File: ERJ-00955-2008, marked revision
Revision 081104

EFFECT OF FORMOTEROL WITH OR WITHOUT BUDESONIDE IN REPEATED LOW-DOSE ALLERGEN CHALLENGE

Barbro Dahlén MD PhD[1,5], Ann-Sofie Lantz RN[1,5], Elisabeth Ihre MD PhD[2], Maria Skedinger MD PhD[1,5], Elisabeth Henriksson BMA[1,5], Leif Jörgensen MSci[3], Tommy Ekström MD PhD[3], Sven-Erik Dahlén MD PhD[4,5], Kjell Larsson MD PhD[4,5]

[1]Division of Respiratory Medicine and Allergy, Department of Medicine at Karolinska University Hospital Huddinge, [2]Cityakuten, Stockholm, [3]AstraZeneca Sweden, [4]Division of Physiology, The National Institute of Environmental Medicine and [5]Centre for Allergy Research at Karolinska Institutet, SE-171 77 Stockholm, Sweden.

Corresponding author: Dr Barbro Dahlén MD, PhD
                          Lung and allergy clinic, M 53
                          Karolinska University Hospital Huddinge
                          SE-141 86 Stockholm, Sweden
                          Tel: +468 5858 1833
                          Fax: +468 711 7306
                          E-mail: barbro.dahlen@ki.se

Short title: Budesonide/formoterol in intermittent asthma

Word count: 2918

ABSTRACT

The use of combination therapy in mild asthma is debated. We evaluated the effects of formoterol alone and formoterol/budesonide combination inhaler on asthma deterioration induced by repeated low-dose allergen exposure.

Fifteen subjects with intermittent allergic asthma inhaled low doses of allergen on 7 consecutive weekdays in a three-period, cross-over, double-blind, double dummy, comparison between formoterol Turbuhaler 4.5µg, budesonide 160µg /formoterol 4.5µg Turbuhaler, and placebo, each taken as two puffs 30 minutes after allergen dosing. Outcome variables were: provocative dose of methacholine causing a 20% fall in FEV_1(PD_{20}), exhaled nitric oxide (F_ENO), sputum eosinophils and prostaglandin D_2, and diary card recordings of symptoms (0-10), short-acting beta$_2$-agonist use and evening FEV_1.

With placebo treatment, allergen exposure caused significant increases in airway hyperresponsiveness (geom mean(CV) PD_{20}: 397(98) µg before vs 168(82) after), F_ENO (mean(SD): 46(31) ppb before vs 73(46) after) and asthma symptom score (mean(SD): 0.39(0.55) before vs 0.68(0.67) after). Budesonide/formoterol abolished these changes and significantly improved baseline FEV_1. Formoterol alone, while providing symptom relief, was no better than placebo in protecting against the allergen-induced increase in airway inflammation.

Signs of deteriorating asthma, provoked by low dose allergen, are prevented by short-term use of budesonide/formoterol but not by temporary use of formoterol alone.

Abstract word count: 200

Clin Trial Reg No: NCT00288379

List of abbreviations:

ICS : Inhaled corticosteroid

LABA : long-acting beta$_2$-agonist

F$_E$NO : Exhaled nitric oxide

FEV$_1$: forced expiratory volume in one second

PD$_{20}$FEV$_1$: provocative dose causing a fall in FEV$_1$ of approximately 20%

PD$_5$FEV$_1$: provocative dose causing a fall in FEV$_1$ of approximately 5%

INTRODUCTION

The goal for successful management of asthma is to achieve and maintain symptom control and to prevent exacerbations [1]. Subjects with intermittent asthma that is in good control may experience periodic worsening after exposure to trigger factors such as allergens, viral infections and pollutants. The deterioration is caused by progressive airway inflammation and associated with enhanced airway hyperresponsiveness [2]. Requirement of increased use of as-needed reliever medication should then prompt the patient to initiate anti-inflammatory treatment with inhaled corticosteroids (ICS) to prevent further and potentially long-lasting deterioration [1]. In practice, timely introduction of ICS often fails due to poor perception of symptoms, lack of patient education and unavailability of medical advice. Thus over-reliance on rapid-acting bronchodilators, may put patients at risk by delaying proper intervention with anti-inflammatory treatment. There is also a concern that use of long-acting beta$_2$-agonists (LABAs), salmeterol or formoterol, particularly as monotherapy, may render the airway inflammation progressively worse [3,4,5]. It has, therefore, been suggested that early use of LABAs should only occur as combination therapy with inhaled corticosteroids [6,7,8,9]. More recently, it has also been established that combination inhalers containing both an ICS and a rapid-onset long-acting [10,11] or short-acting [12] beta$_2$-agonist, can enable patients with persistent asthma to continuously adapt their need for anti-inflammatory treatment according to fluctuations in their disease. So far, only one study has addressed the use of as needed combination therapy in intermittent asthma [13]. The rationale for this strategy in patients whose asthma is mostly well controlled, thus merits

further consideration since airway inflammation has been shown to be a characteristic feature even in very mild disease [14,15].

Repeated low-dose allergen inhalation challenge has been introduced as a method to mimic and standardize natural exposure to environmental allergens [16,17]. In this challenge setting, patients with allergic asthma inhale fixed doses of allergen which are titrated to cause minimal bronchoconstriction and administered once daily on 4-10 consecutive weekdays [17,18]. The procedure generates increased airway hyperresponsiveness to direct bronchoconstrictors, and elevations in exhaled nitric oxide (NO) levels and in inflammatory markers in sputum [17,18]. This occurs despite only few symptoms of asthma being reported by the subjects. Hence the challenge model is particularly suitable to investigate early events in the development of more symptomatic asthma.

The present study is the first to employ the repeated low-dose allergen challenge setting to investigate the effects of either formoterol alone or its fixed combination with budesonide on indices of asthma deterioration that are associated with very mild or no symptoms. The study was conducted as a cross-over, double-blind, double-dummy, three-period comparison between formoterol, budesonide/formoterol in a combination inhaler and placebo in subjects with intermittent asthma. All treatments were administered throughout the course of allergen exposure and their effects on airway responsiveness to methacholine, pulmonary function, symptoms, levels of exhaled NO, sputum eosinophils and prostaglandin D_2 in sputum as mast cell marker, were investigated.

METHODS

The extended version of the methods is available in the on-line supplement.

Subjects

Fifteen nonsmoking subjects with intermittent [1] allergic asthma treated only with a short-acting beta$_2$-agonist prn participated. All had a post-bronchodilator FEV$_1$ greater than 80 per cent of predicted normal value and airway hyperresponsiveness to methacholine (Table 1). Exclusion criteria were significant allergen exposure, COPD or any significant respiratory disease other than asthma, a respiratory tract infection within four weeks and use of glucocorticosteroids within two months prior to the study.

The Ethics Committee at The Karolinska University Hospital approved the study (Dnr 04-470/1-4) and the subjects gave written informed consent.

Study design

The study (NCT00288379) was a three-period, cross-over, double-blind, double dummy comparison (in random order) between formoterol Turbuhaler™ 4.5μg, budesonide 160μg /formoterol 4.5μg Turbuhaler™, and placebo (AstraZeneca, Lund, Sweden) on airway functional and inflammatory changes and symptoms, induced by repeated low-dose allergen exposure (Figure 1). The study medication was taken as two puffs 30 minutes after allergen inhalation on every low-dose challenge day.

The subjects participated in two screening visits prior to randomization, including skin prick test, pre-study spirometry, a methacholine challenge, and a cumulative, high-dose allergen inhalation challenge to establish current sensitivity, expressed as allergen PD$_{20}$FEV$_1$.[19] See on-line supplement.

Each period consisted of nine clinic visits, always in the morning, with methacholine challenges and induced sputum collection (see on-line depository) on visit days 1 and 9, i.e. pre- and post-repeated allergen exposure period. Exhaled NO (NIOX™, Aerocrine AB, Stockholm, Sweden) and FEV_1 (Jaeger MasterScope, IntraMedic Inc, Sweden) were measured daily according to current recommendations [20,21] and the values obtained before methacholine challenge on visit day 1 were taken as pre-allergen exposure, pre-treatment baseline in the respective period.

Allergen (Aquagen™, ALK Laboratories, Copenhagen, Denmark) was inhaled as a single dose on seven consecutive week days, i.e Monday-Friday one week and Mon/Tues next week (visit days 2-8, Figure 1). The allergen dose selected as the low dose, was calculated from the screening allergen challenge as the cumulative dose causing a fall in FEV_1 of approximately 5% (PD_5FEV_1) from post-diluent value (Table I). Spirometry was obtained before and 10, 20 and 30 minutes after allergen inhalation on each occasion. The randomized study treatment was then inhaled under observation before the subject was allowed to leave the clinic.

Diary cards were administered on day 1 in each period and the subjects were asked to record their symptom score on a visual analogue scale (0-10) and their use of short-acting beta-agonist every evening covering the previous 24 hours. In addition, evening measurements of FEV_1 were recorded at home using a pocket spirometer (Spirobank™, IntraMedic Inc, Sweden).

The three periods were separated by a 15 day wash-out, which was extended to a maximum eight weeks in the case of remaining asthma deterioration after the

previous exposure period, or an interfering respiratory tract infection. All study visits were scheduled outside season in pollen-sensitized subjects.

Statistical analysis

Within period changes and treatment differences in log-transformed PD_{20}, FEV_1 and F_ENO values, and diary card data were analyzed using a repeated measures analysis of covariance (ANCOVA) model, with subject, period and treatment as factors, and with baseline pre-allergen, pre-treatment values in each period as covariate. Data are presented as adjusted least square (LS) means (or geometric means for PD_{20}) and 95% CI. Period and carry-over effects of the drug treatments were analyzed by substituting treatment with period and calculating trends throughout the study. The sample size was calculated to be 12 completed patients assuming a standard deviation of log_{10}-transformed PD_{20} to be 0.23, a significance level of 5%, a 80% power, a two-sided alternative hypothesis, and a between-treatment difference (increase) of 83% in PD_{20}. nQuery and SAS Version 8.02 were used for statistical calculations. Differences were considered significant if $p<0.05$.

RESULTS

There were no period or carry over effects of the treatments (on-line supplement).

Airway responsiveness to methacholine

During placebo treatment, repeated low-dose allergen exposure produced an increase in airway hyperresponsiveness to methacholine, with a significant reduction in geometric mean PD_{20} (397µg before versus 168 µg after the exposure period). The reduction corresponded to -1.28 doubling doses (95% CI, -2.1 to -0.49; p=0.01 as least squared mean change) (Figure 2). In contrast, budesonide/formoterol completely prevented allergen-induced deterioration in airway hyperresponsiveness, with a higher post-allergen methacholine PD_{20} (383 µg before versus 660 µg after) corresponding to 0.72 doubling doses (95% CI, -0.07 to 1.51; p=0.07). There was no significant change in airway hyperresponsiveness during formoterol treatment, with a geometric mean PD_{20} 483 µg before and 401 µg after allergen exposure (-0.17 doubling dose; 95% CI, -0.95 to 0.63; p=0.67).

While there was no variability in pre-exposure methacholine responsiveness between the three periods, comparison between treatments demonstrated significant protection by budesonide/formoterol corresponding to 2.7 doubling doses versus placebo (95% CI, 1.3 to 5.5; p=0.01), whereas treatment with formoterol was not statistically significant different from placebo (formoterol versus placebo 1.75 doubling dose; 95% CI, 0.9 to 3.5; p=0.11). Formoterol/budesonide was numerically better than formoterol (1.55 doubling dose) but the difference did not reach statistical significance (95% CI, 0.7 to 3.2; p=0.22).

Correction for drug effects on baseline FEV_1 (see below) in the statistical analysis did not alter the results of the airway hyperresponsiveness assessments (not shown). Furthermore, the change in methacholine PD_{20} did not correlate with initial airway responsiveness to methacholine, allergen sensitivity, baseline symptom score or F_ENO.

Exhaled NO

There was a progressive rise in the concentrations of NO in exhaled air during the placebo-treated allergen exposure (Figure 3), with an adjusted LSmean (95% CI) increase over the exposure period amounting to 25.7 ppb (8.8 to 42.6 ppb; p=0.006). Notably, the levels fell after the weekend pause in exposure, but were raised again when the patients were re-exposed for an additional two days (Figure 3). The allergen-induced rise in F_ENO levels was not inhibited during treatment with formoterol (adjusted LSmean change 22.1 ppb (95% CI, 5.2 to 39.0 ppb; p=0.014)), and the response was closely similar to that of the placebo treatment (Figure 3). In contrast, budesonide/formoterol abolished the allergen-induced rise in F_ENO concentrations (adjusted LSmean change 7.6 ppb (95% CI, -9.3 to 24.6 ppb; p=0.35)).

When comparing budesonide/formoterol with placebo and formoterol, the differences were highly significant with the adjusted LSmean (95% CI) being -18 ppb (-26 to -10 ppb; p=0.0002) and -14.4 ppb (-22 to -6.4 ppb; p=0.0017), respectively. As displayed in Figure 3, there was no difference between treatment with placebo and formoterol alone (adjusted LSmean 3.6 (-4 to 11.2) ppb; p=0.33) on the allergen-induced rise in F_ENO.

Sputum measurements

Eosinophilic granulocytes in induced sputum increased post allergen challenge following formoterol treatment, but not after placebo or budesonide/formoterol treatment (Figure 4). There was a statistically significant difference between budesonide/formoterol and formoterol alone (p= 0.016), while other group comparisons did not show significant differences.

Levels of prostaglandin D_2 likewise increased following formoterol treatment but not significantly after placebo nor budesonide/formoterol (Figure 4).

Lung function

The effect of repeated low-dose allergen exposure on morning baseline FEV_1 measurements is displayed in figure 5. The adjusted LSmean (95% CI) change of FEV_1, including all measurements in the treatment period (Friday before versus mean of Tuesday through Wednesday after), was -0.08 (-0.18 to 0.02) L for placebo (p=0.10), -0.02 (-0.12 to 0.08) L for formoterol (p=0.64) and 0.14 (0.04 to 0.25) L for budesonide/formoterol (p=0.01). Thus, baseline FEV_1 (prior to a repeat allergen dose) improved with budesonide/formoterol during the challenge period, but not with formoterol or placebo.

As a corollary, budesonide/formoterol was significantly superior to treatment with placebo or formoterol on changes in baseline lung function measurements, the adjusted LSmean (95% CI) differences being 0.23 (0.1 to 0.35) L; p=0.002 and 0.17 (0.04 to 0.3)L, p=0.015, respectively (Figure 4). Comparison between placebo and formoterol showed no significant difference (-0.06 (-0.18 to 0.07) L; p=0.33).

The mean (± SD) immediate fall in FEV_1 within 30 minutes after low-dose allergen inhalation (before intake of study medication) was 7.79 (±1.19) %, 7.20

(±1.60) and 6.93 (±1.62) during the respective treatment period with placebo, budesonide/formoterol and formoterol. There was no difference between treatments and no progressiveness over time in the magnitude of immediate responses.

Asthma symptoms, beta$_2$-agonist usage and evening FEV$_1$

Diary card recordings revealed an increase of the average symptom score during placebo-treatment (adjusted LSmean (95% CI) change: 0.31 (0.12 to 0.49); p=0.003) as opposed to treatment with budesonide/formoterol (0.1 (-0.1 to 0.28); p=0.27) and formoterol (0.1 (-0.1 to 0.28); p=0.29) (Figure 6). Accordingly, between-treatment comparisons showed significant protection from symptoms by budesonide/formoterol and formoterol alone (adjusted LSmean difference (95%CI): -0.21(-0.38 to -0.03); p=0.024 for budesonide/formoterol versus placebo, and -0.21 (-0.38 to -0.04); p=0.021 for formoterol versus placebo) with no difference between the two active treatments.

As needed use of beta$_2$-agonist was infrequent, with no difference between treatments and a total number over each exposure period of 24, 14 and 11 puffs for placebo, budesonide/formoterol and formoterol, respectively. There were also no significant changes or between-treatment differences in evening recordings of FEV$_1$ at home (not shown), although a trend for protection by budesonide/formoterol versus placebo (p=0.09) was observed.

DISCUSSION

In this three-period, cross-over treatment study in 15 subjects with intermittent asthma, repeated low-dose allergen exposure in the presence of placebo produced significant increases in airway hyperresponsiveness, exhaled NO and symptom score. Treatment with formoterol alone inhibited the rise in symptoms, but provided no protection against allergen-induced airway inflammation. In contrast, budesonide/formoterol abolished all of these components of asthma deterioration and, moreover, improved baseline pulmonary function.

This is the largest three-period, double-blind, cross-over treatment study performed in the repeated low-dose allergen challenge setting. Our protocol using allergen PD_5 as the target dose administered for seven consecutive week-days was successfully employed. The placebo treated challenge elicited a mean immediate fall in FEV_1 of less than 8% and generated significant increases in the main outcome variables, airway hyperresponsiveness, symptom score and F_ENO. The exquisite sensitivity of exhaled NO measurements as a surrogate marker of the allergen-induced airway inflammation was particularly evident (Figure 3) [17,20]. On the other hand, there was no significant deterioration in morning FEV_1, in patient recorded beta$_2$-agonist usage or evening lung function measurements at home. Analyses of period and carry over effects confirmed that the 15-day washout periods were long enough (on-line repository). Accordingly, the pre-period baselines were very similar for all outcome variables, and statistical calculations yielded the same final results irrespective of adjustments for baseline differences. With a three-period design and four days of allergen PD_5 exposure, Gauvreau et al demonstrated sufficient washout with one week [18].

The drop out rate in the study was less than expected with all 15 subjects who managed the first period, completing the study. Out of the 17 subjects who entered the treatment phase, one was withdrawn early in the first period due to unacceptable increase in allergen sensitivity compared with screening and another because of disc hernia. Of the completing patients, only three subjects had prolongations of wash-out periods due to common colds or in one case markedly increased methacholine responsiveness after the placebo-treated period. It is, thus, felt that our protocol for repeated low-dose allergen exposure is robust and appropriate for use in future intervention studies.

The study medication was always administered under observation 30 minutes after inhalation of the allergen dose. This particular time-point was selected with the intention to mimic the situation of temporary exposure to allergen when patients start to perceive mild symptoms or become aware of the presence of allergen in the environment. Moreover, the supervised administration of study treatment provided full compliance, and the possibly confounding effect of acute bronchodilation before allergen inhalation was avoided.

Budesonide/formoterol combination therapy provided effective protection against the increase in airway hyperresponsiveness, airway inflammation assessed as $F_E NO$, and symptom score, i.e. the elemental components of asthma, despite being administered after allergen exposure. These protective effects of budesonide/formoterol against allergen exposure were observed despite the fact that the study subjects were judged to not require regular treatment with inhaled glucocorticosteroids. The improvement in lung function by budesonide/formoterol did, however, not contribute to the reduction in airway

hyperresponsiveness, since correction for baseline FEV_1 in the statistical analysis did not alter the result.

Formoterol alone, provided relief of symptoms and was numerically better than placebo in protecting against allergen-induced increase in bronchial hyperresponsiveness but produced no improvement in morning baseline lung function. In addition, an increase in sputum eosinophils was seen during formoterol treatment and the rise in $F_E NO$ was identical to that with placebo. Moreover, the marker of mast cell activation, PGD_2 also increased significantly in sputum following treatment with formoterol alone. By providing symptom relief but allowing the underlying inflammation to persist or even worsen, formoterol thereby masked the signs of asthma deterioration.

The budesonide component of budesonide/formoterol has been studied in the repeated low-dose model previously [18,22]. Budesonide 400 μg administered once daily before allergen inhalation prevented the rise in allergen-induced airway hyperresponsiveness [18], sputum eosinophilia [18,22], and $F_E NO$ [22]. However, in previous studies no comparison with a beta$_2$-agonist was done. Since in real life the patients' first treatment is their beta$_2$-agonist, and because of the mounting use of the rapid-acting long-lasting formulation, formoterol, as well as the budesonide/formoterol combination therapy, this provided the rationale for selecting these particular two drugs for investigation.

From a general perspective, the present study was designed to address more in depth two treatment options at the crossing point between the first two steps in the current guidelines for asthma management [1]. This is where patients with intermittent asthma are told to add on regular use of an inhaled corticosteroid on the basis of increasing asthma symptoms, or conversely, if they are well

controlled with no symptoms and normal lung function they may step back from the daily use of inhaled corticosteroids. The number of possibilities for a personalized treatment in patients with mild persistent asthma were recently highlighted [23,24]. Large treatment studies are needed to address at which stage the combination inhalers, containing ICSs and beta$_2$-agonists with various duration and onset of action, are to be introduced in the evolution of asthma. Current guidelines emphasize that frequent use of beta$_2$-agonist on demand should always be accompanied with regular use of an ICS. For the large group of patients who are to be found in the interface between intermittent and mild persistent asthma, this caveat may, however, very well be forgotten.

The present study provides support for the use of the combination inhaler budesonide/formoterol to gain control when intermittent asthma starts to get worse. In contrast, while providing symptom relief but no protection against underlying features of asthma deterioration, the results with formoterol alone are indicative of a risk for masking of inflammation and potential asthma worsening if incorrectly used as monotherapy.

REFERENCES

1. Global Initiative for Asthma. Global Strategy for asthma management and prevention: NHLBI/WHO workshop report. Bethesda, MD:National Heart Lung and Blood Institute, 2006. Available from: www.ginasthma.org.

2. Bousquet J, Jeffery PK, Busse WW, Johnson M, Vignola AM. Asthma. From bronchoconstriction to airways inflammation and remodeling. Am J Respir Crit Care Med 2000;161(5):1720-45.

3. Lazarus SC, Boushey HA, Fahy JV, Chinchilli VM, Lemanske RF Jr, Sorkness CA et al. Long-acting beta2-agonist monotherapy vs continued therapy with inhaled corticosteroids in patients with persistent asthma: a randomized controlled trial. JAMA 2001; 285:2583-93.

4. Nelson HS, Weiss ST, Bleecker ER, Yancey SW, Dorinsky PM; SMART Study Group. The Salmeterol Multicenter Asthma Research Trial: a comparison of usual pharmacotherapy for asthma or usual pharmacotherapy plus salmeterol. Chest 2006;129: 15-26.

5. Pauwels RA, Sears MR, Campbell M, Villasante C, Huang S, Lindh A et al. Formoterol as relief medication in asthma: a worldwide safety and effectiveness trial. Eur Respir J 2003;22:787-794.

6. Greening AP, Ind PW, Northfield M, Shaw G. Added salmeterol versus higher-dose corticosteroid in asthma patients with symptoms on existing inhaled corticosteroid. Lancet 1994;344:219-24.

7. Woolcock A, Lundback B, Ringdal N, Jacques LA. Comparison of addition of salmeterol to inhaled steroids with doubling of the dose of inhaled steroids. Am J Respir Crit Care Med 1996;153:1481-88.

8. Pauwels RA, Löfdahl CG, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ et al. Effect of inhaled formoterol and budesonide on exacerbations of

asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International StudyGroup. N Engl J Med 1997;337:1405-11.

9.   O'Byrne P, Barnes PJ, Rodrigues-Roisin R, Runnerstrom E, Sandstrom T, Svensson K et al. Low dose inhaled budesonide and formoterol in mild persistent asthma: the OPTIMA randomized trial. Am J Respir Crit Care Med 2001;164:1392-1397.

10.   O'Byrne PM, Bisgaard H, Godard PP, Pistolesi M, Palmqvist M, Zhu Y et al. Budesonide/Formoterol combination therapy as both maintenance and reliever medication in asthma.   Am J Respir Crit Care Med 2005;171:129-36.

11.   Rabe KF, Atienza T, Magyar P, Larsson P, Jorup C, Lalloo UG. Effect of budesonide in combination with formoterol for reliever therapy in asthma exacerbations: a randomised controlled, double-blind study. Lancet 2006;368:744-53.

12.   Papi A, Canonica GW, Maestrelli P, Paggiaro P, Olivieri D, Pozzi E et al. Rescue use of beclomethasone and albuterol in a single inhaler for mild asthma. N Engl J Med 2007;356:2040-52.

13.   Haahtela T, Tamminen K, Malmberg LP, Zetterström O, Karjalainen J, Ylä-Outinen H et al. Formoterol as needed with or without budesonide in patients with intermittent asthma and raised NO levels in exhaled air: a SOMA study. Eur Respir J 2006;28:748-755.

14.   Laitinen LA, Laitinen A, Haahtela T. Airway mucosal inflammation even in patients with newly diagnosed asthma. Am Rev Respir Dis 1993;147:697-704.

15. Jeffery PK, Godfrey RW, Adelroth E, Nelson F, Rogers A, Johansson SA. Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma. A quantitative light and electron microscopic study. Am Rev Respir Dis 1992;145:890-9.

16. Ihre E, Zetterstrom O. Increase in non-specific bronchial responsiveness after repeated inhalation of low doses of allergen. Clin Exp Allergy 1993;23: 298-305.

17. Ihre E, Gyllfors P, Gustavsson LE, Kumlin M, Dahlén B. Early rise in exhaled NO and mast cell activation during repeated low dose allergen challenge. Eur Respir J 2006;27:1152-59.

18. Gauvreau GM, Sulakvelidze I, Watson RM, Inman MD, Rerecich TJ, O'Byrne PM. Effects of once daily dosing with inhaled budesonide on airway hyperresponsiveness and airway inflammation following repeated low-dose allergen challenge in atopic asthmatics. Clin Exp Allergy 2000; 30:1235-1243.

19. Roquet A, Dahlén B, Kumlin M, Ihre E, Anstrén G, Binks G et al. Combined antagonism of leukotrienes and histamine produces predominant inhibition of allergen-induced early and late phase airway obstruction in asthmatics. Am J Respir Crit Care Med 1997; 155:1856-1863.

20. Recommendations for standardized procedures for the on-line and off-line measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children. Am J Respir Crit Care Med 1999;160:2104– 2117.

21. American Thoracic Society. Standardization of Spirometry, 1994 update. Am J Respir Crit Care Med 1995;152:1107-36.

22.   De Kluijver J, Evertse CE, Schrumpf JA, van der Veen H, Zwinderman AH, Hiemstra PS et al.   Asymptomatic worsening of airway inflammation during low-dose allergen exposure in asthma. Am J Respir Crit Care Med 2002;166:294-300.

23.   Kraft M, Israel E, O'Connor GT. Treatment of mild persistent asthma. N Engl J Med 2007;356:2096-2100.

24.   The American Lung Association Asthma Clinical Research Centers. Randomized comparison of strategies for reducing treatment in mild persistent asthma. N Engl J Med 2007;356:2027-39.

TABLE 1. Subject characteristics at screening

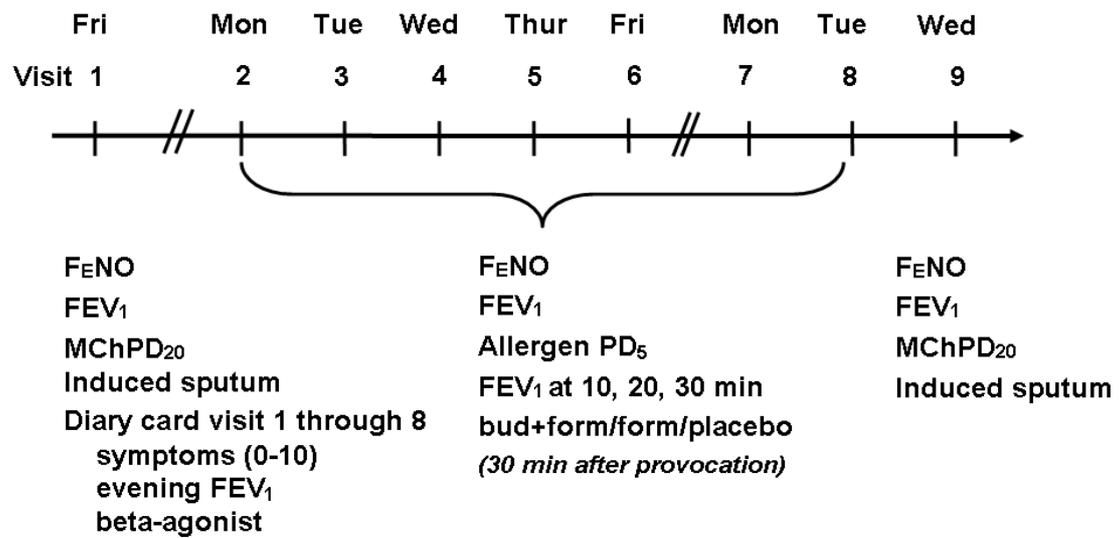| Subject	Sex	Age yrs	FEV_1 % predicted	MChPD$_{20}$* µg	Allergen	Allergen PD$_{20}$ SQ† units	Low-dose Allergen PD$_5$‡ SQ units	F$_E$NO ppb
1	M	25	97	1835	Birch	2070	369	12.3
2	M	25	113	728	Cat	310	42	77.2
3	F	23	114	1082	Cat	1836	213	50.8
4	M	19	113	538	Cat	1991	355	25.0
5	F	39	115	136	Birch	3206	710	18.9
6	F	25	95	222	Birch	362	120	14.4
7	F	36	113	53	Cat	3172	426	50.8
8	M	22	111	1511	Cat	1174	355	25.2
9	M	19	103	220	Timothy	34	14	98.2
10	F	25	91	346	Dog	475	70	76.2
11	M	55	91	93	Birch	128	35	28.8
12	M	39	105	1574	Timothy	804	142	17.1
13	M	31	107	5530	Cat	419	177	36.8
14	F	49	103	165	Cat	2011	710	10.7
15	F	25	107	149	Cat	156	49	97.0

* Methacholine PD$_{20}$FEV$_1$ = provocative dose causing a 20% fall in FEV$_1$
† SQ=standardized quality
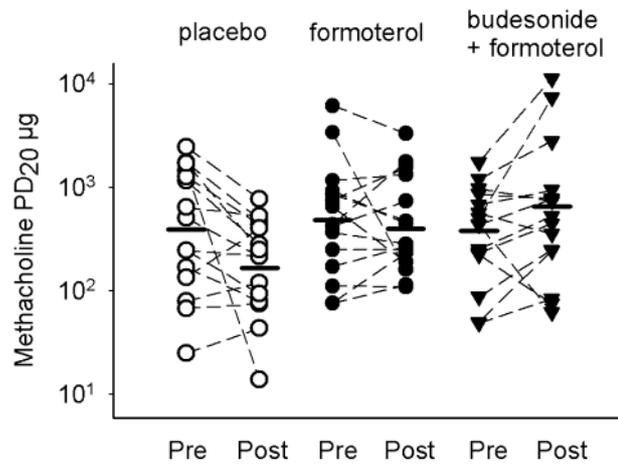‡ Allergen PD$_5$ = provocative dose causing a 5% fall in FEV$_1$

Figure legends

Figure 1

	Fri	Mon	Tue	Wed	Thur	Fri	Mon	Tue	Wed
Visit	1	2	3	4	5	6	7	8	9

F_ENO
FEV_1
$MChPD_{20}$
Induced sputum
Diary card visit 1 through 8
   symptoms (0-10)
   evening FEV_1
   beta-agonist

F_ENO
FEV_1
Allergen PD_5
FEV_1 at 10, 20, 30 min
bud+form/form/placebo
(30 min after provocation)

F_ENO
FEV_1
$MChPD_{20}$
Induced sputum

Design of study.

Figure 2

Individual and geometric mean shift in airway responsivenss to methacholine
expressed as PD_{20} before vs after 7 days of allergen exposure, in a three-period,
crossover, double-blind, randomized treatment study with placebo (open circle),
formoterol (filled circle) and budesonide/formoterol (filled triangle), respectively.
During placebo, there was an increase in airway hyperresponsiveness (p=0.01) which
was inhibited with budesonide/formoterol (p=0.01) but not with formoterol alone when
compared to placebo.

Figure 3
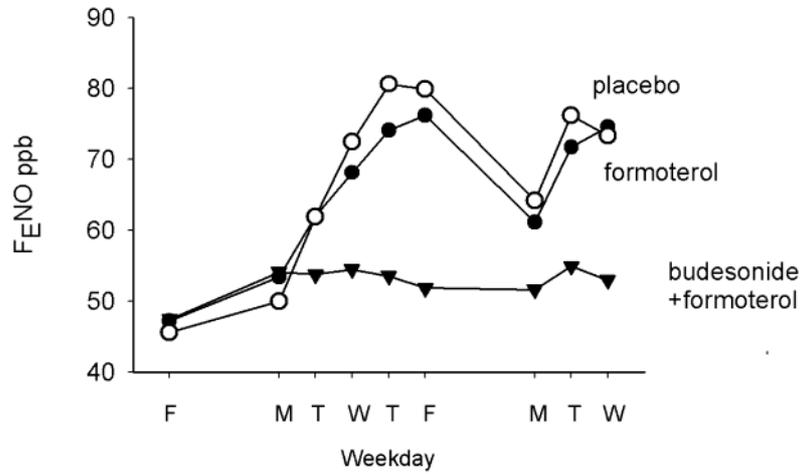
Mean concentrations of NO (ppb) in exhaled air during 7 days of allergen exposure (Monday-Friday plus Monday-Tuesday) in a three-period, crossover, double-blind, randomized treatment study with placebo (open circle), formoterol (filled circle) and budesonide/formoterol (filled triangle), respectively. In the presence of placebo and formoterol, the levels of exhaled NO increased progressively, whereas budesonide/formoterol offered significant protection (p=0.0002 vs placebo).

Figure 4
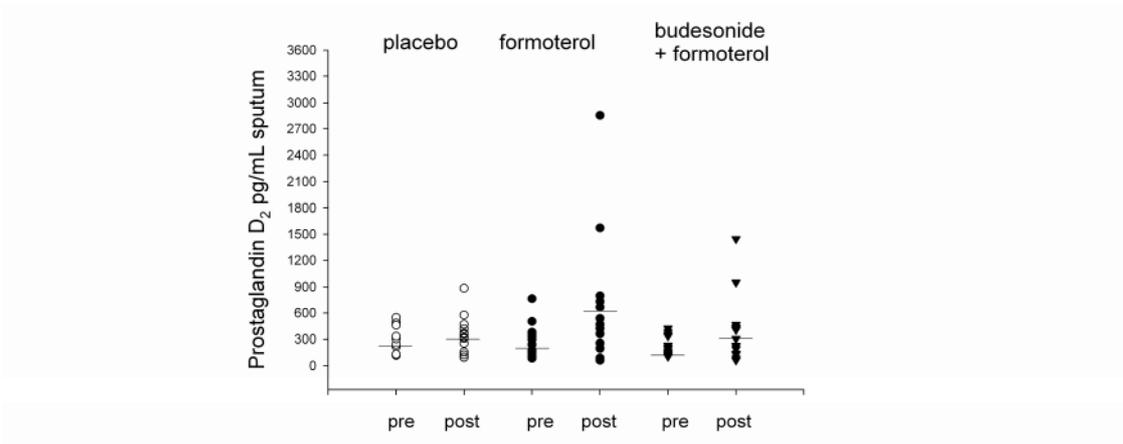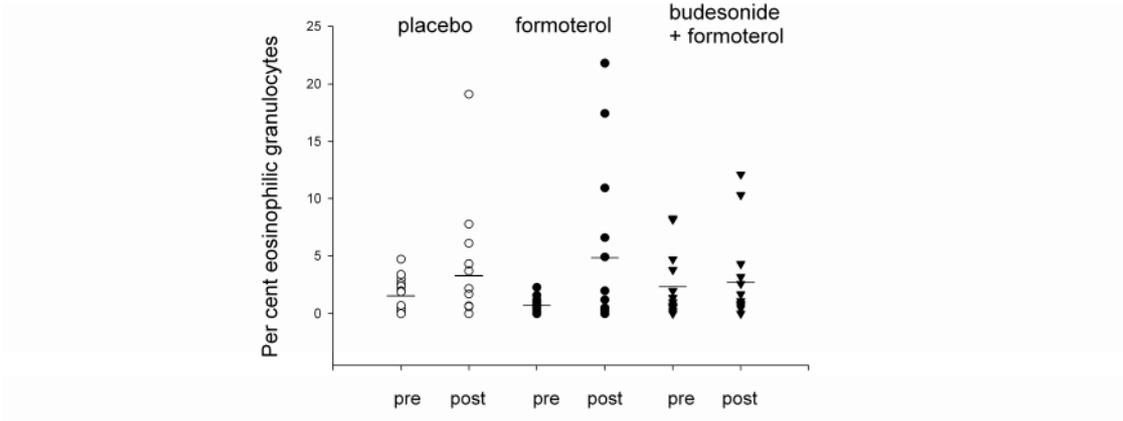
Individual and mean changes of per cent eosinophilic granulocytes (Figure 4 a) and concentrations of prostaglandin D_2 (Figure 4 b) in induced sputum pre vs post 7 days of allergen exposure and concomitant treatment with placebo (open circle), formoterol

(filled circle) and budesonide/formoterol (filled triangle), respectively. With

formoterol, significant increases of sputum eosinophilic granulocytes (p=0.048) as

well as of prostaglandin D_2 concentrations (p=0.005) were seen. With placebo or

budesonide/formoterol, there were no statistically significant increases of either

inflammatory biomarker. However, with placebo, the prostaglandin D_2 concentrations

were close to significance (p=0.060).

Figure 5
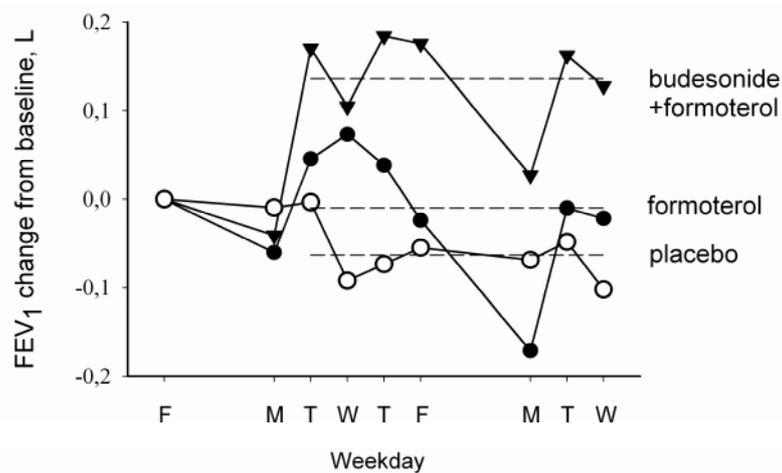
Change from pre-period baseline in FEV_1 during 7 days of allergen exposure

(Monday-Friday plus Monday-Tuesday) and concomitant treatment with placebo

(open circle), formoterol (filled circle) and budesonide/formoterol (filled triangle),

respectively. Solid lines depict the mean change for each treatment. Daily baseline

FEV$_1$ values (prior to a repeat allergen dose) improved with budesonide/formoterol

(p=0.002 vs placebo), but not with formoterol, during allergen challenge.

Figure 6
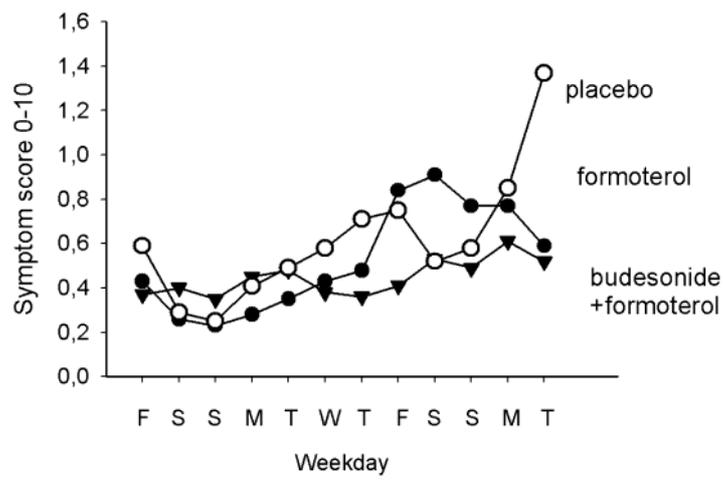
Mean asthma symptom score (0-10) recorded every evening during 7 days of allergen

exposure (Monday-Friday plus Monday-Tuesday). While in the presence of placebo

(open circle) there was an increase in symptom score (p=0.003), both

budesonide/formoterol (filled triangle) (p=0.024) and formoterol alone (filled circle)

(p=0.021) provided protection.