

# Comparison of combination inhalers vs inhaled corticosteroids alone in moderate persistent asthma

Daniel K. C. Lee,<sup>1</sup> Catherine M. Jackson,<sup>2</sup> Graeme P. Currie<sup>1</sup>, Wendy J. Cockburn<sup>1</sup> & Brian J. Lipworth<sup>1</sup>

<sup>1</sup>Asthma & Allergy Research Group, Department of Clinical Pharmacology, Ninewells Hospital and Medical School and <sup>2</sup>Tayside Centre for General Practice, University of Dundee, Dundee DD1 9SY

**Aims** Inhalers combining long acting  $\beta_2$ -adrenoceptor agonists (LABA) and corticosteroids (ICS) are indicated at Step 3 of current asthma guidelines. We evaluated the relative effects of LABA + ICS combination *vs* ICS alone on pulmonary function, bronchoprotection, acute salbutamol recovery following methacholine bronchial challenge, and surrogate inflammatory markers in patients with moderate persistent asthma.

**Methods** Twenty-nine patients with mean FEV<sub>1</sub> ( $\pm$  SEM) of  $78 \pm 3\%$  predicted completed a randomized, double-blind, double-dummy, cross-over study. Patients received either 4 weeks of budesonide 400  $\mu$ g + formoterol 12  $\mu$ g (BUD + FM) combination twice daily followed by 1 week of BUD 400  $\mu$ g alone twice daily, or 4 weeks of fluticasone propionate 250  $\mu$ g + salmeterol 50  $\mu$ g (FP + SM) combination twice daily followed by 1 week of FP 250  $\mu$ g alone twice daily. Measurements were made at baseline and following each randomized treatment.

**Results** FEV<sub>1</sub> increase from pretreatment baseline as mean ( $\pm$  SEM) % predicted was significantly higher ( $P < 0.05$ ) for BUD + FM ( $8 \pm 1\%$ ) *vs* BUD ( $2 \pm 1\%$ ), and for FP + SM ( $8 \pm 1\%$ ) *vs* FP ( $2 \pm 1\%$ ). The fall in FEV<sub>1</sub> following methacholine challenge as percentage change from prechallenge baseline FEV<sub>1</sub> was not significantly different in all four groups; BUD + FM ( $22 \pm 1\%$ ), BUD ( $24 \pm 1\%$ ), FP + SM ( $23 \pm 1\%$ ) and FP ( $23 \pm 1\%$ ). Salbutamol recovery over 30 min following methacholine challenge as area under curve (AUC %·min) was significantly blunted ( $P < 0.05$ ) with BUD + FM ( $486.7 \pm 35.5$ ) *vs* BUD ( $281.1 \pm 52.8$ ), and with FP + SM ( $553.1 \pm 34.1$ ) *vs* FP ( $368.3 \pm 46.7$ ). There were no significant differences between respective combination inhalers or between respective ICS alone. Decreases in exhaled nitric oxide (NO) and serum eosinophilic cationic protein (ECP) from baseline were not significantly different between treatments.

**Conclusions** Combination inhalers improve pulmonary function without potentiating anti-inflammatory effects on exhaled NO and serum ECP as compared with ICS alone, but delay acute salbutamol recovery after bronchoconstriction.

**Keywords:** asthma, budesonide, combination inhalers, fluticasone, formoterol, inflammation, methacholine challenge, pulmonary function, recovery, salbutamol, salmeterol

## Introduction

Current guidelines [1–3] recommend the use of long-acting  $\beta_2$ -adrenoceptor agonists (LABA) such as formoterol (FM) and salmeterol (SM) in conjunction with

inhaled corticosteroids (ICS) to improve asthma control at Step 3 in moderate to severe persistent asthma [4–6]. The functional antagonism attributed by LABA against bronchoconstrictor stimuli (so called ‘bronchoprotection’) may have a role in stabilization of airway smooth muscle and may be a possible explanation for their effects in reducing exacerbations, as they do not exhibit any meaningful anti-inflammatory activity [7, 8]. We have previously shown that both FM 12  $\mu$ g twice daily and SM 50  $\mu$ g twice daily as add on therapy to ICS in mild to moderate asthmatics resulted in significant residual trough protection amounting to less than one doubling dose [9].

Correspondence: Professor Brian J. Lipworth, Professor of Allergy and Respiratory Medicine, Asthma & Allergy Research Group, Department of Clinical Pharmacology, Ninewells Hospital and Medical School, University of Dundee, Dundee DD1 9SY. Tel.: 01382 632983 (direct), 01382 632180 (secretary); Fax: 01382 644972; E-mail: b.j.lipworth@dundee.ac.uk

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The effects of FM and SM as add on therapy in more severe patients are currently unknown, where differences in their  $\beta_2$ -adrenoceptor intrinsic activity may result in differences in their relative bronchoprotection. The pros and cons for using LABA at Step 3 have been extensively reviewed elsewhere [10].

Subsensitivity to the bronchoprotective effects of LABA have been demonstrated irrespective of treatment with ICS [11–14]. Indeed even administering a single 1600  $\mu\text{g}$  bolus of salbutamol (eight times the usual dose) does not overcome this bronchoprotective tolerance induced by LABA [15]. Moreover other studies have shown that the acute response to salbutamol may be blunted in patients treated with LABA and ICS [11, 16–19]. Inhalers combining LABA and ICS are currently advocated in asthma therapy in view of data showing additive effects of LABA and ICS on asthma control which were as effective as increasing the dose of ICS [4, 5, 20].

We therefore aimed to evaluate the relative effects of FM and SM in combination inhalers as compared with ICS alone on pulmonary function, bronchoprotection, salbutamol recovery following methacholine challenge, and surrogate inflammatory markers, in patients with moderate persistent asthma.

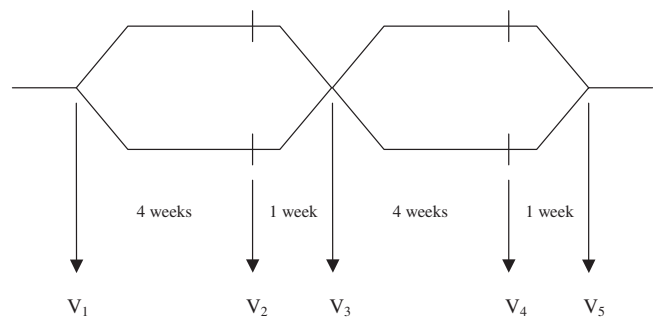
## Methods

### Patients

Eligible patients had stable moderate persistent asthma [1] for at least 3 months prior to the study and none had received a course of oral corticosteroids or antibiotics during this period. All were nonsmokers and were receiving inhaled short-acting  $\beta_2$ -adrenoceptor agonists for symptomatic relief at least once daily and ICS therapy such as beclomethasone dipropionate (BDP) ( $n = 21$ ), budesonide (BUD) ( $n = 4$ ) and fluticasone propionate (FP) ( $n = 4$ ), either alone or in combination with second line controller therapy such as LABA ( $n = 9$ ), theophylline ( $n = 3$ ) or a leukotriene receptor antagonist ( $n = 3$ ). All patients were required to exhibit hyper-responsiveness to methacholine on bronchial challenge testing with a provocative dose causing a 20% reduction from baseline FEV<sub>1</sub> (PD<sub>20</sub>) of less than 500  $\mu\text{g}$ . All gave informed consent and the Tayside Committee on Medical Research Ethics approved the study.

### Study design (Figure 1)

The study was conducted in a randomized, double-blind, double-dummy, cross-over fashion. There was an initial step down period for patients who were receiving more than 800  $\mu\text{g}$  of ICS daily ( $n = 5$ ), which was halved once



**Figure 1** Study flow diagram. Patients had baseline measurements on the first study visit ( $V_1$ ) on their usual ICS therapy and were then randomized to receive either BUD + FM for 4 weeks followed by the same dose of BUD for 1 week, or FP + SM for 4 weeks followed by the same dose of FP for 1 week. Patients then crossed over to the other randomized treatment arm. Measurements were taken at  $V_1 - V_5$ .

every 2 weeks until a maintenance dose of no more than 800  $\mu\text{g}$  day<sup>-1</sup> was achieved. Patients receiving second line controller therapy ( $n = 11$ ) had them stopped during a 1-week period prior to the study.

Upon recruitment, patients had their usual asthma therapy stopped for the remainder of the study period and were given study medications instead. Patients were issued with a standard salbutamol 100  $\mu\text{g}$  per actuation dry powder inhaler which had a dose counter (Asmasal Clickhaler<sup>®</sup>, Celltech Pharmaceuticals Ltd, Slough, UK) as their rescue therapy during the study and were randomized to receive either 4 weeks of inhaled combination of BUD 200  $\mu\text{g}$  + FM 6  $\mu\text{g}$  (Symbicort 200/6 Turbohaler<sup>®</sup>, AstraZeneca UK Ltd, Luton, UK) two puffs twice daily followed by 1 week of BUD 200  $\mu\text{g}$  (Pulmicort 200 Turbohaler<sup>®</sup>, AstraZeneca UK Ltd, Luton, UK) two puffs twice daily or 4 weeks of inhaled combination of FP 250  $\mu\text{g}$  + SM 50  $\mu\text{g}$  (Seretide 250 Accuhaler<sup>®</sup>, Allen and Hanburys Ltd, Uxbridge, UK) one puff twice daily followed by 1 week of FP 250  $\mu\text{g}$  (Flixotide 250 Accuhaler<sup>®</sup>, Allen and Hanburys Ltd, Uxbridge, UK) one puff twice daily. The dose of ICS as FP in Flixotide<sup>®</sup> or Seretide<sup>®</sup> was chosen to be equipotent to that of BUD in Pulmicort<sup>®</sup> or Symbicort<sup>®</sup>. With each active Turbohaler<sup>®</sup>, patients also received a placebo Accuhaler<sup>®</sup> and *vice versa*. All active and placebo Turbohaler<sup>®</sup> and Accuhaler<sup>®</sup> devices were identical in external physical appearance.

### Measurements

Measurements were made at baseline and following each randomized treatment with patients having taken the last dose of study medication 12 h prior to attending the laboratory, i.e. all measurements were made at trough at the end of the dosing interval.

### Nitric oxide

Measurement of exhaled breath nitric oxide (NO) was performed as described by Kharitonov *et al.* [21] using an integrated LR2000® clinical real-time NO gas analyser (Logan Research, Rochester, UK) with a flow rate of 250 ml min<sup>-1</sup> and an accuracy of two parts per billion (p.p.b.) NO with a response time of 2 s. The normal exhaled NO cut-off value in our laboratory is less than 6 p.p.b. for nonatopic nonasthmatic subjects (i.e. < 2 SD from mean).

### Spirometry

Spirometry was performed according to the American Thoracic Society criteria [22] using a Vitalograph® compact spirometer (Vitalograph Ltd, Buckingham, UK) with a computer assisted pneumotachograph head and pressure transducer. The spirometer was calibrated daily with a Vitalograph® 1 l precision syringe.

### Methacholine bronchial challenge

Methacholine bronchial challenge was performed using a standardized computer assisted dosimetric method as previously described by Beach *et al.* [23]. In brief, methacholine was administered at 5 min intervals in doubling cumulative doses from 3.125 µg to 6400 µg until a 20% reduction in FEV<sub>1</sub> was recorded.

### Salbutamol recovery

Immediately following methacholine bronchial challenge, patients received inhaled salbutamol 200 µg (Ventolin 200 Accuhaler®, Allen & Hanburys Ltd, Uxbridge, UK) and then measurements of FEV<sub>1</sub> were recorded at 5 min intervals for 30 min.

### Serum eosinophilic cationic protein

Patients had blood samples taken for serum eosinophilic cationic protein (ECP) prior to any study procedures which were analysed using a radioimmunoassay kit (Pharmacia & Upjohn Diagnostics, Milton Keynes, UK) with an interassay coefficient of variability of 3.1%. The normal serum ECP cut-off value in our laboratory is less than 12 µg l<sup>-1</sup> for nonatopic nonasthmatic subjects (i.e. < 2 SD from mean).

### Peak flow and rescue diary

Patients kept a daily record of morning domiciliary peak expiratory flow (PEF) rates using a Mini-Wright® peak flow meter (Clement Clarke International Ltd, Harlow,

UK) along with documentation of daily rescue inhaler use for the duration of the study.

### Statistical analysis

The study was powered at 80% with alpha error set at 0.05 (2 tailed) and beta error of 0.2, in order to detect a 25% difference in salbutamol recovery (the primary outcome variable) between treatments, with a sample size of 24 patients in a crossover design. An overall analysis of variance and multiple range testing with Bonferroni correction were performed and a probability value of less than 0.05 (two tailed) considered significant. Comparisons were made between treatments as change from baseline. To normalize distribution, data for methacholine PD<sub>20</sub> were logarithmically transformed, and analyses were performed using Statgraphics® statistical software package (STSC Software Publishing Group, Rockville, USA).

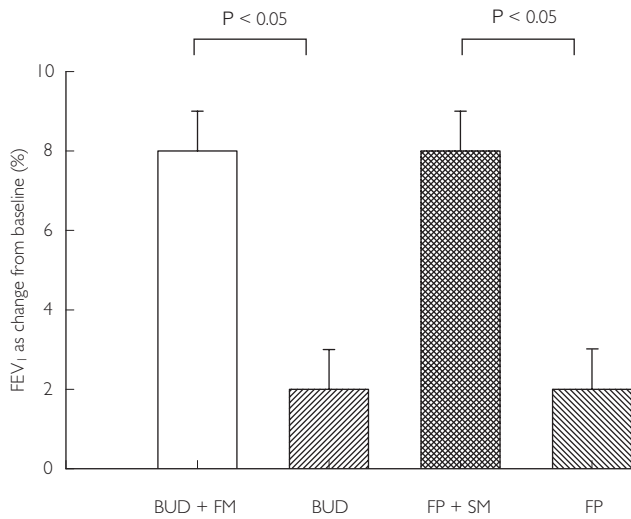
## Results

### Patients

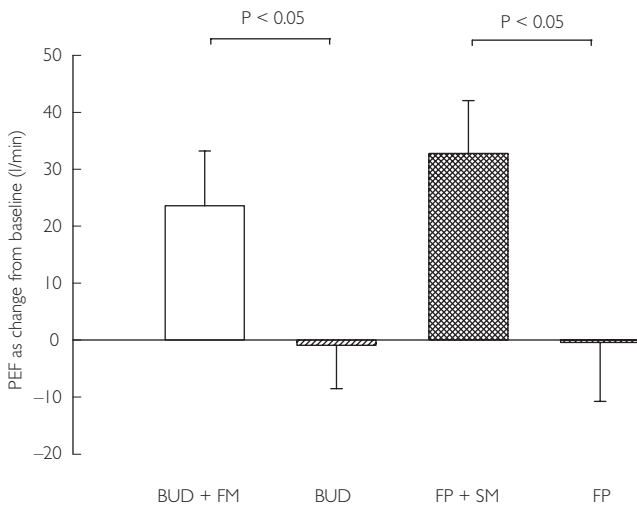
Forty-six patients were initially screened for the study of whom 32 patients fulfilled the eligibility criteria for entry into the study. Following randomization, three patients dropped out due to personal reasons. Twenty-nine patients (16 females and 13 males) with mean age (± SEM) of 46 ± 3 years completed the study per protocol. The mean ICS dose at randomization was 538 ± 46 µg (BDP = 552 ± 41 µg, BUD = 525 ± 63 µg and FP = 475 ± 65 µg).

### Pulmonary function (Figures 2 and 3)

Baseline spirometry at randomization showed mean FEV<sub>1</sub> of 78 ± 3% predicted. FEV<sub>1</sub> increase from baseline was significantly higher ( $P < 0.05$ ) for BUD + FM combination (8 ± 1%) *vs* BUD alone (2 ± 1%): mean difference 6% (95% CI 3, 9%), and for FP + SM combination (8 ± 1%) *vs* FP alone (2 ± 1%): mean difference 6% (95% CI 2, 9%). Baseline mean PEF at randomization was 425 ± 20 l min<sup>-1</sup>. PEF comparisons as change from baseline were significant ( $P < 0.05$ ) for FP + SM (33 ± 9 l min<sup>-1</sup>) *vs* FP (0 ± 10 l min<sup>-1</sup>): mean difference 33 l min<sup>-1</sup> (95% CI 22, 44 l min<sup>-1</sup>), and for BUD + FM (24 ± 10 l min<sup>-1</sup>) *vs* BUD (-1 ± 8 l min<sup>-1</sup>): mean difference 24 l min<sup>-1</sup> (95% CI 14, 35 l min<sup>-1</sup>). There were no significant differences in FEV<sub>1</sub> and PEF for BUD + FM *vs* FP + SM, or BUD *vs* FP. Daily salbutamol rescue use (as puffs per day) was not significantly different for BUD + FM (0.4 ± 0.2) *vs* BUD (1.0 ± 0.3), or FP + SM (0.4 ± 0.2) *vs* FP (0.9 ± 0.3).



**Figure 2** Mean FEV<sub>1</sub> (% predicted) as change from baseline with SEM.



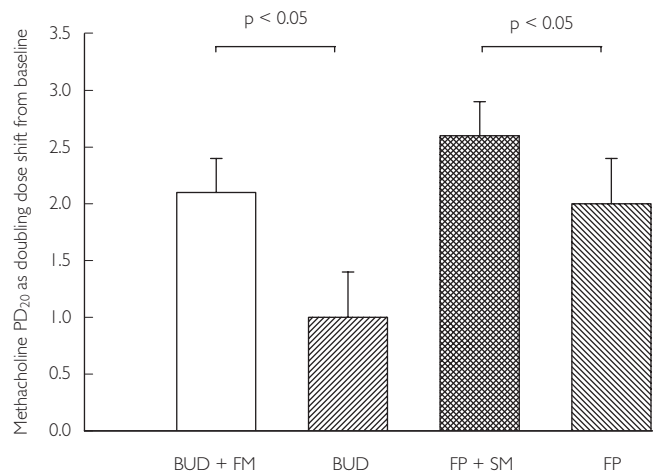
**Figure 3** Mean PEF (l min<sup>-1</sup>) as change from baseline with SEM.

*Bronchial hyper-responsiveness (Figure 4)*

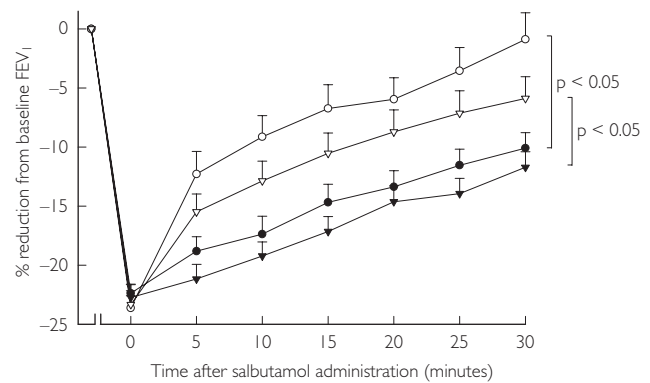
At randomization, patients had a geometric mean baseline methacholine PD<sub>20</sub> of 39 ± 10 µg. Increase in methacholine PD<sub>20</sub> from baseline (as doubling dose) was significantly different (*P* < 0.05) comparing BUD + FM (2.1 ± 0.3) vs BUD alone (1.0 ± 0.4); a 1.2 doubling dose difference, or between FP + SM (2.6 ± 0.3) vs FP alone (2.0 ± 0.4); a 0.5 doubling dose difference. There were no significant differences in methacholine PD<sub>20</sub> comparing BUD + FM vs FP + SM, or between BUD vs FP.

*Salbutamol recovery (Figure 5)*

The fall in FEV<sub>1</sub> following methacholine challenge as percentage change from prechallenge baseline FEV<sub>1</sub> were



**Figure 4** Mean change in methacholine PD<sub>20</sub> as doubling dose shift from baseline with SEM.



**Figure 5** Time profile of salbutamol recovery following methacholine challenge as % mean reduction from baseline prechallenge FEV<sub>1</sub> with SEM. BUD + FM (●); BUD (○); FP + SM (▼); and FP (▽).

not significantly different in all four groups; BUD + FM (22 ± 1%), BUD (24 ± 1%), FP + SM (23 ± 1%) and FP (23 ± 1%). Subsequent salbutamol recovery over 30 min as area under curve (AUC %·min) was delayed significantly (*P* < 0.05) comparing BUD + FM (486.7 ± 35.5) vs BUD (281.1 ± 52.8), or between FP + SM (553.1 ± 34.1) vs FP (368.3 ± 46.7). There were no significant differences in recovery AUC comparing BUD + FM vs FP + SM, or between BUD vs FP.

*Inflammatory markers*

Baseline mean exhaled NO and serum ECP at randomization were 12.7 ± 2.8 p.p.b and 42.7 ± 5.5 µg l<sup>-1</sup>, respectively. Decreases in exhaled NO from baseline were not significant comparing BUD + FM (6.7 ± 2.3 p.p.b) vs BUD (7.5 ± 2.6 p.p.b), or between FP + SM (7.3 ± 2.5 p.p.b) vs FP (7.5 ± 2.5 p.p.b). Similarly, decreases in serum ECP from baseline were also not

significant for BUD + FM ( $11.0 \pm 2.8 \mu\text{g l}^{-1}$ ) *vs* BUD ( $12.0 \pm 2.6 \mu\text{g l}^{-1}$ ), or FP + SM ( $8.0 \pm 2.6 \mu\text{g l}^{-1}$ ) *vs* FP ( $7.4 \pm 3.0 \mu\text{g l}^{-1}$ ). For exhaled NO and serum ECP, there were no significant differences between BUD + FM *vs* FP + SM, or BUD *vs* FP.

## Discussion

Our results showed that each combination inhaler conferred additional bronchodilatation and broncho-protection against methacholine compared with their respective ICS alone. Acute salbutamol recovery after methacholine-induced bronchoconstriction was significantly delayed with each combination inhaler compared with ICS alone. There was no evidence of any potentiation of anti-inflammatory activity on exhaled NO and serum ECP with combination inhalers *vs* ICS alone. Our design employed an initial 4 weeks of combination inhaler followed by withdrawal of LABA for the fifth week in order to compare 5 weeks duration of ICS *vs* 4 weeks of combination therapy.

The bronchodilator properties of LABA as seen with improvement in pulmonary function parameters of FEV<sub>1</sub> and PEF in our patients are in keeping with previous studies with combination inhalers *vs* ICS alone [24–26]. *In vitro* pharmacological differences in  $\beta_2$ -adrenoceptor intrinsic activity between FM and SM [27] were not translated to *in vivo* differences on their effects on bronchodilatation or bronchoprotection in our study. This is consistent with a previous study where there was no difference in bronchoprotection when adding in FM or SM to ICS [9], while down regulation of peripheral blood lymphocyte  $\beta_2$ -adrenoceptors occurs to a comparable degree with both agonists [28].

Our results showed that LABA when taken in combination with ICS did not potentiate anti-inflammatory properties in terms of reduction in exhaled NO and serum ECP. It is important to consider whether we had achieved maximal effects in each of these parameters, in terms of there being further room for improvement with the combination inhalers *vs* their respective ICS alone. For exhaled NO, mean absolute levels had fallen to 5.2 p.p.b. with ICS alone, which is within the range for normal nonasthmatic subjects (i.e. < 6 p.p.b). We have previously reported a plateau response for exhaled NO with 400  $\mu\text{g}$  daily of BUD, while for serum ECP, there was a dose–response effect up to 1600  $\mu\text{g}$  daily in moderate persistent asthmatics [29]. Mean absolute values for serum ECP in our patients had fallen to 31  $\mu\text{g l}^{-1}$  with ICS alone, which is considerably higher than normal values for nonatopic nonasthmatic subjects (i.e. < 12  $\mu\text{g l}^{-1}$ ).

So it would seem that there was further room for suppression of serum ECP but not exhaled NO in our

patients, had there been any potentiation of corticosteroid response with the combination inhalers. In retrospect, we should have perhaps included a comparator group where the dose of BUD and FP alone was doubled to 1600  $\mu\text{g}$  and 1000  $\mu\text{g}$ , respectively, to assess whether there was any further potential for anti-inflammatory suppression. Nonetheless the present data are in agreement with a previous study where we showed no potentiation of serum ECP suppression with FM 12  $\mu\text{g}$  or 24  $\mu\text{g}$  added to BUD 400 or 800  $\mu\text{g}$  daily [30]. *In vitro* data have previously suggested that LABA such as FM or SM may potentiate ligand independent nuclear translocation of the cytosolic glucocorticoid receptor complex, although this also occurs with salbutamol, probably indicating a class effect of  $\beta_2$ -adrenoceptor agonists *per se* [31, 32]. SM has also been shown to be an effective inhibitor of mediators released from human lung mast cells [33], in keeping with *in vitro* data showing LABA to influence cell activation, adhesion, chemotaxis and survival [34]. However, *in vivo*, for mast cell priming using adenosine monophosphate bronchial challenge, with chronic dosing of LABA in ICS treated patients, these effects are lost due to tolerance [13, 14]. We used methacholine bronchial challenge, which reflected the effects on airway smooth muscle. It has been shown with chronic dosing of terbutaline that tolerance was greater for adenosine monophosphate than methacholine bronchial challenge [35]. *In vivo* data in asthmatic patients have shown SM or FM to exhibit little or no clinical meaningful anti-inflammatory activity [7, 8, 14, 36–38]. Nevertheless, we acknowledge that the anti-inflammatory markers used in our study may not have been sufficient alone to assess properly the anti-inflammatory properties of LABA, and that biopsy studies will be required to evaluate this further.

Salbutamol recovery following acute bronchoconstriction was significantly delayed in patients receiving the combination inhalers. As can be seen from the minimal rescue consumption in our patients during the study, any subsensitivity response of salbutamol response can be attributed to effects of the LABA combination inhalers as such. The probable explanations for this phenomenon are due to LABA induced  $\beta_2$ -adrenoceptor down regulation, induced  $\beta_2$ -adrenoceptor uncoupling and associated desensitization, and prolonged  $\beta_2$ -receptor occupancy, all of which have been shown *in vivo* [16, 39–42]. This finding has clinical implications in that asthmatic patients who are on LABA + ICS combination may potentially have a delayed response to salbutamol during an acute episode of bronchoconstriction. Previous *in vivo* data in constricted airways have shown similar interaction between LABA and salbutamol [17, 19, 43], which is not overcome by a high dose (1600  $\mu\text{g}$ ) of the latter [15].

We observed a significantly higher  $PD_{20}$  after each combination inhaler *vs* the respective ICS alone, which may be explained by the residual effects of each LABA on airways smooth muscle, resulting in additional antagonism of methacholine-induced bronchoconstriction. Consequently the higher dose of methacholine administered in the presence of combination inhalers may itself have resulted in delayed recovery. However against this hypothesis would be the finding that the percentage fall in  $FEV_1$  following methacholine challenge was similar in all four groups, i.e. after each combination inhaler and respective ICS alone. If the delay in recovery was merely due to a higher administered dose of methacholine, one would perhaps expect an associated greater fall in  $FEV_1$  with the combination inhalers. Moreover, as this was a cross-over study, one would also anticipate that methacholine sensitivity *per se* to a given dose of ICS would not have changed appreciably.

In conclusion, our results show that combination inhalers improve pulmonary function compared with their respective ICS given alone, but they do not exhibit any potentiation of anti-inflammatory effects on exhaled NO and serum ECP. Furthermore combination inhalers delay recovery following salbutamol when used for relief of acute bronchoconstriction.

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