

Bronchial Diffusing Capacity of Nitric Oxide Is Increased in Patients with Allergic Rhinitis

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Key Words

Allergic rhinitis • Breath condensate • Exhaled nitric oxide • Glucocorticosteroids • 8-Isoprostane

Abstract

Background: Exhaled nitric oxide (NO) measurement at multiple exhalation flow rates allows calculation of flow-independent NO parameters: alveolar NO concentration, bronchial NO flux, bronchial diffusing capacity of NO and bronchial wall NO concentration. **Methods:** In the present study, we measured the flow-independent NO parameters and inflammatory markers in exhaled breath condensate (EBC) and in serum in 14 patients with seasonal allergic rhinitis (AR) without asthma, and in 14 age- and sex-matched healthy volunteers. **Results:** At symptomatic stage before the treatment, patients with AR had higher bronchial wall diffusing capacity of NO than healthy volunteers ($p = 0.024$), but there were no differences in bronchial wall NO concentration or alveolar NO concentration. Patients with AR had also increased 8-isoprostane levels in the EBC ($p = 0.040$), and increased serum levels of IgE ($p = 0.002$) and eosinophil cationic protein (ECP; $p = 0.027$). Two-week treatment with nasal glucocorticoid mometasone decreased symptom scores and serum ECP levels but had no effect on NO parameters or 8-isoprostane levels in EBC. **Conclusions:** Noninva-

sive markers of airway inflammation showed subclinical lower airway inflammation in patients with AR without asthma, but short-term treatment with nasal glucocorticoids did not affect most of the markers of lower airway inflammation.

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Introduction

Exhaled nitric oxide (NO) concentration is elevated in patients with inflammatory airway disorders such as asthma [1, 2], chronic obstructive pulmonary disease [3], allergic rhinitis (AR) [4–6] and in healthy subjects with upper respiratory tract infection [7]. Treatment with inhaled glucocorticoids decreases exhaled NO levels, suggesting that exhaled NO may be a useful marker of lower airway inflammation [8–11].

In most studies exhaled NO concentration has been measured at a single exhalation flow rate of 50 ml/s [12]. If exhaled NO is measured at multiple exhalation flow rates it gives a more profound understanding of pulmonary NO production and its alterations in inflammatory conditions. Based on mathematical models of pulmonary NO dynamics it is possible to calculate the flow-independent NO parameters [alveolar NO concentration ($Calv_{NO}$), bronchial NO flux (Jbr_{NO}), bronchial wall dif-

fusing capacity of NO (Daw_{NO}) and bronchial wall NO concentration (Caw_{NO}) by measuring exhaled NO concentration at several exhalation flow rates [13–15]. This allows separate assessment of peripheral and central components of lower airway inflammation. Increased Jbr_{NO} has been found in asthma, while $Calv_{NO}$ is increased in different forms of alveolitis [16–18]. The increased Jbr_{NO} in asthma is caused by increase in both Daw_{NO} and Caw_{NO} [19].

Collection of exhaled breath condensate (EBC) is a promising noninvasive method for estimating inflammatory markers of the lower airways [20]. Elevated levels of hydrogen peroxide [21, 22], leukotrienes [18, 23, 24], 8-isoprostane [18, 25], nitrotyrosine [24], and nitrite [26] have been measured in EBC in inflammatory airway diseases. AR is a risk factor for asthma and can precede asthma in the atopic march [27]. The 'one airway, one disease' concept predicts that inflammation in the upper airways is linked to inflammatory changes in lower airways [28]. In fact, nasal symptoms, airflow, and markers of inflammation correlate with lower airway markers in patients with concomitant asthma and AR [29]. Many patients with AR without asthma have lower airway hyperreactivity or bronchial hyperresponsiveness which may worsen during the pollen season [27].

The aim of the present study was to investigate if the flow-independent NO parameters or inflammatory markers in EBC are altered in patients with AR without asthma, and to study the effects of nasal glucocorticoid treatment on these markers.

Materials and Methods

Subjects

Fourteen adults with seasonal AR (hay fever; 20–34 years of age) and 14 healthy, age- and sex-matched controls were enrolled into the study. The diagnosis of hay fever was based on a history of typical symptoms, and all of the patients had positive skin prick test to at least timothy or meadow, while the controls reacted to none of the tested allergens (see below). None of the subjects used any regular medication or had lung diseases. Spirometry and bronchodilatation test were normal in all subjects. None of the subjects had symptoms of asthma according to a written questionnaire of asthma symptoms [11]. All study subjects were nonsmokers and they had no symptoms of upper or lower respiratory tract infection in the week preceding the study. Subjects gave their written informed consent and the study protocol was approved by the Ethics Committee of the Tampere University Hospital (Tampere, Finland).

Study Design

Exhaled NO measurements, spirometry, asthma symptoms, hay fever symptoms and inflammatory markers in serum and

EBC were assessed in all subjects. Measurements were conducted once in controls and at four time points in the patients with AR: in asymptomatic stage before pollen season, at the onset of the symptoms in pollen season, and after 1 and 2 weeks of treatment with nasal corticosteroid. None of the patients had used any medication for allergy within 4 weeks preceding the first two measurements. After the second measurement (at the onset of symptoms) the patients started treatment with nasal corticosteroid (mometasone furoate 200 µg once a day divided into both nostrils, Nasonex®, Schering-Plough, Kenilworth, N.J., USA). Before the measurements the subjects were not allowed to drink anything but water or to eat for 2 h.

Exhaled NO Measurement

Exhaled NO concentration was measured with a Sievers NOA 280 analyzer (Sievers Instruments, Boulder, Colo., USA) at exhalation flow rates of 400, 300, 200, 100, 50, 30, 20 and 10 ml/s with a mouth pressure of 5–20 cm H₂O to exclude nasal contamination. The desired exhalation flow rates were achieved by letting the patients exhale through a mass flow meter connected to a computer-controlled adjustable flow restrictor (NOFLA device developed at the University of Tampere). The computer adjusted the flow resistor in real time based on the actual flow rate measured. If the flow rate tended to be higher or lower than the desired flow rate, the flow restriction was increased or decreased in real time to achieve the desired flow rate regardless of changes in the patient's exhalation effort. The accuracy of the flow controller to maintain mean flow rate at target level was $\pm 0.5\%$ or ± 1 ml/s of the set value. A pressure gauge was connected to the system and the subject was asked to maintain exhalation pressure between 5 and 20 cm H₂O during the exhalation.

The analyzer was calibrated daily with NO/N₂ calibration gas [101 parts per billion (ppb), AGA, Lidingö, Sweden] and before every subject with filtered NO-free air. A mean of three consecutive measurements at each flow rate was used in the data analysis. Jbr_{NO} and $Calv_{NO}$ were assessed according to Tsoukias and George [13] by using flow rates of 50–300 ml/s as previously described [17] using the equation $V_{NO} = V \times Calv_{NO} + Jbr_{NO}$, where V_{NO} is NO output (exhaled NO concentration multiplied by exhalation flow rate), and V is exhalation flow rate. Daw_{NO} and Caw_{NO} were assessed according to Silkoff et al. [14] by using all the flow rates of 10–400 ml/s and the equation $FE_{NO} = Caw_{NO}[1 - \exp(-Daw_{NO}/V)] + Calv_{NO} \times \exp(-Daw_{NO}/V)$.

Exhaled Breath Condensate

EBC was collected with EcoScreen condenser (Jaeger, Würzburg, Germany) during 15 min of tidal breathing while wearing a nose clip. Samples were stored in -70°C until assayed. Leukotriene B₄, cysteinyl leukotrienes (Cys-LT) and 8-isoprostane concentrations in the condensate were measured by enzyme immunoassay (EIA; Cayman Chemical Company, Ann Arbor, Mich., USA). Nitrite (NO₂) and nitrite + nitrate (NO_x) concentrations in the condensate were measured by ozone chemiluminescence as previously described [30].

Pulmonary Function and Bronchodilatation Test

Lung function was assessed by flow-volume spirometry at the end of each study session. Results were compared to the normal values in the Finnish population [31]. Bronchodilatation test was performed with inhaled salbutamol (Ventoline®, GlaxoSmith-

Kline) according to the recommendation by the American Thoracic Society [32]. All the subjects had normal lung function and no bronchial reversibility.

Asthma and Allergy Symptoms

Symptoms of AR were scored using a written questionnaire. Sneezing, running, itching and occlusion of nose during the past 2 days were scored as 0 = none, 1 = a little but not disturbing, 2 = disturbing part of the day, 3 = disturbing most of the day. Maximum symptom score was 12. A written questionnaire covering lower airway symptoms was used to exclude asthmatic symptoms [11].

Allergy Screening and Inflammatory Markers

Atopy was tested for by skin prick test. Fifteen common aero- and animal allergens (birch, alder, timothy, meadow, mugwort, dandelion, horse, dog, cat, cow, guinea pig, *Dermatophagoides farinae*, *Dermatophagoides pterigoideus*, *Alternaria alternata*, *Cladosporium herbarum*) with positive and negative controls (i.e. histamine and saline) were tested. A skin wheal diameter ≥ 3 mm was considered positive.

Serum Samples

Serum levels of immunoglobulin E (IgE) and eosinophil cationic protein (ECP) were quantified by using the UniCAP System (Pharmacia Diagnostics, Uppsala, Sweden). Serum levels of eosinophil peroxidase and myeloperoxidase were measured by radioimmunoassay (Pharmacia Diagnostics). The concentrations of interleukin-6 and interleukin-10 in serum samples were determined by EIA (PeliPair ELISA, Sanquin, Amsterdam, The Netherlands).

Statistical Analysis

Normality of the distribution for each parameter was tested with the Kolmogorov-Smirnov test. Differences in NO parameters and inflammatory markers between patients with AR and healthy controls were analyzed with independent sample t test or with Mann-Whitney test, depending on the distribution of the data. Changes in NO parameters, inflammatory markers and allergy symptom score during the treatment were analyzed using repeated-measures analysis of variance or Wilcoxon signed-ranks test. SPSS 11.0 (SPSS Inc., Chicago, Ill., USA) software and Instat 3.05 were used in the statistical analysis. A value of $p < 0.05$ was considered statistically significant. Results are expressed as mean $\pm$ SEM or as median (interquartile range).

Results

Patients at Asymptomatic Stage versus Healthy Controls

In asymptomatic stage before the pollen season, the patients with AR had increased serum levels of IgE [83 kU/l (64–145) vs. 30 kU/l (5–53), $p = 0.001$], but none of the other inflammatory markers in serum, EBC or exhaled air differed significantly from the levels found in the controls.

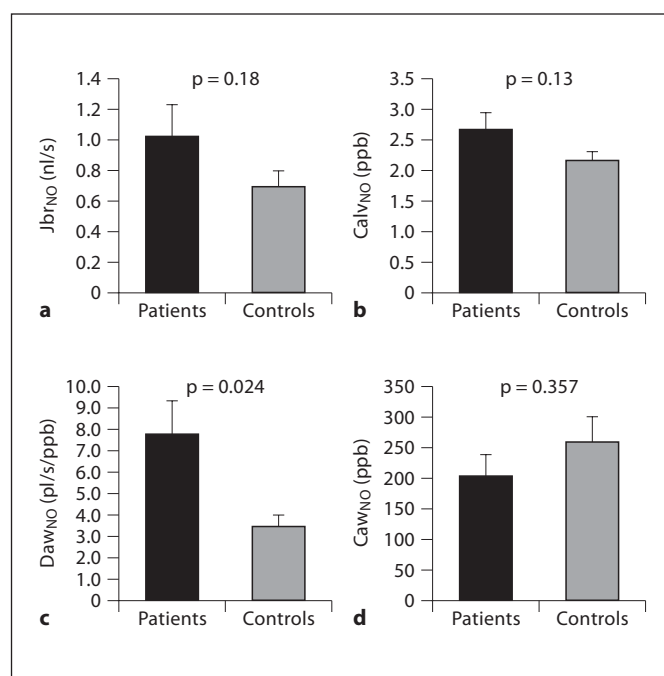


Fig. 1. JbrNO (a), CalvNO (b), DawNO (c) and CawNO (d) in 14 patients with AR at the symptomatic stage before the commencement of treatment with nasal mometasone and in 14 healthy control subjects.

Patients at Symptomatic Stage versus Healthy Controls

Allergy Symptoms

Three subjects with AR were sensitized to seasonal allergens only and 11 subjects were sensitized also to perennial allergens such as house dust mite, mould or dander of household pets. The allergy symptom score was 0.4 ± 0.2 at the asymptomatic stage before the pollen season and increased to 7.4 ± 0.6 at the onset of symptoms. Healthy volunteers had no symptoms and their skin prick test results were negative.

NO Parameters

During the symptomatic stage before nasal corticosteroids, patients with rhinitis had slightly higher JbrNO (1.0 ± 0.2 vs. 0.7 ± 0.1 nl/s, $p = 0.18$; fig. 1a), higher CalvNO (2.7 ± 0.3 vs. 2.2 ± 0.2 ppb, $p = 0.13$; fig. 1b) and lower CawNO (202 ± 38 vs. 258 ± 47 ppb, $p = 0.357$; fig. 1d) as compared to controls, but the differences were not statistically significant. However, DawNO was increased in patients with AR as compared with healthy controls (7.8 ± 1.6 vs. 3.4 ± 0.6 pl/s/ppb, $p = 0.024$; fig. 1c). No difference was found between AR patients and healthy controls in

Table 1. Inflammatory markers in EBC in patients with AR at the symptomatic stage and in healthy controls

	Patients	Controls	p value
8-Isoprostane, pg/ml	13.6 ± 1.4	9.9 ± 0.9	0.040
LTB ₄ , pg/ml	3.6 ± 0.9	3.1 ± 0.5	n.s.
Cys-LT, pg/ml	13.6 ± 1.4	12.2 ± 1.7	n.s.
NO ₂ , µM	1.3 ± 0.3	1.3 ± 0.3	n.s.
NOx, µM	6.9 ± 2.6	3.8 ± 0.5	n.s.

Values are expressed as mean ± SEM. LTB₄ = Leukotriene B₄; n.s. = not significant (p > 0.05).

Table 2. Inflammatory markers in serum/plasma samples in patients with AR at the symptomatic stage and in healthy controls

	Patients	Controls	p value
IgE, kU/l	86.2 (63.6–150.2)	29.7 (4.8–53.0)	0.002
ECP, ng/ml	8.6 ± 1.9	3.6 ± 0.8	0.027
EPX, ng/ml	24.6 ± 4.1	18.3 ± 2.6	n.s.
MPO, ng/ml	273.1 ± 63.0	208.1 ± 34.0	n.s.
IL-6, pg/ml	2.7 ± 0.7	2.4 ± 0.7	n.s.
IL-10, pg/ml	1.8 (0.8–8.3)	1.9 (1.0–2.9)	n.s.

Values are expressed as mean ± SEM or median (interquartile range). EPX = Eosinophil peroxidase; n.s. = not significant (p > 0.05); MPO = myeloperoxidase.

NO concentrations measured at the recommended flow rate of 50 ml/s according to ATS/ERS guidelines [12].

Inflammatory Markers in EBC and Serum

In symptomatic stage before the steroid treatment, patients with AR had increased levels of 8-isoprostane in EBC, but the other markers in EBC were not different as compared with controls (table 1). Serum levels IgE and ECP were also increased in the subjects with AR (table 2).

Effects of Treatment

Allergy symptom scores were highest at symptomatic stage before treatment. A 2-week treatment with nasal mometasone decreased symptom scores and serum ECP levels. Daw_{NO} and 8-isoprostane in EBC also decreased but the changes were not statistically significant. There were no changes in other exhaled NO parameters or measured inflammatory markers during the 2 weeks' treatment with nasal mometasone (table 3).

Discussion

In the present study, we found increased Daw_{NO} and elevated levels of 8-isoprostane in EBC in patients with AR without asthma during the symptomatic stage before the commencement of the treatment with nasal corticosteroid mometasone. Treatment with nasal mometasone had no statistically significant effects on exhaled NO parameters or inflammatory markers in EBC although symptoms of AR were reduced.

Exhaled NO concentration at a single exhalation flow rate of 50 ml/s is a sensitive marker of inflammation in

central parts of lower airways, but it is quite insensitive to inflammation in the small peripheral airways or lung parenchyma. We measured exhaled NO at several exhalation flow rates and calculated Calv_{NO} and Jbr_{NO} according to Tsoukias and George [13]. This method allows separate assessment of central and peripheral components of lung inflammation. By using the same method we have previously found increased Jbr_{NO} in patients with asthma and increased Calv_{NO} in patients with alveolitis or asbestosis [16–18]. When exhaled NO is measured also with lower flow rates, it is possible to calculate Daw_{NO} and Caw_{NO} [14, 15].

In patients with AR without asthma, Calv_{NO} and Jbr_{NO} were slightly but not significantly higher than in normal controls. By dividing Jbr_{NO} into its two components, we found increased Daw_{NO} but normal mucosal concentration of NO in these patients. These results are in line with those published by Högman et al. [19].

Normal mucosal concentration of NO in bronchial wall suggests that iNOS activity in the bronchial wall of rhinitis patients is close to normal. In healthy subjects, most of the bronchial NO production originates from the proximal large airways [33]. Increased Daw_{NO} in patients with AR suggests that either the NO-producing area of the bronchial tree is larger in these subjects, or that physical diffusivity of NO from mucosa to luminal air is altered due to changes in the physical properties of bronchial mucosa. This might reflect changes induced by subclinical lower airway inflammation supported by eosinophilia and increased collagen deposition found in bronchial biopsies from patients with AR [34]. It has also been suggested that increased Daw_{NO} would be an early manifestation in asthmatic pathophysiology representing endogenous protection against bronchoconstriction

Table 3. Effects of nasal mometasone treatment on the levels of NO parameters, on inflammatory markers in EBC and in serum/plasma, and on symptom score in patients with AR

	At the onset of symptoms	Treatment with mometasone		p value ^a
		1 week	2 weeks	
Symptom score	7.4 ± 0.6	4.2 ± 0.8	2.9 ± 0.8	0.001
NO parameters				
Jbr _{NO} , nl/s	1.0 ± 0.2	1.0 ± 0.2	0.9 ± 0.2	n.s.
Calv _{NO} , ppb	2.7 ± 0.3	2.2 ± 0.2	2.4 ± 0.2	n.s.
Daw _{NO} , pl/s/ppb	7.8 ± 1.6	7.3 ± 1.1	8.1 ± 1.3	n.s.
Caw _{NO} , ppb	202.4 ± 36.3	181.0 ± 33.8	142.4 ± 17.1	n.s.
EBC				
8-Isoprostane, pg/ml	13.6 ± 1.4	12.8 ± 3.0	9.3 ± 1.7	n.s.
LTB ₄ , pg/ml	3.6 ± 0.9	4.0 ± 0.8	3.8 ± 0.6	n.s.
Cys-LT, pg/ml	13.6 ± 1.4	15.2 ± 1.8	12.9 ± 1.9	n.s.
NO ₂ , µM	1.3 ± 0.3	1.3 ± 0.2	1.0 ± 0.1	n.s.
NO _x , µM	6.9 ± 2.6	4.1 ± 0.2	4.2 ± 0.6	n.s.
Serum/plasma markers				
IgE, kU/l	86.2 (63.6–150.2)	89.1 (66.0–147.9)	84.8 (62.4–138.2)	n.s.
ECP, ng/ml	8.6 ± 1.9	7.0 ± 1.5	5.4 ± 0.8	0.045
EPX, ng/ml	24.6 ± 4.1	24.8 ± 3.4	21.8 ± 2.7	n.s.
MPO, ng/ml	273.1 ± 63.0	222.0 ± 22.2	229.5 ± 25.9	n.s.
IL-6, pg/ml	2.7 ± 0.7	2.6 ± 0.7	2.8 ± 0.7	n.s.
IL-10, pg/ml	1.8 (0.8–8.3)	1.4 (0.6–8.7)	2.2 (0.8–8.7)	n.s.

Values are expressed as mean ± SEM or median (interquartile range). n.s. = Not significant (p > 0.05); LTB₄ = leukotriene B₄; EPX = eosinophil peroxidase; MPO = myeloperoxidase.

^a Difference between onset of symptoms and 2 weeks' treatment.

by increasing the mucosal surface area producing NO by constitutive isoforms of NOS [14].

Increased levels of 8-isoprostane, a marker of lipid peroxidation, have been found in EBC in subjects with asthma, cystic fibrosis, chronic obstructive pulmonary disease, and asbestosis, and in healthy smokers [18, 25, 35–37]. Elevated EBC levels of 8-isoprostane and increased Daw_{NO} in subjects with AR without asthma in the present study further support the idea of subclinical lower airway inflammation in AR even in the absence of asthma and offers further support to the 'one airway, one disease' concept [28], where inflammation in the upper airways is associated with inflammatory changes in the lower airways.

Exhaled NO concentration at 50 ml/s was not significantly different in patients with AR without asthma as compared with healthy controls. These results are similar to those reported by Kharitonov et al. [38] and Aronsson et al. [39]. However, Henriksen et al. [4] found elevated exhaled NO levels in patients with seasonal or perennial AR outside the pollen season with a more significant increase further in the pollen season. The same kinds of

results were shown by Gratziou et al. [5]. The reason for discrepancies between studies may be related to the severity of the disease. Our patients were asymptomatic before the pollen season, and their mean symptom score during the pollen season before treatment was 7.4 ± 2.1 (the maximum value being 12) and only 2 patients had very severe symptoms (symptom score 11 or 12). In addition, all patients had normal spirometry and bronchodilatation test.

Increased serum levels of IgE and ECP in patients with AR in the present study are in line with results by Alvarez et al. [40]. ECP is a cytotoxic protein secreted by activated eosinophils, and its concentration is increased in sputum, nasal mucosa and nasal lavage fluid in patients with AR [41–43].

Treatment with nasal mometasone relieved patients' symptoms but had no statistically significant effects on markers of lower airway inflammation (exhaled NO parameters and markers in EBC), but reduced serum ECP levels. In a previous study, nasal triamcinolone for 4 weeks decreased exhaled NO levels in patients with AR with concomitant asthma [44], suggesting that nasal glu-

cocorticoids may have an anti-inflammatory effect even in lower airways in case of a significant lower airway inflammation. It is also possible that treatment with nasal mometasone for 2 weeks in our study was not long enough to cause a significant anti-inflammatory effect in lower airways because a tendency to lowered Daw_{NO} and 8-isoprostane levels in EBC was found. In addition, inhaled steroids have been shown to reduce increased exhaled NO levels in AR patients without asthma and to decrease increased bronchial NO output in asthma [5, 11]. In the literature we did not find any studies about the effects of nasal corticosteroids on flow-independent parameters of pulmonary NO production in patients with AR without asthma. In the present study, nasal steroids decreased serum levels of ECP, and this is likely related to relieved inflammation in nasal mucosa.

In conclusion, our results suggest that patients with AR have a subclinical lower airway inflammation even in the absence of asthmatic symptoms or impaired lung function which can be measured as increased Daw_{NO} and as elevated 8-isoprostane concentrations in EBC. Short-term treatment with nasal corticosteroids seemed not to affect these changes.

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