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Inhaled steroids modify bronchial responses to hyperosmolar saline

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ABSTRACT: We investigated the effects of inhaled beclomethasone dipropionate (BDP) on airway sensitivity (provocative dose producing a 20% fall in forced expiratory volume in one second (FEV_1) from baseline (PD_{20})) and reactivity (slope of the dose-response curve) to inhaled aerosols of hyperosmolar (4.5%) saline, and histamine or methacholine.

This was an open study on 13 patients referred to the laboratory by their respiratory physician for investigation of their asthma. These challenges were performed on separate days before (initial visit) and 8.8±0.8 (SD) weeks (range 5.6–12.4 weeks) after (visit 1) a treatment period with BDP (dose range 600–1,500 $\mu g \cdot day^{-1}$).

At visit 1 there was a significant reduction in sensitivity to 4.5% NaCl and histamine/methacholine and in reactivity. The PD_{20} increased 5.6 fold for 4.5% NaCl and 4.1 fold for histamine/methacholine. All patients remained responsive to histamine/methacholine and a fall in FEV_1 >20% to 4.5% saline was documented in 10 of the 13 patients.

We conclude that treatment with BDP reduces sensitivity and reactivity to both osmotic and pharmacological challenge.

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Patients with symptoms of asthma, who are either receiving treatment with aerosol corticosteroids or are about to commence treatment with steroids, are frequently referred to the pulmonary function laboratory for assessment of bronchial responsiveness. Bronchial provocation with inhaled histamine and methacholine are the most commonly requested tests for assessing airway sensitivity and reactivity [1] to inhaled stimuli. These pharmacological agents are thought to act at specific receptor sites on bronchial smooth muscle, causing it to contract. In recent years, bronchial provocation tests using aerosols of hyperosmolar saline have been developed [2, 3]. Hyperosmolar challenges are thought to cause the release of chemical mediators from mast cells in response to a change in airway osmolarity [4–9]. Thus, hyperosmolar challenge could be a useful technique to assess response to treatment with a corticosteroid, a therapy which is thought to reduce mast cell numbers [10].

Inhaled beclomethasone dipropionate (BDP) and budesonide, when taken regularly, reduce airway sensitivity to histamine and methacholine, as demonstrated by an increase in the dose of these agents required to cause a 20% reduction in FEV_1 [11–14]. The effects of inhaled corticosteroids on airway reactivity, that is the slope of the dose-response curve, has not been studied formally although KRAAN *et al.* [12] noted that patients remained "hyperreactive during the treatment".

The effects of treatment with aerosol corticosteroids on sensitivity and reactivity to hyperosmolar saline has not been reported. If the presence of inflammatory cells contributes to the airway responses observed with hyperosmolar aerosols, and if the activity of these cells is reduced by inhaled corticosteroids, then it would be expected that sensitivity and reactivity to hyperosmolarity would also be reduced. If so, hyperosmolar challenge may be a useful laboratory test to evaluate the effects of corticosteroids.

The aim of this pilot study was to evaluate the effect of regular treatment with aerosol beclomethasone dipropionate on the airway responses to hyperosmolar (4.5%) saline. The responses were compared with those obtained from bronchial challenge with histamine or methacholine aerosols, which are now used to document changes in airway sensitivity after long-term treatment with corticosteroids.

Patients and methods

Thirteen asthmatic patients, who had asthma as defined by the American Thoracic Society [15] and who

had been referred to the Pulmonary Function laboratory by their physician for bronchial provocation tests, were studied. The patients were included in the study if they had a provoking dose of histamine or methacholine which caused a 20% fall in FEV_1 (PD_{20}) of <4.0 μmol and a PD_{20} to 4.5% saline <15 ml.

The patients were selected because they were either about to commence (Group 1, n=5), or had commenced, regular treatment within the last 6 weeks with inhaled beclomethasone (Group 2, n=5), or they had been instructed by their referring physician to increase their dose of inhaled beclomethasone (Group 3, n=3), based on a worsening of their asthma symptoms.

Anthropometric details, lung function [16], dose of inhaled beclomethasone and the PD_{20} to 4.5% saline and histamine and methacholine are given in table 1.

Symptoms were controlled by beta-adrenoceptor agonists, anticholinergics and sodium cromoglycate. These medications were withheld for at least 6 h before the bronchial provocation test. Patients did not take corticosteroids systemically for the duration of the study. Patients gave their informed consent and the protocol was approved by the Ethics Committee of the Royal Prince Alfred Hospital. There was no placebo control in this study because it was considered unethical to change the treatment schedule of the referring physician.

Patients were challenged with 4.5% NaCl and histamine or methacholine on two separate days within a period of 8 days. They were challenged again 8.8±0.8 (SD) weeks later on two separate days.

Histamine or methacholine aerosol challenges

Histamine or methacholine challenges were used because either one or the other was requested by their physician. We continued to assess airway responsiveness during treatment with corticosteroids with the same challenge that was initially requested. The potency of histamine and methacholine are similar, such that the PD_{20} for these challenges, expressed in molecular weight units, has been shown to be similar when compared in the same patient [17].

The method used to deliver the histamine and methacholine aerosol was developed by YAN *et al.* [18], although the maximum cumulative dose of histamine delivered to each patient was increased to approximately 6.4 μmol, which is higher than that described in the original method.

The challenge was stopped either when FEV_1 fell >20% from the post-saline (0.9%) value, or after the maximum dose of histamine or methacholine was administered.

4.5% NaCl aerosol challenge

The method of delivery of the 4.5% NaCl aerosol from an ultrasonic nebulizer ($Mist O_2 gen$, Timeter, Penn, USA) is described by SMITH and ANDERSON [3]. Patients inhaled 4.5% NaCl for increasing periods (0.5, 1.0, 2.0, 4.0 and 8.0 min). Some patients who were particulary sensitive to this challenge inhaled the 4.5% saline for only part of the time indicated. FEV_1

Table 1. – Anthropometric details, lung function, provoking dose of 4.5% NaCl and histamine or methacholine (Hist/Mch) on patients initial visit, and daily doses of inhaled beclomethasone dipropionate (μg) (BDP)

Pts	Age yr	Sex	Ht cm	Initial FEV_1 % pred	4.5% NaCl ml	Hist/Mch μmol	BDP μg	Rx
Group 1								
NS	21	F	157	84	4.94	0.54	800	S
GL	24	M	172	76	1.31	0.05	1500	S
PB	40	M	174	86	1.69	0.02	1000	S
TR	28	M	173	63	0.09	0.03	800	S
DS	32	M	163	75	4.25	0.3	600	S
Group 2								
HC	23	M	182	103	8.15	2.03	800	S
PM	23	M	193	98	2.87	0.7	1000	S,C
DA	38	F	164	85	2.57	0.03	800	S
SR	19	F	170	72	0.11	0.29	1500	S
JM	20	M	177	60	0.71	0.02	1000	S,C
Group 3								
LT	21	F	166	88	0.2	0.1	600/1000	S
AP	16	M	180	67	1.04	0.31	400/800	S,I,C
LH	25	F	161	69	1.72	0.03	1000/1500	S

Group 1 was commencing BDP; Group 2 had commenced taking BDP in the 6 weeks before the initial visit; and Group 3 had their regular dose of BDP increased. Other regular asthma medications are given in the Rx column. Rx: prescription; S: salbutamol; I: ipratropium; C: sodium cromoglycate; Pts: patients; FEV_1: forced expiratory volume in one second. The FEV_1 values represent the mean of the values measured on the initial 4.5% saline and Hist/Mch days. For Group 3, the initial dose of BDP and the increased dose of BDP are reported.

was measured 1 min after each inhalation period. If, at the end of the 8 min challenge, a patient recorded a fall in FEV_1 which was >10% but <20%, then the aerosol challenge was extended for another 4 or 8 min. The nebulizer bowl and tubing were weighed (Sartorius 1216MP, Gottingen, Germany) before and after the completion of the challenge to calculate the output and, thus, dose of 4.5% NaCl delivered to each patient.

Bronchodilators were administered at the completion of the challenges either by metered dose inhaler, or *via* a jet nebulizer.

Expression of results

To evaluate the effect of the treatment on lung function, the values for FEV_1, expressed as a percentage of the predicted normal value, were compared before the provocation challenge on each test day.

To compare changes in sensitivity to the inhaled aerosols, the doses of aerosol (μmol or ml) required to provoke a 20% fall in FEV_1 (PD_{20}) were compared initially and after treatment with beclomethasone. The PD_{20} after treatment was expressed as a ratio of PD_{20} observed on the initial visit. As an additional indicator of change in sensitivity the lowest values for FEV_1, expressed as a percentage of predicted, measured after the same dose of the challenge aerosol had been given, were compared on the initial visit and again on visit 1.

To determine if improvement in PD_{20} was related to change in baseline FEV_1, the ratio of

visit 1 FEV_1 % predicted : initial visit FEV_1 % predicted

was compared with the ratio

visit 1 $PD_{20}FEV_1$: initial visit $PD_{20}FEV_1$

To assess changes in reactivity in response to treatment, an index relating change in FEV_1 in response to a unit dose of the aerosol used for challenge was compared before and after the treatment period. This has been termed the reactivity index and is a measure of the slope of the dose-response curve [1]. Where possible, this index was measured over the same range of FEV_1 when FEV_1 was expressed as a percentage of the predicted value.

For example:

$$\text{Reactivity index} = \frac{\text{Change in } FEV_1 \text{ (\% predicted)}}{\text{Dose of 4.5\% NaCl (ml), histamine or methacholine (mmol)* required to produce this change in } FEV_1}$$

*: mmol rather than μmol was used.

Thus, for example, for 4.5% NaCl:

$$\text{Reactivity index}_{\text{(before treatment period)}} = \frac{82\text{-}49}{2.95} = 11.18\% \text{ predicted units of } FEV_1 \cdot ml^{-1}$$

$$\text{Reactivity index}_{\text{(after treatment period)}} = \frac{82\text{-}49}{10.99} = 3.0\% \text{ predicted units of } FEV_1 \cdot ml^{-1}$$

A comparison was made between sensitivity as measured by the PD_{20} and reactivity as measured by the reactivity index.

Individual dose-response curves were constructed relating % fall in FEV_1 and FEV_1 (% predicted) to the cumulative dose of aerosol delivered to the patient.

Statistical analysis

The statistical analysis has been made on the group of 13 patients in order to assess approximately 8 weeks of therapy.

The values for FEV_1 (% predicted) were compared using a paired t-test. Values for PD_{20} and the change in FEV_1 (% predicted) per unit dose (*i.e.* the reactivity index) were log transformed and compared using a paired t-test. The geometric mean and 95% confidence limits are reported.

A Spearman's rank correlation coefficient rho (r_s) [19] was used to compare: 1) sensitivity to 4.5% NaCl and histamine/methacholine using PD_{20} values on the two challenges; 2) sensitivity (PD_{20}) and reactivity (reactivity index) for each patient for both types of challenge; 3) the effect of beclomethasone on the different provocation tests, *i.e.* the ratio for the

PD_{20} visit 1 : the PD_{20}

on the initial visit measured when 4.5% NaCl was compared with the same ratio measured after histamine or methacholine; 4) to assess the relationship between the ratio of the

% predicted FEV_1 visit 1 : initial visit

and the ratio

PD_{20} visit 1 : initial visit.

The fold difference was used to assess shifts in the PD_{20} to 4.5% NaCl and histamine/methacholine over the treatment period. It was calculated by taking the antilog value of the mean of the differences of the log PD_{20} values for the group of 13 patients.

Results

Initial visit

The individual and mean values for FEV_1 (% predicted), the lowest value recorded for FEV_1 (% predicted) at a dose of the challenge aerosol which was common to the test days before and after the treatment period, the PD_{20} and the change in FEV_1 (% predicted) per unit dose (reactivity index), and the statistical findings are illustrated in figures 1 and 2.

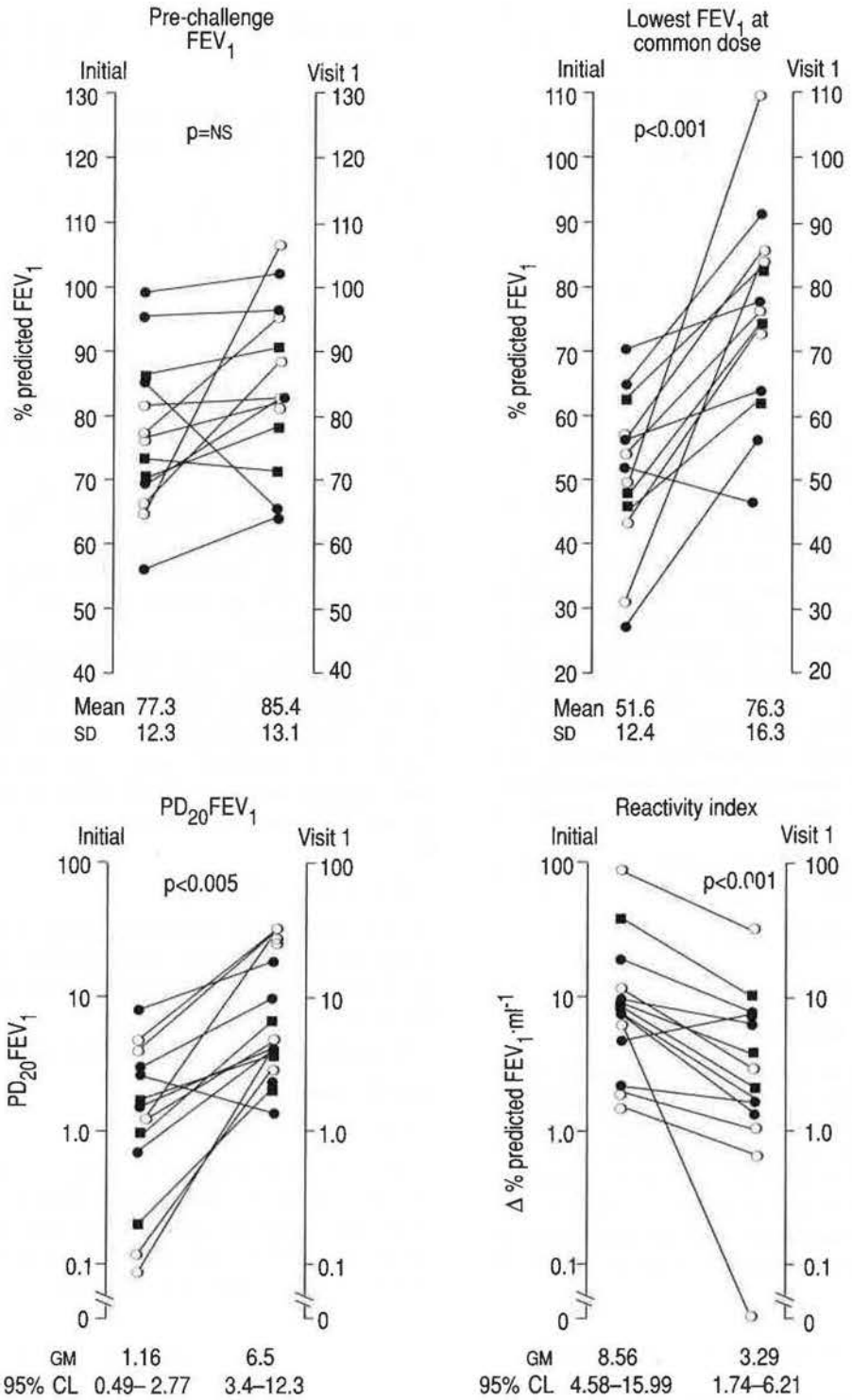

Fig. 1. – Results for 4.5% NaCl challenge performed on the initial visit and repeated on visit 1, approximately 2 months later. In patient GL the reactivity index to 4.5% NaCl equalled zero after the treatment period. To calculate the geometric mean, GL was given a value less than the second lowest reactivity index for the group, *e.g.* GL=0.6, second lowest=0.69. FEV_1: forced expiratory volume in one second; $PD_{20}FEV_1$: provocative dose producing a 20% fall in FEV_1 from baseline; CL: confidence limit; SD: standard deviation; GM: geometic mean. ○: Group 1; ●: Group 2; ■: Group 3.

The lung function of the patients varied widely as reflected in the FEV_1. For the whole group the FEV_1 (% predicted), ranged from 56–111%. However, there was no significant difference between the pre-challenge FEV_1 (% predicted) on the days when 4.5% saline (77.3±12.3%) and histamine/methacholine were performed (79.6±15.7%).

When the sensitivity to 4.5% NaCl was compared with the sensitivity to histamine/methacholine the correlation was not significant when the PD_{20} values for

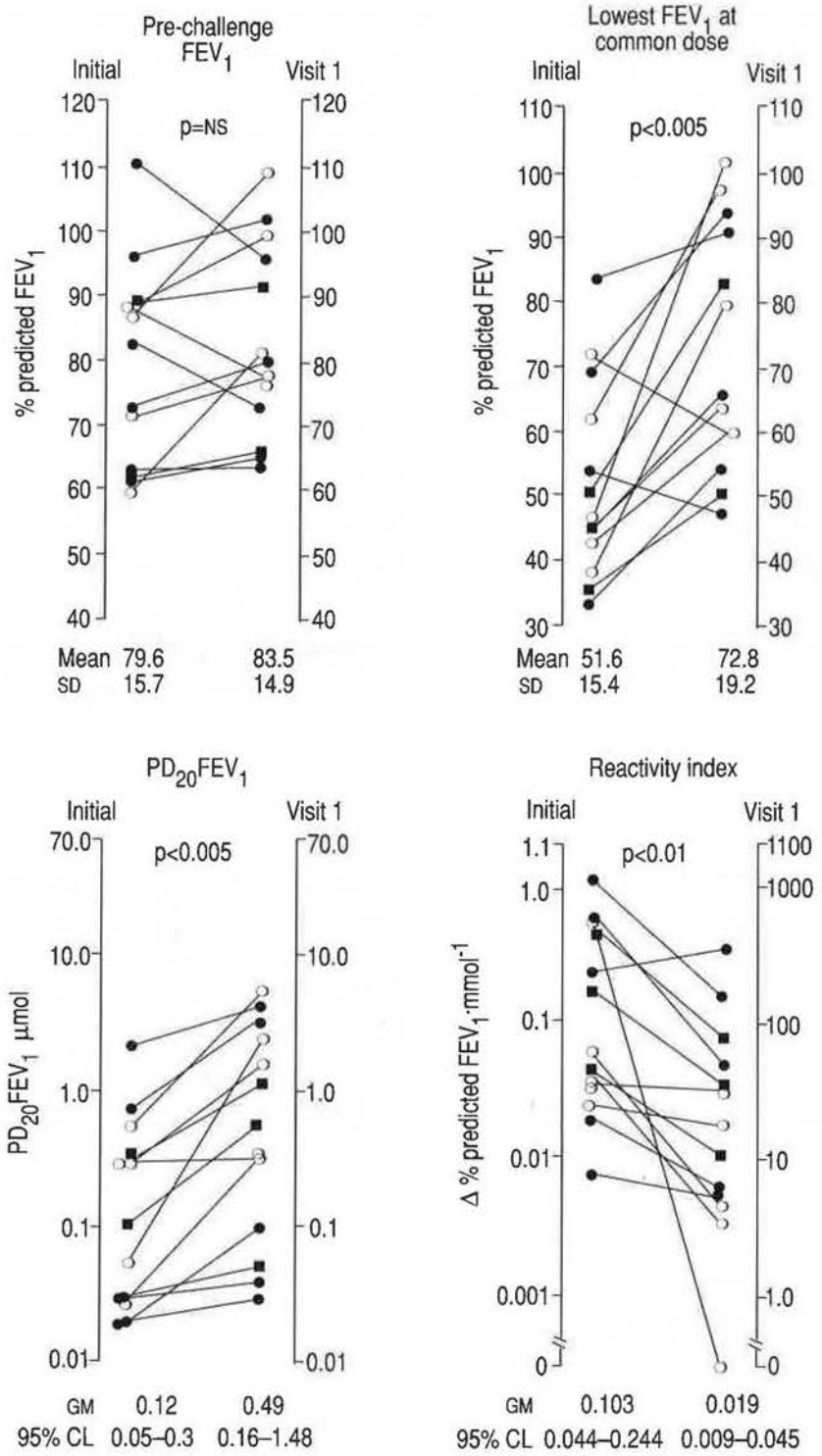

Fig. 2. – Results for histamine/methacholine challenges performed on the initial visit and repeated on visit 1, approximately 2 months later. In patient TR the reactivity index to histamine equalled zero after the treatment period. To calculate the geometric mean, TR was given a value less then the second lowest reactivity index for the group, *e.g.* TR=3.5, second lowest value=3.53. For abbreviations and key see legend to figure 1.

the two tests were ranked (r_s=0.53, p=NS, n=13). There was a highly significant relationship between the sensitivity and reactivity to 4.5% NaCl (r_s=0.92, p<0.01) and between the sensitivity and reactivity to histamine/methacholine (r_s=0.72, p<0.01).

Visit 1 approximately 8 weeks later: 4.5% NaCl challenge

The PD_{20} measured 8 weeks later was expressed as a ratio of the PD_{20} recorded on the initial visit and the

values ranged from 0.63–45.7 with a median value of 5.5. There was a 5.6 fold increase in the dose of 4.5% NaCl required to induce a 20% fall in FEV_1.

There was a significant relationship between the sensitivity and reactivity index (r_s=0.85, p<0.01).

The individual dose-response curves are given in figure 3a and b. It should be noted that the FEV_1 continued to fall beyond a level of 20% in most patients as they continued to be challenged with 4.5% saline. Only three patients had <20% fall in their FEV_1 (15, 15 and 18%). For these three patients the values for $PD_{20}FEV_1$ were given as the maximum dose (27, 28.7, 18.7 ml) or calculated by extrapolation.

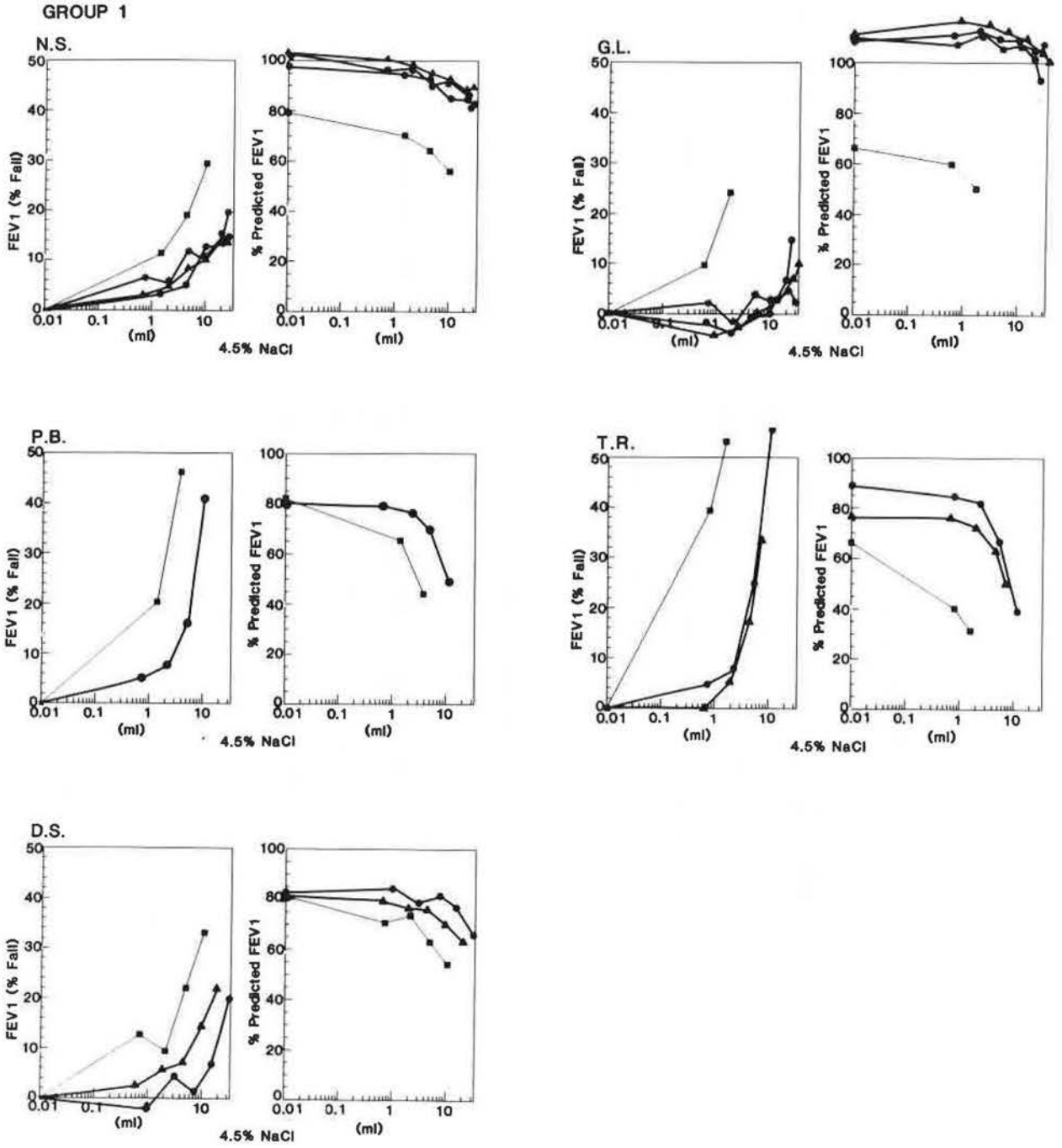

Fig. 3. – Individual dose-response curves for 4.5% NaCl aerosol challenges in the 13 asthmatic subjects. The results are expressed as the % fall in FEV_1 (left column) and the % predicted FEV_1 (right column) in relation to the cumulative dose of 4.5% NaCl (ml) delivered) Group 1 (n=5): Subjects who were about to commence BDP (NS, GL, PB, TR, DS). Continued on next page.

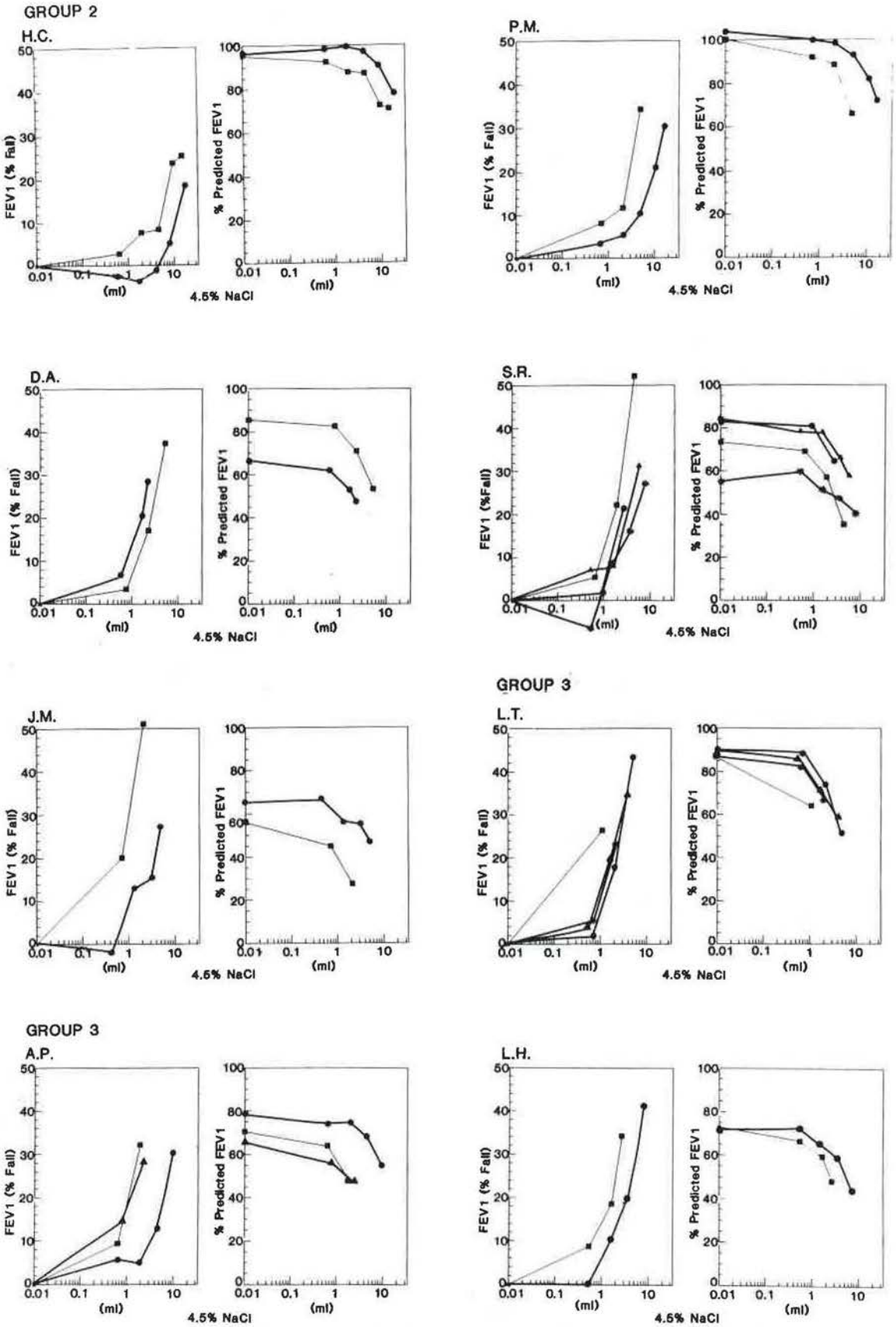

Fig. 3b. – Group 2 (n=5): had commenced BDP (HC, PM, DA, SR, JM) in the last 6 weeks. Group 3 (n=3) were subjects who had increased their daily dose of BDP (LT, AP, LH). —■—: Initial visit; —●—: Visit 1; —▲—: Visit 2; —✳—: Visit 3 N.B. 0.01 on the X-axis represents pre-challenge values. BDP: Beclomethasone diproprionate.

There was no significant relationship between the ratio of

visit 1 : initial visit FEV_1 % predicted

and the ratio of

visit 1 : initial visit PD_{20} (r_s=0.49, p=NS, n=13).

Thus, the increase in PD_{20} was not related to increase in FEV_1.

Histamine/methacholine challenge

The PD_{20} improved in all patients and the ratio of the PD_{20} at 8 weeks to PD_{20} on the initial visit ranged from 1.07–44.0 with a median value of 4.94. This was a 4.1 fold increase in the dose of histamine/methacholine required to induce a 20% fall in FEV_1.

There was a significant relationship between sensitivity and the reactivity index (r_s=0.79, p<0.01).

There was a significant relationship between the ratio of the

visit 1 : initial visit FEV_1 % predicted

and the ratio of the

visit 1 : initial visit PD_{20} (r_s=0.84, p<0.01, n=13).

Thus, the increase in FEV_1 was related to the increase in PD_{20}.

The FEV_1 continued to fall when the challenge was continued with increasing concentrations of histamine/methacholine in all patients.

Responses to 4.5% NaCl compared with histamine and methacholine

There was no significant difference between the values for FEV_1 recorded before both challenges (figs 1 and 2). When the values for PD_{20} recorded after 8 weeks were compared, there was a significant relationship observed between the sensitivity to 4.5% NaCl and histamine/methacholine (r_s=0.75, p<0.01). Thus, those patients who were most sensitive to the effects of 4.5% NaCl were now most sensitive to the effects of inhaled histamine and methacholine, a relationship which was not evident on the initial visit. There was not a significant relationship in the change in sensitivity between 4.5% NaCl and histamine/methacholine (r_s=0.44, p=NS, n=13).

Discussion

The results of this study provide evidence that treatment with beclomethasone dipropionate reduces the sensitivity to both osmotic and pharmacological challenge in asthmatic patients. The change in sensitivity occurred both when patients were treated with inhaled beclomethasone for the first time and when the dose was increased. This confirms the findings of previous studies, which used histamine and methacholine for the bronchial provocation test [11–13]. The magnitude of the shift in sensitivity in the present study is similar or better than that reported by others using the same technique [13]. This study extends these findings by demonstrating that a decrease in sensitivity also occurs in response to an osmotic challenge. Our patients, with some exceptions, however, remained responsive to these aerosol challenges even though they were less sensitive than they had been before the treatment period.

We do not think that the changes in sensitivity to inhaled 4.5% NaCl can be accounted for by an increase in FEV_1 after the treatment period. Although there was a relationship between changes in lung function and PD_{20} for challenge with histamine/methacholine, this was not observed with 4.5% NaCl. Even at a time when there were only small increases in baseline FEV_1, there were marked increases in PD_{20} and a reduction in the lowest FEV_1 recorded in response to the same dose of aerosol. This suggests that the measurement of FEV_1 alone does not reflect the benefits of regular treatment with BDP.

Other studies have confined their measurements to the documentation of PD_{20}. In this study, for the majority of patients the challenge aerosol was continued and we recorded a >20% fall in FEV_1 in response to 4.5% NaCl in 10 patients. Airway responses to histamine/methacholine and 4.5% NaCl were measured on a number of occasions after the 8 week treatment period in 7 of the 13 patients. Of these patients, only one (GL) had achieved a normal response [2] to 4.5% NaCl but this occurred only after 8 months of daily treatment with beclomethasone. The other 6 patients remained responsive to 4.5% NaCl.

The documentation of falls in FEV_1 >20% in patients who are considered to be well controlled, by criteria commonly accepted as reflecting severity of disease (*i.e.* resting lung function, PD_{20}, symptoms) is important and should alert the patient and doctor to the possibility of severe attacks occurring in response to continuous exposure to a known irritant, for example exercise [20].

It is possible that higher doses of beclomethasone or a longer period of treatment, may have resulted in more patients achieving falls <20%. However, the average dose of a 1,000 $\mu g \cdot day^{-1}$ and the duration of treatment used here is commonly prescribed in clinical practice.

We consider that measuring the slope of the dose-response curve (reactivity) over the same change in % predicted FEV_1 is superior to measuring it over the same absolute change in FEV_1. We believe that this technique makes it valid to compare the before and after treatment values for reactivity.

In a study such as this, where a drug treatment is being investigated, it is useful to know the effect of the drug on both airway sensitivity and reactivity to the stimulus. While sensitivity gives the dose of a stimulus causing the airways to narrow by a set amount, reactivity is a measure of the rate of change of lung function in response to the stimulus.

Thus, these indices give information about both the position and the slope of the dose-response curve and are both important in assessing bronchial responsiveness [21].

The change in sensitivity and reactivity to aerosols of hyperosmolar saline after treatment with aerosol steroids has not previously been reported. The findings in this study suggest that this challenge may be useful for documenting improvement in response to treatment. Furthermore, there are a number of reasons to suggest that challenge with a non-isotonic aerosol may be preferable to challenge with histamine or methacholine. First, an increase in osmolarity of the airways is a natural stimulus and one that is probably commonly encountered in daily life during exercise or hyperventilation [22, 23]. Second, the airway response is likely to be a result of the endogenous release of chemical mediators in response to a change in osmolarity. Third, the drugs used in the treatment of asthma such as sodium cromoglycate and nedocromil sodium [24, 25] prevent the response to these challenges and, thus, their potential use and their dose may be better identified in patients by using an osmotic challenge.

The precise mechanism whereby an increase in osmolarity leads to acute airway narrowing in patients with asthma is not known. It is generally accepted that mast cell release of mediators is involved [4–6, 26]. Whilst histamine is an important mediator of the response it is unlikely to be the only mediator involved [6–9]. Recent studies by UMENO *et al.* [27] suggest that tachykinin release from sensory nerve endings may also be important in hyperosmolar challenge. Furthermore, there is evidence of interaction between mast cell release of mediators and sensory nerve stimulation [28].

On the basis of the PD_{20} values to histamine and methacholine the patients included in this group would be assessed clinically as having moderate to severe bronchial hyperresponsiveness before treatment and mild to moderate bronchial hyperresponsiveness after treatment [29]. The findings in this study that airways of these asthmatics remained responsive with continued exposure to the inciting stimulus supports the contention by STERK and BEL [21] that there is a need to distinguish between changes in sensitivity and airway narrowing in response to treatment. The suggestion by WOOLCOCK *et al.* [30] that the demonstration of a plateau may be the important feature determining a reduction in risk from severe asthma is also supported by the findings in this study.

Aerosol corticosteroids are commonly prescribed by many physicians as first line treatment in mild as well as moderate and severe asthma. It is often difficult to assess the patient in the laboratory before the commencement of treatment. Although this study was not placebo-controlled we think that documentation of change in severity can be measured adequately even when the patient has recently commenced treatment or had a change in dose. We have shown for the first time that changes in sensitivity and reactivity in response to treatment with steroids can be assessed by using a challenge with hyperosmolar saline. These changes do not appear to be dependent on improvement in resting lung function. The observation that patients may have significant improvement in sensitivity and reactivity but remain responsive was an unexpected finding. We would now recommend, for the laboratory assessment of asthma severity, that the duration of the provoking stimulus is increased in order to document the potential for the airways to narrow.

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