide a picture of an abundance of reactive oxygen and nitrogen biochemical reactions in the asthmatic airway, and the potential downstream pathologic consequences.

6. NO in the S-nitrosylation reaction in asthma

The covalent addition of NO to certain protein moieties, such as thiol residues of cysteines, is termed nitrosation and can promote altered expression or function of enzymes, structural and signaling proteins [128,129]. Not all cysteine residues are equally susceptible to S-nitrosylation. In fact, the majority of proteins are modified at a single critical cysteine residue [130]. The sources of nitrosylating

groups might include nitrogen oxide species that originate exogenously or endogenously. For example, NOS activity can lead to SNO formation; each NOS isoform is capable of producing SNOs in multiple tissues, cells and subcellular compartments. SNO formation appears to depend on the microenvironment of the protein itself [131–135]. On the other hand, NO also may be exchanged between transition metal ion and thiols in proteins [136,137]. In addition, binding of NOS to adaptor proteins may localize nitrosylation reactions, but there are many more nitrosylated substrates than NOS binding partners. Recent work by Kornberg et al. suggests that the S-nitrosylated GAPDH functions as a nuclear nitrosylase via a redox signal transduction cascade mechanism [138,139].

Nitrosothiols (RSNO) are important reservoirs of bioactive NO, able to take up and store NO for later release as needed. There are several mechanisms for the decomposition of RSNO to produce NO, including nonenzymatic catalysis with copper or reaction with ascorbate [140–143], as well as through enzymatic pathways that act on R-SNO, such as thioredoxin reductase [144], xanthine oxidase [145], Cu/Zn superoxide dismutase [146]. GSNO reductase [GSNOR; alcohol dehydrogenase 5 (EC 1.1.1.1)] catalyzes the denitrosylation of S-nitrosoglutathione (GSNO), but not other protein SNO [128,147]. The decomposition of GSNO leads to formation of NH₃ and oxidized glutathione (GSSG).

RSNO are found in the human airway lining fluid, with the most common derivative GSNO at levels of 0.2 to 0.5 μ M [148,149]. GSNO has clearly defined physiologic roles in the airways, including regulation of ciliary beat frequency, immune response and bronchodilation. The bronchodilatory effects of GSNO are largely cGMP independent [148,150] and result from S-nitrosylation of ion channels, receptor systems, and other myocyte proteins [150–153]. SNOs also have pulmonary vascular smooth muscle relaxant effects [152,154,155], and thus serve as a mechanism to couple perfusion via vasodilation to ventilation via bronchodilation [155,156]. GSNO also contributes to airway homeostasis by antimicrobial and anti-inflammatory properties [157].

Many studies now definitively link GSNO deficiency to asthma pathophysiology [149,158,159]. RSNO are present at lower than normal levels in the tracheal aspirates of children with acute asthma exacerbations [149,160] as well as in the bronchoalveolar lavage fluid of well-controlled asthmatic individuals [14]. Accelerated catabolism of GSNO in asthma results in the overall lower levels of GSNO and contributes to the consequent higher release of free NO as measured in exhaled breath of asthmatics [149]. Most accumulating evidence points to greater enzymatic catabolism of GSNO via GSNOR in asthma [161]. GSNOR-deficient mice, which are unable to break down GSNO and thus have high airway levels, are protected from airway hyper-reactivity in the murine ovalbumin asthma model [158]. Recently, Que et al. have also shown that the low level of GSNO in asthmatic bronchoalveolar lavage fluid is inversely correlated with GSNOR expression in lung lavage cell lysates [159]. Furthermore, the GSNOR activity in bronchoalveolar lavage samples was higher in asthma as compared to healthy control subjects and was inversely related to the degree of airway hyperresponsiveness [159,161]. Altogether, studies suggest that GSNO/NO metabolism is a determinant of airway reactivity in asthma.

In addition to effects on bronchial smooth muscle and airway reactivity, nitrosylation also impacts pro-inflammatory signal transduction in asthma. NO attenuates NF- κ B p50–p65 heterodimer DNA binding and inhibits pro-inflammatory gene transcription through S-nitrosylation of the p50 subunit [162]. The p65 binding partner of p50 is also found to be S-nitrosylated in cytokine stimulated cells [163]. S-nitrosylation of p65 inhibits NF- κ B-dependent gene transcription, and nuclear levels of S-nitrosylated p65 correlate with decreased DNA binding of the p50–p65 heterodimer [163]. Very recently Olson et al. have tested the therapeutic benefit of S-nitrosothiols in the murine model of ovalbumin induced allergic inflammation [164]. They found

that instillation of GSNO suppressed NF-B activation, but did not significantly affect overall markers of inflammation or mucus metaplasia, which may suggest that its bronchodilatory effects outweigh its anti-inflammatory properties.

7. Summary

There is definitive evidence for alterations in NO synthetic and catabolic pathways in asthma. There are greater levels of toxic RNS, and equally important, lower levels of beneficial RSNO in asthmatic airways. In contrast to stable nitration products, the labile nature of RSNO has presented challenges to comprehensive identification of *in vivo* targets in asthma. However, mass spectrometry and derivatization techniques are providing exciting insight into the wide array of events controlled by nitrosylation chemistry in the cell. In parallel, physiologic studies have identified that arginine/NO metabolic processes regulate airway hyper-reactivity and inflammation, key components that define asthma. Detailed understanding of the biochemical pathways that control NO metabolism and its physiologic functions will enable future rational design of strategies to optimize levels of beneficial NO products, while limiting the formation of more damaging species, in the treatment of asthma.

Acknowledgements

The authors acknowledge grant support from the NIH [RO1 HL69170, PO1 HL081064, RC1 HL099303, UL1 RR024989 and KL2 RR024990]. SCE is a Senior Fellow of the American Asthma Foundation Sandler Program for Asthma Research.

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