Persistent elevation of exhaled nitric oxide and modification of corticosteroid therapy in asthma

Tsunahiko Hiranoa, Kazuto Matsunagaa,*, Hisatoshi Sugiuraa, Yoshiaki Minakataa, Akira Koarai, Keiichiro Akamatsu, Tomohiro Ichikawa, Kanako Furukawa, Masakazu Ichinose

Third Department of Internal Medicine, Wakayama Medical University, School of Medicine, 811-1 Kimiidera, Wakayama 641-0012, Japan
Department of Respiratory Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan

Abstract

Background: Persistent airway inflammation, detected by fractional exhaled nitric oxide (FeNO), is occasionally observed in asthmatic patients, even in those treated with inhaled corticosteroids (ICS). However, improvement in residual airway inflammation and pulmonary function through modification of corticosteroid therapy has not been proven.

Methods: Thirteen asthmatic patients whose FeNO levels were over 40 parts per billion (ppb), despite dry-powder ICS therapy, were enrolled. A 3-step change in steroid treatment was undertaken until FeNO was less than 40 ppb. In the first step, the powder formula was changed to an ultra-fine particle compound as an equipotent ICS dose. In the second step, the ICS dose was doubled. In the third step, oral corticosteroids were added. We measured pulmonary function and FeNO and alveolar NO concentrations (CaNO).

Results: Doubling the ICS dose and changing the ICS formula significantly improved FVC (p < 0.001), FEV1 (p < 0.05), the slope of the single nitrogen washout curve (dN2) (p < 0.01), FeNO (p < 0.001), and CaNO (p < 0.05), relative to baseline. The reductions in FeNO were significantly associated with the improvement in airway limitation assessed by dN2 (r = 0.73, p = 0.007). The remaining FeNO elevation, even after doubling the ICS dose, did not decrease after oral corticosteroid administration.

Conclusions: These results suggest that modification of ICS therapy can suppress residual FeNO elevation, and that reduction in FeNO levels is associated with improvement in airway limitation. However, steroid-resistance mechanisms may exist in some asthmatic patients with sustained FeNO elevations.

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1. Introduction

Airway inflammation is a very important part of the pathophysiology of asthma [1,2]. Fractional exhaled nitric oxide is increasingly being used as a surrogate marker for airway inflammation because the values provide information about the cause of asthma in these patients [3]. Several previous studies have shown that increased levels of FE\(_{\text{NO}}\) are related to a loss of asthma control and an accelerated decline in pulmonary function [4–7]. More recently, it has been suggested that persistently high FE\(_{\text{NO}}\) level in steroid-treated asthmatic individuals may also reflect a highly reactive phenotype, and such patients should be managed with caution [8,9]. However, the effectiveness of sequential measurement of FE\(_{\text{NO}}\) to guide adjustment of anti-inflammatory therapy for asthma is controversial [10,11].

Corticosteroids effectively suppress eosinophilic inflammation, and inhaled corticosteroids (ICS) are widely used for long-term management of asthma [12]. Furthermore, ICS/long-acting \(\beta_2\)-agonist (LABA) combinations have also been developed, their efficacy is more pronounced than that of previous therapies [13]. However, some asthma patients show persistent airway inflammation and pulmonary dysfunction despite ICS treatment [14,15]. Thus far, the ability of the modification of corticosteroid therapy to improve airway inflammation and pulmonary function in asthmatic patients with sustained FE\(_{\text{NO}}\) elevations has not been fully elucidated.

In this prospective interventional study, modification of steroid therapy included three steps: changing the powder formula to an ultra-fine particle compound, doubling the dose, and administering oral prednisolone. We assessed airway inflammation by using measurement of exhaled NO (eNO) and pulmonary function by using spirometry and single nitrogen-washout curve to determine the relationship between the changes in eNO and pulmonary function facilitated by modification of steroid therapy.

2. Methods

2.1. Study subjects

Thirteen stable patients with asthma were recruited from the outpatient clinic at Wakayama Medical University Hospital. All subjects were diagnosed with asthma by a pulmonologist and had documented reversible airflow limitation. All subjects had adequate inhalation and good adherence to asthma therapy. Patients were included in the study if their eNO levels at a flow rate of 50 mL/s (FE\(_{\text{NO}}\)) were persistently over 40 parts per billion (ppb) despite receiving conventional asthma therapy, including dry powder-inhaled (DPI) corticosteroids. Subjects were excluded if they had an exacerbation of asthma 3 months prior to the study; if they had other pulmonary disease, including chronic obstructive pulmonary disease, allergic bronchopulmonary aspergillosis, and allergic and granulomatous angitis; or if they had esophageal reflux, vocal cord dysfunction, or bronchiectasis that could influence asthma control. All patients received an explanation of the study protocol and gave written informed consent. This study was performed in conformance with the Declaration of Helsinki and was approved by the local ethics committee at Wakayama Medical University (IRB #526; February 15, 2008). The study was registered at the University Hospital Medical Information Network (UMIN 000008401).

2.2. Study design

This was a prospective interventional study for assessing the effect of the augmentation of steroid therapy on airway inflammation and pulmonary function in asthmatic patients with persistently high FE\(_{\text{NO}}\) (Fig. 1). The augmentation of steroid treatment included three steps: (1) changing the powder formula to an ultra-fine particle compound, (2) increasing the dose two-fold, and (3) administering oral corticosteroids in addition to changing the ICS formula. In step 1, ciclesonide, which is formulated as a solution to be delivered via a hydrofluoroalkane-134a metered-dose inhaler, was given at a dose equivalent to the regularly used DPI corticosteroid dose at the beginning of the study. In step 2, the ciclesonide dose was increased two-fold if FE\(_{\text{NO}}\) was still over 40 ppb after step 1. In step 3, if FE\(_{\text{NO}}\) was still over 40 ppb after step 2, oral prednisolone (0.5 mg/kg) was administered for 2 weeks. Based on our recent study [9], we selected 40 ppb as the cutoff point for high and low FE\(_{\text{NO}}\) in this analysis, a value that was within previously published cutoff points [8,16–18].

2.3. Exhaled NO measurement

The level of eNO was measured in accordance with the recommendations of the current guidelines [19]. We measured eNO in triplicates prior to spirometry at four separate, constant expiratory flow rates (50, 100, 175 and 370 mL/s) by using a chemiluminescence-based exhaled NO analyzer (NA-623 N, Chest Co. and Kimoto Electric Co., Tokyo, Japan), and the mean of three values was reported. Measurements were included if an adequate NO plateau could be measured or if the NO levels were above the detection limit. The NO analyzer was calibrated monthly with a known concentration (748 ppm) and before examining each patient with NO-free air. The technique of Tsoukias and George was used to calculate the peripheral airway/alveolar NO concentration (CA\(\text{LVNO}, \ \text{ppb}\) (slope) by using a linear regression line for each subject with a minimum of three expiratory flow rate data points [20]. To adjust for possible spurious overestimation of values for peripheral lung CA\(\text{LVNO}\), the initial, uncorrected large airway NO flux (\(\text{nL/s}\)) was divided by a correction factor and subtracted from the initial uncorrected small airway/alveolar CA\(\text{LVNO}\) [21]. Because FE\(_{\text{NO}}\) is perhaps one of the fastest responding markers and the decrease in FE\(_{\text{NO}}\) levels after corticosteroid use is rapid [22], we selected 4-and 2-week intervals for the examination.

2.4. Pulmonary function testing

Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV\(_1\)) were measured as previously described [23]. Single-breath nitrogen (SB\(_{\text{N}}\)) test was performed (CHESTAC-7800, Chest Co., Tokyo, Japan) according to previously described methods [24]. In order to minimize inter-observer variability,
a single reader measured closing volume and the slope of the alveolar N\textsubscript{2} plateau. The SBN\textsubscript{2} test was performed on two occasions to train subjects to perform the closing volume maneuver correctly. The diffusion capacity of the lung for carbon monoxide (DL\textsubscript{CO}) was also measured using the single-breath method. The person recording the measurements was blinded to the treatment of patients.

### 2.5. Statistical analysis

Data are expressed as mean \pm SD. Wilcoxon’s signed rank sum test was used to compare the effect of treatment on eNO and pulmonary function. Spearman’s correlation analysis was used to assess the correlation between changes in eNO and those in pulmonary physiologic parameters. A value of \( p < 0.05 \) was considered to be significant.

### 3. Results

Baseline demographics and medications are shown in Tables 1 and 2. The mean value of the baseline % predicted FEV\textsubscript{1} was 89.7%. The mean FE\textsubscript{NO} and CA\textsubscript{NO} levels were 73.1 ppb and 6.7 ppb, respectively. Nine patients were treated with ICS/LABA combination drugs and four patients with dry-powder ICS.

Although the FE\textsubscript{NO} levels in four of 13 subjects decreased to below 40 ppb after changing to an equivalent dose of ciclesonide (step 1), the change in mean FE\textsubscript{NO} level was not significant (n.s.) (73.1 ppb vs. 60.5 ppb, n.s., Fig. 2A). The FE\textsubscript{NO} levels in five of the remaining nine subjects whose FE\textsubscript{NO} levels were still over 40 ppb decreased to below 40 ppb after doubling the dose of ciclesonide (step 2), significantly improving the mean FE\textsubscript{NO} level (71.9 ppb vs. 52.7 ppb, \( p < 0.01 \), Fig. 2A).

However, the mean CA\textsubscript{NO} level was not changed by step 1 (6.7 ppb vs. 5.6 ppb) or by step 2 (6.6 ppb vs. 6.1 ppb) (Fig. 2B). Finally, augmenting with 8 weeks of ICS therapy after step 1, followed by step 2, resulted in a significant reduction in both the mean FE\textsubscript{NO} level (73.1 ppb vs. 45.3 ppb, \( p < 0.001 \), Fig. 2C) and CA\textsubscript{NO} level (6.7 ppb vs. 4.8 ppb, \( p < 0.05 \), Fig. 2D), relative to baseline values.

The mean FVCs did not change after step 1 but significantly improved after step 2 (3.70 L vs. 3.83 L, \( p < 0.05 \)). The mean FEV\textsubscript{1} values did not change after step 1 or step 2. The mean dN\textsubscript{2} values were significantly reduced by step 1 therapy (1.39% vs. 1.18%, \( p < 0.05 \)), but not changed by step 2. Significant improvement in FVC (3.70 L vs. 3.85 L, \( p < 0.001 \)), FEV\textsubscript{1} (2.72 L vs. 2.80 L, \( p < 0.05 \)), and dN\textsubscript{2} (1.39% vs. 1.06%, \( p < 0.01 \)) after augmentation with ICS therapy was also seen (Table 3). No significant difference in other lung function parameters was observed.

In the subsequent analysis, eNO values and pulmonary function after modification of ICS therapy (changing the ICS formula and doubling the dose of ICS) were compared to baseline values. As shown in Fig. 3, the magnitude of the reduction in the FE\textsubscript{NO} levels was significantly associated with the degree of improvement in dN\textsubscript{2} (\( r = 0.73, p = 0.007 \)) after step 1 for 8 weeks and step 2. Comparison of entry values with the 4-week values after step 1 for 13 subjects showed statistically significant relationships between the reduction in FE\textsubscript{NO} levels and the improvement in FEV\textsubscript{1} (\( r = -0.73, p = 0.007 \)) after step 1 for 8 weeks and step 2. Comparison of entry values with the 4-week values after step 1 for 13 subjects showed statistically significant relationships between the reduction in FE\textsubscript{NO} levels and the improvement in FEV\textsubscript{1} (\( r = -0.73, p = 0.007 \)).

There was no other correlation between the changes in pulmonary physiologic parameters and eNO levels.

The FE\textsubscript{NO} levels in the remaining four patients were still over 40 ppb even after doubling the dose of ICS. Administration of oral corticosteroids did not alter the FE\textsubscript{NO} and CA\textsubscript{NO} levels in these patients (Fig. 4). The changes in FVC (3.70 L vs. 3.71 L), FEV\textsubscript{1} (2.58 L vs. 2.65 L), and dN\textsubscript{2} (1.64% vs. 1.32%) after step 3 were also not significant. Response to our therapeutic strategy did not differ with baseline medication (nine out of 13 patients on ICS/LABA with the remaining on ICS alone).

### 4. Discussion

The present study provides evidence that the modification of ICS therapy by changing the formula of ICS and increasing

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**Table 1**

<table>
<thead>
<tr>
<th>Entry</th>
<th>4 weeks</th>
<th>8 weeks</th>
<th>10 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>FE\textsubscript{NO}</td>
<td>73.1 ppb</td>
<td>60.5 ppb</td>
<td>52.7 ppb</td>
</tr>
<tr>
<td>CA\textsubscript{NO}</td>
<td>6.7 ppb</td>
<td>5.6 ppb</td>
<td>6.1 ppb</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Baseline Medication</th>
<th>FP or SFC</th>
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<tr>
<td>Step 1</td>
<td>If FE\textsubscript{NO} &gt; 40 ppb at entry, change powder formula to fine-particle compounds (Equivalent dose of ciclesonide or equivalent dose of ciclesonide + salmeterol)</td>
</tr>
<tr>
<td>Step 2</td>
<td>If FE\textsubscript{NO} &gt; 40 ppb even after 4 weeks of step 1 treatment, increase the dose twofold (Double the dose of ciclesonide or double the dose of ciclesonide + salmeterol)</td>
</tr>
<tr>
<td>Step 3</td>
<td>If FE\textsubscript{NO} &gt; 40 ppb even after 8 weeks of step 2 treatment, add oral prednisolone</td>
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**Fig. 1 – Study design.**
the dose of ICS can suppress residual FE\textsubscript{NO} elevations, and that reduction in the FE\textsubscript{NO} levels is associated with improvement in airflow limitation. However, steroid-resistant mechanisms may exist in some asthmatic patients with sustained FE\textsubscript{NO} elevations, irrespective of whether they received conventional ICS therapy.

Endogenous NO plays a critical role in the physiological regulation of airway function. NO is a gaseous signaling molecule that is generated by NO synthase (NOS). Inducible NOS is not constitutively expressed but is induced by inflammatory and infectious stimuli producing large amounts of NO independently of calcium ion influx. Indeed, exhaled NO is elevated in patients with asthma, is reduced by treatment with inhaled corticosteroids, and correlates with eosinophilic airway inflammation detected in induced sputum [25,26]. Occasionally, persistent airway inflammation detected by FENO is observed in asthmatic subjects regardless of ICS treatment. This might reflect mechanisms such as (1) limited drug delivery to the small airways, (2) an insufficient dose of ICS, or (3) steroid-resistant inflammatory processes in the airway [27–30]. Although in these cases, modification of steroid therapy may be performed, it is not clear which strategy can improve the airway inflammation and pulmonary function in asthmatic patients with sustained FE\textsubscript{NO} elevations [31–36]. In our study, a strategy of modification of steroid treatment indicated that these mechanisms are involved in persistent airway inflammation in treated asthmatics.

The findings of our study suggest that increasing the dose of ICS rather than changing the formula to ultra-fine particles for such patients may leave room for further improvement of pulmonary function. Indeed, doubling the dose of ICS (step 2) significantly improved persistent airway inflammation (FE\textsubscript{NO}) and airway caliber (FVC). Although it has been suggested that the aerosol particle size influences the extent, distribution, and site of inhaled drug deposition within the airways [37], whether specifically targeting the small airways can lead to a further clinical benefit is still not elucidated [31–36]. A previous study showed that 4-week ciclesonide treatment is
effective in asthma control [33]. However, our study showed a lack of significant efficacy, except in the measurement of dN₂ after changing to ciclesonide. One possible reason for this discrepancy is that there was no withdrawal period after the powder formula ICS administration, and another is that our study was performed in patients with relatively mild, stable asthma. Overall, changing the formula of ICS and increasing the dose of ICS significantly improved the parameters that represent not only airway inflammation (FENO, CAlvNO) but also airway caliber (FVC, FEV₁, and dN₂). Furthermore, the magnitude of reduction in FE NO levels was significantly correlated with the improvement in dN₂. These results indicate that suboptimally controlled airway inflammation may remain in a subset of asthmatic patients with persistently high FENO, even with optimal management efforts and that modification of ICS for such patients may leave room for further improvement of airway inflammation and pulmonary function.

Early studies have shown that exacerbations of asthma are associated with increased levels of FE NO, especially when spirometry is abnormal [6] and the increase in FE NO levels are indicative of the loss of asthma control [4,38], suggesting that sequential FE NO measurement may improve asthma management. More recent evidence shows that the subgroups with high FE NO are the most reactive and worrisome phenotype [8,9]. These studies and ours have suggested that sustained high FE NO might be an important therapeutic target and can be a useful guide for adjusting anti-inflammatory therapy. In contrast, in our study, the improvement in alveolar NO was not related to improvement in lung function. A larger-scale study might be needed to confirm the role of alveolar NO in the management of asthma.

Fig. 2 – Effect of the modification of inhaled corticosteroid therapy on FE NO (A) (C) and CAlvNO (B) (D) (n = 13). Horizontal bars indicate mean values. Dotted line represents a FE NO level of 40 ppb. Step 1: changing ICS powder formula to ultra-fine-particle compound. Step 2: increasing the dose two-fold. Step 1+step 2: Step 1 after 8 weeks plus step 2. (A) and (B): step 1 for 13 subjects with comparison between entry and 4 weeks, and step 2 for nine subjects with comparison between 4 weeks and 8 weeks. (C) and (D): step 1 after 8 weeks for four subjects and step 2 for nine subjects with comparison between entry and 8 weeks. n.s., not significant.
Systemic steroid therapy did not significantly change the FENO levels or pulmonary function of the subjects whose FENO levels were over 40 ppb, even after doubling the dose of ICS. Among patients with refractory asthma, it is speculated that some proportion of individuals are truly steroid resistant, which is defined as no clinical improvement after treatment with systemic steroids [39]. Van Veen et al. [40] reported that patients on chronic oral steroid therapy require additional anti-inflammatory treatment. Taken together, the results of our study may suggest that steroid-resistant inflammatory processes in asthmatic airways might exist in patients who have high eNO levels despite high-daily-dose ICS therapy. Several distinct molecular mechanisms that contribute to decreased anti-inflammatory effects of glucocorticoids have now been identified [41]. These include glucocorticoid receptor modification, defective glucocorticoid receptor binding and translocation, transcription factor activation, abnormal histone acetylation, and constitutive NOS sources that are steroid insensitive. However, ten Brinke et al. [42] reported that persistence of sputum eosinophilia despite treatment is not a refractory phenomenon and is sensitive to high-dose systemic therapy.

| Table 3 – Changes in pulmonary function during augmentation of ICS therapy. |
|-----------------------------|-----------------------------|
| FVC (L) | 3.70 ± 0.75 | 3.85 ± 0.77*** |
| FEV1 (L) | 2.72 ± 0.63 | 2.80 ± 0.62* |
| CV/VC (%) | 14.6 ± 8.9 | 14.6 ± 6.2 |
| CC/TLC (%) | 52.3 ± 6.0 | 53.6 ± 8.6 |
| dN2 (%) | 1.39 ± 0.68 | 1.06 ± 0.52** |
| % DLco/VA (%) | 102 ± 17 | 106 ± 19 |

Values are mean ± SD. Step 1, changed FP or SFC to ciclesonide or ciclesonide plus salmeterol; step 2, increased dose of ciclesonide twofold; step 1 + step 2, step 1 after 8 weeks plus step 2; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; CV, closing volume; CC, closing capacity; TLC, total lung capacity; dN2, slope of phase III of the washout curve; DLco, diffusion capacity for carbon monoxide; DLco/VA, DLco divided by alveolar volume.

* p < 0.05.
** p < 0.01.
*** p < 0.001 compared to entry values.

Fig. 3 – Relationship between changes in exhaled nitric oxide and pulmonary function mediated by the modification of inhaled corticosteroid therapy (n = 12). Post: step 1 after 8 weeks plus step 2. Pre: entry. The line and p value correspond to the fitted regression equation. * r, correlation coefficient; n.s., not significant.

Fig. 4 – Effect of modification of inhaled corticosteroid therapy and systemic steroid therapy on FENO (A) and CAlvNO (B) of four patients whose FENO levels were over 40 ppb even after doubling the dose of ICS. Horizontal bars indicate mean values. Dotted line represents a FENO level of 40 ppb. Step 2: increasing the dose two-fold. Step 3: administering oral corticosteroids. n.s., not significant.
corticosteroids. Therefore, further study is needed to confirm whether truly steroid resistant inflammatory processes exist in asthmatic airways with persistently high eNO levels.

There are some limitations to our study. First, the sample size was relatively small. Second, a selection bias may have been present because ours was not a randomized controlled trial. Finally, the effect of augmentation of ICS therapy on the parameters for asthma control, including symptoms and need for rescue treatment, should be further quantified.

5. Conclusion

Our results show that modification of ICS therapy can suppress residual FE\textsubscript{NO} elevations, and that reduction in FE\textsubscript{NO} levels is associated with improvement in airflow limitation. However, steroid-resistant mechanisms may exist in some asthmatic patients with sustained FE\textsubscript{NO} elevations.

Conflict of interest

Authors have no potential conflict of interest.

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