Anti-inflammatory effects of salmeterol compared with beclomethasone in eosinophilic mild exacerbations of asthma: A randomized, placebo controlled trial

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BACKGROUND: Salmeterol is a potent long acting beta-agonist that is effective in relieving the symptoms and airflow limitation of asthma.

OBJECTIVE: To determine whether the effect of salmeterol on clinical parameters in a mild eosinophilic exacerbation of asthma was similar to that of beclomethasone dipropionate (BDP) and, thus, is due to an anti-inflammatory property.

PATIENTS AND METHODS: Thirty-four asthmatics with a persistent increase in symptoms for at least two weeks and an increase of sputum eosinophils of 4% or more were randomized in a double-blind fashion to one of three groups that received daily treatment with 100 μg salmeterol, 1 mg BDP or placebo in divided doses using identical pressurized inhalers. Patients were treated with study medications for three weeks, followed by one week of open label BDP (500 μg bid). Patients were seen at weekly intervals, and sputum and blood were obtained on each visit. The primary outcome measure was a change in sputum eosinophils, and secondary outcomes were changes in blood eosinophils, eosinophilic cationic protein (ECP) and clinical parameters. Three patients (one in each group) could not produce any sputum after randomization and were excluded from the analysis.

RESULTS: Twelve patients received salmeterol, 10 received BDP and nine received placebo. Salmeterol treatment had no effect on sputum eosinophils geometric mean, (from 35.5 [24.9] to 26.9% [25.8]), blood eosinophils (from 7.6 [4.8] to 7.2% [3.9]) or ECP (from 33.1 [18.1] to 27.8 [16.3] mg/L) but improved morning peak expiratory flow (PEF) and diurnal variation of PEF, and decreased the use of rescue medication more than placebo ($P<0.05$ for all comparisons). In contrast, BDP improved both inflammatory indexes (sputum eosinophils from 22.5 [17.9] to 5.7% [6.8], blood eosinophils from 9.0 [5.5] to 2.1% 1.0, and serum ECP from 36.5 [22.0] to 16.1 [10.1] mg/L) as well as clinical parameters.

CONCLUSIONS: These results show that salmeterol improves the symptoms and airway function of patients with asthma, but has no effect on eosinophilic airway infiltration. These findings support current asthma guidelines, which rec-

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ommend the initial use of inhaled steroid to maximize clinical improvement. While salmeterol also produces clinical improvement, it does not suppress sputum eosinophilia. The analysis of induced or spontaneous sputum for inflammatory indexes may be a valuable clinical test to guide the use of inhaled steroid and/or a long acting beta-agonist.

Key Words: *Airway inflammation, Asthma, Beclomethasone, Salmeterol, Sputum eosinophils, Sputum examination*

Les effets anti-inflammatoires du salmétérol par rapport au béclométhasone dans les légères exacerbations asthmatiques à éosinophiles : Un essai aléatoire contrôlé contre placebo

RÉSUMÉ : Le salmétérol est un puissant béta-agoniste à action prolongée qui soulage les symptômes et la diminution des débits respiratoires dont souffrent les asthmatiques.

L'OBJECTIF : Établir si l'effet du salmétérol sur les paramètres cliniques d'une légère exacerbation asthmatique à éosinophiles est semblable à celui du dipropionate de béclométhasone (DBM) et que cet effet est donc attribuable à ses propriétés anti-inflammatoires.

LES PATIENTS ET LES MÉTHODES : Trente-quatre asthmatiques présentant un accroissement persistant de leurs symptômes depuis au moins deux semaines et une augmentation des éosinophiles dans les expectorations d'au moins 4 % ont été placés au hasard dans l'un des trois groupes qui recevaient quotidienneement 100 μg de salmétérol, 1 mg de DBM ou un placebo en doses divisées, au moyen d'inhalateurs doseurs identiques et ce, selon la méthode à double insu. Les patients ont reçu les médicaments à l'étude pendant trois semaines, puis un traitement ouvert de DBM pendant une semaine (500 μg bid). Ils étaient vus toutes les semaines et remettaient un échantillon de sang et d'expectoration à chaque visite. Le principal indicateur de résultat consistait en une modification des éosinophiles des expectorations, et les indicateurs secondaires étaient des modifications des éosinophiles sanguins, de la protéine cationique à éosinophiles (PCÉ) et des paramètres cliniques. Trois patients (un dans chaque groupe) ne réussissaient pas à produire d'expectoration après la répartition au hasard et ont donc été exclus de l'analyse.

LES RÉSULTATS : Douze patients ont reçu du salmétérol, 10, du DBM et neuf, un placebo. Le traitement au salmétérol n'a eu aucun effet sur la moyenne géométrique (DGS) des éosinophiles des expectorations (de 35,5 % [24,9] à 26,9 % [25,8]), des éosinophiles sanguins (de 7,6 % [4,8] à 7,2 % [3,9]) ou de la PCÉ (de 33,1 mg/l [18,1 % mg/l] à 27,8 mg/l [16,3 mg/l]), mais a amélioré à la fois le débit expiratoire de pointe (DEP) matinal et les variations diurnes du DEP, ainsi que l'utilisation d'un médicament de secours davantage que le placebo ($p<0,05$ selon toutes les comparaisons). Par contre, le DBM a amélioré à la fois les indices inflammatoires (éosinophiles des expectorations de 22,5 % [17,9] à 5,7 % [6,8], éosinophiles sanguins de 9,0 % [5,5] à 2,1 % [1,0] et PCÉ sérique de 36,5 mg/l [22,0 mg/l] à 16,1 mg/l [10,1 mg/l]) et les paramètres cliniques.

LES CONCLUSIONS : Ces résultats démontrent que le salmétérol réduit les symptômes et améliore le débit respiratoire des patients asthmatiques, mais qu'il n'a aucune répercussion sur l'infiltration des éosinophiles dans les voies aériennes. Ces observations soutiennent les directives actuelles sur l'asthme, qui recommandent l'utilisation initiale de stéroïdes par aérosol pour porter l'amélioration clinique au maximum. Bien que le salmétérol produise aussi des améliorations cliniques, il ne supprime pas les éosinophiles des expectorations. L'analyse des expectorations induites ou spontanées pour les mesures des indices inflammatoires pourrait représenter une épreuve clinique précieuse afin de guider l'utilisation des stéroïdes par aérosol ou d'un béta-agoniste à action prolongée.

Salmeterol is a selective and potent long acting beta-agonist bronchodilator (1) that was initially considered to have added anti-inflammatory effects. This position, however, has been controversial (2-4). A single dose of salmeterol causes prolonged bronchodilation, protects against a variety of bronchoconstrictive stimuli for at least 12 h (5-8) and prevents or attenuates allergen-induced early and late responses (3,9,10). In mild to moderate asthmatics already using an inhaled steroid, regular use of salmeterol improves symptoms (11) and quality of life (12).

Airway inflammation is considered to be a determinant of asthma exacerbations. Asthma treatment guidelines recommend that exacerbations be treated by adding an inhaled corticosteroid or by increasing the daily dose (13-15). Recently, two large multicentre trials have shown that the addition of salmeterol produces a greater improvement of symptoms and lung function than doubling the dose of inhaled steroid (16,17). This improvement may be due to salmeterol's prolonged bronchodilator effect, to an anti-inflammatory effect or to both actions.

In this study, we examined the clinical and anti-inflammatory effects of salmeterol treatment in mild eosinophilic exacerbations of asthma. We compared these effects with those of inhaled beclomethasone dipropionate (BDP) and placebo in a double-blind, randomized, parallel group study. The anti-inflammatory effects were measured relatively noninvasively by sputum eosinophil counts, peripheral blood eosinophil counts and serum eosinophil cationic protein (ECP). The clinical effects were investigated by recording symptoms, morning and diurnal variation in peak expiratory flow (PEF) and rescue beta-agonist use.

PATIENTS AND METHODS

Subjects: Adults with a mild exacerbation of asthma were recruited from the Firestone Regional Chest and Allergy Clinic, Hamilton, Ontario, family doctor referrals and advertisements (Table 1). All patients had asthma as indicated by episodic wheezing, chest tightness and/or dyspnea, and either reversible airflow limitation (predicted forced expiratory volume in 1 s [FEV_1] less than 70% or FEV_1 to forced vital capacity [FVC] ratio less than 70% increasing by 15% after salbutamol 200 μg, n=10) or methacholine airway hyper-responsiveness (methacholine provocation challenge causing a 20% fall in FEV_1 [PC_{20}] less than 8 mg/mL, n=21) when the spirometric measurements were 70% or more. The asthma was mildly exacerbated, as defined by a history of increased symptoms for at least two weeks that were not spontaneously improving, and/or an increased need for an inhaled short acting beta-agonist, and an FEV_1 after bronchodilator of more than 75% of predicted or of previous best, and sputum eosinophilia of 4% or more (normal less than 2% [18]). All patients were on treatment with inhaled beta-agonist

TABLE 1
Characteristics of subjects at baseline

	Salmeterol	BDP	Placebo	P*
Subjects n	12	10	9	NS
Age (years)	42.0 (15.1)	36.0 (14.9)	38.0 (16.2)	NS
Sex (male)	5	3	4	NS
Smoking (current to ex-smoker)	0:4	1:4	2:1	NS
Number of atopic subjects	8	10	8	NS
On inhaled steroid	4	4	3	NS
Inhaled steroid dose (μg/day)	125 (260)	185 (320)	172 (277)	NS
Symptom score	5.5 (1.2)	6.3 (0.8)	5.7 (1.7)	NS
FEV_1 (L)	2.62 (0.6)	2.72 (0.7)	2.63 (0.9)	NS
FEV_1 (% predicted)	81.1 (13.9)	76.1 (7.9)	69.2 (28.3)	NS
FEV_1:FVC (%)	72 (11)	69 (7)	70 (15)	NS
PC_{20} (range) mg/mL	0.40 (0.03-1.7)	0.25 (0.03-1.31)	0.29 (0.08-1.81)	NS

*Data are expressed as mean and standard deviation. *P is equal to differences between groups. Atopic defines as one or more positive allergy skin prick tests. Inhaled steroid was beclomethasone dipropionate (BDP) or budesonide. Forced expiratory volume in 1 s (FEV_1) predicted values from Crapo (reference 16) or previous best in the last two years. Methacholine provocation challenge causing a 20% fall in FEV_1 (PC_{20}) geometric mean. NS Not significant. VC Vital capacity*

when needed and either no steroid or low to moderate inhaled steroid doses (1000 μg/day or less BDP or equivalent). Patients had to have had no exacerbations of asthma requiring prednisone treatment, emergency room attendance or hospitalization in the previous three months. None had purulent sputum or had used antibiotics during the previous month. None were exposed to relevant seasonal allergens. The hospital research committee approved the study, and all subjects gave written informed consent.

Study design: This was a single centre, randomized, double-blind, parallel group trial with two active and one placebo treatment groups. After stratification by percentage of sputum eosinophils in the initial sputum sample – less than 50%, or 50% or more – suitable subjects were randomized to receive salmeterol (25 μg per actuation, two puffs twice daily), BDP (250 μg per actuation two puffs twice daily) or placebo (two puffs twice daily) for three weeks. Identical pressurized inhalers were used for the delivery of study medication. Subjects were followed for one week after discontinuing the study medications with open label BDP (500 μg twice daily). The randomization sequence was computer generated off-site and administered by the hospital research pharmacist. Patients were seen before randomization (baseline visit) and at weekly intervals for four weeks. At the baseline visit symptoms, medications, spirometry with salbutamol reversibility or a methacholine inhalation test (if the prebronchodilator FEV_1 was 70% or more), allergy skin prick tests, sputum examination for the proportion of eosinophils and blood for eosinophil, and ECP measurements were obtained. Patients were instructed to continue their prestudy treatment; to take the study medication with a large volume spacer device (Ventahaler 750 mL, Glaxo Canada); to record symptoms, medication use, and morning and evening PEF in a daily diary; and to contact a study physician if symptoms worsened or a deterioration of PEF occurred. The four subsequent weekly visits were held at the same time of the day, and the study medication was withheld for 12 h. Diary cards were reviewed, and symptoms, spirometry, blood and sputum measurements were repeated. Patients were withdrawn from the study for noncompliance, if they were unable to produce sputum, if there was a worsening of symptoms requiring increased short acting beta-agonist of eight or more puffs per day, if nocturnal symptoms disturbed sleep and/or if the postbronchodilator FEV_1 fell by 10% or more below the baseline value.

Clinical methods: Patient characteristics were documented by history and a structured questionnaire that graded symptoms and their severity (19). Spirometry, methacholine inhalation tests and allergy skin prick tests were performed using standard procedures (20-23). The diary recorded symptoms of wheeze, chest tightness, dyspnea and cough using a Likkert scale (scores ranged from 1 for most severe to 9 for absent symptoms) (19). The symptom score at each visit was calculated as the average of the scores for individual symptoms. PEF was measured twice daily before taking the study medication with a Mini-Wright peak flow meter (Clement-Clarke International Limited, London, United Kingdom), and the best of three measurements was recorded. Diurnal variation of PEF was calculated as

$$[(\text{high} - \text{low})/\text{high}] \times 100\%$$

Medications were recorded as number of actuations of study medication, short acting beta-agonist and inhaled steroids (if used at the baseline).

Sputum was obtained spontaneously or induced by the inhalation of an aerosol of hypertonic saline as described by Pin et al (24). Sputum selected from saliva was processed as described by Pizzichini et al (25). Venous blood was collected in an EDTA tube, and a differential white blood cell count was obtained using an automated Coulter counter meter (Coulter STKS, Coulter Corporation, Florida). Serum ECP (μg/L) was determined using a sensitive radioimmunoassay (RIA, Kabi Pharmacia Diagnostics AB, Uppsala, Sweden).

Statistical analysis: Sputum, blood and clinical variables were summarized by means and standard deviations except for PC_{20} data, which was log transformed and summarized

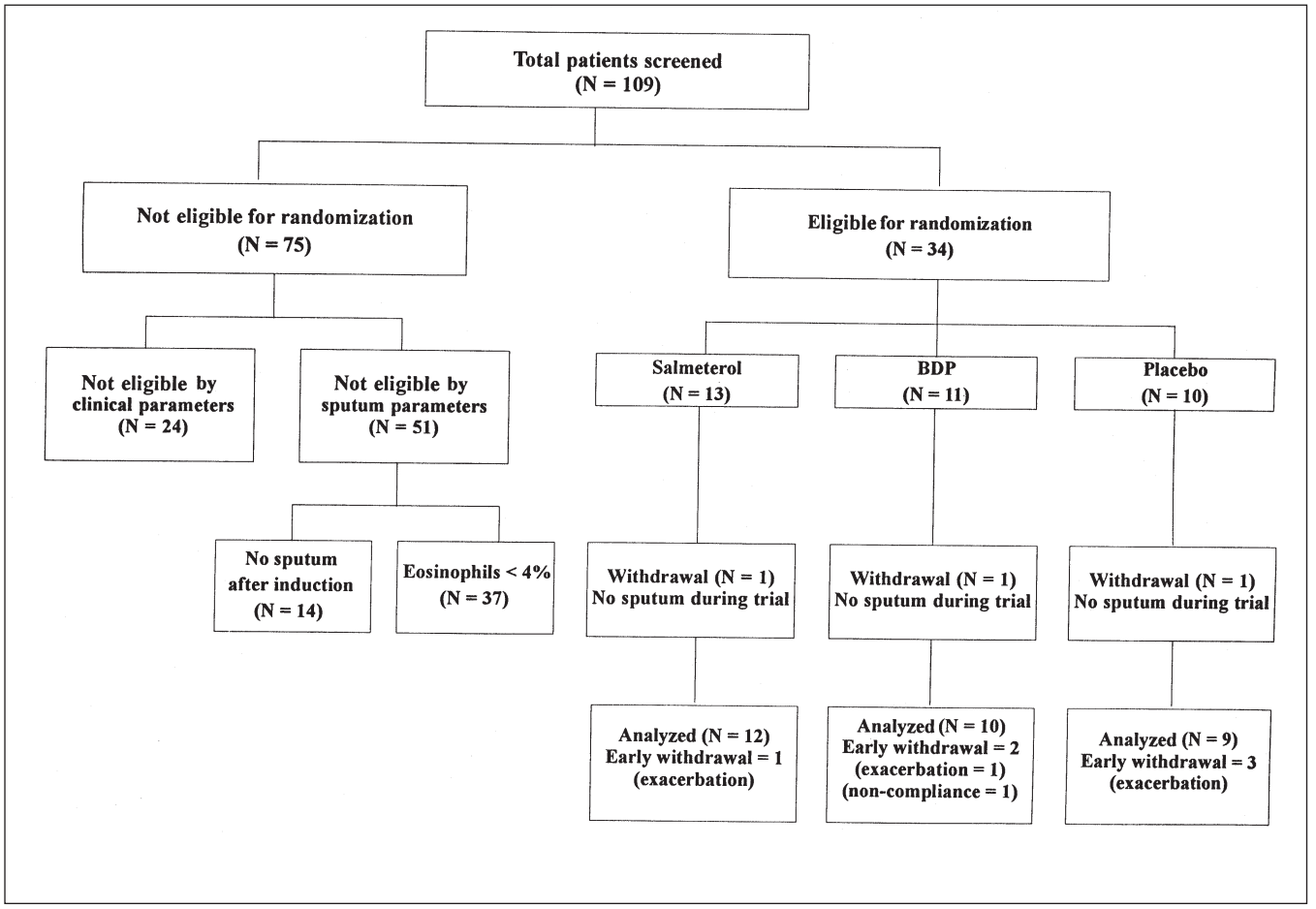

Figure 1) *Flow diagram of randomized participants and withdrawals. BDP Beclomethasone dipropionate*

by geometric means. The results were compared at week three or at the final visit before early withdrawal and at week four (except for blood measurements). The effects of treatment on sputum and blood parameters were compared via ratios of the means of week 3 adjusted for baseline values. Ratio estimates resulted from fitting a generalized linear model (GLM), with a log link function and constant coefficient of variation, using the GENMOD procedure of SAS software (SAS Institute Inc, North Carolina). This method provides good estimates of ratios of population means under a variety of distributions and, if log normality is assumed, it estimates the ratio of population medians. The effects of treatment on clinical variables were compared via differences in week 3 means. Differences were obtained from a linear model using the GLM procedure of SAS. For spirometry and symptom scores, a baseline covariate was included in the model, while, for PEF and diurnal variation, the model was adjusted for age, sex and age-by-sex. Correlations between variables were estimated by Pearson's correlation coefficient. All statistical tests were two-sided, and significance was accepted at the 5% level.

RESULTS

Randomization and withdrawals: Thirty-four eligible patients were randomly assigned to the following three-week treatments: 13 received salmeterol, 11 received BDP and 10 received placebo (Figure 1). Three patients (one in each group) could not produce sputum after randomization and were excluded from the analysis. Of the remaining 31 patients, seven were withdrawn before the end of the three-week treatment period: six due to treatment failure (three placebo, two BDP and one salmeterol) and one (BDP) because of noncompliance. These patients had been on treatment for a mean (SD) of 8.7 (4.5) days, and all results available before withdrawal were used for the analysis.

Changes in inflammatory indexes: Treatment with BDP decreased sputum eosinophils significantly. The mean percentage reduction (95% CI) in sputum eosinophils caused by BDP compared with placebo was 63% (29 to 85) and compared with salmeterol was 67% (20 to 83). In contrast, the effect of salmeterol was not different from that of placebo (Table 2, Figure 2). After discontinuing the study medications at week three and adding open label BDP 1000 μg/day to the 24 patients followed for another week, sputum eosinophils fell further in each group. The changes in the percentage of peripheral blood eosinophils and serum ECP were qualitatively similar to those of sputum eosinophils.

Changes in clinical parameters: The effects of salmeterol on clinical parameters were similar to those produced by BDP (Table 3). However, only the effects of salmeterol on

TABLE 2
Effects of treatment on inflammatory parameters in sputum and peripheral blood

	Means (SD)			Ratios of adjusted means (95% CI) for final treatment week		
	Salmeterol	BDP	Placebo	BDP:salmeterol	Salmeterol:placebo	BDP:placebo
Sputum eosinophils (%)						
Baseline	35.5 (24.9)	22.5 (17.9)	28.9 (18.5)			
Final	26.9 (25.8)	5.7 (6.8)	15.1 (4.8)	0.33 (0.15, 0.71)**	1.14 (0.52, 2.48)	0.37 (0.17, 0.80)*
Follow-up	10.8 (12.5)	4.7 (6.8)	5.2 (7.0)			
Blood eosinophils (%)						
Baseline	8.0 (4.7)	9.1 (5.5)	5.5 (2.1)			
Final	7.1 (3.7)	2.2 (1.0)	7.4 (3.3)	0.32 (0.19, 0.46)***	0.82 (0.52, 1.37)	0.26 (0.15, 0.42)***
Serum ECP (μg/L)						
Baseline	33.1 (18.1)	36.5 (22.0)	28.5 (24.1)			
Final	27.8 (16.3)	16.1 (10.1)	26.6 (20.2)	0.56 (0.35, 0.88)*	0.99 (0.62, 1.57)	0.55 (0.34, 0.90)*

**P<0.05; **P<0.01; ***P< 0.001. BDP Beclomethasone dipropionate; ECP Eosinophil cationic protein*

rescue medication, morning PEF and diurnal variation were significantly different from the changes observed in the placebo treatment group. Open treatment with BDP improved symptoms and rescue medications in the salmeterol and placebo groups, and morning PEF in the latter group. Neither active treatment significantly improved the FEV_1, FEV_1 per cent predicted or FEV_1:FVC. However, changes in FEV_1 were inversely correlated with the changes in sputum eosinophils (r=–0.65, P<0.001) (Figure 3), and positively correlated with change in symptom scores (r=0.50, P=0.008).

DISCUSSION

We have compared the effects of three weeks of salmeterol treatment on asthmatic eosinophilic airway inflammation with inhaled corticosteroid treatment. We have shown that salmeterol improves symptoms, morning PEF, diurnal variation of PEF and decreases the need for rescue medication in patients with mildly exacerbated asthma and active airway eosinophilic inflammation. However, these effects were not paralleled by suppression of inflammation measured by sputum and blood eosinophils and serum ECP. In contrast, treatment with BDP 1000 μg/day improved symptoms and morning PEF, and effectively suppressed the inflammatory indexes. These results show that the improvement in the clinical parameters produced by salmeterol may mask the effects of ongoing active eosinophilic airway infiltration.

This is the first randomized, controlled trial designed to investigate the anti-inflammatory treatment effects of salmeterol in patients with mild exacerbations of asthma. The strengths of the study include the use of sputum to measure indexes of airway inflammation directly, the selection of patients with sputum eosinophilia to permit detection of anti-inflammatory effects, and inclusion of a BDP treatment group as a positive control and of a placebo group to discriminate treatment effects from the natural variability of asthma and regression to the mean (26). Although some patients were already using an inhaled steroid before randomization, they were still poorly controlled at that dose and were included in the study because this is a common situation in clinical practice. Any prestudy treatment with inhaled steroid

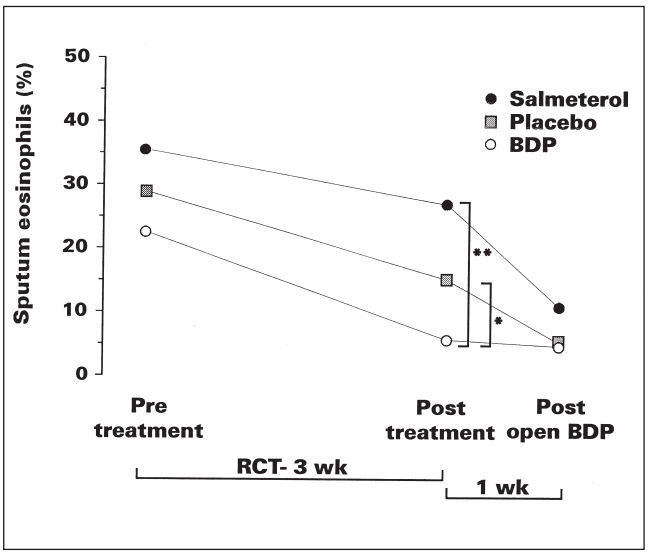

Figure 2) *Effects of three weeks' treatment with two active drugs and placebo and after one week follow-up with open label beclomethasone dipropionate (BDP) (1 mg/day) on sputum eosinophils. After three weeks of treatment, there was a fall in sputum eosinophils in the three treatment groups. Salmeterol effect on sputum eosinophils was worse than the placebo effect. *P<0.05 for differences between BDP and placebo; **P<0.01 for BDP verus salmeterol, adjusted means. RCT Randomized controlled trial*

was left unchanged in each treatment group (Table 1). It is possible that the inclusion of these patients in the study resulted in increased compliance with the previous treatment, and one could argue that this affected the results. However, if there was any effect this was obviously minimized by the study design with a random allocation of subjects for each treatment. Furthermore, a possible increase in compliance with the previous inhaled steroid treatment would have attenuated the differences of salmeterol or placebo in relation to beclomethasone anti-inflammatory effects. We included the last available sputum and clinical measurements of the six subjects who were withdrawn early because of worsening asthma to minimize any potential bias due to severity. The power of the study (despite the relatively small sample size) to detect a fourfold difference in sputum eosinophil means

TABLE 3
Effects of treatment on clinical parameters

	Means (SD)			Differences of adjusted means (95% CI) for final treatment week		
	Salmeterol	BDP	Placebo	BDP-salmeterol	Salmeterol-placebo	BDP-placebo
Symptoms (score)						
Baseline	5.5 (1.2)	6.3 (0.8)	5.7 (1.7)			
Final	6.8 (1.5)	7.0 (1.7)	5.9 (2.1)	–0.12 (–1.66, 1.42)	0.96 (–0.53, 2.45)	0.84 (–0.77, 2.46)
Follow-up	7.4 (1.0)	7.0 (0.9)	7.0 (1.4)			
Rescue medication (puffs)						
Final	1.6 (2.2)	3.4 (3.7)	4.8 (3.1)	2.14 (–0.47, 4.76)	–2.89 (–5.54, –0.24)*	–0.74 (–5.53, 2.05)
Follow-up	0.4 (0.5)	2.7 (2.3)	3.6 (2.3)			
Morning PEF (L/min)						
Final	441 (82)	444 (77)	397 (89)	–4.2 (–61.1, 53.7)	60.1 (2.4, 117.7) *	55.9 (–4.9, 116.6)
Follow-up	438 (82)	487 (40)	438 (92)			
Diurnal variation (%)						
Final	6.0 (3.5)	7.6 (4.3)	11.0 (8.3)	2.6 (–2.4, 7.6)	–5.4 (–10.5, -0.41)*	–2.8 (–8.2, 2.5)
Follow-up	5.4 (2.3)	4.4 (1.8)	5.3 (3.4)			
FEV_1 (L)						
Baseline	2.62 (0.61)	2.72 (0.65)	2.63 (0.86)			
Final	2.64 (0.49)	2.85 (0.57)	2.70 (0.72)	0.16 (–0.25, 0.57)	–0.06 (–0.47, 0.35)	0.10 (–0.33, 0.52)
Follow-up	2.70 (0.70)	3.22 (0.51)	2.73 (0.69)			

**P<0.05. BDP Beclomethasone dipropionate; FEV_1 Forced expiratory volume in 1 s; PEF Peak expiratory flow*

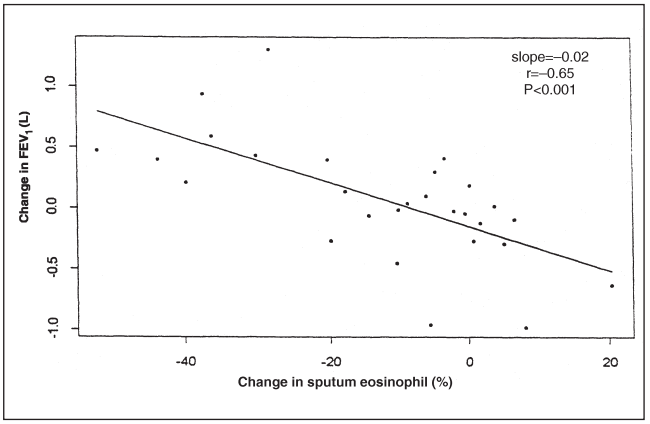

Figure 3) *Relationship between improvement in forced expiratory volume in 1 s (FEV_1) and decrease in sputum eosinophils. A decrease in sputum eosinophils by 10% was associated with an improvement in FEV_1 by 0.2 L*

between two treatments, with a significance level of 5%, was 80%. The study was considered ethical because the exacerbation of asthma was mild, and the patients were closely observed and had 24 h access to a study physician.

The method of sputum examination used in this study is reliable, valid (25) and responsive (27). It has the advantage of being noninvasive and, therefore, of being applied repeatedly to follow the effects of treatment (27). Sputum examination has previously been shown to be more accurate in detecting airway eosinophilic inflammation than blood eosinophils or serum ECP (18). In the present study, blood eosinophils and ECP were more responsive to anti-inflammatory treatment with BDP than in other studies, probably due to patient selection.

Salmeterol's lack of an effect on sputum eosinophil counts and serum ECP is in keeping with previous studies that examined the effect of long acting beta-agonists in allergen-induced inflammatory and asthmatic responses compared with the effects of placebo and BDP (10,28). In one of these studies (12), a single dose of salmeterol (50 μg) produced complete protection of the late asthmatic response and the heightened methacholine airway responsiveness at 24 h, but had no effect on the observed increase in proportion of sputum eosinophils. However, the results were inconclusive because a single dose of BDP 500 μg only partially inhibited the late response and had no effect on sputum eosinophils. Regular treatment with budesonide 400 μg/day for seven days before allergen inhalation did inhibit the allergen-induced increase in sputum eosinophils (29). Gardiner et al (30) found that eight weeks of treatment with salmeterol in nine asthmatics receiving maintenance inhaled steroid improved PEF, but had no effect on bronchoalveolar lavage cell profile or on proportion of activated lymphocytes. In the present study, three weeks of salmeterol treatment also had no measurable effect on sputum eosinophils, while BDP significantly reduced sputum and blood eosinophils. The findings, therefore, support previous results that suggested that salmeterol has no effect on airway eosinophilic inflammation. However, it is still possible that salmeterol may have other anti-inflammatory effects on eosinophil or other cell activation, microvascular leakage, or bronchoconstrictive mediator release; these possibilities require further investigation.

We also examined the anti-inflammatory effects of salmeterol indirectly by studying changes in peripheral blood eosinophils and serum ECP. Similar to sputum eosinophils, these measurements were unaffected by salmeterol. This observation differed from that of Lorenzo et al (31), who reported a significant fall in serum ECP after a week of salmeterol treatment compared with salbutamol in atopic asthmatics during a pollen season. However, this was an open study, and it is unknown whether the pollen counts were

the same at the times of ECP measurements in the two groups.

The clinical effect of salmeterol was greater than placebo but not as much as beclomethasone. This finding contrasts with those of two large multicentre studies (16,17) in which uncontrolled asthma was improved more by inhaled salmeterol than by an increase in inhaled steroid treatment. The observed differences may be due to our selection of symptomatic asthmatics with definite airway inflammation. In our study, the severity of airflow limitation was mild, and this would, therefore, be less influenced by salmeterol. In addition, all our subjects had airway eosinophilia that was diminished by BDP. In contrast, in the multicentre trials, there was more severe airflow limitation, and it is unknown whether airway eosinophilia was present. As a result, salmeterol would be expected to act in most patients with airflow obstruction, while the increase in inhaled steroid would only be expected to be effective in some and, even then, not to the same degree because of the lack of bronchodilator effect. This bronchodilator effect may be responsible for a steroid sparing effect of salmeterol and formoterol, another long acting beta-agonist, in reducing exacerbations of asthma (32,33).

We conclude that in mild exacerbations of asthma, salmeterol is as effective as BDP at improving symptoms and expiratory airflow rates, but it has no effect on ongoing airway eosinophilic inflammation. These results raise concerns about adding salmeterol to asthma therapy before evaluating the benefit of increasing the dose of inhaled steroid. It is reassuring that the use of long acting beta-agonists in several recent studies of six to 12 months' duration did not result in the deterioration of asthma control or more frequent exacerbations (32-34). However, our findings identify a need to investigate whether long term treatment with salmeterol in asthmatics with ongoing airway eosinophilic inflammation affects airway remodelling, resulting in more severe structural changes. The measurements of airway inflammation by sputum analysis emphasize the potential benefit of this test in guiding therapy in clinical practice and for evaluating other parameters of the efficacy of asthma medications. Our results also provide evidence to support current guidelines (13), which recommend initially treating mild asthma exacerbations by first introducing an inhaled steroid or increasing the daily dose. This approach improves airway eosinophilia as well as clinical features.

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