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Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients

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Changes in the dose of inhaled steroid affect exhaled nitric oxide levels in asthmatic patients. S.A. Kharitonov, D.H. Yates, K.F. Chung, P.J. Barnes. ©ERS Journals Ltd 1996.

ABSTRACT: An increased concentration of nitric oxide (NO) in the exhaled air of asthmatic patients may reflect inflammation of the airways, and exhaled NO may, therefore, be useful in monitoring asthma control and the optimal use of anti-inflammatory treatment.

We have studied the effect of reducing and then increasing the dose of inhaled steroid on exhaled NO, lung function and symptoms in 14 asthmatic patients treated with twice daily budesonide. Baseline measurements were made at the end of a 2 week run-in period, 2 weeks after the daily dose of budesonide was reduced by 200 µg daily, and 2 weeks after the dose was then increased by 200 µg daily.

Exhaled NO increased significantly compared with baseline after the dose was reduced by 200 µg daily (from 122±13 to 246±52 ppb); whereas, there was no significant decrease in spirometry or change in peak flow variability. There was also a significant increase in symptoms at night, but no change during the day or in the number of rescue doses of inhaled β_2-agonist. The level of exhaled NO decreased when the dose of inhaled steroids was increased, and this was associated with a reduction in diurnal variability of peak expiratory flow, and in nocturnal symptoms.

Our study suggests that exhaled nitric oxide may be a useful means of monitoring control of asthma. Further longitudinal studies in patients of differing asthma severity are now indicated.

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There is now general agreement that inhaled steroids are the most effective treatment for chronic asthma and that they control asthma by suppressing eosinophilic inflammation in the airways [1]. Inhaled steroids are now recommended for the treatment of asthma in any patients who need to use an inhaled β_2-agonist more than once daily [2–4]. It is recommended that the dose of inhaled steroid should be increased until asthma symptoms are controlled and lung function is optimal. The dose of inhaled steroid should be stepped down once asthma has been controlled for several months, until the minimal dose that maintains control is achieved. Asthma control is normally monitored by frequency of symptoms, inhaled β_2-agonist use or by measurement of serial peak expiratory flow (PEF). However, these clinical measurements may not reflect the degree of airway inflammation, and it may, therefore, be difficult to determine whether the prescribed dose of inhaled steroid is adequate to control asthmatic inflammation. Inflammation in the airways may be reflected by increased airway responsiveness to inhaled histamine or methacholine. Inhaled glucocorticoids reduce airway hyperresponsiveness, although airway responsiveness rarely returns to normal values and the change with inhaled steroids is often very small (less than one dilution) [5–7]. This indicates that measurement of airway hyperresponsiveness may not be a useful clinical means of following control of asthmatic inflammation.

Recently, asthmatic patients have been found to exhale increased concentrations of nitric oxide (NO) [8–10]. This increase in exhaled NO may reflect the increased expression of the inducible form of NO synthase (iNOS) in epithelial cells of asthmatic patients [11]. Human epithelial cells have been shown to express this enzyme in response to proinflammatory cytokines [12]. There is also an increase in NOS enzyme activity in the lungs of patients with asthma [13]. Furthermore, glucocorticoids inhibit the induction of iNOS in epithelial cells *in vitro* [12], and in asthmatic patients *in vivo* [14]. This is reflected by a reduction in exhaled NO in asthmatic patients after oral or inhaled steroids [15, 16]. This suggests that exhaled NO may be a useful marker of chronic inflammation in the airways, and may also be useful in monitoring the response to anti-inflammatory treatment, such as inhaled steroids. Exhaled NO is a simple noninvasive test that would be applicable to repeated measurements in patients, even when lung function is impaired.

We have, therefore, studied the effect of reducing and then increasing the dose of inhaled steroids on exhaled NO in asthmatic patients already established on regular inhaled steroid therapy.

Materials and methods

Patients

Fourteen asthmatic patients were studied (7 males and 7 females) with a mean age of 34 (range 23–63) yrs. All had clinical features of asthma, with previously documented reversibility of forced expiratory volume in one second (FEV_1) of >15%. All patients had positive skin tests for at least two common aeroallergens (cat dander, house dust mite, grass pollen, *Aspergillus fumigatus*) and had increased bronchial hyperreactivity to methacholine, defined by a provocative concentration of methacholine causing a 20% fall in FEV_1 (PC_{20}) <8 $mg \cdot mL^{-1}$ (table 1).

All of the patients were receiving regular treatment with inhaled budesonide (Pulmicort, Astra) *via* a multiple-dose dry powder delivery system (Turbohaler®, Astra) in a dose range of 200–2,000 $\mu g \cdot day^{-1}$, and on inhaled β_2-agonists as required for symptom control. There was no history of upper respiratory tract infection for at least 4 weeks before the study and PEF recordings over the 2 weeks prior to study were stable with <15% variability. None of the patients were smokers.

Study design

Two sets of observations were made: the effect of steroid dose reduction by 200 µg (n=14), and the effect of increasing the dose by the same amount (n=12). The duration of the study was 8 weeks and involved an initial assessment and four follow-up visits.

There was a 2 week run-in period with no changes in prescribed treatment, followed by a 2 week period on reduced treatment. The patients were then prescribed their original dose of inhaled steroid and remained on this for a further 2 weeks. They then entered the final 2 week period, during which the dose of inhaled steroid was increased. At each study visit, exhaled NO and lung function were measured. Symptoms scores were recorded throughout the study.

The protocol was approved by the hospital Medical Ethics Committee, and written consent from each patient was obtained.

Lung function

FEV_1 and forced vital capacity (FVC) were measured with a dry spirometer (Vitalograph Ltd, Buckingham, UK). The best value of three manoeuvres was expressed as a percentage of the predicted value.

PC_{20} was measured by inhalation methacholine challenge, with doubling concentrations of methacholine (0.06 to 32 $mg \cdot mL^{-1}$) delivered by dosimeter (Mefar, Bovezzo, Italy), with an output of 100 µL. The aerosols were inhaled at tidal breathing, wearing a noseclip. A total of five inhalations of each concentration were administered (inhalation time 1s, breathholding time 6 s), and FEV_1 was measured 2 min after the last inhalation, until there was a fall in FEV_1 of 20% compared with the control inhalation (0.9% saline solution) or until the maximal concentration was inhaled. The PC_{20} value was calculated by interpolation of the logarithmic dose-response curve.

Nitric oxide measurements

Exhaled NO was measured on a chemiluminescence analyser (Dasibi Environmental Corporation, Glendale,

Table 1. – Clinical characteristics of the patients

Pt No.	Age yrs	Sex	Atopic status +/-	Duration of asthma yrs	FEV_1 % pred	FEV_1/FVC %	PC_{20} $mg \cdot mL^{-1}$
1	27	M	+	26	98	99	0.22
2	36	M	+	14	82	105	1.74
3	41	M	-	8	83	94	0.64
4	34	M	+	19	105	108	2.44
5	63	M	-	6	77	92	-
6	42	F	+	12	66	86	-
7	24	F	+	12	82	94	0.64
8	24	F	+	4	93	103	2.39
9	24	M	+	13	69	68	1.15
10	33	F	+	25	92	91	0.23
11	27	M	+	21	114	114	2.14
12	54	F	+	49	62	100	-
13	23	F	+	6	79	91	2.21
14	29	F	+	27	72	78	-
Mean	34			17	84	95	1.01†
SEM	±3			±3	±4	±3	±0.29

Pt: patient; FEV_1: forced expiratory volume exhaled in one second; FVC: forced vital capacity; PC_{20}: provocative concentration of methacholine producing a 20% fall in FEV_1. Mean±SEM values are presented. †: Geometric mean (+SEM).

California, USA) sensitive to NO from 2 to 4,000 parts per billion (ppb, by volume), adapted for on-line recording of NO concentration, as described previously [9]. This feature obviates the need for collection in a reservoir, with its variable loss of reactive NO, and gives greater sensitivity and reproducibility. Subjects wore a noseclip and were asked to produce a slow vital capacity manoeuvre over 30–45 s into wide-bore Teflon tubing. NO was sampled continuously at a rate of 250 mL·min^{-1} and the measurement of NO was linear over the range measured. The baseline value at each visit was measured after at least 15 min of quiet rest. Three successive recordings were made, and the highest value of three readings made at 2 min intervals was used in analysis. Results were displayed on a chart recorder and compared with the signal generated from a calibration mixture of NO (89 ppb) in nitrogen. The area under the curve of concentration traces was highly correlated with the peak value (r=0.98); peak values were, therefore, used in all calculations. Ambient air NO was recorded before each breath and subtracted. Exhaled NO was measured at each of six visits (at the beginning and end of the run-in and each treatment period).

Symptom maximal diurnal variability

Diary cards were completed every morning and evening by all patients to record asthma symptoms, PEF, time of taking inhaled corticosteroids and β_2-agonist use. At every visit to the clinic, the information from the self-assessment diary card was collated. The symptom enquiry included assessment of symptoms during the day and night, using a four point scale (0=no symptoms; 3=severe symptoms). The scores were added to form an overall score (maximum=9).

Diurnal variability of PEF was calculated as follows:

$$\frac{\text{Maximum PEF - Minimum PEF}}{\text{Maximum PEF}} \times 100\%$$

Statistics

Values of FEV1, FVC, PC20, NO and symptom scores during steroid reduction were compared with values obtained during steroid increase, using Student t-tests for paired observations. Comparisons between groups were made by analysis of variance (ANOVA). All results were expressed as the mean±SEM. A p-value of less than 0.05 was considered significant.

Results

Exhaled NO

Reduction in the dose of inhaled budesonide in 14 patients from a mean±SEM of 714±152 to 514±152 µg for 2 weeks was associated with a significant increase in exhaled NO level from 122±13 to 246±52 ppb (p<0.05) (table 2 and fig. 1). In 12 patients in whom the dose of

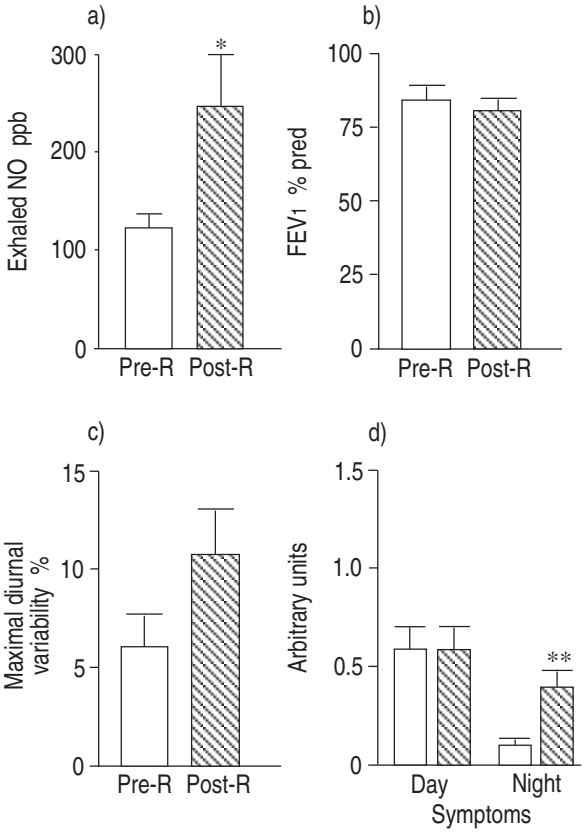

Fig. 1. – Effect of reducing the dose of inhaled steroids in asthmatic patients (n=14). a) exhaled NO; b) FEV1; c) diurnal variability in PEF; d) asthma symptoms. Values are presented as mean±SEM. FEV1: forced expiratory volume in one second; PEF: peak expiratory flow; R: reduction. **: p<0.001, compared to baseline.

Table 2. – The effect of reduction in the dose of inhaled budesonide on exhaled nitric oxide, lung function and symptom scores in 14 patients with asthma

	NO ppb	FEV1 % pred	FVC % pred	PEF variability %	Symptoms scores Day	Symptoms scores Night	Rescue medication puffs·day^{-1}	Budesonide µg·day^{-1}
Pre-Red.	122±13	84±4	95±3	6±1	0.6±0.11	0.1±0.03	2±0.5	714±152
2 weeks post-Red.	246±52*	80±4	90±3	11±2	0.6±0.11	0.4±0.08**	3±0.1	514±152

ppb: parts per billion; Day: asthma during the day; Night: asthma last night; PEF variability: maximal diurnal variability of peak expiratory flow rate; Rescue medication: β_2-agonists used; Budesonide: dose of inhaled budesonide. Red.: reduction. For further abbreviations see legend to table 1. Values are presented as mean±SEM. Significant differences *vs* baseline. *: p<0.05; **: p<0.01 (analysis of variance (ANOVA), Student's t-test).

Table 3. – The effect of increase in the dose of inhaled budesonide on exhaled nitric oxide, lung function and symptom scores in 12 patients with asthma

	NO ppb	FEV_1 % pred	FVC % pred	PEF variability %	Symptoms scores Day	Symptoms scores Night	Rescue medication puffs·day^{-1}	Budesonide µg·day^{-1}
Pre-I	167±25	74±5	89±4	12±2	0.9±0.18	0.5±0.16	4±0.7	800±152
2 weeks post-I	101±10*	79±3	90±4	6±1*	0.7±0.17	0.1±0.04*	3±0.1	1117±164

Values are presented as mean±SEM. Significant differences *vs* baseline. *: $p<0.05$ (ANOVA, Student's t-test). I: increase. For further abbreviations see legend to tables 1 and 2.

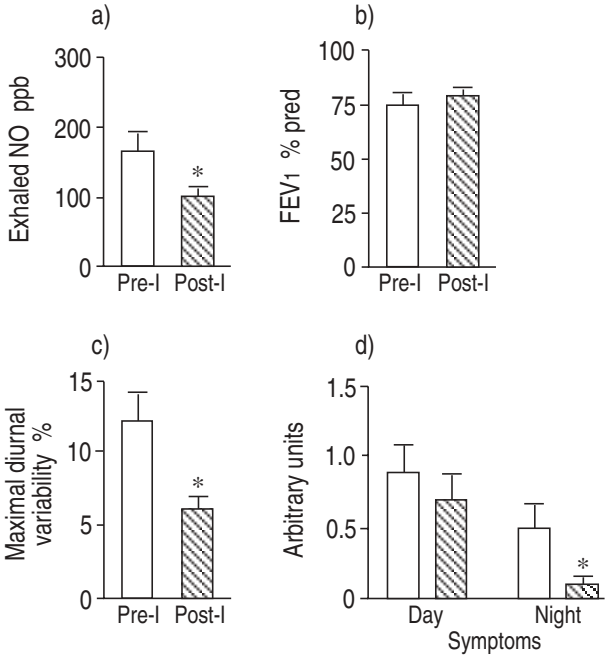

Fig. 2. – Effect of increasing the dose of inhaled steroids in asthmatic patients (n=12) who had an initial reduction in dose. a) Exhaled NO; b) FEV_1; c) diurnal variability; d) symptoms. Values are presented as mean±SEM. I: increase; FEV_1: forced expiratory volume in one second; ppb: parts per billion. *: $p<0.05$, compared to baseline.

budesonide was then increased from 800±152 to 1,117±164 µg, the exhaled NO level decreased from 167±25 to 101±10 ppb ($p<0.05$) (table 3 and fig. 2) after 2 weeks of treatment. Of the initial 14 patients who completed the steroid reduction limb, two were withdrawn due to development of upper respiratory tract infections.

Lung function

Lung function and β_2-agonist usage in both treatment groups remained unchanged (FEV_1 84±4% predicted prior to steroid reduction and 80±4% pred after reduction; FEV_1 74±5% pred prior to steroid increase and 79±3% after increase in dose (tables 2 and 3, figs. 1 and 2).

Symptoms

In the group of 14 patients, the reduction in the dose of inhaled budesonide resulted in an increase in clinical scores, but this was not significant, except for nocturnal symptoms which increased from 0.1±0.03 to 0.4±0.08 units ($p<0.001$) (table 1, fig. 1). In 12 patients whose dose of budesonide was then increased, symptoms scores were not significantly affected by the change in the treatment, except for diurnal variation (12±2% before and 6±1% after) ($p<0.05$) (table 2, fig. 2) and nocturnal symptoms (0.5±0.16 before and 0.1±0.04 after, $p<0.05$). There was no change in the use of inhaled β_2-agonists during any treatment period.

Discussion

This study has demonstrated that the concentration of NO increases in the exhaled air of asthmatic patients when the dose of inhaled steroids is reduced. This was not accompanied by any change in FEV_1 or FVC, and although there was an increase in diurnal variability of PEF, this did not reach statistical significance. There was, however, an increase in asthma symptoms at night, although asthma symptoms during the day did not increase. This suggests that exhaled NO may be a more sensitive index of deterioration in asthma than either symptoms or lung function. Furthermore, when the dose of inhaled steroids was increased again there was a significant reduction in exhaled NO, which returned to baseline values after 2 weeks.

These results suggest that exhaled NO might be a useful means of monitoring inflammation in asthmatic airways. It is likely that inhaled steroids reduce exhaled NO by inhibiting the induction of iNOS in epithelial and inflammatory cells in the airways [17, 18]. This may be a direct effect on the transcription factors, such as nuclear factor-kappa B (NF-kβ) which increases transcription of the iNOS gene [19, 20], or by inhibiting the synthesis of proinflammatory cytokines, such as interleukin-1β (IL-1β) and tumour necrosis factor-α (TNF-α), that may induce iNOS expression in asthma. Reduction in the dose of inhaled steroids presumably allows an increase in proinflammatory cytokines and also has a direct inducing effect on iNOS expression by increasing activation of repressed transcription factors.

In order to monitor the control of asthma it would be desirable to monitor inflammation in the airways. Monitoring of symptoms may be misleading, as bronchodilators relieve symptoms without treating the underlying inflammatory process. Airway hyperresponsiveness has been used as a marker of airway inflammation, but it is relatively difficult to perform in a clinical setting; the changes

in airway responsiveness after inhaled steroids are modest and even when asthma is optimally controlled the values often remain abnormal. Diurnal variability in peak flow may not accurately reflect airway inflammation and may also be confounded by concomitant treatment with bronchodilators. More direct measurements of airway inflammation include bronchial biopsy, bronchoalveolar lavage and induced sputum. Bronchial biopsies and bronchoalveolar lavage are invasive procedures and are clearly unsuitable for clinical monitoring of inflammation in asthma [21]. Furthermore, it has proved difficult to quantify inflammatory changes in biopsies and lavage fluid. Induced sputum is a useful means of assessing airway inflammation in asthma, but not all patients are able to produce satisfactory samples and there are also problems in quantifying the abnormalities [22].

The measurement of exhaled NO may overcome some of these problems, as the measurement can be made easily in all patients, even when there is severe airflow limitation. Furthermore, the measurement can be repeated in order to study the time course of any treatment or manoeuvre, and the measurement is quantifiable. However, further studies are needed to define the relationship between airway inflammation and exhaled NO. In a recent study of allergen challenge in asthmatic patients, we have demonstrated that exhaled NO increases during the late phase, which is associated with inflammation in the airways, but is not increased during the immediate bronchoconstrictor response to allergen [23l.

The source of the NO in exhaled air is still a matter of debate. In normal individuals, exhaled NO may be derived predominantly from the upper respiratory tract [24, 25], although studies in perfused pig lungs have suggested that it may also be derived from the alveoli [26]. In asthmatic patients, who show elevated levels of NO in exhaled air, the increase in NO is likely to derive from the lower respiratory tract, as it is reduced by inhaled steroids [16] and inhaled NOS inhibitors [15, 27]. More importantly, direct measurements from the lower respiratory tract *via* a bronchoscope indicate elevations in exhaled NO in asthmatic patients, with similar values recorded from the lower respiratory tract as in orally exhaled air [28]. It is likely that the increased exhaled NO in asthmatic patients is derived from increased expression of iNOS in airway epithelial cells of asthmatic patients [11]. Furthermore, an inhaled steroid reduces the elevated exhaled NO in asthmatic patients back to normal [16], and is associated with reduced expression of iNOS in epithelial cells of asthmatic patients.

Our study lends further support to the view that exhaled NO may be a useful means of monitoring the control of airway inflammation in asthma. Further controlled longitudinal studies are now needed in asthmatic patients of differing severity in order to confirm this impression. NO analysers are not widely available at the present time and are expensive. However, in the future it is likely that such devices will be minaturized and will become more widely available. It is even possible to conceive of personal monitors that may be used in conjunction with home peak flow meters. One disadvantage of exhaled NO as a monitor of asthma is that levels are also elevated in the exhaled air in other conditions, such as bronchiectasis and during upper respiratory tract infections [29, 30]. NOS activity in the lung is increased, not only in asthma, but also in cystic fibrosis and obliterative bronchiolitis [13]. Thus, exhaled NO measurements may not be of diagnostic value, but they may be useful in monitoring control of asthma and the response to anti-inflammatory treatments in individual asthmatic patients.

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