Exhaled nitric oxide; relationship to clinicophysiological markers of asthma severity

M. K. AL-ALI, C. EAMES, AND P. H. HOWARTH

University Medicine, Southampton General Hospital, Southampton, U.K.

Bronchial asthma is an airway disorder associated with bronchial hyperresponsiveness, variable airflow obstruction and elevated levels of nitric oxide (NO) in exhaled air. The variables all reflect, in part, the underlying airway inflammation in this disease. To understand their interrelationships we have investigated the relationship between exhaled NO levels and clinicophysiological markers of asthma severity.

Twenty-six steroid naive atopic asthmatics participated in the analysis. All were given diary cards and were asked to record their peak expiratory flow (PEF) rates twice daily together with their asthma symptom scores and β-agonist use. Diary cards were collected 2 weeks later and measurements of exhaled NO levels, FEV₁, and histamine bronchial hyperreactivity (PC₂₀, histamine) were undertaken.

Exhaled NO levels were significantly higher in our study population than in normal control subjects and correlated negatively with PC₂₀ histamine (r = -0.51; P=0.008) and positively with PEF diurnal variability (r=0.58; P=0.002), but not with symptom scores, β-agonist use or FEV₁ (%).

We conclude that a significant relationship exists between exhaled NO levels and the two characteristic features and markers of asthma severity, namely bronchial hyperreactivity and PEF diurnal variability. The lack of correlation between symptom score and β-agonist use, or FEV₁ (%) predicted and exhaled NO suggests that these measures are reflective of differing aspects of asthma.

Introduction

Bronchial asthma is an inflammatory disease of the airways associated with bronchial hyperresponsiveness (BHR) and variable airflow obstruction (1,2). A number of inflammatory cells have been implicated in the pathogenesis of this disease through their ability to synthesize and release various mediators and pro-inflammatory cytokines (2-4).

Nitric oxide (NO) is a free radical gas produced endogenously from the amino acid L-arginine through the action of the enzyme nitric oxide synthase (NOS) (5), of which at least three isoforms are known, two being constitutive and one inducible (6-8). The inducible isoform (iNOS) is upregulated by pro-inflammatory cytokines (9) and there is evidence of increased expression of iNOS in asthmatic airways (10). This increased epithelial expression of iNOS is likely to be a significant factor underlying the increased levels of exhaled NO seen in asthmatics (11-13).

Exhaled NO levels are increased in allergen-induced late asthmatic reactions (14), while neither early reactions nor acute changes in airway calibre, induced by methacholine or salbutamol, are associated with significant changes in exhaled NO levels (15). Furthermore, inhaled steroids which are known to reduce airway inflammation (16) and BHR (17,18), are associated with lower levels of exhaled NO in asthmatics (15,19). All these observations suggest that exhaled NO levels in asthmatics are a reflection of the underlying airway inflammation and thus potentially provide a valuable clinical measure. There are, however, no detailed studies investigating the relationship between this marker and other markers of disease activity in asthma.

Thus to explore the relationship between exhaled NO levels and clinicophysiological markers of asthma severity, as reflected by symptom scores, β-agonist use, lung function and BHR, we have made these measurements in a group of steroid naive asthmatics only receiving treatment with β-agonists.

Materials and Methods

PATIENTS

Twenty-six asthmatic patients were studied (15 males and 11 females) with a mean age of 27 years (range 18-47 years) (Table 1). All were non-smokers, had a clinical diagnosis of...
Table 1. Patient characteristics, clinical indices of asthma severity, PC20, histamine and exhaled NO levels

<table>
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<th>Patient no.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>FEV1 (% predicted)</th>
<th>PEF variability*</th>
<th>Symptom score†</th>
<th>β-Agonist use‡</th>
<th>PC20§ (mg ml⁻¹)</th>
<th>NO¶ (ppb)</th>
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*PEF variability expressed as the amplitude as a percentage of the mean over 7 days.
†Total symptom scores over 7 days.
‡Number of puffs of β-agonist used over 7 days.
§Cumulative concentration of histamine (mg) that induces a 20% fall in FEV1, expressed as the geometric mean (range).
¶Exhaled NO levels parts per billion, results expressed as median (range).

asthma and were atopic as evidenced by positive epicutaneous skin prick test (>3 mm wheal diameter) to one or more of the common inhalant allergens (Dermatophagoides pteronyssinus, tree pollen, grass pollen, cat fur, dog hair and Aspergillus fumigatus: Bayer Corporation, Pharmaceutical Division, Elkhart, U.S.A). None of these patients had received disease modifying therapy, their sole medication being p.r.n. short-acting β-agonists as bronchodilators. No patient had experienced an upper or lower respiratory tract infection or acute exacerbation of their asthma within 8 weeks prior to the study. The study was approved by the Southampton Hospitals and University Ethics Committee and all patients gave written informed consent.

STUDY DESIGN

Patients with a clinical diagnosis of asthma and prescribed β-agonists by their general practitioner were provided with peak flow meters and diary cards and asked to keep daily records over a 2 week period. Peak expiratory flow rates (PEF) were measured twice daily, morning (06:00–09:00 hours) and evening (18:00–21:00 hours), and at the same time symptom scores and β-agonist usage in the preceding day or night were entered in the diary cards. The diary cards were collected at the end of this 2 week period and at this visit measurements were made of exhaled NO levels, lung function and bronchial reactivity by histamine inhalation challenge.

The study was approved by the Southampton Hospital and University Joint Ethical Committee, and a written informed consent was given by each patient.

LUNG FUNCTION

Peak expiratory flow was measured by patients using a Wright mini peak flow meter (Wright, Derby, U.K.). Morning and evening measurements were made at the same time of day with the morning values being recorded prior to any
β-agonist use on waking. For each PEF measurement the best of three readings was entered in the diary card. Data from the 7 days with complete PEF measurements closest to the date of the second visit were used for analysis.

At the second visit, following abstinence from inhaled β-agonists for at least 6 h, FEV₁ was measured using a dry spirometer (Vitalograph Ltd, Buckingham, U.K.). The best of three measurements was expressed as a percentage of the predicted value [FEV₁ (%)] and used for the comparative analysis. PEF variability was expressed as the diurnal PEF variation (amplitude as a percentage of the mean) (20) and defined as [(highest PEF-lowest PEF)/(mean value of these two)] × 100% over a period of 7 days.

SYMPTOM SCORES AND β-AGONIST USAGE

Diary cards were completed every morning and evening by entering the symptom scores for the preceding 12 h using a four-point scale (0, no symptoms; 1, mild, 2, moderate; 3, severe), with a maximum score of 42 for 1 week. At the same time β-agonist usage for the preceding 12 h was entered as the number of puffs used. Data collected from the same days used for PEF evaluation were used for analysis.

NO MEASUREMENTS

Exhaled NO levels were measured as a mixed exhaled air sample using a chemiluminescent analyser (model 9841A, Siemens Plessey, Dorset, U.K.) with a detection threshold of 1 part per billion (ppb). The analyser was calibrated daily using NO-free air and a certified gas cylinder of 525 ppb of NO (MG Gas Products Ltd, Surrey, U.K.). The method was described previously (21). Briefly, while quietly seated and wearing nose clips, patients who were tidally breathing at a normal respiratory rate were asked to take a full inspiration and then immediately to exhale through a mouthpiece to fill a 500 ml reservoir bag attached to the analyser. Once the reservoir bag was filled, the sample was continuously analysed until the bag was empty. The peak concentration of NO was recorded and the mean of three readings was considered for evaluation.

HISTAMINE BRONCHIAL CHALLENGE

BHR was assessed in all patients, after they omitted their bronchodilator therapy for at least 6 h, using a five-breath inhalation technique modified from that of Chai et al. (22). Histamine acid phosphate (Sigma, Dorset, U.K.) dissolved in 0.9% saline was administered in increasing doubling concentrations (0.03 mg ml⁻¹–8.0 mg ml⁻¹) from an inspiron mini nebulizer (CR Bard International, Sunderland, U.K.) following a saline control inhalation as described previously (23). PC₂₀ was derived as the cumulative concentration of histamine (mg) that induced a 20% fall from the post-saline FEV₁ and obtained by linear interpolation between the last two points of the log (concentration) response curve.

Results

Results of diary card recordings, lung function and BHR to histamine are presented in Table 1. The average symptom score for 1 week was 11.7 (maximum possible 42) and the average β-agonist usage was 32 puffs week⁻¹. The mean FEV₁ (% predicted) was 88% and the mean PEF diurnal variability was 10.2%. The group geometric mean cumulative PC₂₀ histamine (range) was 0.73 mg ml⁻¹ (0.04–8.77 mg ml⁻¹).

Exhaled NO levels were elevated in our study population with a mean ± SEM of 19.7 ± 1.9 ppb compared with values recorded in normal subjects of 11.1 ± 0.59 ppb (P < 0.0001) using the same technique (21).

There was a significant positive correlation between PC₂₀ histamine and FEV₁ (% predicted) (r = 0.44; P = 0.026) (Fig. 1). Exhaled NO levels correlated negatively with PC₂₀ histamine (r = 0.51; P = 0.008) and positively with diurnal PEF variability (r = 0.58; P = 0.002) (Fig. 2). A strong positive correlation existed between symptom scores and β-agonist use (r = 0.85; P < 0.0005) (Fig. 3). There was no significant correlation between exhaled NO levels and β-agonist use, symptom scores or FEV₁ (% predicted).

Discussion

In this study we have demonstrated that steroid-naive asthmatics exhale significantly higher levels of NO than normal control subjects and that such levels correlate negatively with BHR as assessed by the PC₂₀ histamine and positively with the PEF diurnal variability expressed as the amplitude as a percentage of the mean.

BHR and variable airflow obstruction are characteristic features of asthma and are a consequence of the underlying
EXHALED NO AND ASTHMA SEVERITY

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Fig. 2. Relationship between exhaled NO levels and (a) PC_{20} histamine \((r=-0.51; P=0.008)\) and (b) PEF diurnal variation \((r=0.58; P=0.002)\).

Fig. 3. Relationship between symptom scores and \(\beta\)-agonist usage \((r=0.85; P<0.0005)\).

Airway inflammation (1), and both have been used as markers for assessment of asthma severity. Studies in patients with asthma have shown that bronchial reactivity is increased in relation to the clinical severity of the disease (24-26), daily requirement for medications to control symptoms (24,25,27) and lung function as assessed by PEF variability (28) or FEV\(_1\) (% predicted) (24,26). Our finding of a significant correlation between PC\(_{20}\) histamine and FEV\(_1\) (% predicted) (Fig. 1) is consistent with these observations. Recently, bronchial biopsy studies in asthma (16,29,30) have demonstrated a relationship between the degree of airway inflammation and bronchial reactivity (29) as well as PEF variability (30). Furthermore, following treatment with inhaled corticosteroids the reduction in the degree of airway inflammation was paralleled by reduction in bronchial reactivity and improvement in lung function (16). All these observations suggested that bronchial reactivity and PEF variability might be useful markers for monitoring asthma severity. However, measurement of airway hyperresponsiveness can be distressing to the patient owing to the induced bronchoconstriction and relatively difficult to perform in clinical practice. In addition, this measure is influenced by concurrent-bronchodilator medication and, even with optimal control of asthma, improvements in bronchial reactivity are often modest and still remain abnormal. Similarly, although serial measurements of PEF are easy to perform, the results may not reflect the underlying airway inflammation as the results of these measurements will also be affected by the use of inhaled or oral bronchodilators. An improved indirect marker of airway inflammation is thus required that is easy to measure and can be applied clinically. NO measures in exhaled air potentially fulfil such criteria. NO is generated by NOS and enhanced expression of an inducible form of this enzyme is evident in the airways in asthma. This enzyme is induced by the cytokines (31) such as interleukin-1\(\beta\), tumour necrosis factor-\(\alpha\) and interferon-\(\gamma\). All these cytokines have enhanced potential expression in asthma together with other pro-inflammatory cytokines (32-34). NO is thus likely to reflect inflammatory events indirectly and our identification of elevated levels of NO is consistent with this concept and with the results of other studies (11-13).

Our findings of a strong association between exhaled NO levels and the two characteristic features of asthma, namely bronchial hyperreactivity and PEF diurnal variability, suggest that measurements of exhaled NO levels in asthmatics might be a useful surrogate for these markers in monitoring asthma severity which overcomes the limitations attached to these markers as the test is easy to perform and the results are not affected by bronchodilator use (15). Indeed, Kharitonov et al. in a recent report (35) have shown that exhaled NO levels are more sensitive than either symptom scores or lung function in detecting deterioration in asthma control associated with changes in the dose of inhaled corticosteroids. Currently, NO analysers are expensive and not widely available, but in the future cheaper and potentially portable machines might become available.

The other observation we have made is the strong association between symptom scores and \(\beta\)-agonist usage (Fig 3). The patients were using more than four puffs of a \(\beta\)-agonist per day and thus were inadequately treated and should be receiving regular inhaled prophylactic therapy (34). However, their average weekly symptoms score was only 11.7, which gives the impression that the study population have mild disease. The mean FEV\(_1\) (% predicted) of 88% gives the same impression. The use of \(\beta\)-agonists suggests, however, that their disease is not mild (36) and suggests that most patients are underestimating their symptoms, making symptom scores an unreliable marker for monitoring asthma severity and lending further support.
to the use of a more objective measure such as exhaled NO levels in monitoring asthma.

Finally, although FEV₁ measurements indirectly reflect airway pathology, PEF diurnal variation over 1 week is more likely to reflect the underlying airway mucosal inflammation than measurement of FEV₁ at a single time point as this will also be influenced by structural airway changes unrelated to current inflammatory events. The strong association between exhaled NO levels and PEF diurnal variability but not with FEV₁ (% predicted) would support this tenue.

In conclusion, we have demonstrated that asthmatics exhale significantly higher levels of NO than normal control subjects and that there is a strong association between exhaled NO levels and two characteristic features of asthma; bronchial hyperreactivity and diurnal PEF variation. These findings lend further support to the suggestion that measurement of exhaled NO levels is likely to be a valuable technique for the monitoring of the current status of airway inflammation in asthma and an aid in the determination of the current inhaled anti-inflammatory therapy requirements.

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References


