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Newer Insights into the Role of Nitric Oxide (NO) in Bronchial Asthma: An Experimental Study

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Abstract

Bronchial asthma is an obstructive airway disease characterized by airway inflammation, and hyperresponsiveness to a variety of stimuli and airflow obstruction resulting in variable respiratory symptoms viz. cough, wheezing and shortness of breath. The pathophysiology of asthma involves the complex interactions among inflammatory cells, cytokines and mediators of stress. Nitric oxide (NO), initially identified as a vascular endothelial regulator, is being implicated for its role in obstructive airway diseases. The study was planned to evaluate the effects of NO modulators on ovalbumin (OVA) induced bronchial hyperresponsiveness, airflow resistance and airway inflammation in rats. Asthma was experimentally induced in rats by Ovalbumin (OVA) immunization (day 0) followed by OVA aerosol challenge (from day 8 to day 15) for 20 min, on each day. In this disease model, bronchial hyperresponsiveness, airway inflammation and airflow restriction was classically expressed and served as the disease control (DC). All drugs (L-arginine, ABH, and prednisolone) were administered 30 minutes prior to aerosol challenge, daily for 8 days. To assess pulmonary function, 24 hours after the last OVA challenge, baseline p-enh, a marker of airway resistance and hyperresponsiveness was assessed in response to increasing doses of methacholine (spasmogen) aerosol using whole-body plethysmography. Following this, blood and BAL fluid samples were collected from different treatment groups and assayed for biomarkers of airway inflammation and oxidative stress. The results showed that in OVA immunized and challenged rats (DC), there was marked increase in p-enh values as compared to normal controls. Pre-treatment with both NO precursor, L-arginine, as well as arginase inhibitor, ABH, attenuated the raised p-enh values seen in the DC group. Assay of BAL fluid showed that eosinophil counts and OVA specific IgE, levels were differentially raised in the DC group and these were attenuated by L-arginine and ABH treatments, which were comparable to the standard drug, prednisolone. Further, oxidative stress markers, like MDA was elevated in DC rats, which was also attenuated after L-arginine and ABH treatments. Thus, it is inferred that NO may play a crucial regulatory role in the pathophysiology of bronchial asthma by regulating bronchial hyperresponsiveness and airway inflammation and that interactions with oxidative stress may be involved in these effects.

Keywords: *Bronchial Asthma; Airway Hyperresponsiveness; Airway Inflammation; Nitric Oxide; L-Arginine; Arginase Inhibitor*

Introduction

Bronchial asthma is a heterogenous respiratory disease characterized by chronic airway inflammation, airway hyper responsiveness and reversible airway obstruction. It is an obstructive lung disease with clinical manifestations of dyspnea, bouts of excessive coughing and wheezing. Complexly interacting cellular and humoral components contribute to the pathophysiology of the disease. Pathogenesis of asthma includes Th2 and IgE mediated responses to allergens. Other mediators of Type-1 Hypersensitivity such as histamine, bradykinin, leukotrienes, prostaglandins, IL-4, IL-5 and IL-13 also play important roles in the disease process [1]. The histopathology of asthma is characterized by epithelial detachment, mucus gland hyperplasia, subepithelial fibrosis, inflammatory cell infiltrate, bronchial smooth muscle hypertrophy, and vascular changes [2]. Many molecular mechanisms have been proposed for the pathophysiology of bronchial asthma and the role of nitric oxide (NO) as a modulator/effector substance has been the subject of considerable contemporary research.

Nitric Oxide (NO) is a simple, ubiquitous, gasotransmitter molecule with complex actions. NO signaling has pleiotropic roles in biology and a crucial function in cardiovascular homeostasis, which led to the Nobel Prize in 1998. In addition to its key role in regulating the cardiovascular function, NO has been reported to be involved in the pathological processes of a variety of human diseases, including cardiovascular diseases, metabolic diseases, inflammatory diseases, cancer, and neurological diseases [3]. Several studies have shown the role of endogenous NO in regulating airways physiology and its involvement in respiratory diseases. NO initially identified as a vascular endothelial regulator is being implicated for its role in obstructive airway diseases. Nitric oxide is produced from oxidative metabolism of L-arginine by nitric oxide synthase (NOS), which has one inducible (iNOS) and two constitutive isoforms (eNOS and nNOS). In hypoxic situations, NO is generated by the alternative, NOS independent, Nitrate-NO pathway. NO and its metabolites participate in various physiological processes such as smooth muscle relaxation, inflammation and nitrosative stress. NO plays a role in regulation of both pulmonary vascular tone as well as broncho-motor tone through effects on relaxation of smooth muscle. Nitric Oxide causes smooth muscle relaxation by binding and activating soluble Guanylate cyclase and thus increasing the production of cGMP in the smooth muscle. NO acts by binding to the haem moiety of the soluble guanylate cyclase (sGC) enzyme. The cGMP reduces the affinity of Myosin Light Chain Kinase (MLCK) for Ca^{2+}-Calmodulin complex and consequently leads to smooth muscle relaxation [4].

Barnes (1996) first suggested that NO has beneficial effects in asthma, causing bronchodilation by relaxing airway smooth muscle [5]. In addition, NO participates in inflammation and host defence against infection via alterations in vascular permeability, changes in epithelial barrier function and repair, cytotoxicity, upregulation of ciliary motility, altered mucus secretion, and inflammatory cell infiltration [6]. These multiple functions of NO have been implicated in the pathogenesis of chronic inflammatory airway diseases such as asthma. Further, a positive correlation between nitrite/nitrate levels in sputum and bronchial remodeling was seen [7]. Studies have also found a strong association between increased nitric oxide levels with increased asthma risk, and severity as well as response to bronchodilator agents [8].

Experimental and clinical data have indicated that Nitric oxide may be crucial in the pathophysiology of asthma. On one hand, NO has been reported to produce bronchoprotective effects in asthma by airway smooth muscle relaxation and inhibition of smooth muscle proliferation. On the other hand, higher levels of Fractional exhaled NO (FeNO) have been reported in asthma. Moreover, higher levels of FeNO have been associated with increased expression of both, arginase and iNOS enzymes in the airway epithelium [9]. Arginase is an enzyme that hydrolyzes L-arginine by breaking it down into urea and L-ornithine and thus cleaves the molecule and reducing availability of the precursor for synthesis of NO. Thus, arginase and NOS compete for the common substrate L-arginine, impacting the generation of NO in the lung. The inducible nitrogen oxide synthase (iNOS) is an enzyme that synthesises NO in huge amounts. It is paradoxical that two enzymes acting in opposite directions, both are enhanced in bronchial asthma. Thus, this study was designed to evaluate the role of NO in bronchial asthma. The pharmacological tools i.e. NO modulators (NO precursor and arginase inhibitor) were used to evaluate their effects on airway inflammation, bronchial hyperresponsive-ness and oxidative stress in an OVA-induced experimental model of bronchial asthma in rats.

Materials and Methods

Inbred Wistar rats of either sex (180 - 250g) were used for this study (n = 6 per group). Animals were kept in an environmentally controlled room (22 ± 2°C, 12 hours light and dark cycles). Animals were fed on standard laboratory food pellets and water *ad libitum.* Animal care was as per the guidelines for the care and use of animals in scientific research prepared by the Indian National Science Academy (INSA), New Delhi, with approval of the Institutional Animal Ethics Committee (IAEC).

Rats were divided into following 07 groups (n = 6/group): (1) Normal group; and (2) Disease control: rats were administered vehicle (isotonic saline) daily. (3 and 4) L-Arginine 250 and L-Arginine 500 groups received L-arginine 250 mg/kg and 500 mg/kg in isotonic saline, i.p, respectively. (5 and 6) ABH 200 and ABH 400 groups received 200 µg/kg and 400 µg/kg of ABH hydrochloride in isotonic saline i.p. each, respectively. (7) Rats were treated with prednisolone 10 mg/kg, i.p., served as positive control.

All rats, except the normal group, were immunized and challenged with OVA according to the procedure described by Abdureyim., *et al.* [10]. All groups were immunized with ovalbumin (40 mg/rat, i.p.) adsorbed to 2 mg of aluminium hydroxide on day 1. Fourteen days after immunization, the animals were challenged with 1% ovalbumin aerosol in 0.9% saline, for 20 min./day for 8 consecutive days. All drugs and vehicles were administered 30 minutes prior to 1% ovalbumin aerosol challenge, daily for 8 days. The doses for L-arginine and ABH were selected on the basis of doses mentioned in existing literature [11,12].

Assessment of bronchial hyper-responsiveness and airflow restriction by whole body plethysmography (WBP)

After twenty-four hours of the last OVA challenge, i.e. on day 23, pulmonary function and airflow resistance were assessed using BUXCO unrestrained whole body plethysmography system, and expressed as enhanced pause (P-enh) [13]. Whole body plethysmography (WBP) system is used for the measurement of airway resistance as well as bronchial hyperresponsiveness in experimental animals. WBP provides the measurement of lung functions in conscious animals and provides an idea about the structural changes occurring in the lungs. WBP is used to assess various parameters such as total lung capacity, peak inspiratory flow, peak expiratory flow, tidal volume, pause, and enhanced pause (P-enh) in conscious animals.

WBP system consists of three chambers- (i) nebulization chamber, spasmogen is nebulized to the conscious animal for a specific period; (ii) unrestrained observation chamber, where the breathing changes in animals are observed and compared with (iii) third reference chamber. Enhanced pause (P-enh), a marker of airway hyperresponsiveness was assessed using whole body plethysmography. Animals were placed in the nebulization chamber, where animals were exposed to different doses of inhaled methacholine (0-20 mg/ml) aerosol for 3 minutes. Animals were then placed in the second chamber where the difference in the breathing pressure between the animal chamber and the reference chamber was recorded using a transducer connected to the amplifier. Changes in the air volume leading to changes in pressure resulted in the changes in box pressure (reference chamber). The resultant waveform provides an estimation of variation in the respiratory cycle, tidal volume, enhanced pause, etc. Enhanced pause (Penh) is a dimensionless value that acts as a marker of bronchial resistance as well as bronchial hyperresponsiveness. Enhanced pause (Penh) is also calculated by multiplying the ratio of peak expiratory height to peak inspiratory height with pause [13,14].

The readings were recorded and averaged for 3 minutes after each nebulization. Between successive doses of Methacholine challenge, an interval of 10 minutes was maintained. At the conclusion of the experiment, the animal was removed from the chambers and data was analyzed using Ponemah physiologic data acquisition and analysis system developed by Data Sciences International.

Bronchoalveolar lavage (BAL) fluid collection and biomarker assays

After the assessment of pulmonary function and airflow resistance using WBP, rats were anesthetized and blood was collected by cardiac puncture. Blood was centrifuged at 4°C (3000 rpm) for 10 minutes and the serum was separated and stored at -80°C.

BAL fluid was collected by conducting a bronchoalveolar lavage through a tracheal cannula with 0.9% Sodium Chloride solution and centrifuged at 1500 rpm at 4°C for 10 minutes. The supernatant from the BAL fluid was recovered and stored at -80°C for assay of various biochemical markers. The precipitated pellets were resuspended in 100 µl of normal saline.

Cell counts

Total leukocyte count, eosinophils (%) and neutrophils (%) in the blood and BAL fluid was carried out using Neubauer's chamber as per Abdureyim., *et al*. [10]. Wright-Giemsa stain was used for differential counts.

OVA-specific IgE assay

OVA-specific IgE levels were measured using commercially available ELISA kit manufactured by Qayee Bio-Technology Co. Ltd., China (Cat. No. - QY-E11195). The assay was performed as per the manufacturer's instructions. Summarily, 50 µl of varying concentration of standard was added to the standard wells. Similarly, 40 µl of special diluent provided in the kit was added to the test wells along with 10 µl of the sample. 50 µl of horseradish peroxidase was further added to all wells except the blank wells. The plate was further sealed and incubated for 60 minutes at 37°C. The wells were then washed as per the manufacturer's instructions and chromogen solution was added to the wells. The plate was then incubated in the dark for 10 minutes at 37°C. The reaction was arrested by adding 50 µl of Stop solution provided with the kit. Absorbance was read at 450 nm. Standard curve was generated for 0 - 100 µg/ml concentrations of OVA-specific IgE.

Malondialdehyde (MDA) assay

MDA was measured using the method described by Ohkawa., *et al*. [15]. In a 0.5 ml serum sample, 1.5 ml of acetic acid (20%, pH adjusted to 3.5 with NaOH), 1.5 ml of thiobarbituric acid (0.8%) and 0.2 ml of sodium lauryl sulfate (8.1%) were added. The mixture was heated at 100°C for 1 hour in boiling water bath and cooled with tap water. 5 ml of butanol: pyridine (15:1% v/v) and 1 ml of distilled water were added in the mixture. The mixture was then centrifuged at 4000 rpm for 10 minutes. Thereafter, absorbance of the separated organic layer was measured at 532 nm using a spectrophotometer and concentration expressed as nmol/ml. 1,1,3,3-tetramethoxypropane (TMP) was used as the standard.

Statistical analysis

Data was expressed as Mean ± S.E.M. It was analysed using one-way Analysis of Variance (ANOVA) followed Dunnett's test for inter-group comparisons. $P < 0.05$ has been considered statistically significant.

Results

Effects of NO-modulators on airway hyperresponsiveness to methacholine challenge in OVA-induced model of bronchial asthma in rats

After treatment with various NO-modulators in OVA immunized and challenged rats, the pulmonary function and airflow resistance were assessed using BUXCO unrestrained whole body plethysmography system, and expressed as enhanced pause Enhanced Pause (P-enh). P-enh, a marker of airway resistance was measured using whole-body-plethysmography (WBP). OVA-challenged rats showed a statistically significant increase in P-enh values, as compared to that of the rats in normal group ($p < 0.001$). Inhibition of arginase with ABH hydrochloride significantly decreased the P-enh values ($p < 0.05$) in a dose-dependent manner as compared to that of the disease control rats. Administration of L-arginine was also able to show statistically significant reduction ($p < 0.05$) in P-enh values as compared to disease control rats, but only at lower Methacholine challenge doses. Prednisolone group ($p < 0.05$) had significantly lower P-enh values indicating less hyperresponsiveness. The results are depicted in figure 1.

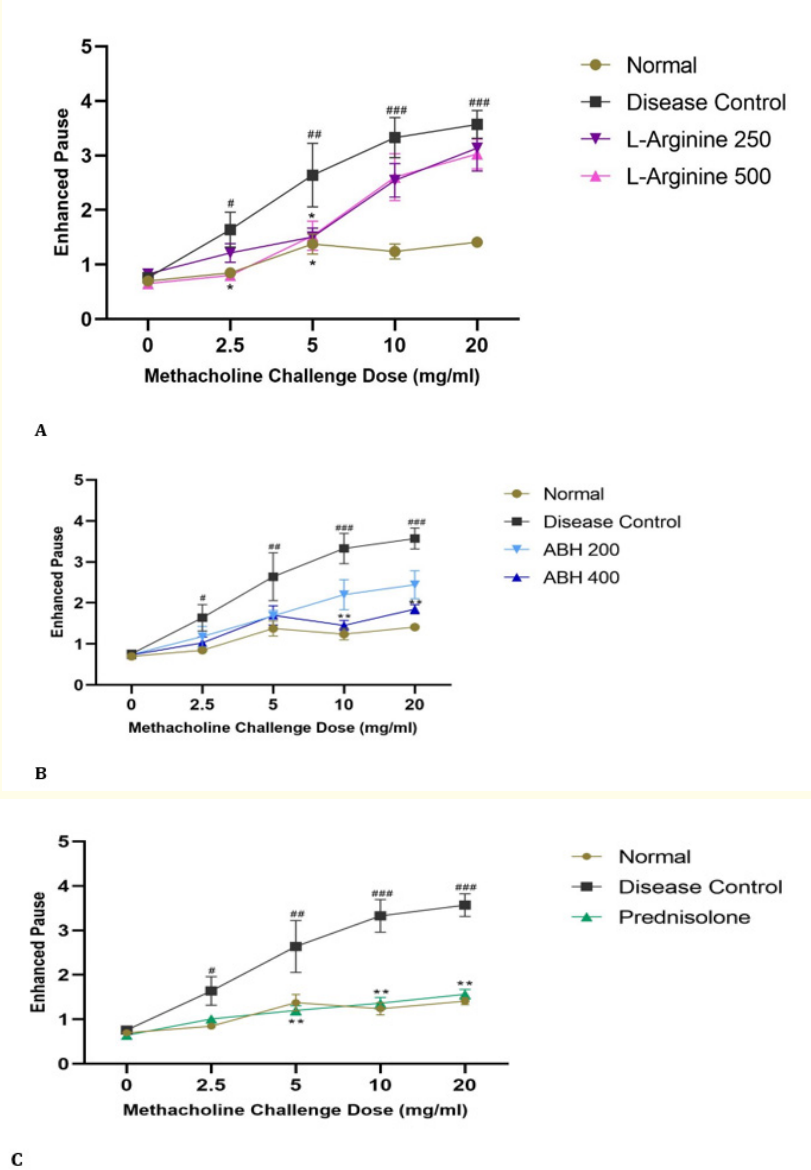

Figure 1: *Effect of NO modulators on Enhanced Pause (P-enh) in ovalbumin immunized and challenged rats. A: L-Arginine 250 and 500 mg/kg i.p.; B: ABH [2(S)-Amino-6-boronohexanoic acid hydrochloride] 200 and 400 µg/kg i.p.; C: Prednisolone 10 mg/kg i.p. #: p < 0.05; ##: p < 0.01; ###: p < 0.001 when compared to normal group; *: p < 0.05; **: p < 0.01 vs disease controls.*

Effect of nitric oxide modulators on BAL fluid biomarkers in OVA-induced model of bronchial asthma in rats

Inflammatory cell counts

The results showed that challenge with ovalbumin aerosol from day 15 to day 22, led to a statistically significant ($p < 0.01$) increase in the blood leukocyte count in disease control rats as compared to the normal rats. Administration of L-arginine and ABH in separate groups caused a reversal of this change and significantly reduced the leukocyte counts ($p < 0.05$). The results were comparable with standard drug prednisolone. The ovalbumin-challenged rats also showed a significant increase ($p < 0.01$) in blood eosinophil count as compared to the normal rats. The eosinophil count was reduced significantly ($p < 0.05$) by the higher dose of ABH as compared to the disease control rats, and the results were comparable with prednisolone. Blood neutrophil count was unaffected by the ovalbumin challenge or pre-treatment with either of the nitric oxide modulators ($p > 0.05$). The results are shown in table 1.

	Total WBC Count (cells/mm^3)	Eosinophils (%)	Neutrophils (%)
Normal	2633 ± 373.0	1.83 ± 0.13	28.33 ± 2.29
Disease Control	7417 ± 389.4$^{\#\#}$	10.67 ±1.33$^{\#\#}$	37.50 ± 2.12
L-Arginine 250	5050 ± 491.8*	7.46 ± 0.32	35.00 ± 1.82
L-Arginine 500	4633 ± 374.8**	7.33 ± 0.37	33.67 ± 3.60
ABH 200	4867 ± 488.3*	7.03 ± 0.45	29.00 ± 2.67
ABH 400	4067 ± 88.19**	5.06 ± 0.63*	29.17 ± 1.64
Prednisolone	3683 ± 484.7**	3.350 ± 0.45*	31.00 ± 1.98

Table 1: *Effect of NO modulators on total leukocyte count, eosinophils (%) and neutrophils (%) in blood of ovalbumin immunized and challenged rats.*

*All data is expressed as Mean ± SEM. ##: $p < 0.01$ vs normal group. *: $p < 0.05$; **: $p < 0.01$ vs disease control group.*

Rats challenged with ovalbumin developed an increase in the WBC count in BAL fluid vs. that of normal rats ($p < 0.01$). This change was attenuated by the 400 µg/kg ABH ($p < 0.01$). The effects were comparable to prednisolone. L-arginine also showed a reduction of inflammatory cell count but it did not achieve the level of statistical significance. Eosinophil content in the BAL fluid was also significantly increased with ovalbumin challenge as compared to normal group ($p < 0.01$). Pre-treatment with ABH markedly decreased the eosinophil count as compared to the disease control group ($p < 0.01$). These changes were similar to the changes observed in prednisolone group. As observed earlier in blood samples, ovalbumin challenge as well as NO modulators did not produce any significant change in the neutrophil count in BAL fluid. The results are depicted in table 2.

	Total WBC count (cells/mm^3)	Eosinophils (%)	Neutrophils (%)
Normal	783.3 ± 113.8	0.93 ± 0.13	32.50 ± 2.11
Disease Control	2817 ± 253.5$^{\#\#}$	12.67 ± 1.6$^{\#\#}$	38.83 ± 0.47
L-Arginine 250	2350 ± 220.2	12.77 ± 1.8	35.00 ± 2.6
L-Arginine 500	1967 ± 194.4	7.83 ± 0.36	35.5 ± 3.1
ABH 200	1933 ± 92.8	6.53 ± 0.43	33.67 ± 2.10
ABH 400	1425 ± 168.2**	4.23 ± 0.59*	32.17 ± 1.97
Prednisolone	1092 ± 111.4**	3.217 ± 0.52**	33.00 ± 2.35

Table 2: *Effect of NO modulators on total leukocyte count, eosinophils (%) and neutrophils (%) in BAL fluid of ovalbumin immunized and challenged rats.*

*All data is expressed as Mean ± SEM; ##: $p < 0.01$ vs normal group. *: $p < 0.05$; **: $p < 0.01$ vs disease control group.*

OVA-specific IgE levels in serum and BAL fluid

Antigen-specific IgE antibodies are crucial to the initiation as well as progression of asthma pathology. OVA-specific IgE were raised in blood and BAL fluid of rats exposed to ovalbumin aerosol as compared normal rats ($p < 0.01$). Pre-treatment with L-arginine, ABH and prednisolone reduced serum levels of OVA-specific IgE antibodies compared to the disease control rats ($p < 0.01$). However, no statistically significant effect on IgE levels was observed in the BAL fluid of rats receiving either of the NO modulators. A statistically significant reduction of IgE levels in serum and BAL fluid was observed in rats receiving the standard drug prednisolone. Results are depicted in table 3.

	OVA-specific IgE levels	
	Serum (µg/ml)	BAL Fluid (µg/ml)
Normal	0.38 ± 0.07	0.18 ± 0.06
Disease Control	1.05 ± 0.073##	0.55 ± 0.05##
L-Arginine 250	0.53 ± 0.07**	0.41 ± 0.02
L-Arginine 500	0.49 ± 0.07**	0.44 ± 0.017
ABH 200	0.40 ± 0.04**	0.44 ± 0.02
ABH 400	0.41 ± 0.04**	0.40 ± 0.08
Prednisolone	0.36 ± 0.02**	0.28 ± 0.05*

Table 3: *Effect of NO modulators on OVA-specific IgE levels (µg/ml) in serum and BAL fluid of ovalbumin sensitized and challenged rats. All data is expressed as Mean ± SEM. ##: p < 0.01 vs normal rats. *: p < 0.05; **: p < 0.01 vs disease control group.*

Effects of NO-modulators on oxidative stress marker in serum and BAL fluid in OVA-induced model of bronchial asthma in rats

Malondialdehyde (MDA) is a product of lipid peroxidation and thus serves as a marker of oxidative stress. Serum MDA levels were raised in response to ovalbumin challenge in disease control rats compared to the normal rats ($p < 0.05$). Groups receiving pre-treatment with ABH had significantly lesser MDA levels in response to ovalbumin challenge as compared to the disease control group ($p < 0.05$), similar to the standard drug. No significant changes were observed in MDA levels in rats receiving L-arginine.

MDA levels in BAL fluid were also observed to be raised in response to ovalbumin challenge in the disease control group as compared to the normal rats ($p < 0.05$). Pre-treatment with high dose of 400 µg/kg of ABH reduced MDA levels to lower than those in the disease control group ($p < 0.01$). Prednisolone alleviated the response as compared to the disease control group ($p < 0.05$). Results are shown in table 4.

	MDA levels	
	Serum (nmol/ml)	BAL Fluid (nmol/ml)
Normal	3.26 ± 0.41	0.94 ± 0.30
Disease Control	7.33 ± 0.94#	3.21 ± 0.93#
L-Arginine 250	4.16 ± 0.84	1.45 ± 0.47
L-Arginine 500	3.77 ± 0.97	1.16 ± 0.27*
ABH 200	3.08 ± 0.78*	1.30 ± 0.25
ABH 400	2.61 ± 0.59*	0.59 ± 0.14**
Prednisolone	3.02 ± 0.84*	0.90 ± 0.31*

Table 4: *Effect of NO modulators on MDA levels in serum and BAL fluid of ovalbumin sensitized and challenged rats. All data is expressed as Mean ± SEM. #: p < 0.05 vs normal rats; *: p < 0.05; **: p < 0.01 vs disease control group.*

Discussion

Bronchial asthma is a respiratory disease characterized by airway obstruction, airway inflammation as well as airway hyperresponsiveness mediated through complex cellular and humoral events. A number of therapeutic agents have been successfully employed in the symptomatic treatment of asthma. However, some of the issues viz. difficult-to-treat asthma, adverse effects from conventional medications, affordability of biological agents and inability to arrest or slow down remodeling presented unmet clinical needs that necessitate further research into the pathophysiology of asthma.

Nitric oxide has been extensively studied as a fundamental component of basal metabolism and cellular function and is implicated in numerous pathophysiological fields including aging, apoptosis, diabetes, inflammation, neurodegenerative pathology, etc. [16]. However, the role of nitric oxide in the pathophysiology of asthma has been elusive. The complex interaction of intermediates of L-arginine metabolism through the arginase and nitric oxide synthase pathways, has generated multiple questions about the role of NO in asthma.

Thus, this study was designed to evaluate the relative roles of arginase and NOS in inflammation, bronchial responsiveness and oxidative stress in an experimental model of asthma. A validated model of asthma employing intraperitoneal immunization and challenge with aerosolized ovalbumin was used [10]. The rats in Disease Control group showed significant increase in airway hyper-responsiveness and markers of inflammation and oxidative stress.

Airway hyper-responsiveness is characterized by increased airway resistance in response to exposure to various stimuli. Methacholine, a short acting cholinergic agent, is commonly used as a stimulus to assess bronchoconstriction response. P-enh, a marker of airway resistance, was raised significantly in the disease control group when compared to the normal rats. Challenge with higher doses of methacholine successively raised the P-enh values in the asthmatic rats. The elevated P-enh values were consistent with previously observed results in ovalbumin induced rat model of asthma [17,18]. In the present study, L-arginine administration caused a reduction in P-enh values indicating reduced airway hyperresponsiveness. Previously, Maarsingh., *et al.* [19] have observed that L-arginine administration reduced airway hyperresponsiveness in guinea pigs. Hypoargininemia has also been shown to exacerbate hyperresponsiveness in a murine model of asthma [20]. Studies in isolated tracheal preparations have shown that L-NAME inhibited the L-arginine mediated reduction in hyperresponsiveness [21]. This suggests a role of NO generation in the attenuation of airway hyperresponsiveness by L-arginine. In the current study, P-enh values were decreased significantly by the inhibition of arginase. The results are supported by an earlier study by [22], wherein endogenous arginase activity attenuated NO mediated smooth muscle relaxation in isolated guinea pig tracheal preparation. Arginase inhibition has also been shown to reduce airway hyperresponsiveness in a murine model of chronic asthma [20,23]. Arginase overexpression, as observed in asthma, decreases the availability of L-arginine for NOS. It appears that NOS-derived NO plays an important role in preventing hyperresponsiveness and thus bronchoconstriction.

Asthma is a complicated inflammatory disease that involves many different stages with airway infiltration by various cell types, cytokines, and mediators which remain incompletely understood. Depending on the stage, different inflammatory mediators are released from the various cell types, with implications for NOS/arginase activity and airway function. Mast cells and basophils are involved in the early asthmatic reaction (EAR) and secrete histamine and TNF-α. Eosinophils play an important role in the late asthmatic reaction (LAR) and contribute to ongoing inflammation through secretion of cytotoxic major basic protein and eosinophilic cationic protein. Eosinophils are found in both the lung tissue and the bronchoalveolar lavage (BAL) fluid of asthmatic patients [24,25]. Eosinophils typically predominate the inflammatory infiltrate, and the degree of eosinophilia has been correlated with disease severity in some asthmatic patients [25,26]. Eosinophil recruitment follows the release of prostaglandins, cysteinyl leukotrienes, cytokines and chemokines such as monocyte chemotactic protein.

In the present study, ovalbumin challenge increased total leukocyte counts and percentage of eosinophils in blood and BAL fluid compared to the normal rats. These results were consistent with other observations in animal models of asthma [27]. Inhibition of arginase with ABH reduced the total white cell and eosinophil count dose-dependently in both blood and BAL fluid. In earlier studies, increased inflammatory cell count has been reported with arginase inhibition in female mice with allergen induced airway inflammation [28]. However, the contradictory results can be due to gender and species differences from that of our study. In the present study, L-arginine administration also reduced the total white blood cell count. The results are supported by the study of Arıkan-Ayyıldız., *et al*. [29], who found a reduction in BAL eosinophilia with L-arginine administration and arginase inhibition.

Antigen-specific IgE antibodies are crucial to the pathophysiology of asthma. Pre-treatment with L-arginine and ABH were shown to decrease the serum IgE levels. Our results are corroborated by the findings of Mabalirajan., *et al*. [36] who observed that high dose of L-arginine reduced OVA-specific IgE levels in a murine model of asthma. Arginase inhibition with BEC (S-(2-boronoethyl)-l-cysteine) has also been shown to decrease OVA-specific IgE in mice [27], thus supporting our findings with ABH in ovalbumin induced asthma in rats.

Complex cellular and molecular mechanisms are involved in asthma pathophysiology and oxidative stress plays pivotal role. Oxidative stress impacts airway by increases in airway hyper reactivity, airway smooth muscle contraction, mucus overproduction, epithelial detachment and increased vascularity. Moreover, reactive oxygen species induces cytokine and chemokine secretion by activation of oxidative stress-sensitive transcription of NF-κB in airway epithelium. Imbalance between pro- and anti-oxidant forces results in raised concentration of ROS and results in oxidative damage to a variety of cellular structures like RNA, DNA and cell membrane. Free radicals generated during inflammatory response are short lived in circulation and they are difficult to identify and thus they are assessed indirectly by measuring free radical-mediated lipid peroxidation. Lipid peroxidation is the most important pathophysiological feature resulting in production of secondary metabolite malondialdehyde (MDA), which is marker of oxidative damage in cell membrane. The extent of lipid peroxidation correlates with asthma severity [37,38].

In the present study a significant increase in lipid peroxidation, as evidenced by increased MDA levels, was observed in the disease control group when compared to the normal rats. Inhibition of arginase significantly decreased MDA levels, thus indicating reduced lipid peroxidation. These results are in agreement of earlier findings in a rat model of pneumoperitoneum, where ABH administration decreased MDA levels [39]. Similarly, L-arginine also attenuated the MDA levels. These results are supported by the findings of an earlier study in which L-arginine supplementation was able to reduce urinary MDA levels in patients with diabetes, and that the NO precursor stimulates glutathione synthesis via the activation of nuclear factor erythroid 2–related factor 2 (Nrf2) pathway [40,41]. Taken together, the results suggest that administration of both NO precursor and inhibitor of arginase mitigated the airway hyper-responsiveness and airway-inflammation, in bronchial asthma. Interactions of NO with oxidative stress could have contributed the observed effects with NO modulators. Such studies are of translational significance as they may help in developing NO-based therapeutic strategies for the treatment of bronchial asthma.

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Conflict of Interest

The authors declare that there is no conflict of interest involved in this manuscript.

Bibliography

1. Kumar V., *et al*. "Robbins and Cotran pathologic basis of disease". 10th edition. Philadelphia (Pa.): Elsevier Saunders (2015): 683-686.

2. Hamid Q. "Gross pathology and histopathology of asthma". *Journal of Allergy and Clinical Immunology* 111.2 (2003): 431-432.

3. Ray A., *et al*. "Translating nitric oxide research to therapeutics: a critical appraisal". In: Arunabha Ray, Kavita Gulati (Eds.) `Nitric oxide: from research to therapeutics`, Springer-Nature, Switzerland (2023): 1-14.

4. Gulati K and Ray A. "Nitric oxide as a diagnostic and therapeutic tool in respiratory diseases". In: Arunabha Ray, Kavita Gulati (Eds.) 'Nitric oxide: from research to therapeutics', Springer-Nature, Switzerland (2023): 223-248.

5. Barnes PJ. "NO or no NO in asthma?" *Thorax* 51.2 (1996): 218-220.

6. Redington AE. "Modulation of nitric oxide pathways: Therapeutic potential in asthma and chronic obstructive pulmonary disease". *European Journal of Pharmacology* 533.1-3 (2006): 263-276.

7. Gabazza EC., *et al*. "Role of nitric oxide in airway remodelling". *Clinical Science* 98.3 (2000): 291-294.

8. Erzurum SC. "New insights in oxidant biology in asthma". *Annals of the American Thoracic Society* 13.1 (2016): S35-S39.

9. Gulati K and Ray A. "Current trends in nitric oxide research and its clinical applications". In: Molecular Medicine and Biomedical Research in the Era of Precision Medicine: New Technologies and Personalized Drug Therapies for Patient Care. (Eds. Sobti RS, Wilson DW, Buttar HS, Telessy IG, Sobti A.) Elsevier (2025).

10. Abdureyim S., *et al*. "Anti-inflammatory, immunomodulatory, and heme oxygenase-1 inhibitory activities of ravan napas, a formulation of Uighur traditional medicine, in a rat model of allergic asthma". *Evidence-Based Complementary and Alternative Medicine* (2011): 725926.

11. Mitani Y., *et al*. "Prolonged administration of L-arginine ameliorates chronic pulmonary hypertension and pulmonary vascular remodeling in rats". *Circulation* 96.2 (1997): 689-697.

12. Krause BJ., *et al*. "Chronic intermittent hypoxia-induced vascular dysfunction in rats is reverted by N-acetylcysteine supplementation and arginase inhibition". *Frontiers in Physiology* 9 (2018): 901.

13. Hamelmann E., *et al*. "Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography". *American Journal of Respiratory and Critical Care Medicine* 156.3 (1997): 766-775.

14. Criee CP., *et al*. "Body Plethysmography: Its principles and clinical use". *Respiratory Medicine* 105.7 (2011): 959-971.

15. Ohkawa H., *et al*. "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction". *Analytical Biochemistry* 95.2 (1979): 351-358.

16. Ray A., *et al*. "Current trends in nitric oxide research". *Cellular and Molecular Biology* 53.1 (2007): 3-14.

17. Lv H Yan., *et al*. "Rutin has anti-asthmatic effects in an ovalbumin-induced asthmatic mouse model". *Tropical Journal of Pharmaceutical Research* 16.6 (2017): 1337-1347.

18. Yu B., *et al*. "*Selaginella uncinata* flavonoids ameliorated ovalbumin-induced airway inflammation in a rat model of asthma". *Journal of Ethnopharmacology* 195 (2017): 71-80.

19. Maarsingh H., *et al*. "Arginase inhibition protects against allergen-induced airway obstruction, hyperresponsiveness, and inflammation". *American Journal of Respiratory and Critical Care Medicine* 178.6 (2008): 565-573.

20. Cloots RHE., *et al*. "Hypoargininemia exacerbates airway hyperresponsiveness in a mouse model of asthma". *Respiratory Research* 19.1 (2018): 98.

21. Meurs H., *et al*. "Increased arginase activity underlies allergen-induced deficiency of cNOS-derived nitric oxide and airway hyperresponsiveness". *British Journal of Pharmacology* 136.3 (2002): 391-398.

22. Maarsingh H., *et al*. "Arginase attenuates inhibitory nonadrenergic noncholinergic nerve-induced nitric oxide generation and airway smooth muscle relaxation". *Respiratory Research* 6.1 (2005): 23.

23. North ML., *et al*. "Functionally important role for arginase 1 in the airway hyperresponsiveness of asthma". *American Journal of Physiology-Lung Cellular and Molecular Physiology* 296.6 (2009): L911-L920.

24. Kay AB. "The role of eosinophils in the pathogenesis of asthma". *Trends in Molecular Medicine* 11.4 (2005): 148-152.

25. Lemière C. "The use of sputum eosinophils in the evaluation of occupational asthma". *Current Opinion in Allergy and Clinical Immunology* 4.2 (2004): 81-85.

26. Bousquet J. "Relating inflammatory changes in asthma to clinical status". *Respiratory Medicine* 94 (2000): S32-S33.

27. Azadmehr A., *et al*. "Protective effects of *Scrophularia striata* in Ovalbumin-induced mice asthma model". *DARU Journal of Pharmaceutical Sciences* 21.1 (2013): 56.

28. Ckless K., *et al*. "Inhibition of arginase activity enhances inflammation in mice with allergic airway disease, in association with increases in protein S-nitrosylation and tyrosine nitration". *Journal of Immunology* 181.6 (2008): 4255-4264.

29. Arıkan-Ayyıldız Z., *et al*. "Beneficial effects of arginase inhibition and inhaled L-arginine administration on airway histology in a murine model of chronic asthma". *Allergologia et Immunopathologia* 42.4 (2014): 316-323.

30. Mabalirajan U., *et al*. "Beneficial effects of high dose of L-arginine on airway hyperresponsiveness and airway inflammation in a murine model of asthma". *Journal of Allergy and Clinical Immunology* 125.3 (2010): 626-635.

31. Rahal A., *et al*. "Oxidative stress, prooxidants, and antioxidants: The interplay". *BioMed Research International* (2014): 76126.

32. Wood LG and Gibson PG GM. "Biomarkers of lipid peroxidation, airway inflammation and asthma". *European Respiratory Journal* 21.1 (2003): 177-186.

33. Cho JS., *et al*. "The effects of arginase inhibitor on lung oxidative stress and inflammation caused by pneumoperitoneum in rats". *BMC Anesthesiology* 15 (2015): 129.

34. Lubec B., *et al*. "L-Arginine reduces lipid peroxidation in patients with diabetes mellitus". *Free Radical Biology and Medicine* 22.1-2 (1997): 355-357.

35. Liang M., *et al*. "l-Arginine induces antioxidant response to prevent oxidative stress via stimulation of glutathione synthesis and activation of Nrf2 pathway". *Food and Chemical Toxicology* 115 (2018): 315-328.

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