Atopy Influences Exhaled Nitric Oxide Levels in Adult Asthmatics*

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Study objective: To examine whether atopy influences exhaled nitric oxide (NO) levels in adults with established asthma.

Setting: Specialist respiratory unit in a university teaching hospital.

Patients: Twenty-eight asthmatics (mean FEV1, 85.7%) receiving short-acting inhaled bronchodilators and a range of inhaled steroids (0 to 4,000 μg/d).

Interventions: Subjects were studied on two occasions, 5 to 7 days apart, between September and March.

Measurements and results: On the first day, FEV1, exhaled NO, and histamine challenge were performed. On the second day, exhaled NO, total IgE, and skin-prick testing to six common allergens were conducted. Exhaled NO was measured with the single exhalation method. We found exhaled NO levels to correlate positively with total IgE (r = 0.43, p = 0.02) and number of positive skin-prick tests (p = 0.002). By contrast, there was no significant correlation between exhaled NO and FEV1 or the provocative concentration causing a 20% fall in FEV1. Subanalyses of steroid-treated and steroid-naive patients in this group revealed the same findings.

Conclusion: Exhaled NO levels in asthmatics correlate more closely with atopy than with bronchial hyperreactivity and lung function.

Key words: asthma; atopy; exhaled nitric oxide

Abbreviations: HEW = histamine-equivalent wheal; iNOS = inducible nitric oxide synthase; NO = nitric oxide; PC20 = provocative concentration causing a 20% fall in FEV1; ppb = parts per billion

Nitric oxide (NO) is a highly reactive molecule with multiple biological and pathophysiologic functions. In the airways, it is involved in mediating inflammatory processes, vasodilation, and bronchodilation. NO production and inducible NO synthase (iNOS) expression in the asthmatic airways are known to be increased, and studies suggest that exhaled NO levels correlate positively with bronchial hyperactivity in steroid-naive asthmatics. However, the biological relevance of increased NO production in asthma has not been fully elucidated. Airways NO levels are increased in conditions such as allergic and perennial rhinitis in the absence of asthma. In a community study, Frank and colleagues found that atopic children with asthma have increased levels of exhaled NO compared with nonatopic asthmatics. These studies suggest that atopy exerts an effect on exhaled NO levels regardless of the presence of asthma. We questioned whether these findings might be seen in adults with established asthma and what, if any, is the relationship between exhaled NO levels and atopy, bronchial hyperresponsiveness, and lung function in such patients.

Materials and Methods

Subjects

Twenty-eight asthmatics were recruited. The inclusion criteria were as follows: (1) diagnosis of asthma by a respiratory physician in accordance with the American Thoracic Society guidelines, (2) treatment with short-acting inhaled bronchodilator with or without inhaled steroids only, (3) clinically stable, ie, no change in asthmatic symptoms in last 3 months, and (4) previous documentation of positive histamine challenge test (provocative concentration causing a 20% fall in FEV1 [PC20] < 16 mg/mL). Atopy was defined according to levels of circulating IgE and skin-prick testing to six common allergens as described below. Subjects

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were characterized as atopic if they had at least one positive skin-prick test (a positive test refers to any response equal or greater than that to histamine alone). Smokers and those with upper respiratory tract infection during or within 4 weeks of the study, perennial rhinitis, active allergic rhinitis, and concomitant lung diseases were excluded. We measured exhaled NO in 25 healthy nonsmokers to provide a normal range for exhaled NO levels. These subjects were screened by a physician for (1) symptoms of asthma, allergic or perennial rhinitis, (2) allergic symptoms to animals or specific materials, (3) history of eczema or hay fever, and (4) any other medical conditions. Anyone with any of these features was excluded.

Study Protocol

Asthmatic subjects were asked to attend on two occasions, 5 to 7 days apart. On the first day, clinical history, exhaled NO, FEV1, and PC20 on histamine challenge were measured. On the second visit, exhaled NO was measured again, venipuncture was performed for total plasma IgE, and skin-prick testing was conducted. Exhaled NO levels were measured before spirometric maneuvers and histamine challenge.

Exhaled NO levels obtained on respective days of measurement were compared with measures of atopy (histamine-equivalent wheal [HEW] as described below, total IgE, and number of positive skin-prick tests), FEV1, and PC20.

Normal subjects were asked to attend on 1 day when exhaled NO and FEV1 were measured. Since NO levels are known to be affected by seasonal exposure to airborne allergens, we conducted the study between September and March to eliminate this possible confounding factor. The study was approved by the regional ethics committee (Lothian Health Ethics Committee).

Exhaled NO Measurement

Exhaled NO was measured using a chemiluminescence analyzer (LR 2000; Logan Research Ltd; Kent, UK). The analyzer provides online continuous measurement of NO in a single exhalation with a detection limit of 0.3 parts per billion (ppb) of NO. The analyzer was calibrated daily with NO/N2 calibration gas containing 103 ppb of NO (BOC; Guildford, UK). All subjects exhaled at a constant rate (15 L/min), maintaining a constant mouth pressure of 5 cm H2O by observing a visual display of this pressure. As previously described, the 95% confidence interval for each measurement is ±1.72 ppb. The subject’s NO level was recorded as the mean of three consecutive readings taken at the plateau of the exhalation profile. The method conformed optimally with European Respiratory Society guidelines on exhaled NO measurements.

Skin-Prick Testing

Cutaneous response to allergens was assessed by skin-prick testing on the forearm to the following allergens (BioDiagnostics; Worcestershire, UK): cat and dog dander, house dust mite (Dermatophagoides pteronyssinus), grass pollen, tree pollen, and Aspergillus fumigatus, compared with a saline solution negative control and a histamine positive control. After 15 min, the wheal diameter was measured against the positive control. All measurements equal to or greater than the positive control were considered as positive responses. Histamine equivalent wheal (HEW) was calculated as the maximum diameter of the allergen wheal minus the maximum diameter of the histamine control wheal. All the HEWs on one subject were then summed to give a numerical representation of cutaneous allergic response for that subject.

Total IgE

Circulating total IgE in the plasma was measured using a fluorometric enzyme immunoassay (UniCAP; Pharmacia & Upjohn; Milton Keynes, UK). The normal value with this method is <144 kU/L.

Histamine Challenge

Histamine challenge was performed using a tidal breathing method. Histamine (Tayside Pharmaceuticals; Dundee, UK) was delivered using a breath-activated dosimeter in doubling concentration from 0.25 mg/mL until at least a 20% decrease in FEV1, compared with baseline (0.9% saline solution inhalation), was recorded. PC20 was determined by linear interpolation between the last two data points on the log concentration-response curve.

Statistical Analysis

Where data were normally distributed with constant variance, correlations were measured using Pearson’s moment product correlation. Otherwise, the Spearman rank sum correlation was used. For comparisons of exhaled NO between groups with increasing number of positive skin-prick tests, one-way analysis of variance on ranks (Kruskal-Wallis method) was used, and Dunn’s method was used subsequently for pairwise comparisons. Comparison of exhaled NO between normal and asthmatic groups was performed using the Mann-Whitney test inasmuch as exhaled NO in both groups followed a nonparametric distribution. Statistical significance was assumed at p < 0.05, and data are expressed as mean ± SD.

Results

Demographic Data

The mean FEV1 in the asthmatic group was 85.7 ± 22.0% of predicted, and the mean age of the group was 41.3 ± 11.5 years (Table 1). Seventeen of 28 patients were receiving inhaled steroids, mean dose, 1,094.1 ± 1,049 µg/d. This cohort of patients had a range of disease activity. Nineteen patients had “well-controlled” asthma, defined as patients having no nocturnal wakening and less than twice-daily use of rescue medications in the 4 weeks before the study. The rest of the study patients had “poorly controlled” asthma, in which there was at least one nocturnal wakening per week and more than twice-daily use of rescue therapy.

Table 1—Demographic Data of Asthmatic and Normal Groups*

<table>
<thead>
<tr>
<th>Groups</th>
<th>FEV1, %</th>
<th>Age, yr</th>
<th>Male/Female, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroid-naïve asthmatics</td>
<td>83.5 ± 13.9</td>
<td>38.1 ± 7.2</td>
<td>3/8</td>
</tr>
<tr>
<td>Asthmatics receiving inhaled steroids</td>
<td>80.6 ± 25.0</td>
<td>43.4 ± 13.4</td>
<td>4/13</td>
</tr>
<tr>
<td>Normal subjects</td>
<td>101 ± 10.5</td>
<td>32.7 ± 5.8</td>
<td>9/16</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD unless otherwise indicated.
Exhaled NO Levels in Asthmatics Compared With Normal Subjects

In keeping with published data, exhaled NO levels in asthmatics (mean of the two visits) were higher than those of the normal subjects (median [interquartile range], 10.3 [6.3 to 17.0] ppb compared with 5.9 [3.7 to 7.1] ppb; p < 0.001; Mann-Whitney rank sum test, U = 565).

Exhaled NO and Atopic Measurements

Exhaled NO within the asthmatic population was found to correlate positively with HEW (r = 0.73; p < 0.001; Spearman rank correlation; Fig 1, right). There was a highly significant difference in exhaled NO between those subjects with no positive skin-prick test and those with one to two and three to four positive tests (p = 0.002; Kruskal-Wallis analysis of variance on ranks; Fig 1, left).

There was significant positive correlation between exhaled NO levels and total IgE levels (r = 0.43; p = 0.02; Pearson product moment correlation; Fig 2).

Exhaled NO and FEV₁ and Bronchial Hyperactivity

No significant correlation was found between exhaled NO and FEV₁ (r = -0.01; p = 0.97; Spearman rank sum correlation test) or PC₂₀ (r = -0.04; p = 0.82; Spearman rank sum correlation test).

Subanalysis of Steroid-Naïve Asthmatics and Those Receiving Inhaled Steroids

We subanalyzed the data by assessing the patients who were not receiving inhaled steroids (n = 11) and those who were receiving inhaled steroids (n = 17). The results were similar to the group as a whole (Table 2).

Discussion

Our results show that exhaled NO levels in asthmatics correlate with atopic indexes rather than FEV₁ and bronchial hyperreactivity. This study adds to a body of studies, mainly in children, which have shown significant relationships between exhaled NO
levels and atopy. Apart from the study by Frank et al., another group has also recently reported exhaled NO levels to be higher in healthy nonasthmatic children with positive skin-prick reactions compared with those without positive skin-prick reactions. In both children and adult nonasthmatics and asthmatics, exhaled NO levels are higher in those with positive skin-prick test to grass pollen, with further increase during exposure to grass pollen. It is legitimate to question, therefore, whether allergic airways inflammation is the cause of increased exhaled NO levels in asthmatics.

During the preparation of this article, a similar study was published by Simpson et al., showing almost identical results. Our study extends their findings by demonstrating that the relationship between exhaled NO and atopy holds even for asthmatics receiving inhaled steroids. Because iNOS is steroid sensitive, the explanation for this observation is unclear. Could steroid-induced modification in exhaled NO correspond to steroid-induced reduction in atopic activity? This is unlikely because although exhaled NO would be lowered by inhaled steroid use, it would not affect mast cell activity at sites other than the area of application and therefore should not influence skin-prick positivity and total IgE count. Further, we found no correlation between steroid dose and severity of atopy. Thus, it would seem that the relationship between exhaled NO and atopy shows a positive correlation, which is not the case with inhaled steroids and exhaled NO. This may be because the effect of atopy is much stronger than that of inhaled steroids and therefore overrides that of inhaled steroids. The suggestion could be that allergy-mediated production of NO, for example, from eosinophils or T helper-2 activation may be greater than that from neutrophils and inflamed epithelial cells and not as responsive to steroid treatment. However, it could also be hypothesized that iNOS expression in atopic asthmatics is less responsive to inhaled steroids, and therefore they continue to produce high levels of NO in the airways.

Putting our findings and those from the other studies in context of data on sputum and peripheral eosinophilia, it can be suggested that increased exhaled NO levels reflect a specific type of inflammation, that of T helper-2–driven inflammatory response rather than nonspecific airway inflammation. Increasing evidence suggests that airway inflammation in atopic asthma is associated with a T helper-2 cytokine profile, characterized by interleukin-4 and interleukin-5 production. These cytokines promote switching of B-cell isotypes to IgE production and activation of eosinophils, respectively. Interleukin-4 also appears to prolong the expression of iNOS in airway epithelium, providing a possible explanation for increased production of NO from the airways.

The idea that increased levels of exhaled NO are related to a specific type of airway inflammation would be in keeping with lack of increase in other airway inflammatory disorders, such as ARDS, cystic fibrosis, and COPD, in which there is excess neutrophilic inflammation. This is somewhat surprising, as neutrophils are thought to produce far greater amounts of NO than the epithelium, endothelium, or eosinophils. The lack of increase in these conditions may be attributed to the substantial release of other reactive species, such as superoxide anion, which then chemically remove NO from detection in the exhaled gas. Asthma may therefore be the unique inflammatory airway condition that demonstrates increased exhaled NO levels by virtue of its underlying atopic nature. This could also explain why some untreated asthmatics do not have increased NO levels, because not all asthma has an atopic basis.

In conclusion, our study showed that within an
asthmatic population, exhaled NO levels correlated with atopy in both steroid-treated and steroid-naive subgroups. This suggests that in asthmatics, increased NO production may be predominantly a feature of atopy.

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