

Arginine Metabolism in Asthma



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KEYWORDS

• Arginine metabolism • Nitric oxide synthase • Arginase • Agmatine • Citrulline
• Ornithine • Polyamine • ADMA

KEY POINTS

- L-Arginine metabolism via the arginase and nitric oxide synthase pathways is important in maintenance of airways muscular tone.
- Imbalance in the L-arginine metabolism leading to nitric oxide deficiency and airway constriction can occur through increased arginase activity or by accumulation of endogenous nitric oxide synthase inhibitors such as asymmetric dimethylarginine (ADMA).
- ADMA may represent a novel therapeutic target in correcting the enhanced airways responsiveness in asthma.

INTRODUCTION

The semiessential amino acid, L-arginine, serves many functions in cellular and organ homeostasis. Of particular relevance to physiologic function is the production of nitric oxide (NO) from L-arginine by the nitric oxide synthase (NOS) family of isozymes, which serves to relax airway smooth muscle cells through direct interaction with the heme core of soluble guanylate cyclase. The production of NO can be modified in disease by increased competition between NOS and the arginase pathway, which produces urea and ornithine. The authors, and other investigators, have previously reported that the airways hypercontractility in animal models of asthma is at least in part related to imbalances in the production of NO by the neuronal NOS,¹ as well as increased competition for substrate L-arginine with the arginases.^{2–4} It has more recently become appreciated that the production of the endogenous NOS inhibitor, asymmetric dimethylarginine (ADMA), as well as accumulation of the L-ornithine-derived

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polyamines, downstream of the arginase pathway, may also modify NO production and airway tone in asthma.^{5,6}

ADMA in the Lung

Synthesis of ADMA (protein arginine methyltransferases)

The first step of arginine incorporation into proteins is through the attachment of arginine to the corresponding transfer RNA (tRNA) anticodon via aminoacyl tRNA synthetase. The arginine residue is inserted into the forming protein by the ribosomal complex and the formation of a peptide bond. Methyl groups are then added to the arginine residues in proteins by a family of protein arginine methyltransferases (PRMTs).⁷ The 2 classes of PRMTs, type I and II, exhibit differential kinetics for the formation of asymmetric (ADMA) versus symmetric dimethylarginine (SDMA), respectively, in addition to synthesis of monomethyl arginine (MMA).⁷ Yildirim and colleagues⁸ (2006) reported that PRMTs are expressed in the mouse lung within the bronchiolar and alveolar epithelium and that PRMT2 can be induced by chronic hypoxia. Bulau and colleagues⁹ subsequently reported a comprehensive expression profile of the PRMTs in mouse lung and demonstrated expression of both type I (PRMT1, PRMT3, PRMT4, PRMT6) and type II PRMTs (PRMT5, PRMT7). Although alterations in PRMT expression have not yet been demonstrated in human asthma, Ahmad and colleagues^{10,11} reported increased expression of PRMT2 protein levels in bronchial epithelium from their mouse model of ovalbumin (OVA)-induced allergic airways inflammation. Sun and colleagues^{12,13} also comprehensively examined PRMT expression in their rat OVA model of allergic airways inflammation and demonstrated significant upregulation of PRMT1, PRMT2, and PRMT3 and downregulation of PRMT4 in the lungs. PRMT1 expression was quantified in immunostained sections and shown to be upregulated in the bronchi and alveolar epithelial cells. They further demonstrated that administration of an arginine methyltransferase inhibitor (AM-1) before the OVA exposure led to a reduction in inflammation, as assessed by eosinophil cell counts from bronchoalveolar lavage (BAL) fluid, semiquantitative scoring of histologic sections of the lungs, interleukin 4 (IL-4) messenger RNA expression, and quantification of immunoglobulin (Ig) E and OVA-specific IgG1 in serum.¹³ Given the timing of the AM-1 inhibitor administration, the investigators posited that IL-4 induced PRMT1 expression, which led to the expression of eotaxin in the inflamed airways. Given the complex interaction of PRMTs with arginine metabolism, it would be very interesting to examine the changes in ADMA and the pathophysiologic/functional impact of administration of AM-1 in established disease (ie, is PRMT a good candidate for therapeutic intervention in asthma?). The role of altered expression of PRMTs and the impact on protein and physiologic functions in asthma is clearly an area that requires further investigation.

ADMA, SDMA, and MMA are liberated on proteolytic cleavage of proteins, allowing access of these derivatives to the intracellular milieu. SDMA has been reported to inhibit the cationic amino acid transporters (CAT1, *SLC7A1*; CAT2, *SLC7A2*), which are the primary means of cellular uptake of L-arginine.^{14,15} In addition to competition at the cationic amino acid transporters, MMA and ADMA also act directly as competitive inhibitors of NOS.¹⁶ The accumulation of these endogenous NOS inhibitors likely leads to impaired NOS function, despite the presence of “normal” levels of L-arginine; this has been described as the “arginine paradox”.¹⁶

Degradation of ADMA

ADMA is metabolically degraded to L-citrulline and dimethylamine by dimethylarginine dimethylaminohydrolases (DDAH).^{17,18} The 2 members of this family of enzymes, DDAH1 and DDAH2, are differentially expressed in tissues, including the kidney, liver,

lung, and heart. Bulau and colleagues⁹ reported that DDAH1 was expressed at low levels, and DDAH2 was expressed at high levels in lungs from normal mice; however, DDAH enzymatic conversion of ADMA to L-citrulline was the highest in liver and kidney, suggesting a significant potential for circulating ADMA derived from the lung to manifest systemic effects. By contrast, SDMA is not metabolized appreciably *in vivo*, with heart, lung, kidneys, and liver being tested by Bulau and colleagues,⁹ supporting its predominant renal excretion on liberation.

Klein and colleagues¹⁹ (2010) reported that DDAH1-overexpressing transgenic mice exhibit an attenuated OVA-induced increase in pulmonary inflammation compared with wild-type controls. This effect on allergic airways inflammation was recently corroborated by Kinker and colleagues²⁰ (2014) using a house dust mite model in DDAH1-overexpressing mice, in which the attenuation of BAL inflammatory cells was completely attributable to a reduction in eosinophils, but not neutrophils. However, although Kinker and colleagues²⁰ reported decreased expression of both DDAH1 and DDAH2, the authors demonstrated that DDAH enzyme activity was altered neither in the acute OVA-induced allergic airways inflammation model in mice nor in lung tissue from human asthma.⁵ Thus, the rate-limiting factor of ADMA metabolism in asthma would appear to be the presence of functional DDAH.

Additional evidence supporting an important role of DDAH in lung disease has also been shown in a mouse model of acute lung injury. Aggarwal and colleagues²¹ recently reported that overexpression of DDAH2 in a mouse model of acute lung injury led to a reduction in ADMA levels as well as indices of oxidative/nitrosative stress in lungs and ameliorated the lung leak, injury, and functional changes following treatment with *E. coli* lipopolysaccharide (LPS). Thus, modification of ADMA metabolism by DDAH represents a potential therapeutic target in both bacteria- and allergy-induced pulmonary inflammation.

Functional Consequences of ADMA Liberation in the Lung

ADMA *in vitro*

Wells and Holian²² (2007) reported that ADMA treatment of LA-4 lung epithelial (adenoma) cells, which had been primed with an LPS exposure, led to the production of reactive nitrogen and oxygen species, thus contributing to overall oxidative stress in these cells. These data suggested that ADMA may play an important role in airway inflammation and led to *in vivo* studies of allergic airways inflammation.

Animal studies

Wells and colleagues²³ (2009) furthermore treated normal BALB/c mice with ADMA via osmotic pump to achieve a chronic (2-week) systemic exposure and demonstrated increased total respiratory resistance and reduced dynamic compliance, thus evidencing altered lung function in response to ADMA. These changes were attributed to increased collagen production via the arginase pathway, leading to increased levels of hydroxyproline, a precursor for collagen biosynthesis. Interestingly, these changes in respiratory function were reversed after withdrawal of ADMA for 4 weeks, supporting the potential therapeutic benefit of targeting this pathway. They further demonstrated increased arginase enzyme activity with ADMA, both *in vitro* and *in vivo*, in the absence of increased expression of arginase. They posited that this increase was due to disinhibition of arginase by the lack of production of the intermediate of the NOS biosynthetic pathway NO-hydroxy-arginine, which inhibits arginase. Klein and colleagues¹⁹ (2010) went on to demonstrate that exogenous systemic ADMA (also delivered by osmotic pump) further augmented pulmonary eosinophilic and neutrophilic inflammation in the OVA model of allergic airways inflammation.

Consistent with the findings of Wells and colleagues²³ and Ahmad and colleagues,¹⁰ the authors reported elevated levels of both ADMA and SDMA in an acute model of OVA-induced allergic airways inflammation.⁵ These observations were extended to demonstrate increased airways contractility to methacholine in response to the acute administration of levels of ADMA similar to those observed in the model mice, thus demonstrating that ADMA at levels observed in the OVA model could achieve the degree of airway hyperresponsiveness observed in disease.⁵

Recently the ratio of L-arginine:ADMA has been postulated in the cardiovascular literature to explain what is called the “arginine paradox”, the apparent dysfunction of NOS activity even in the presence of “normal” levels of L-arginine.¹⁶ As such, the normal L-arginine:ADMA ratio would be in the 100 to 200 range, with attenuation indicated in the presence of cardiovascular disease.¹⁶ Bulau and colleagues⁹ (2007) reported similar L-arginine:ADMA ratios in BAL and serum from control mice and human subjects (range: 108 ± 20 and 163 ± 26 for BAL from mice and humans, respectively; and 124 ± 14 and 166 ± 29 for serum from mice and humans, respectively), thus supporting that peripheral indices of L-arginine:ADMA changes reflect alterations within the lungs in both species. Interestingly, the authors demonstrated similar ratios in normal mice and human tissues, which were attenuated in their mice with allergic airways inflammation and in human asthma.⁵

Ahmad and colleagues¹⁰ (2010) reported increased ADMA levels in their OVA model of allergic airways inflammation, with concomitant elevation of peroxynitrite and nitro-tyrosine levels in mitochondria and lung cytosol, respectively. Ahmad and colleagues¹⁰ further demonstrated increased bronchial epithelial cell expression of PRMT2 and decreased expression of DDAH2 by Western blotting and immunohistochemical staining. Ahmad and colleagues¹¹ (2011) subsequently reported that simvastatin administration during the allergen challenge phase of the acute OVA model led to induction of endothelial NOS expression and attenuation of inducible NOS expression, apoptosis, inflammation, and Th2 cytokines (IL-4, IL-5, IL-13) and more importantly that the augmented methacholine responsiveness in these mice was attenuated compared with the vehicle-treated OVA-inflamed mice.

Increased arginase activity results in increased production of proline, a precursor of collagen, and formation of the L-ornithine-derived polyamines putrescine, spermidine and spermine. Inhibition of NOS and increased L-arginine:ADMA ratio may result in increased consumption of L-arginine by arginase and increased formation of polyamines. The authors have observed increased polyamines in mouse lungs from the acute OVA-induced allergic airways inflammation model.²⁴ The importance of this observation lies in the role of spermine as an inhibitor of NOS, because nebulization of spermine into mouse airways resulted in a decrease in NO formation and increase in airway hyperresponsiveness to methacholine.²⁴

Clinical studies

The authors reported that ADMA levels were elevated 2.4-fold in sputum from pediatric patients with asthma compared with control subjects. Sputum ADMA levels and L-arginine:ADMA ratios correlated significantly with fractional exhaled NO (FeNO) in the patients with asthma, suggesting physiologic relevance of ADMA as NOS inhibitor in human asthma airways. Similarly, the L-arginine:ADMA ratios were also significantly reduced in asthma lung tissues compared with control lung tissues, matching the observation in mouse lung tissue.⁵

Increased levels of ADMA were also found in exhaled breath condensate (EBC) from children with asthma. There was no significant difference in EBC ADMA between children treated or not treated with inhaled corticosteroids. In this study, there were no

significant correlations between EBC ADMA with serum ADMA levels, FeNO, or lung function parameters.^{5,25}

Further evidence of a potential role of ADMA in airway responsiveness comes from a study of adult patients with mild, eosinophilic asthma where ADMA was measured in induced sputum before and after a controlled inhaled allergen challenge. Sputum ADMA was increased significantly in these patients 7 and 24 hours after the challenge, along with increases in sputum eosinophils and changes in pulmonary function testing consistent with airflow limitation and airway hyperresponsiveness.²⁶

Seemingly conflicting data exist in regards to systemic levels of ADMA in asthma. In a case-control study of 50 pediatric patients with mild allergic asthma, FeNO was found to be increased, as expected, whereas mean plasma ADMA and L-arginine concentrations were significantly lower than in healthy controls.²⁷ Although the reduced systemic L-arginine concentrations confirmed results of an earlier study by Morris and colleagues in a mixed population of pediatric and adult patients with asthma, and may be explained by increased concentrations of free arginase in blood, low circulating levels of ADMA and L-arginine were not confirmed in a cohort of 8-year-old children with asthma. However, the patients in this particular study had very mild asthma, were no different in post-bronchodilator spirometry (forced expiratory volume in the first second of expiration [FEV₁], forced vital capacity [FVC], or FEV₁/FVC) from their age-matched nonasthmatic controls, and did not have increased mean FeNO.²⁸

There is growing evidence that obesity is associated with the development of asthma.^{29,30} Obesity is an important factor among female patients whose asthma occurs after childhood and who have less atopy, using cluster analyses,^{31,32} and this cluster was also associated with lower airway eosinophils and FeNO.^{32,33} The inverse association between body mass index (BMI) and FeNO³⁴ may be explained by an imbalance between L-arginine and ADMA, because ADMA concentration and the L-arginine:ADMA ratio in plasma were reported to have similar associations with FeNO as for sputum among late onset asthma.^{6,35,36} Specifically, subjects with late-onset asthma had higher ADMA and lower L-arginine plasma levels compared with early onset, respectively. The log plasma L-arginine:ADMA ratio was inversely correlated with BMI in late-onset but not the early-onset asthma. Although FeNO was inversely associated with BMI in the late-onset phenotype, the relationship was lost after adjusting for L-arginine:ADMA ratio. Also in this phenotype, a reduced L-arginine:ADMA ratio was associated with less serum IgE, increased respiratory symptoms, lower lung volumes, and worse asthma quality of life.⁶

These studies not only provided evidence that ADMA may be a link between obesity and asthma, but also demonstrated that clinical-biological phenotyping in asthma may help unravel pathways beyond inflammation that lead to asthma and potentially to new asthma treatments.³⁷

TREATMENT OPTIONS

As both animal and human studies suggested that the L-arginine:ADMA ratio is important in the development of NO-mediated airway hyperresponsiveness and asthma, therapies that would result in increased availability of L-arginine for NOS could potentially be beneficial in asthma.

Mabalirajan and colleagues³⁸ examined the effect of a high dose of L-arginine (250 mg/kg, twice daily) on airway hyperresponsiveness in the mouse OVA-model of allergic airways inflammation and demonstrated a reduction of the methacholine-induced airways resistance changes in treated mice compared with untreated

OVA-stimulated animals. Interestingly, the high-dose L-arginine treatment also led to a reduction of pulmonary arginase activity, which might be attributable to increased production of the arginase inhibitor N^{ω} -hydroxy-L-arginine as an intermediate metabolite of the NOS pathway. Administration of high-dose L-arginine also further augmented expired NO from the allergically inflamed mice, which reinforces the role of the different compartments within the lungs, airways, and infiltrating inflammatory cells in the disease pathogenesis.

Supplementation with L-arginine should theoretically improve the L-arginine:ADMA ratio but the effectiveness of this approach may be limited by the high first-pass mechanism of L-arginine in the gut and liver. In addition, recent clinical trials have suggested that long-term supplementation with this amino acid may also result in an increase in ADMA formation.^{39,40} Delivery of L-citrulline, which serves a substrate for *de novo* synthesis of L-arginine, may help circumvent some of these problems as L-citrulline supplementation has been shown to lead to increased L-arginine levels and beneficial effects in heart failure and sepsis.^{41,42}

However, as arginase is upregulated in asthma,^{2,4,43} strategies that increase L-arginine concentrations may ultimately result in increased arginase activity and the formation of polyamines and proline. The polyamine spermine was recently shown to contribute to airway hyperresponsiveness in asthma by inhibiting NOS,²⁴ and proline may contribute to airway remodeling, because it is a precursor of collagen formation.^{24,44,45} Therefore, arginase inhibition may be a more elegant approach than L-arginine or L-citrulline supplementation, because it would result not only in an increase in

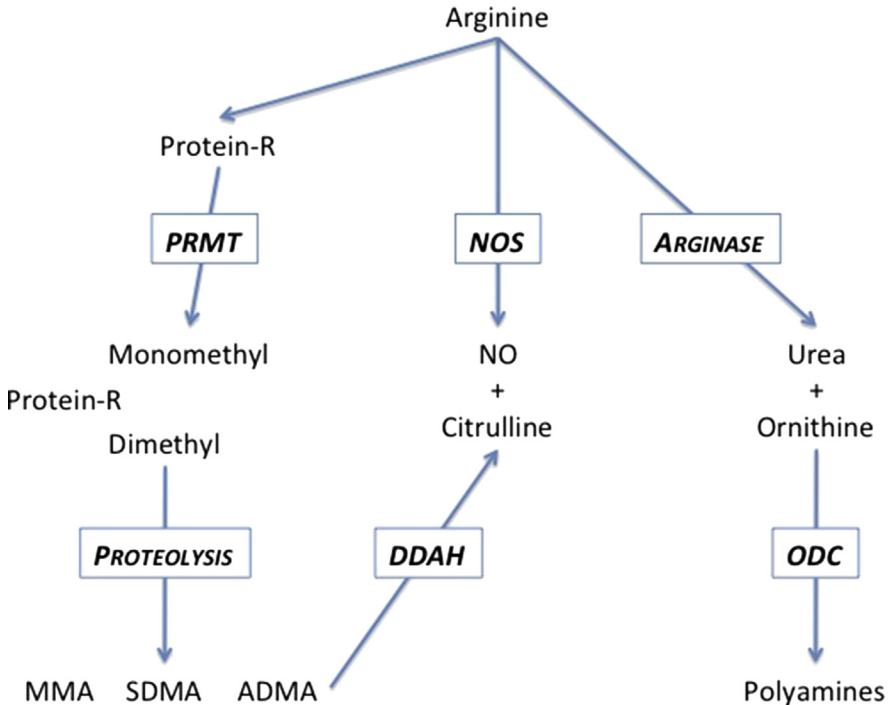


Fig. 1. Metabolism of L-arginine via the PRMT, NOS, and arginase pathways. ADMA, asymmetric dimethylarginine; DDAH, dimethylarginine dimethylaminohydrolase; MMA, monomethylarginine; NO, nitric oxide; NOS, nitric oxide synthase; ODC, ornithine decarboxylase; PRMT, protein arginine methyltransferase; SDMA, symmetric dimethylarginine.

L-arginine availability for NOS but also a decrease in the production of factors that contribute to airway remodeling.^{2,46,47}

Future strategies may include a reduction of ADMA synthesis by inhibiting PRMTs or enhancing metabolic breakdown by increasing the enzymatic activity of DDAH.

FUTURE CONSIDERATIONS/SUMMARY

Current evidence suggests that L-arginine metabolism is altered in asthma. Changes in the balance of the L-arginine metabolism result in reduced L-arginine availability for NOS and impairment of the enzyme, which has significant functional consequences. Further studies will have to elucidate the role of other L-arginine metabolizing enzymes, including the arginine decarboxylase-argmatine-argmatinase pathway. A better characterization of the L-arginine metabolome in well-phenotyped patient populations may lead to a better understanding of the pathways involved and may offer new targets for therapeutic interventions (**Fig. 1**).

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