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Baseline airway hyperresponsiveness and its reversible component: role of airway inflammation and airway calibre

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Baseline airway hyperresponsiveness and its reversible component: role of airway inflammation and airway calibre. M. Ichinose, T. Takahashi, H. Sugiura, N. Endoh, M. Miura, Y. Mashito, K. Shirato. ©ERS Journals Ltd 2000.

ABSTRACT: Airway hyperresponsiveness (AHR), in which airway inflammation has been reported to be a key factor, is an important component of asthma. However the precise role of inflammation in AHR is still unclear.

In this report, airway inflammatory changes were assessed using hypertonic saline-induced sputum examination and exhaled nitric oxide analysis, and the relation between AHR to methacholine, airway calibre forced expiratory volume in one second (FEV_1) and airway inflammatory indices examined. Furthermore, the changes in these variables were also examined by means of 8 weeks' open uncontrolled inhaled steroid administration (800 $\mu g \cdot beclomethasone \cdot day^{-1}$).

Asthmatic subjects had higher eosinophil counts and bradykinin concentration in induced sputum and higher exhaled NO levels, and showed AHR to methacholine. Baseline AHR significantly correlated with FEV_1 but not with indices of inflammation in sputum or exhaled air. Steroid inhalation therapy was associated with a reduction in eosinophil and bradykinin concentration in sputum and NO levels in exhaled air and an improvement in FEV_1 and AHR. The changes in FEV_1 and AHR were significantly related to changes in markers in sputum and exhaled air ($p<0.01$ for each).

These results suggest that baseline airway hyperresponsiveness can be predicted from the airway calibre but not from inflammatory parameters in sputum or exhaled air. In contrast, the reversible component of airway hyperresponsiveness appeared to be associated with the reduction in airway inflammation.
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In asthma, an important component underlying the instability of the airways is airway hyperresponsiveness (AHR), which is the presence of an exaggerated bronchoconstrictor response to a wide variety of exogenous and endogenous stimuli [1]. Although airway inflammatory changes have been reported to be a key factor in AHR, conflicting findings have been obtained by the investigative groups who have studied the relationship between airway inflammation and airway responsiveness [2]. Some groups have shown a strong relationship between the above two phenomena [3–6], whereas others have failed to establish such a relationship [7–11].

Recently, some new noninvasive and highly reproducible methods for the detection of airway inflammatory changes have been developed. One of the new methods is hypertonic saline-induced sputum examination, which can be used in subjects without sputum in the basal condition, enabling airway inflammatory changes to be assessed *via* inflammatory cell counts and mediator analysis in the sputum [12–14]. Another is exhaled nitric oxide analysis. This technique has also been reported to reveal the inflammatory changes in asthmatic airways [15, 16].

The aim of this report is to elucidate the relationship between airway responsiveness and inflammatory parameters. Airway responsiveness was assessed by methacholine inhalation challenge, and airway inflammatory changes were analysed *via* the above-mentioned noninvasive methods. Concerning induced sputum examination, this study focused on bradykinin concentration, which has been reported to be relevant in asthmatic airway inflammation [17, 18], as well as on eosinophil counts. Furthermore, the relationship between steroid inhalation-mediated changes in airway inflammatory parameters and in responsiveness were studied.

Methods

Subjects

Eighteen patients with asthma took part in the study after giving informed consent. The study was approved by the local ethics committee. All patients satisfied the American Thoracic Society criteria for asthma [19]. The clinical characteristics of these subjects are shown in table 1. Forced expiratory volume in one second (FEV_1) was measured using a dry rolling-seal spirometer (OST 80A; Chest Co., Tokyo, Japan). All patients were stable and had been without steroid therapy for $\geq$6 months before the study.

Table 1. – Characteristics of study subjects

Subject No.	Age yrs	Sex	FEV1		PD35 mg·mL^{-1}		NO ppb		Sputum examination: Bradykinin ng·mL^{-1}		Sputum examination: Eosinophil %		Drug treatment
			Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
1	29	M	78.4	79.7	1.4	5.2	46	21	4.3	2.6	7.0	1.0	None
2	29	M	98.0	98.5	1.4	2.0	70	40	4.9	5.3	16	1.0	None
3	72	F	133	160	2.2	4.6	40	23	4.2	1.8	80	3.0	A
4	68	F	89.2	99.4	6.2	8.2	72	20	4.2	3.3	40	0	None
5	65	F	65.1	91.0	0.50	0.50	90	22	4.8	3.0	9.0	0	None
6	38	F	92.4	98.2	6.0	6.2	198	60	9.6	2.9	50	20	A
7	20	M	67.4	79.5	0.47	2.0	128	35	8.7	1.9	60	0	A
8	63	F	88.2	116	0.34	0.73	140	52	11	9.7	10	0	A
9	66	F	123	124	25	25	98	60	10	9.0	8.0	0	A, AC
10	59	F	118	118	1.1	1.2	60	30	7.0	6.0	18	0	None
11	29	M	48.5	95.6	0.11	9.3	198	30	47	1.0	80	5.0	A
12	46	M	96.2	107	6.0	12	320	250	40	30	70	15	A, B
13	29	M	72.9	80.6	0.25	0.60	193	46	6.6	2.2	50	2.0	None
14	28	M	85.0	110	0.47	1.1	95	20	8.3	1.2	67	1.0	None
15	46	F	63.3	85.7	0.73	4.1	200	50	84	11	86	15	None
16	32	M	128	129	2.9	3.2	20	16	2.5	2.0	5.0	0	None
17	40	F	100	149	2.1	21	282	51	5.0	0.5	71	0	None
18	28	M	80.0	120	1.0	5.5	178	30	10	1.1	7.0	0	None
Mean	43.7		90.4	107.8	3.2	6.2	134.9	47.6	15.2	5.2	40.8	3.5	
SEM	4.2		5.7	5.6	1.4	1.7	20.6	12.7	5.1	1.7	7.4	1.5	

FEV1: forced expiratory volume in one second; PD35: cumulative provocative dose of methacholine causing a 35% increase in respiratory resistance; ppb: parts per billion; pre: before steroid therapy; post: after steroid therapy; M: male; F: female; A: aminophylline; AC: anticholinergic agent; B: β-adrenergic agent.

Measurement of nitric oxide concentration

Exhaled NO levels were measured using a chemiluminescence analyser (Sievers Model 280NOA; Sievers, Boulder, CO, USA). Subjects were asked to perform a slow vital capacity manoeuvre at a constant flow of 2.5 L·min^{-1} and to maintain a constant expiratory oral pressure (30 cmH_2O) to close the velum, thus excluding nasal NO [20]. The exhaled air was absorbed at a sample flow of 200 mL·min^{-1}. NO signals were simultaneously displayed on a chart recorder (Hewlett-Packard HP3396 SERIES III Integrator; Hewlett-Packard, Palo Alta, CA, USA) and compared with the signal generated from a calibration mixture of NO in nitrogen. At least two successive recordings at 2-min intervals were made and the mean of the peak values of two reproducible readings was used in the analysis of the results.

Sputum induction and examination

Fifteen minutes after fenoterol (400 μg, inhalation) pretreatment, hypertonic saline (4%) inhalation was performed using an ultrasonic nebulizer (MU-32; Sharp Co., Osaka, Japan). Since the samples contained saliva, this contamination was eliminated by both visual inspection and inverted microscope examination [12, 13]. Hypertonic saline inhalation was continued for 15–30 min until the sputum volume was ~1 mL. The nebulizer generated particles with a mean mass median aerodynamic diameter of 5.4 μm at an output of 2.2 mL·min^{-1}.

Ten microlitres of homogenized sputum was used for the eosinophil count. The remaining sputum samples were immediately mixed with saline, boiled to avoid degradation of bradykinin and stored at -70°C until assay. The sputum samples (1 mL) for bradykinin assay were mixed with isopropyl alcohol (2.5 mL) and centrifuged for 10 min at 790 × *g* at 4°C. The supernatant was mixed with petroleum ether (2 mL) and the upper layer aspirated; this manipulation was repeated. The lower layer was evaporated under nitrogen at 790 × *g* at 55°C, mixed with 50 mM tris (hydroxymethyl)aminomethane (Tris)-HCl (pH 7.6) (500 μL) and centrifuged for 5 min at 790 × *g* at 4°C. The sample (400 μL) or standard bradykinin (Peptide Institute Inc., Osaka, Japan) was mixed with antibradykinin (100 μL; SRL Inc., Tokyo, Japan) and incubated overnight at 4°C. Next, they were incubated with ^{125}I-tyr^{8}-bradykinin (100 μL; DuPont NEN Research Products, Boston, MA, USA) for 4 h at 4°C. The mixtures were mixed with 1% γ-globulin (100 μL) and 25% polyethylene glycol 6000 (mean molecular weight 7,500 Da; Wako Pure Chemical Industries, Osaka, Japan) (700 mL), and centrifuged for 10 min at 790 × *g* at 4°C, to separate the antibody-bound peptide from the free form. The supernatant was discarded, and the radioactivity in the pellet was measured using a gamma counter (1261 Multi Gamma; Wallac Oy, Turku, Finland). The sensitivity of this assay to immunoassayable bradykinin in saline was 1.0–3,000 pg·mL^{-1}. If the bradykinin concentrations were above this range, the samples were diluted and measured again.

The percentage eosinophil reduction was determined from the difference in eosinophil counts before and after steroid therapy.

Methacholine inhalation challenge

Airway responsiveness to inhaled methacholine was measured using a device (Astograph TCK-6000CV; Chest Co.) that displays respiratory resistance (R_{rs}) measured *via*

the forced oscillation method during tidal breathing with continuous inhalation of the aerosolized drug [21]. Briefly, it consists of an aerosol delivery system, a loudspeaker box system which generates a constant-amplitude sine wave pressure at 3 Hz, and a system for measuring R_{rs} automatically from mouth flow and mouth pressure. Aerosols were generated by a Bird nebulizer (Bird Corp., Palm Springs, CA, USA), each containing 4 mL of solution driven with a constant airflow of 6 $L \cdot min^{-1}$ by an air compressor to elicit an output of ~0.15 $mL \cdot min^{-1}$. The output was determined by measuring the change in weight of the nebulizer chamber. Methacholine (Sigma Chemical Co., St Louis, MO, USA) was prepared in 0.9% saline in two-fold increasing concentrations ranging 0.049–25 $mg \cdot mL^{-1}$. After it was confirmed that a 1-min inhalation of saline did not change the baseline R_{rs}, each concentration of methacholine solution was inhaled for 1 min until R_{rs} reached approximately twice the baseline value or until the maximum concentration was administered. The index of the airway responsiveness was defined as the cumulative provocative dose of methacholine causing a 35% increase in R_{rs} (PD_{35}).

Study protocol

On the first day, after exhaled NO and FEV_1 measurement, sputum induction was carried out. The methacholine inhalation challenge test was performed on a separate day. After baseline value assessment, open unblinded uncontrolled steroid inhalation therapy (800 μg $beclomethasone \cdot day^{-1}$) was administered to all subjects for 8 weeks, and then the same examination was repeated. All bronchodilator therapies were stopped ≥24 h before the examination.

Statistical analysis

A linear regression analysis was performed using the method of least squares. The PD_{35} was log-transformed in the analysis. A p-value of <0.05 was considered significant.

Results

Baseline airway calibre, inflammatory indices and cumulative provocative dose of methacholine causing a 35% increase in respiratory resistance

The baseline FEV_1 of the enrolled subjects were normal to moderate (48.5–133% of the predicted value) and all subjects showed enhanced airway responsiveness (PD_{35} 0.25–25 $mg \cdot mL^{-1}$) (table 1). All airway inflammatory indices, namely bradykinin concentration, percentage of eosinophils in the induced sputum and exhaled NO concentration, were elevated in all subjects (table 1).

The results of multiple regression analysis of airway responsiveness (PD_{35}) and baseline airway calibre (FEV_1 % pred) against inflammatory indices are summarized in table 2. The FEV_1 was inversely related to both exhaled NO levels ($p<0.05$) and bradykinin concentration in the induced sputum ($p<0.05$) but not to the percentage of eosinophils in the induced sputum. In contrast, the PD_{35} had no significant correlation with any of the airway inflammatory indices.

Table 2. – Correlation between airway inflammatory indices and physiological parameters

	PD_{35}		FEV_1	
	Pre	Post	Pre	Post
Exhaled NO concentration	-0.17 NS	0.32 NS	-0.53 <0.05	-0.04 NS
Bradykinin concentration in induced sputum	-0.22 NS	0 NS	-0.48 <0.05	-0.22 NS
Eosinophils (%) in induced sputum	-0.30 NS	0.26 NS	-0.30 NS	-0.21 NS

PD_{35}: cumulative provocative dose of methacholine causing a 35% increase in respiratory resistance, which was log transformed in the analysis; FEV_1: forced expiratory volume in one second (percentage of the predicted value); pre: before steroid therapy; post: after steroid therapy; NS: nonsignificant.

As shown in figure 1, baseline FEV_1 andPD_{35} correlated positively ($p<0.01$). However, there was no significant correlation between PD_{35} and any of the AIIs.

Effect of steroid inhalation therapy

Eight weeks′ steroid inhalation therapy improved FEV_1 and PD_{35} as well as airway inflammatory indices (table 1). In contrast to the data before steroid administration, the significant correlation between FEV_1 (% pred) and inflammatory parameters such as exhaled NO levels and bradykinin concentration in the sputum disappeared after steroid treatment (table 2). The percentage increase in FEV_1 after steroid therapy correlated significantly with the reduction in bradykinin concentration ($p<0.01$) and eosinophil counts ($p<0.01$) in the induced sputum and exhaled NO concentration ($p<0.01$) (fig. 2). The degree of PD_{35} improvement after steroid treatment also correlated significantly with the reductions in the above three airway inflammatory indices ($p<0.01$ for each) (fig. 3). Further, the steroid-mediated PD_{35} improvement correlated significantly with the percentage increase in FEV_1 after steroid therapy ($p<0.01$) (fig. 4).

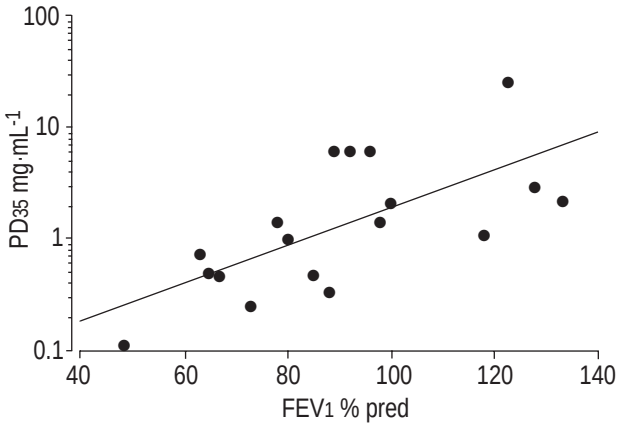

Fig. 1. – Baseline forced expiratory volume in one second (FEV_1) and airway responsiveness to methacholine (cumulative provocative dose of methacholine causing a 35% increase in respiratory resistance (PD_{35})). The line corresponds to the fitted regression equation ($r=0.67$, $p<0.01$).

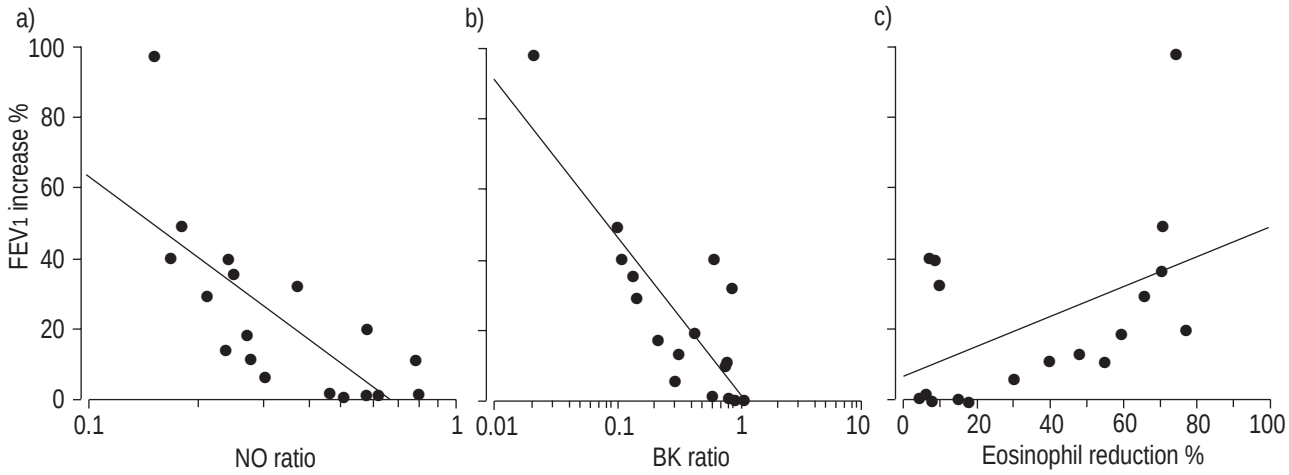

Fig. 2. – Relationship between steroid-mediated improvement in airway calibre (percentage increase in forced expiratory volume in one second (FEV_1)) and airway inflammatory indices: the ratio of a) nitric oxide (NO) in exhaled gas ($r=-0.73$, $p<0.001$) and b) bradykinin (BK) in induced sputum ($r=-0.84$, $p<0.001$) (post-steroid level/pre-steroid level); and c) percentage eosinophil reduction in induced sputum (difference in eosinophil counts before and after steroid therapy) ($r=0.48$, $p<0.05$). The lines correspond to the fitted regression equation.

Discussion

It has been demonstrated that, in asthmatic subjects: 1) airway responsiveness to methacholine correlated positively with baseline airway diameter but not with airway inflammatory indices such as exhaled NO levels, eosinophil counts and bradykinin concentration in induced sputum; 2) 8 weeks′ uncontrolled steroid inhalation therapy improved both airway calibre and responsiveness in proportion to the reduction in the airway inflammatory parameters; and 3) the improvement in airway responsiveness after steroid therapy significantly correlated with the improvement in airway diameter.

Asthma is defined by three characteristic features, namely intermittent reversible airway obstruction, AHR and airway inflammation [2]. In the present study, baseline airway responsiveness correlated significantly with baseline airway calibre, in agreement with a previous study [22], suggesting that airway geometric factors are involved in the mechanisms of AHR in asthmatics. The underlying mechanisms of the airway calibre reduction observed in asthmatic subjects may be due to airway inflammation [1, 2]. In the present study, it was observed that two of three inflammatory indices, *i.e.* exhaled NO levels and sputum bradykinin concentration, showed a significant inverse relation with airway calibre, supporting the above hypothesis.

In contrast to the significant relationship between airway calibre and responsiveness, the three airway inflammatory parameters employed in the present study, namely sputum eosinophil counts, exhaled NO levels and bradykinin concentration in sputum, exhibited no significant relationship with airway responsiveness. To date, the relationship between airway responsiveness and inflammatory cells, such as eosinophil recruitment into the airways, has been the subject of much controversy. Some reports have shown

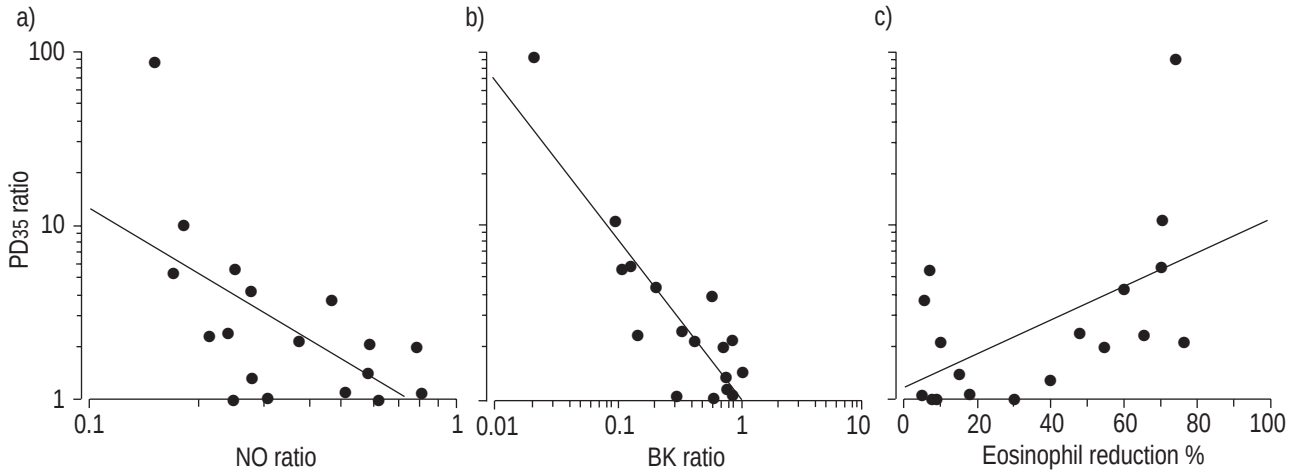

Fig. 3. – Relationship between steroid-mediated improvement in airway responsiveness to methacholine (cumulative provocative dose of methacholine causing a 35% increase in respiratory resistance (PD_{35})) and airway inflammatory indices: the ratio of a) nitric oxide (NO) in exhaled gas ($r=-0.61$, $p<0.01$) and b) bradykinin (BK) in induced sputum ($r=-0.88$, $p<0.001$) (post-steroid level/pre-steroid level); and c) percentage eosinophil reduction in induced sputum (difference in eosinophil counts before and after steroid therapy) ($r=0.55$, $p<0.05$). The PD_{35} ratio was the ratio of post-steroid PD_{35} to pre-steroid PD_{35}. The lines correspond to the fitted regression equation.

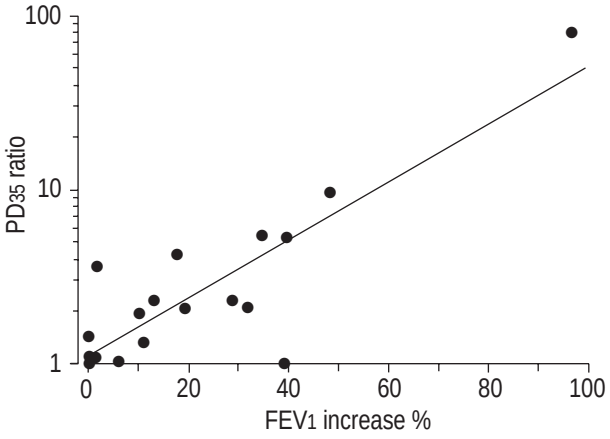

Fig. 4. – Relationship between steroid-mediated improvement in airway responsiveness to methacholine (cumulative provocative dose of methacholine causing a 35% increase in respiratory resistance (PD_{35}), post-steroid PD_{35}/pre-steroid PD_{35}) and airway calibre (percentage increase in forced expiratory volume in one second (FEV_1)). The line corresponds to the fitted regression equation ($r=0.85$, $p<0.001$).

a positive relationship between the presence of inflammatory cells and enhanced airway responsiveness [3–6], whereas others have failed to establish such a relationship [7–11]. The present data is compatible with the latter reports.

It has been reported that NO production is exaggerated in asthmatic airways [15], and there is increasing evidence that high concentrations of NO may contribute to asthmatic airway inflammation *via* development of the T-helper 2 lymphocyte response and through chemotaxis of eosinophils [23, 24]. A recent report has demonstrated that exhaled NO levels show a significant correlation with airway responsiveness, *i.e.* asthmatic subjects with higher NO concentrations show more enhanced airway responsiveness [25], which is in contrast to the present results.

Bradykinin has potent inflammatory action [26–28] and its upregulation has been reported in asthmatic airways [17, 18]. The present study is the first report examining the relationship between airway responsiveness and bradykinin concentration in the airways, and no significant relation between the two was found.

A possible explanation for the lack of association between airway inflammation and responsiveness may be as follows. Airway inflammation seems to cause airway hyperresponsiveness *via* two mechanisms [2]. One mechanism is through the release of chemical mediators end the other is *via* cytokine and chemokine mechanisms. The former mechanism affects airway function for a short duration. In contrast, the later mechanism works for a relatively longer duration *via* recruitment of additional inflammatory cells and modification of airway resident cells, such as epithelial cells and airway smooth muscle cells, resulting in airway remodelling [2]. Baseline airway responsiveness is likely to be determined by two factors, one of which is active inflammation and the other airway remodelling. Airway inflammation assessment by means of exhaled NO levels and sputum examination (eosinophil counts and bradykinin concentration) may well reflect the former component but not the latter. Therefore, although airway inflammation contributes greatly to airway hyperresponsiveness in asthmatic subjects [1], the two phenomena have often appeared dissociated. This hypothesis may be supported by the present steroid treatment data.

In the present study, 8 weeks′ uncontrolled steroid treatment largely improved airway responsiveness, as has also been shown in previous studies [29, 30]. The improvement in airway responsiveness after steroid therapy correlated well with each inflammatory parameter change after steroid therapy, suggesting that the steroid-sensitive component, in other words the reversible component of airway responsiveness, in asthmatic subjects, depends upon the degree of reduction in airway inflammatory changes. Although steroid treatment largely reduced airway responsiveness as well as inflammatory indices, airway hyperresponsiveness was still observed even after treatment. The persistent airway hyperresponsiveness may be due to airway remodelling and other mechanisms such as those due to genetic factors.

In conclusion, it has been shown that airway responsiveness exhibits significant correlation with baseline airway calibre but not with airway inflammatory parameters such as exhaled NO levels, eosinophil counts and bradykinin concentration in the airways. The improvement in airway diameter and responsiveness during uncontrolled therapy with inhaled steroids depends upon the degree of reduction in the above three airway inflammatory indices. Because the changes in airway calibre and responsiveness show a significant positive relationship, steroid therapy may change airway responsiveness, at least in part, *via* a reduction in airway narrowing. The latter requires further, placebo-controlled study.

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