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Effect of inhaled budesonide on seasonal changes in sensitivity and maximal response to methacholine in pollen-sensitive asthmatic subjects

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Effect of inhaled budesonide on seasonal changes in sensitivity and maximal response to methacholine in pollen-sensitive asthmatic subjects. L. Prieto, J.M. Bertó, V. Gutiérrez, C. Tornero. ©ERS Journals Ltd 1994.

ABSTRACT: The aim of this study was to investigate the effect of inhaled budesonide on modifications of the provocative concentration of agonist causing a 20% fall in forced expiratory volume in one second (FEV_1) (PC_{20}) and maximal response plateau to inhaled methacholine during the pollen season in pollen-sensitive subjects with mild asthma.

The effects of inhaled budesonide (800 μg·day^{-1}) on the threshold value (PC_{20}) and maximal response plateau to inhaled methacholine were studied in 28 pollen-sensitive subjects with mild asthma during a pollen season in a randomized, double-blind, placebo-controlled parallel fashion. They were challenged with methacholine (up to 200 mg·ml^{-1}) in February (preseasonal assessment) and during the middle of the pollen season (in May and again in June).

Subjects treated with budesonide (n=13) were protected from the decrease in PC_{20} seen in the placebo (n=15) group (geometric mean PC_{20} placebo group: preseasonal=8.51, May=2.19 and June=1.78 mg·ml^{-1}; budesonide group: preseasonal=3.71, May=3.23 and June=2.40 mg·ml^{-1}; intergroup differences in doubling concentrations, $p<0.05$). Among 10 subjects in the placebo group who reached plateau during the preseasonal assessment, seven lost the plateau in May and six in June. In the budesonide group, among seven subjects who reached plateau during the preseasonal assessment, three lost the plateau in May and four in June. Between-group differences were not significant.

We conclude that in pollen-sensitive subjects with mild asthma, inhaled budesonide blocks the effects of natural allergen exposure on the methacholine threshold value. However, we were not able to show a significant protection against the pollen-induced changes on the maximal response plateau.

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There is convincing evidence [1, 2] that, in sensitized asthmatic subjects, natural allergen exposure during a pollen season results in increased airway responsiveness, and drugs with anti-inflammatory properties, such as methylprednisolone [2], sodium cromoglycate [3], nedocromil sodium [4], and nasal beclomethasone [5] protect against this seasonal increase in airway responsiveness. In these studies, the bronchoconstriction induced by the pharmacological agent was measured in a dose-response fashion, and the airway responsiveness was expressed as the provocative concentration of agonist causing a 20% fall in forced expiratory volume in one second (FEV_1) (PC_{20}). However, from the clinical standpoint, airway hyperresponsiveness can be regarded as the tendency of the airways to narrow too easily and too much. Thus, assessment of bronchial responsiveness requires not only a threshold response to an inhaled agonist but an overall assessment of the shape of the curve [6, 7].

Inhaled glucocorticosteroids have proved to be at least as effective as nedocromil sodium or sodium cromoglycate at reducing the provoked increase in airway responsiveness following allergen challenge in the laboratory [8]. However, it is not known whether inhaled corticosteroids protect against the seasonal increase in bronchial responsiveness induced by natural allergen exposure.

In a previous study [9], we have shown that in pollen-sensitive asthmatic patients the cessation of exposure to pollen is associated with a significant increase in methacholine PC_{20} and decrease in the maximal response-plateau level. In the current study, we investigate the effect of inhaled corticosteroid therapy on modifications of PC_{20}, and maximal response to inhaled methacholine during the pollen season in sensitized subjects with asthma.

Subjects and methods

Subjects

Thirty nonsmoking subjects (8 males) aged 15–52 yrs, from our out-patient Allergy Clinic, were studied, having met the following inclusion criteria: a history of mild seasonal asthma [10] for at least 2 yrs; an FEV_1 greater than 80% of the predicted value; a positive skin-prick test (≥3 mm wheal diameter) to grass and/or Parietaria pollens and no skin sensitization for other allergens tested, namely olive, house dust mites, Alternaria, Aspergillus, Neurospora, Penicillium, Cladosporium, and cat and dog dander (Abelló S.A., Madrid, Spain). Patients with significant renal, hepatic or cardiovascular disease, pregnant women, and subjects having experienced chest infections within 4 weeks before the start of the study, were excluded. Out of the pollen season, these subjects had no asthma symptoms and none had taken bronchodilators, inhaled steroids (nasal or bronchial) or other respiratory anti-inflammatory drugs within 5 months before the first evaluation.

The study was approved by the Hospital Medical Assays Committee and all subjects gave their informed consent.

Study design

In our region [9, 11], the concentration of Urticaceae (Parietaria) pollen increases in April, and peaks between May and the beginning of September. Data on grass pollen indicate that the concentration increases in April and plateaus between May and July.

We used a randomized, double-blind, placebo-controlled, parallel study design. Subjects were first evaluated in February 1993, before the pollen season (preseasonal assessment), when baseline pulmonary function and methacholine bronchial challenge were performed. They were then randomized to receive budesonide (Olfex Bucal, Aristegui, Bilbao, Spain) 400 µg *b.i.d.* or placebo, which they commenced taking in March. Budesonide and placebo were administered using a metered-dose inhaler (MDI) with a 750 ml spacer device (Inhalventus, Aldo-Union, Barcelona, Spain). One puff of the preparation contained 200 µg of budesonide. Patients were instructed to take their study medication upon awakening and approximately 12 h later, and were trained during the first and every follow-up visit to use the MDI and spacer correctly. New study drugs were dispensed at each visit. At subsequent visits the canisters were returned and weighed. Treatment compliance was reported as the actual canister weight loss as a percentage of the expected weight loss. The latter was estimated from the known weight loss per dose and the number of doses prescribed.

Salbutamol MDI (Ventolin, Glaxo, Madrid, Spain) and/or terfenadine (Rapidal, Aristegui, Bilbao, Spain) and/or sodium cromoglycate eye drops (Cusicrom, Cusi S.A., Barcelona, Spain) were used on an "as needed" basis to control their pulmonary, nasal, or eye symptoms, respectively. Subjects were asked not to take salbutamol for at least 8 h and terfenadine for at least 72 h before each challenge.

Subjects returned to the laboratory at the height of the pollen season (seasonal assessment) in May and again in June (after 8 and 12 weeks of treatment), having taken their last dose of trial medication the evening before. Spirometry and methacholine bronchial challenges were performed by personnel blinded to the earlier challenge results.

Measurement of bronchial responsiveness

Spirometry was performed [12] on a dry rolling seal spirometer (PFT Horizon System Two, Sensormedics Co., Anaheim, CA, USA). On each test day, baseline FEV_1 and forced vital capacity (FVC) were determined as the best of three consecutive measurements that agreed within 5%. Reference values of CRAPO *et al.* [13] were used for young patients (15–25 yrs of age) and those of the European Community for Coal and Steel [14] for subjects aged over 25 yrs. Each patient was required to have an FEV_1 of at least 80% of the predicted value and no more than a 7% fall in FEV_1 after inhalation of phosphate-buffered saline (PBS). Moreover, the variation in the prechallenge FEV_1 on the three study days had to be within 10%.

Methacholine provocation tests were performed using a tidal breathing method adapted from COCKCROFT *et al.* [15]. Aerosols were delivered by a Hudson 1720 nebulizer (Temecula, CA, USA) with 2 ml of test solution in the container and operated by oxygen (mean±SD nebulizer output, 0.19±0.018 $ml \cdot min^{-1}$). The nebulizer was connected directly to a mouthpiece, a noseclip was worn, and the aerosol was inhaled through the mouth by tidal breathing for 2 min. After inhalation of PBS, subjects inhaled doubling concentrations of methacholine (Sigma Chemical, St. Louis, MO, USA) from 0.095 to 200 $mg \cdot ml^{-1}$, diluted in PBS. Subjects were experienced at performing forced expiratory manoeuvres, and a single determination of FEV_1 was taken 60–90 s after each methacholine concentration [16], unless the trace was uninterpretable because of cough. The challenge was stopped when the FEV_1 dropped by more than 50% from the post-PBS value or when the highest concentration of methacholine had been administered.

Dose-response curves were plotted for each challenge test as the percentage fall in FEV_1 against the log methacholine concentration and were characterized by their threshold value (PC_{20}), and if possible, by their maximal response plateau.

Data analysis

Methacholine PC_{20} was calculated from the log concentration-response curves by linear interpolation of the two adjacent data points. A PC_{20} value of 200 $mg \cdot ml^{-1}$ was assigned to six subjects (three in each group) in the preseasonal assessment, and to two subjects (budesonide

group) in May, in whom FEV_1 dropped less than 20% even when the highest concentration of methacholine was used. All PC_{20} values were log-transferred before analysis. Changes in PC_{20}-methacholine between the three study periods were also expressed in terms of doubling concentrations of methacholine calculated as $\Delta\log PC_{20}/\log 2$. Changes in prechallenge FEV_1, and methacholine PC_{20} were assessed by analysis of variance of repeated measurements within each randomized group, and by an unpaired t-test to compare between groups.

A maximal response plateau was defined when three or more of the highest concentrations of methacholine produced no further fall in FEV_1 (within 5% response ranges). The level of the maximal response was obtained [17] by averaging the data points on the plateau. The prevalence of plateau in each period was compared by the Fisher's exact test. Based on previous data [9], the power of our analysis to detect a 50% difference in the proportion of patients who lost the plateau during the pollen season was 65% with a level of significance of 0.05.

Results

Of the 30 subjects initially recruited, two (budesonide group) were withdrawn from the study before the completion of the first month of treatment. One patient declined the previous acceptance of participation, whilst the other withdrew due to pregnancy. Only the data for the remaining 28 subjects who completed the study period were included in the final analysis. Of those who completed, 13 were randomized to receive budesonide and 15 to receive placebo. The two groups (table 1) did not differ with respect to age, sex, pulmonary function, skin sensitization, or bronchial responsiveness (PC_{20} and prevalence or level of plateau). Those taking budesonide started with greater bronchial responsiveness (PC_{20} and level of plateau), although the differences were not significant.

Table 1. – Subject characteristics at enrolment

Characteristic	Placebo	Budesonide
Subjects n	15	13
Age yrs*	35±2	33±3
Sex M/F	4/11	4/9
Skin sensitization		
Grass	7	5
Parietaria	6	5
Grass + Parietaria	2	3
FEV_1 % pred*	102±3	101±3
PC_{20} mg·ml^{-1}#	8.5 (0.42–200)	3.7 (0.15–200)
Plateau yes/no	10/5	7/6
Level of plateau* % FEV_1	24.8±4.8	26.0±3.1

PC_{20}: provocation concentration of methacholine producing a 20% fall in FEV_1; FEV_1: forced expiratory volume in one second; level of plateau: average of the data points on the plateau in the seven subjects of the budesonide group and 10 subjects of the placebo groups in whom plateau was detected. *: data presented as mean±SEM; #: data presented as geometric mean and range in parenthesis.

Table 2. – FEV_1 (l) values before methacholine challenge in each period and treatment compliance for the two treatment groups

Subj. No.	Preseasonal assessment	Seasonal assessment May	Seasonal assessment June	Compliance % expected
Placebo group				
1	3.10	2.92	2.93	92
2	4.34	3.97	4.16	81
3	4.52	4.52	4.39	70
4	2.70	2.90	2.92	86
5	2.83	3.02	3.11	101
6	3.68	3.79	3.68	66
7	3.61	3.48	3.46	99
8	3.63	3.73	3.73	85
9	3.29	3.28	3.12	68
10	3.34	3.40	3.26	89
11	4.30	4.30	4.32	97
12	2.95	2.74	2.69	91
13	4.27	4.56	4.48	83
14	2.63	2.62	2.64	137
15	3.30	3.31	3.40	95
Mean	3.50	3.50	3.48	89
SEM	0.16	0.16	0.16	
Budesonide group				
16	2.86	2.85	2.69	98
17	2.60	2.52	2.70	91
18	4.72	4.62	4.72	92
19	3.96	4.26	4.27	103
20	3.35	3.65	3.38	88
21	3.28	3.18	3.08	98
22	2.80	2.60	2.62	102
23	3.85	3.76	3.75	70
24	2.70	2.62	2.80	80
25	3.68	3.59	3.47	91
26	2.82	2.82	2.81	105
27	2.60	2.40	2.61	87
28	3.27	2.98	3.18	94
Mean	3.27	3.22	3.24	92
SEM	0.18	0.19	0.18	

Subj.: subject; FEV_1: forced expiratory volume in one second.

Individual values for FEV_1 in each period before methacholine challenges are shown in table 2. Baseline FEV_1 was not significantly different for all visits ($p>0.05$). Mean treatment compliance rate (table 2) was 89% in the placebo group and 92% in the budesonide group. No subject experienced an exacerbation for which prednisolone was prescribed.

Values of PC_{20} before and during the pollen season for the two treatment groups are shown in table 3. Geometric mean (range) methacholine PC_{20} decreased in the placebo group from 8.51 (0.42–200) during the preseasonal assessment to 2.19 (0.13–47.1) and to 1.78 (0.10–35.0) mg·ml^{-1} in May and June, respectively ($p<0.01$). In the budesonide group, there was a decrease from 3.71 (0.15–200) in the preseasonal assessment to 3.23 (0.23–200) and to 2.40 (0.19–145.8) mg·ml^{-1} in May and

Table 3. – Individual values of PC_{20} methacholine ($mg \cdot ml^{-1}$) before and during the pollen season for the two treatment groups

Subj. No.	Preseasonal assessment	Seasonal assessment May	June
Placebo group			
1	0.80	0.16	0.33
2	6.88	8.15	1.80
3	1.67	0.80	1.09
4	7.35	3.46	3.79
5	0.74	0.28	0.20
6	1.94	1.27	2.01
7	3.87	1.33	1.67
8	35.0	8.94	13.2
9	200	8.64	11.2
10	45.5	40.6	35.0
11	5.72	2.11	3.12
12	200	2.21	1.05
13	200	47.1	14.8
14	0.42	0.13	0.10
15	7.72	0.91	0.42
Geometric mean	8.51	2.19	1.78
Budesonide group			
16	200	200	145.8
17	1.33	0.89	0.76
18	1.80	1.73	2.76
19	0.36	0.35	0.63
20	0.16	0.41	0.76
21	3.95	23.3	2.20
22	4.99	1.59	2.99
23	200	1.73	1.88
24	6.59	7.23	1.22
25	200	200	43.7
26	0.43	1.70	1.74
27	0.15	0.23	0.19
28	3.09	3.06	3.05
Geometric mean	3.71	3.23	2.40

Subj.: subject; PC_{20}: provocative concentration of agonist causing a 20% fall in forced expiratory volume in one second.

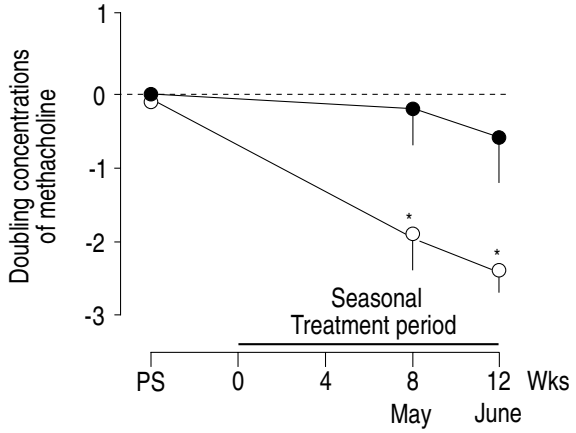

Fig. 1. – Mean±SEM change in PC_{20} (doubling concentrations of methacholine) during the pollen season for the two treatment groups. —●—: budesonide; —○—: placebo. PS: preseasonal assessment; PC_{20}: provocative concentration producing a 20% fall in FEV_1; FEV_1: forced expiratory volume in one second. *: $p<0.05$ compared to the budesonide group.

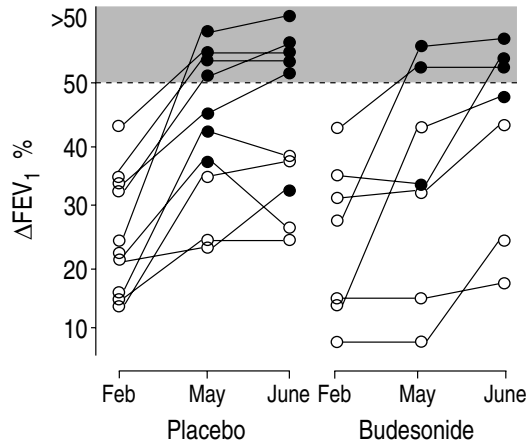

Fig. 2. – Maximal percentage fall in forced expiratory volume in one second (ΔFEV_1) during each period in 10 subjects of the placebo group and 7 subjects of the budesonide group in whom plateau was detected during the preseasonal period. —○—: with plateau; —●—: without plateau. Shaded area: Δ >50%.

June, respectively ($p>0.05$). Between-group analysis (fig. 1) showed that in the budesonide group, the PC_{20} decreased by (mean±SEM) 0.19±0.60 and 0.63±0.64 doubling concentrations in May and June, respectively, as compared with 1.95±0.44 and 2.25±0.52 doubling concentrations in the placebo group ($p<0.05$).

In the placebo group, a maximal response plateau on the concentration-response curves was detected in 10 subjects during the preseasonal assessment, in three subjects in May ($p<0.05$), and in four individuals in June ($p<0.05$). In the budesonide group, seven subjects showed plateau during the preseasonal assessment, four in May and three in June ($p>0.05$). The proportion of subjects (fig. 2) who lost the plateau after natural pollen exposure was not different in each group ($p>0.05$, power =65%). Moreover, in five subjects who reached a maximal response plateau in all three periods, the level of plateau increased (fig. 3) during the seasonal assessment both in the placebo group (two subjects) and in the budesonide group (three subjects).

Discussion

In this study, we have shown that in pollen-sensitive subjects with mild asthma, treatment with inhaled budesonide blocks the effects of natural allergen exposure on the methacholine threshold value, and yet has no effect on changes in the maximal response plateau. Furthermore, this study confirms and extends earlier observations [1, 9] that natural antigenic exposure is associated with significant modifications both in the threshold (PC_{20}) and shape (maximal response plateau) of the concentration-response curves to inhaled methacholine.

In pollen-sensitive asthmatic subjects, natural exposure to pollen has been shown [1, 2] to decrease the threshold value of the dose-response curves to inhaled pharmacological agents. In a previous article, we reported that, in pollen-sensitive asthmatic patients, the cessation of exposure to pollen is associated with a significant decrease of the maximal response-plateau level to inhaled

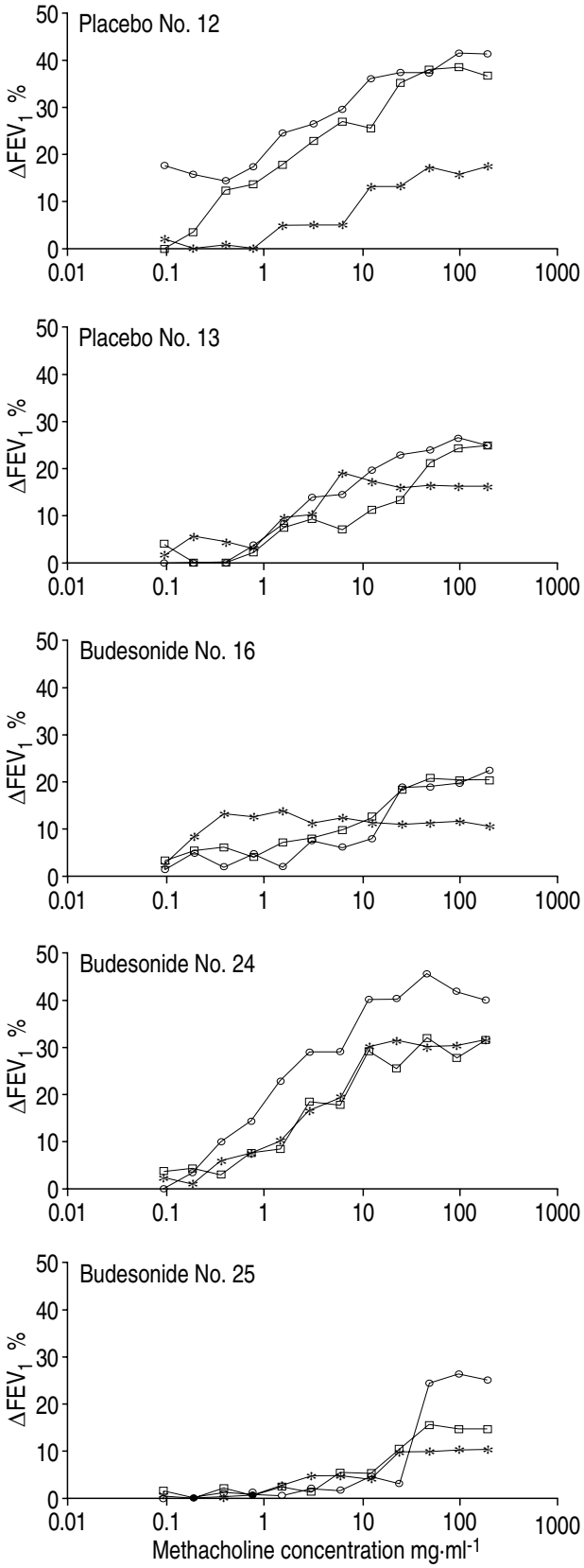

Fig. 3. – Concentration-response curves to methacholine in subjects who reached a maximal response plateau in all three challenges. The level of plateau increased during the pollen season in two subjects of the placebo group (Nos. 12 and 13) and in three subjects of the budesonide group (Nos. 16, 24 and 25). —✳—: preseasonal; —□—: May; —○—: June. For abbreviations see legend to figure 2.

methacholine [9], but this is the first study to document that natural pollen exposure causes the loss of the plateau in a considerable number of subjects.

A primary goal of this investigation was to determine the effect of inhaled budesonide on modifications of concentration-response curves to inhaled methacholine after natural allergen exposure. Increased airway responsiveness has been shown [18–21] after allergen-induced early and/or late asthmatic reactions in the laboratory. Moreover, the development of a late asthmatic response is preceded by an increased airway responsiveness [22], suggesting that the airway changes that increase responsiveness start before the late asthmatic reaction is clinically evident. Drugs, such as inhaled steroids [23, 24], sodium cromoglycate [8], and nebulized salbutamol [25] have been shown to prevent increases in airway responsiveness following exposure to specific allergens in the laboratory. However, provocation with nebulized allergen extract is entirely different from natural exposure to pollen grains. Under conditions of natural exposure during a pollen season, the allergen load may be greater and the duration of exposure is longer. Therefore, a single inhalation of an allergen in the laboratory can cause a mild increase in nonspecific airway responsiveness, whereas repeated allergen exposures, such as exposures that occur during the pollen season, can cause greater increases in airway responsiveness.

In a study by BONER *et al*. [26], it was observed that a 2 month treatment with beclomethasone dipropionate increased the methacholine PC_{20}, even during a period of maximal allergen exposure. Moreover, a 7 day treatment with oral methylprednisolone (16 mg·day^{-1}) was shown [2] to reverse the seasonal decrease in provocative dose of agonist causing a 20% fall in FEV_1 (PD_{20})-carbachol in patients allergic to grass pollen. This suggested that the decrease in the threshold value observed during the period of allergen exposure could be reversed or prevented by oral and (probably) by inhaled steroid therapy. Recently, CORREN *et al*. [5] showed that treatment with nasal beclomethasone dipropionate prevented the decrease in methacholine PC_{20} associated with seasonal allergen exposure in pollen-sensitive patients with mild asthma, but this was not confirmed in a study by ARMITAGE *et al*. [27]. To the best of our knowledge, there are no studies that have investigated the effects of treatment with inhaled corticosteroids on the increase in airway responsiveness during the pollen season in pollen-sensitive asthmatic subjects. The results of our study demonstrate that in pollen-sensitive asthmatic patients, inhaled budesonide abolishes the effects of natural allergen exposure on the threshold response to inhaled methacholine. Interestingly, this was independent of any improvement in spirometry.

On the other hand, airway responsiveness to pharmacological agonists is usually measured in terms of the PD_{20} or PC_{20} histamine or methacholine. Beneficial effects of inhaled corticosteroids on threshold response to inhaled pharmacological agents have already been observed in asthmatic subjects [28, 29]. However, bronchial hyperresponsiveness is an *in vivo* phenomenon, in which the concentration-response curve shows three characteristics

[7]: decreased threshold value; steepened slope; and increased maximum bronchoconstriction. The only functional characteristic that can limit the degree of airway obstruction in asthmatic patients is a plateau at mild degrees of airway narrowing and, thus, the effect of drugs on the maximal response plateau is considered to be relevant [30]. It has been shown [31] that in asthmatic subjects not exposed to the relevant allergens, the maximal response can be altered by inhaled budesonide without changing the threshold of the dose-response curve. Moreover, although the plateau correlates with the threshold value [32], changes in the characteristics of the dose-response curve may be at least partially independent of each other [7]. In our placebo group, the increase or loss of the plateau during the pollen season was associated with a significant decrease of the PC_{20}. However, budesonide protects against the seasonal modifications in the threshold, but has no effect on pollen-induced changes in the maximal response plateau. This supports the hypothesis [31, 33] that the plateau and the threshold response to inhaled methacholine are not modulated by the same mechanisms.

Although, in this study, it was not possible to assess the clinical benefit of inhaled budesonide on seasonal exacerbations of asthma in pollen-sensitive subjects, our observations are relevant to the pharmacological management of pollen-induced asthma. A drug which is able to reduce the effect of natural allergen exposure on the threshold value of the methacholine dose-response curve could be useful in the treatment of seasonal asthma [34]. However, it has been suggested that the asthma therapy should not be limited to increasing the threshold value, but also towards restoring the mechanism (plateau) which protects against excessive bronchoconstriction [30, 33]. Therefore, our results suggest that inhaled budesonide can be insufficient for protection of patients with seasonal asthma during the pollen season. However, the insufficient protection of inhaled budesonide on seasonal modifications in the plateau should be interpreted with caution. Although we intentionally chose patients with mild seasonal asthma in order to safely document a maximal response plateau to methacholine during the preseasonal assessment, this was not possible in 11 out of 28 patients at entry into the study. Moreover, plateau levels could be measured in only five patients in the three periods, and this small number of subjects precluded comparison of the plateau levels. We were not able to show a significant protection of inhaled budesonide against the pollen-induced modifications in the plateau, but this may be due to the limited number of subjects in whom plateau could be compared. The findings of our study would obviously need to be extended to a larger number of subjects with plateau.

In summary, in this study we have shown that in pollen-sensitive asthmatic subjects, a 12 week treatment with inhaled budesonide (800 $\mu g \cdot day^{-1}$) blocks the effect of natural pollen exposure on the threshold value of the concentration-response curves to inhaled methacholine, but we were not able to show a significant protection against the pollen-induced modifications in the maximal response plateau.

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