

Anti-inflammatory Effect of Arginase Inhibitor and Corticosteroid on Airway Allergic Reactions in a *Dermatophogoides farinae*-induced NC/Nga Mouse Model

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Abstract—The present study was aimed to investigate the effect of an arginase inhibitor, *N*-hydroxy-nor-L-arginine (nor-NOHA) and a corticosteroid, prednisolone, in an intranasal mite-induced NC/Nga mouse model of asthma. The treatment with nor-NOHA and prednisolone inhibited the increase in airway hyperresponsiveness, the number of bronchoalveolar lavage fluid cells, protein expression of arginase I and arginase II, messenger RNA (mRNA) expression of nitric oxide synthase (NOS)2 and Th2 cytokines such as interleukin (IL)-4, IL-5, and IL-13, and the pathological inflammatory changes of the lung. NOx levels in the lung were not changed in mice treated with prednisolone and elevated in mice treated with nor-NOHA or prednisolone plus nor-NOHA despite suppressed NOS2 mRNA expression. The study concluded that anti-inflammatory effect by nor-NOHA might be dependent on NO supply from depleted NO by downregulated arginine availability of arginase and was not related with the anti-inflammatory mechanisms by prednisolone.

KEY WORDS: experimental asthma; nitric oxide; arginase inhibitor; corticosteroid.

INTRODUCTION

In the respiratory system, generation of nitric oxide (NO) occur in a variety of cells such as airway epithelial cells, airway nerve cells, vascular epithelial cells, and inflammatory cells. NO is involved in airway relaxing via cGMP-dependent [1] or in-dependent pathways via *S*-nitrosoglutathione (GSNO) and contributes to a remission of oxidative stress in asthma. Usually, GSNO is eliminated by GSNO reductase (GSNOR). A lack of GSNOR resulted in protection from airway hyperres-

ponsiveness (AHR) in OVA-exposed mice [2]. In human asthma, depletion of GSNO and increased expression of GSNOR were observed [3, 4]. Although several types of nitric oxide synthase (NOS) are involved in experimental asthma [5] and human asthma [6], NOS2-derived NO is thought to be important in the pathophysiology of allergic airway disease [1].

Recent studies have demonstrated that alterations in L-arginine homeostasis by the induction of arginase play a major role in allergen-induced NO deficiency and AHR in experimental asthma models and asthmatic patients [7–11] because only arginase I was highly expressed among expressed proteins such as L-arginine-related enzyme pathways and L-arginine-related transporters in both human asthma and murine asthma models [12], although high concentrations of exhaled NO were observed in asthmatic patients [13]. The inhibition of experimental asthma using arginase inhibitors has been investigated in only four studies [9, 10, 14, 15]. Three of the studies revealed inhibition of AHR or airway inflammation in guinea pigs [9] and mice [10,

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[15]. In previous study, we demonstrated direct inhibition of arginase by an inhibitor, *N*^w-hydroxy-nor-L-arginine (nor-NOHA). Attenuated AHR, bronchoalveolar lavage fluid (BALF) cells number, expressions of Th2 cytokines, eotaxin-1, eotaxin-2, NOS2, and histological inflammation were probably dependent on anti-inflammatory action of increased NO by increased L-arginine supply to NOS from arginase [15]. However, precise mechanisms for anti-inflammatory action of nor-NOHA were unknown. Moreover, in human asthmatic patients, increased serum arginase I levels were not changed by inhalation steroid therapy [16]. Although upregulation of arginase activity in rat airway fibroblasts induced by interleukin (IL)-4 and IL-13 was found to be inhibited by dexamethasone [17], there are few *in vivo* studies that demonstrated in the mechanisms of glucocorticoids on the regulation of arginase I.

Therefore, the aim of this study was to investigate effect of steroid on the expression of arginase and to compare anti-inflammatory action between nor-NOHA and corticosteroid in a mite-challenged NC/Nga mouse model that develops allergic asthma-like responses by intranasal exposure to *Dermatophagoides farinae* (Df) extract as common asthma allergens without adjuvant [15, 18, 19].

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents without annotation were of analytical reagent grade and purchased from Wako Pure Chemical Industries (Osaka, Japan).

Animals

Male NC/Nga mice, 7 weeks old, were obtained from Charles River Laboratories Japan (Yokohama, Japan). The mice were maintained under specific pathogen-free conditions with a 12-h light/12-h dark cycle and had free access to standard laboratory food and tap water. They were acclimatized for at least 1 week before the experiments. The care and handling of the animals were in accordance with the Guidelines for the Care and Use of Laboratory Animals at Shikata Campus of Okayama University and approved by the Okayama University Institutional Animal Care and Use Committee.

Intranasal Administrations

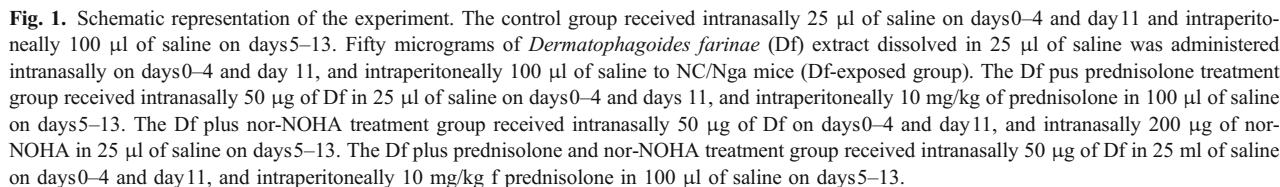
NC/Nga mice were sensitized to mite crude extract (Cosmo Bio, Tokyo, Japan) based on a previously described protocol [18] as in Fig. 1. Briefly, the anesthetized mice were intranasally instilled with Df crude extract (50 µg/25 µl saline) for five consecutive days (days 0–4), and on day 11, Df crude extract was administered. For control group, saline was administered instead of Df. To observe the effect of prednisolone or an inhibitor of arginase *N*-hydroxy-nor-L-arginine (nor-NOHA) on Df-induced asthma, prednisolone sodium succinate (10 mg/kg, twice a day) was given intraperitoneally from days 5 to 13 for nine consecutive days or nor-NOHA (100 µg/10 µl saline) was given intranasally from day 11 to 13 for three consecutive days or given with both prednisolone sodium succinate and nor-NOHA.

Measurement of AHR

On day 14, the degree of bronchoconstriction was measured according to the overflow method [20]. Briefly, mice were anesthetized with pentobarbital (80 mg/kg) and connected to an artificial ventilator following surgical incision of the trachea. A pulmotor system was constructed with a rodent ventilator (model 132; New England Medical Instrument, Medway, MA, USA), a bronchospasm transducer (model 7020; Ugo Basile, Comerio-Barese, Italy), and a DATA recorder (Omniace II data acquisition system, model RA1300; NEC San-ei, Tokyo, Japan). Gallamine triethiodide (350 µg/mouse) was intravenously administered immediately to eliminate spontaneous respiration and followed by administrations of acetylcholine with stepwise increases in the concentration from 62.5 to 2,000 µg/kg. Dose–response curves for acetylcholine in anesthetized, mechanically ventilated mice were obtained. Bronchoconstriction was expressed as the respiratory overflow volume provoked by acetylcholine as a percentage of the maximal overflow volume (100 %) obtained by totally occluding the tracheal cannula [20].

Bronchoalveolar Lavage Fluid

Immediately after the assessment of acetylcholine-induced AHR, the lungs were lavaged with 1-ml aliquots of cold Hanks' balanced salt solution without calcium and magnesium, containing 0.05 mM EDTA [Hank's balanced salt solution (HBSS)]. The collected BALF was then centrifuged at 150×g for 10 min at 4 °C. The supernatant was stored at –80 °C for further analysis, and the cell pellet was resuspended in HBSS. An aliquot



MP (Takara Bio, Ohtsu, Japan) with oligo-dT primers according to the manufacturer's instructions. Primer sets and PCR conditions are detailed in Table 1. PCR products were detected by agarose gel electrophoresis and ethidium bromide staining. PCR bands were quantified by ImageJ software (National Institutes of Health, Bethesda, MD, USA) and normalized against GAPDH.

Western Blotting

The protein expression of arginase was evaluated by Western blotting [21]. Aliquots of lung tissue homogenate with or without 1 % Triton X-100 were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis, and proteins were transferred onto polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). After blocking with 5 % dried skimmed milk in Tris-buffered saline containing 0.5 % Tween 20 (TBS-T), blots were incubated with appropriate primary antibodies (polyclonal rabbit, 1:100 to 1:500 dilutions; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and a horseradish peroxidase-conjugated secondary antibody (1:2,000 to 1:4,000; Sigma-Aldrich, St. Louis, MO, USA). Antibody-specific bands were detected using an enhanced chemilu-

After the total RNA of each sample was extracted with ISOGEN (Nippon Gene, Tokyo, Japan), reverse transcription PCR was performed using TakaRa RNA PCR kit AMV, ver. 3.0, and a Takara PCR thermal cycler

Table 1. List of Primers and Reverse Transcription PCR Conditions

Target gene	Sense	Antisense	Denaturation	Amplification	Cycle	Elongation
IL-4	CTAGTTGTGATCCTGCTCTTCTTT	CTTTAGGCTTTCCAGGAAGTCTTT	94 °C, 3 min	94 °C, 60 s	72 °C, 75 s	72 °C, 7 min
IL-5	GTGAAAGAGACCTTGACACAGCTG	CACACCAAGGAACCTTTGCAGGTA	94 °C, 3 min	94 °C, 60 s	72 °C, 75 s	72 °C, 7 min
IL-13	CTCCCTCTGACCCTTAAGGAG	GAAGGGCCCGTGGCGAAACAG	94 °C, 3 min	94 °C, 60 s	72 °C, 75 s	72 °C, 7 min
IFN- γ	TGTTACTGCCACGGCACAGTCATT	GTGGACCACTCGGATGAGCTCATT	94 °C, 3 min	94 °C, 60 s	72 °C, 75 s	72 °C, 5 min
GAPDH	ACCACAGTCCATGCCATCAC	TCCACCACCTGTTGCTGTA	94 °C, 3 min	94 °C, 45 s	60 °C, 45 s	72 °C, 5 min

minescence Western blot detection system (Perkin-Elmer, Boston, MA, USA) and quantified by Scion Image software (Scion, Frederick, MD, USA).

Measurement of Arginase Activity

An aliquot of the lung tissue homogenate with 1 % Triton X-100 was ultracentrifuged at 105,000 $\times g$ for 60 min at 4 °C (Beckman TL-100; Beckman Coulter, Fullerton, CA, USA). Arginase activity was determined as described previously [22]. Briefly, arginase was activated for 10 min at 55 °C in the presence of $MnCl_2$, followed by the addition of arginine and incubation for 60 min at 37 °C. The hydrolysis of arginine was stopped with acid, and the reaction product, urea, was measured by a colorimetric assay at 560 nm after incubation with α -isonitrosopropiophenone for 45 min at 100 °C.

Measurement of NOx Concentrations

To estimate NO production, the NOx concentration in the lung tissue homogenate was determined with an NO analyzer (model 280i NOA with the Purge Vessel; Sievers, Boulder, CO, USA) [23]. An aliquot of the

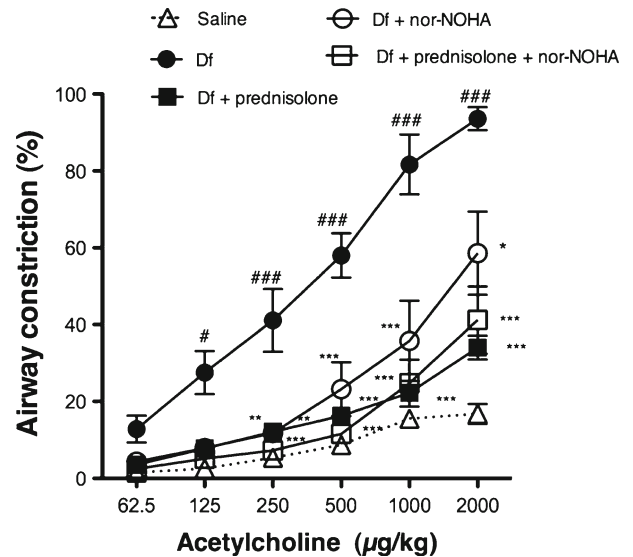


Fig. 2. Airway hyperresponsiveness (AHR) to acetylcholine. Effect of prednisolone, nor-NOHA, and prednisolone plus nor-NOHA on Df-induced increase in AHR in NC/Nga mice. Data were obtained from seven to eight animals/group. Bronchoconstriction (%) is expressed as mean $\pm$ SE. Two-way ANOVA showed an interaction between acetylcholine concentration and mouse groups. The results of a multiple comparison test are shown as follows. $^{\#}P < 0.05$ and $^{###}P < 0.001$ Df group vs. saline group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ Df plus prednisolone group, Df plus non-NOHA group or Df plus prednisolone and nor-NOHA vs. Df group.

homogenate was treated with nitrate reductase (Sigma-Aldrich) to convert nitrate to nitrite for 30 min at room temperature. Nitrite was further reduced to NO in a Purge Vessel containing the reducing agent potassium iodide in acetic acid, and NO was subsequently detected by the ozone-chemiluminescence method.

Histopathological Observations

The fixed lung tissues were dehydrated, embedded in paraffin, and sectioned. Hematoxylin and eosin (H&E) staining was used to assess the degree of inflammation [24]. Periodic acid–Schiff (PAS) stainings were also done to observe goblet cell hyperplasia, respectively [19]. The levels of inflammation in peribronchial and perivascular spaces of the lung were determined by an ordinal scale ranging from 0 to 3, as described elsewhere [25]. A value of 0 meant no inflammation was detectable; a value of 1, occasional cuffing with inflammatory cells; a value of 2, most bronchi or vessels were surrounded by a thin layer (1–5 cells thick) of inflammatory cells; and a value of 3, most bronchi or vessels were surrounded by a thick layer (more than five cells thick) of inflammatory cells. The proportion of goblet cells in the bronchial epithelium was digitized by Image J software.

Statistical Analysis

Data, unless otherwise noted, are expressed as means $\pm$ SD and were analyzed using Student's unpaired *t* test, one- or two-way ANOVA with a multiple comparison test. A $P < 0.05$ was considered statistically significant. Statistical analyses were performed using the GraphPad Prism 5.0c for Mac (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

Effect of Prednisolone, nor-NOHA, and Prednisolone Plus nor-NOHA on AHR

Mice treated with Df showed an increasing AHR from acetylcholine concentrations 125 to 2,000 $\mu\text{g/kg}$ compared to saline control. Two-way ANOVA showed an interaction between dose of acetylcholine and treated mice groups. Remarkable attenuation of AHR was demonstrated by the administration of prednisolone, nor-NOHA, and prednisolone plus nor-NOHA to Df-induced airway constriction. However, AHR of each acetylcholine administration was not significantly differ-

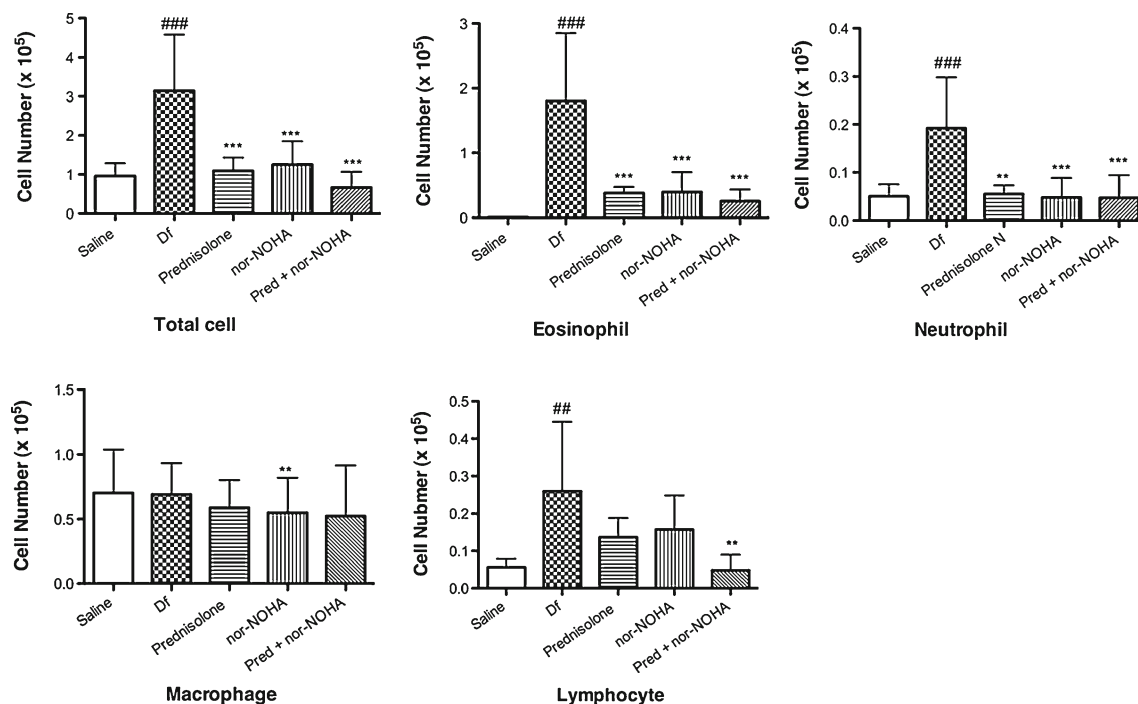


Fig. 3. Effect of prednisolone, nor-NOHA, and prednisolone plus nor-NOHA on BALF cell numbers. Data obtained from seven to eight animals/group are shown. The total cell, eosinophil, neutrophil, macrophage, and lymphocyte are expressed as means $\pm$ SD. # $P < 0.05$ and ## $P < 0.01$ vs. saline group. ** $P < 0.01$ and *** $P < 0.001$ vs. Df group.

ent among the groups of prednisolone, nor-NOHA, and prednisolone plus nor-NOHA (Fig. 2).

Effect of Prednisolone, nor-NOHA, and Prednisolone Plus nor-NOHA on BALF Cells

The increased cell numbers in BALF observed in Df-exposed mice were significantly reduced by treatment with prednisolone, nor-NOHA, and prednisolone plus nor-NOHA (Fig. 3). Significant reduction was observed in eosinophils and neutrophils but not observed in macrophage. Increased lymphocyte was significantly reduced by only prednisolone plus nor-NOHA.

Effect of Prednisolone, nor-NOHA, and Prednisolone Plus nor-NOHA on the Activity and Protein Expression of Arginase

The effect of prednisolone, nor-NOHA, and prednisolone plus nor-NOHA on arginase activity and protein expression of arginase are shown in Fig. 4a–d. By Df exposure, arginase activity and protein expression of arginase (I and II) were significantly augmented.

Augmented activity of arginase and protein expressions of arginase (I and II) were reduced to normal level by prednisolone. Arginase activity and arginase I expression were significantly reduced by nor-NOHA. However, reduction rate by nor-NOHA was not so high compared to that by prednisolone. Synergic reduction of arginase activity and arginase protein expression was not observed by simultaneous administration of prednisolone and nor-NOHA. If anything, reduction of arginase activity and arginase protein expression by prednisolone was weakened by simultaneous administration of nor-NOHA.

Effect of Prednisolone, nor-NOHA, and Prednisolone Plus nor-NOHA on mRNA Expression of Th2 Cytokines

Messenger RNA levels of the Th2 cytokines IL-4, IL-5, and IL-13 increased after the treatment of Df (Fig. 5). However, significant reduction of messenger RNA (mRNA) levels were observed in IL-4 by nor-NOHA and prednisolone plus nor-NOHA, in IL-5 by prednisolone, nor-NOHA and prednisolone plus nor-NOHA, and in IL-13 by prednisolone and nor-NOHA.

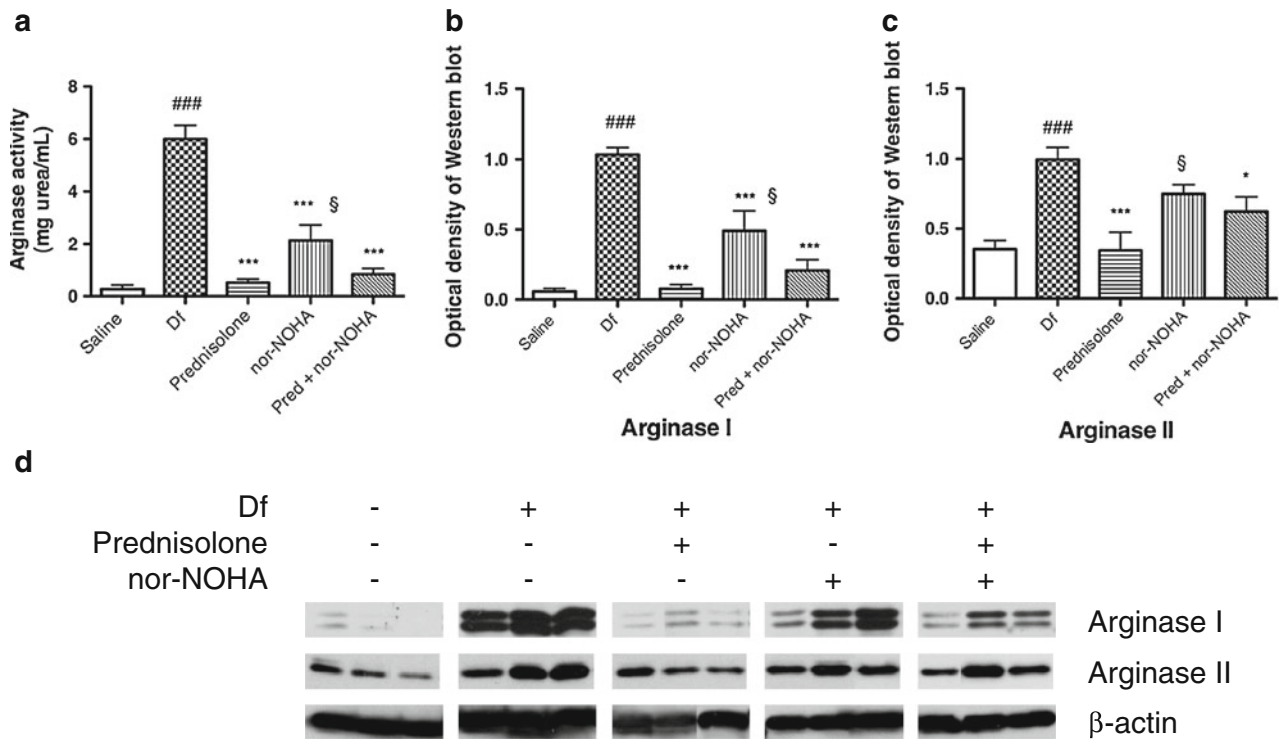


Fig. 4. Effect of prednisolone, nor-NOHA, and prednisolone plus nor-NOHA on arginase expression. Total arginase activity (a), a graphic representation of optical density of Western blot: arginase I (b) and arginase II (c) were expressed as means $\pm$ SD for seven to eight mice. Actual X-ray film of Western blot of arginase I, arginase II, and β -actin (d). ^{###} $P < 0.001$ vs. saline group. ^{*} $P < 0.05$ and ^{***} $P < 0.001$ vs. Df group. ^{\$} $P < 0.05$ vs. Df plus prednisolone group.

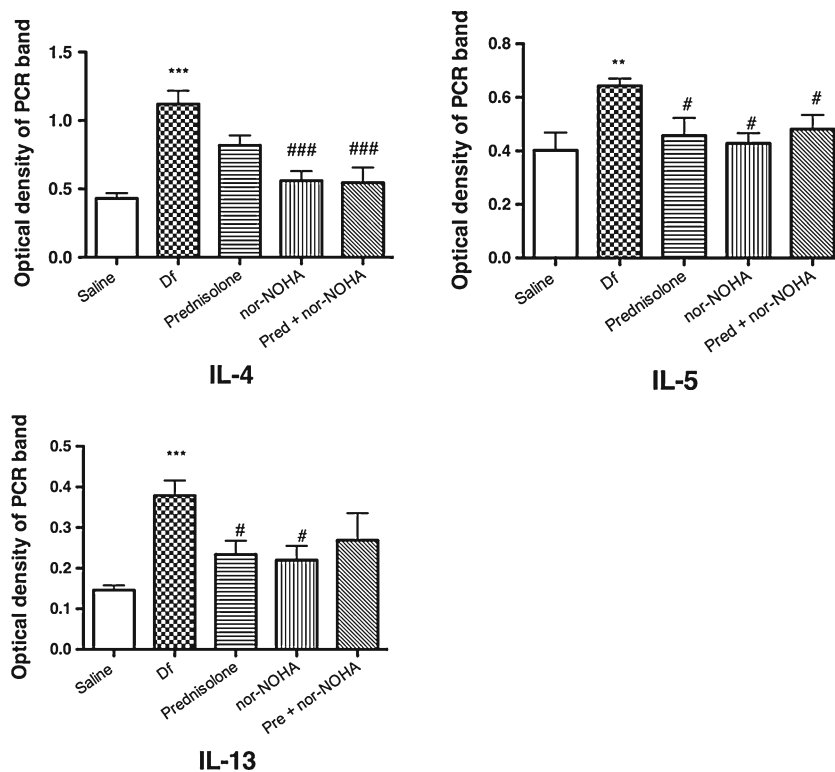


Fig. 5. Effect of prednisolone, nor-NOHA, and prednisolone plus nor-NOHA on Df-induced mRNA expression of Th2 cytokines. Each value is the mean $\pm$ SD for seven to eight mice. ** $P < 0.01$ and *** $P < 0.001$ vs. saline group. # $P < 0.05$ and ### $P < 0.001$ vs. Df group.

Effect of Prednisolone, nor-NOHA, and Prednisolone Plus nor-NOHA on mRNA Expression of NOS Isoforms and NOx Concentrations

The effects of prednisolone, nor-NOHA, and prednisolone plus nor-NOHA on NOS isoforms and NOx concentration in Df-exposed mice are shown in Fig. 6. The mRNA expression for NOS2 was upregulated by Df treatment, whereas NOS1 and NOS3 levels were unchanged. Df-induced upregulation of NOS2 mRNA expression was significantly reduced by prednisolone, nor-NOHA, and prednisolone plus nor-NOHA. The concentration of NOx increased by Df treatment. Df-induced increase in NOx concentration was not changed by additional treatment with prednisolone. However, NOx levels increased significantly after additional treatment with nor-NOHA and prednisolone plus nor-NOHA, respectively.

Effect of Prednisolone, nor-NOHA, and Prednisolone Plus nor-NOHA on Histopathological Findings

The histopathological changes in the lung by treatment with Df, showing high inflammation scores

and increased PAS staining cells, were observed in Fig. 7a and e. However, additional treatment with prednisolone, nor-NOHA, and prednisolone plus nor-NOHA to Df reduced inflammatory appearance in H&E staining (Fig. 7b–d) and goblet cell numbers in PAS staining (Fig. 7f–h). High inflammation scores of perivascular area and peribroncheal space in Df-exposed mice were reduced by prednisolone and prednisolone plus nor-NOHA. However, nor-NOHA reduced perivascular inflammation scores but did not reduce peribroncheal inflammation. Increased PAS staining cells in Df-exposed mice were significantly attenuated by prednisolone, nor-NOHA, and prednisolone plus nor-NOHA.

DISCUSSION

The present study demonstrated a reduction in arginase activity caused by a steroid hormone, prednisolone, and an inhibitor of arginase, nor-NOHA, which attenuated pathophysiological findings of asthma such as AHR and airway inflammation in a Df-exposed animal model via the airways [15]. There are few *in vivo* studies

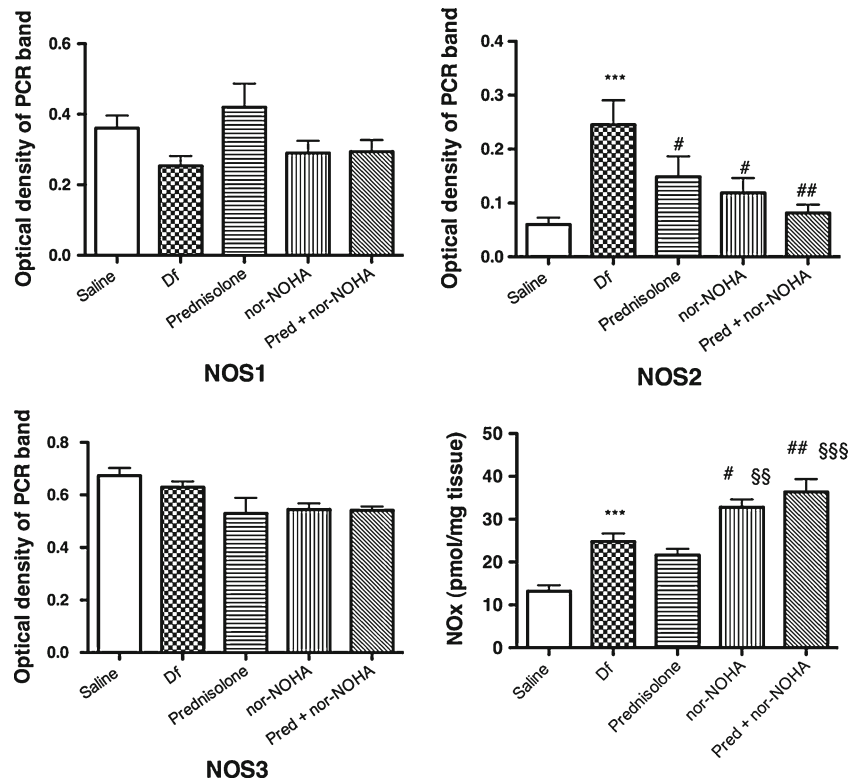


Fig. 6. Effect of prednisolone, nor-NOHA, and prednisolone plus nor-NOHA on Df-induced mRNA expression of NOS (1–3). Each value represents the mean $\pm$ SD for 7–8 mice. *** P <0.001 vs. saline control. # P <0.05 and ## P <0.01 vs. Df group. \$\$ P <0.01 and \$\$\$ P <0.001 vs. Df plus prednisolone group.

that have investigated the effect of glucocorticoids on the regulation of arginase I. We demonstrated no changes in serum arginase I levels by inhalation steroid therapy [16]. Upregulation of arginase activity in rat airway fibroblasts induced by IL-4 and IL-13 was inhibited by dexamethazone [17]. In this study, upregulated levels in AHR, BALF cells number, arginase activity, mRNA expressions of arginase I and II, IL-4, IL-5, IL-13, NOS2, and inflammatory scores by Df-exposure were significantly attenuated by prednisolone, nor-NOHA and prednisolone plus nor-NOHA.

Generally, corticosteroids exhibit anti-inflammation by two main mechanisms [26]. One is that high dose corticosteroids, which are binding to glucocorticoid receptor (GR), affect on glucocorticoid response elements in the promoter region of glucocorticoid-responsive genes and promote the generation of anti-inflammatory protein by the interaction of the DNA-bound GR and transcriptional coactivator molecules such as CREB-binding protein (CBP), which have intrinsic histone acetyltransferase (HAT) activity and cause acetylation of core histones. Other is that activated GR by low dose of corticosteroids inhibit HAT activity

of CBP or p300/CBP-activating factor directly, which is transferred by nuclear factor κ B (NF- κ B) signals generated by various inflammatory stimuli, and recruit histone deacetylase-2, which reverse histone acetylation leading in suppression of these activated inflammation genes. Administration of prednisolone 10 mg/kg twice a day in this study may show maximal anti-inflammatory effect because $C_{\max}$ for prednisolone is considered to be 10 mg/kg and maximal concentration of prednisolone in serum after administration of 10 mg/kg prednisolone was 1 μ g/ml (unpublished data). Therefore, generation of GR-related anti-inflammatory proteins may be involved in anti-inflammatory effect of prednisolone. However, reduction of AHR by prednisolone may not be associated with arginine availability and NO synthesis because of little changes of NOx by the treatment with prednisolone.

On the other hand, anti-inflammatory effect of nor-NOHA, an inhibitor of arginase, was demonstrated previously in the reduction of AHR and BALF cell numbers in Df-induced NC/Nga mice [19] and OVA-exposed C57BL/6 mice [12], although precise mechanisms were not clear. Moreover, in previous and this

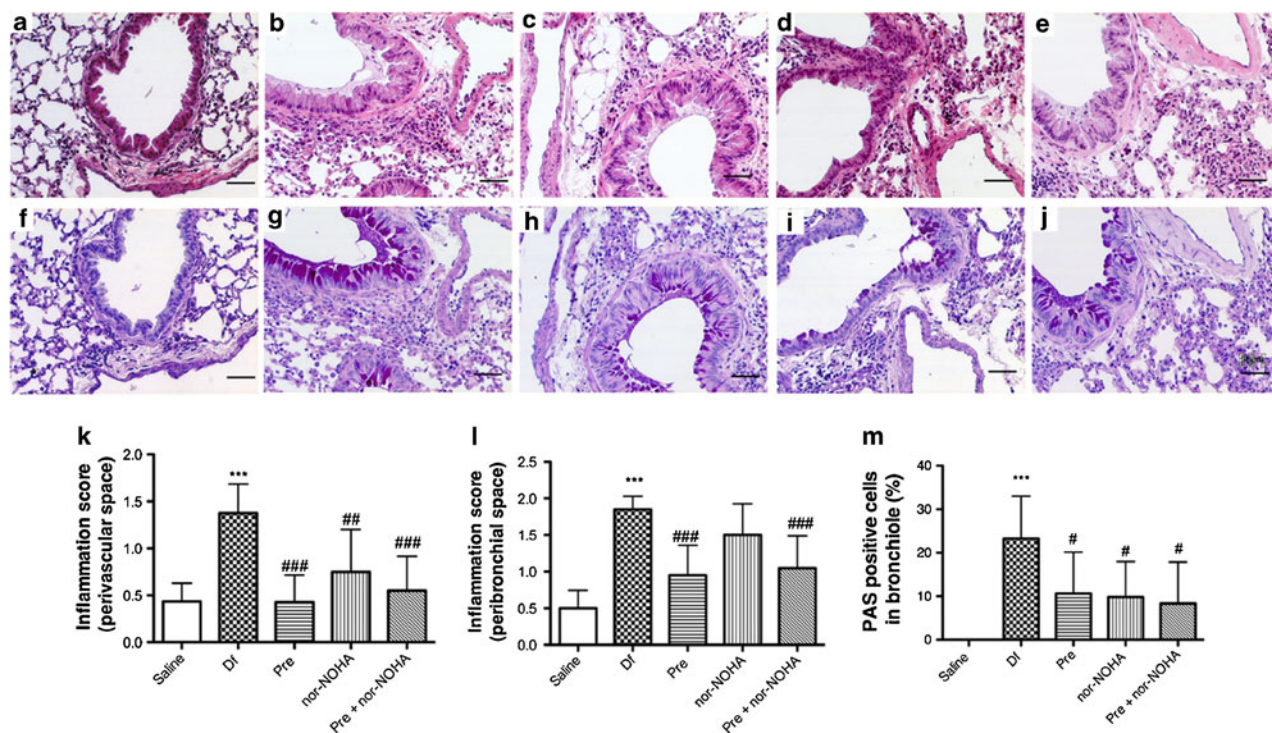


Fig. 7. Histopathology of H&E stained lungs or periodic acid-Schiff (PAS)-stained lungs from Df group (a, e), Df plus prednisolone group (b, f), Df plus nor-NOHA group (c, g), and Df plus prednisolone and nor-NOHA group (d, h). The accumulation of inflammatory cells shown by arrowheads in peribronchial space and perivascular space were clarified by score (i, j). The ratio of PAS-positive cells was determined with Image J software (k). Each value represents the mean $\pm$ SD for seven to eight mice. *** $P < 0.001$ vs. saline group. # $P < 0.05$, ### $P < 0.001$ vs. Df group.

study, increased expressions of arginase I, Th2 cytokines such as IL-4, IL-5, and IL-13, and NOS2 by Df were attenuated. Reduction of arginase I may be associated with attenuated expression of IL-4 and IL-13 because both IL-4 and IL-13 can stimulate expression of arginase I via STAT6, a nuclear transcription factor [27]. Reduction of NOS2 expression may be associated with S-nitrosylation and inactivation of NF- κ B by NO [28]. Inhibition of arginase may lead to an increase in the bioavailability of L-arginine to NOS. Finally, elevated NO levels resulted in the augmentation of NOx expression. In addition, an increase in NOS2 mRNA levels was observed in Df-treated mice, and the levels dropped following treatment with nor-NOHA. The transcription factor NF- κ B is involved in the regulation of NOS. NO modulates NF- κ B activity by S-nitrosylation of the p50 subunit [29]. This modification is related to suppressed phosphorylation and subsequent degradation of I κ B. Therefore, the significant reduction in NOS2 levels caused by nor-NOHA in our study suggests the involvement of S-nitrosylation and inactivation of NF- κ B by NO. According to the inhibitory expression of IL-4 and IL-

13 by nor-NOHA, there was no evidence to show S-nitrosylation of STAT6.

Degree of attenuation by prednisolone was significantly large in AHR, arginase activity, and protein expressions of arginase I and II compared to that by nor-NOHA. These differences were not observed in BALF cells number, mRNA expressions of Th2 cytokines and NOS, and NOx. Especially, reduced protein expressions of arginase I and II by prednisolone were weakened by additional treatment with nor-NOHA. In this phenomenon, glucocorticoid resistance may be involved. Anti-inflammation effect of prednisolone is associated with GR. GR is modified by NO via S-nitrosylation reaction and reduce binding affinity for glucocorticoids [30]. Therefore, combinative use of arginase inhibitors and high dose of corticosteroid for the strategy of asthma therapy may promote steroid resistance.

There are several evidences *in vivo* that arginase inhibitors attenuate allergic pathophysiological changes [9, 15, 31]. Pretreatment with an arginase inhibitor, 2(s)-amino-6-boronoheptanoic acid, inhibited early and late-phase asthmatic reactions, AHR, and inflammatory cell infiltration in an OVA-induced asthmatic model in

guinea pigs [9]. Another arginase inhibitor, S-(2-boronoethyl)-L-cysteine (BEC), attenuated AHR in acute and chronic asthmatic mouse model [10]. In our study, although the experimental animal model and allergens were different from previous studies, treatment with nor-NOHA, an arginase inhibitor used by two other reports [32, 33], had the same inhibitory effect on AHR. However, there is a report that BEC did not inhibit AHR, but augmented inflammation in another OVA-induced model of allergic asthma in mice, although BEC inhibited IgE and IL-4 [14]. The dose of BEC inhaled was 2.7 $\mu\text{g}/\text{mouse}$, lower than that of 800 $\mu\text{g}/\text{mouse}$ in the study mentioned above [10]. An inadequate dose of arginase inhibitor was suggested from data indicating that the inhibition rate for arginase activity by BEC worsened from 24 to 48 h [14]. However, to resolve this discrepancy, comparative studies for evaluating these inhibitors in the same experimental model are needed.

Asymmetric dimethylarginine (ADMA) is a naturally generating inhibitor of NOS by type 1 protein-arginine methyltransferases [34] and metabolize by dimethylarginine dimethylamino hydrolase to citrulline and dimethylamine. A study of ADMA showed augmentation of AHR and collagen deposits in the absence of inflammation [35]. Increased ADMA levels and protein expression of RPMT was demonstrated in OVA-exposed asthmatic mice [36]. Since the level of ADMA in the lungs was not determined in mice in the present study, the contribution of ADMA is unknown.

In this study, we demonstrated improvement of AHR and anti-inflammatory effect of nor-NOHA and prednisolone in Df-induced NC/Nga mice. Anti-inflammatory mechanism of nor-NOHA and high dose of prednisolone was different each other and synergic effect by combinative administration of nor-NOHA and prednisolone was not observed. More investigations for anti-inflammatory effect of nor-NOHA were needed in future.

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Conflict of Interest. No conflicts of interest, financial or otherwise, are declared by the author(s).

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