Advances in Asthma and COPD Treatment: Combination Therapy with Inhaled Corticosteroids and Long-Acting β_2-Agonists

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Abstract: Asthma treatment guidelines advocate the use of long-acting β_2-agonists (LABA) in addition to inhaled corticosteroids (ICS) in patients whose asthma is uncontrolled by ICS alone, thereby addressing two processes fundamental to asthma: bronchoconstriction and inflammation. Superior control – including a reduction in severe exacerbations – of asthma and COPD by ICS/LABA combination therapy has been demonstrated. Results from clinical studies suggest additive and potentially synergistic effects when the two agents are used in combination. No new safety-related issues have been identified with ICS/LABA compared with the monocomponents.

The exact mechanisms for the enhanced efficacy of ICS/LABA combinations are under investigation but likely include drug interactions at the receptor level and interwoven signalling pathways, which may result in improved function of β_2-adrenoceptors and steroid receptors. Data from preclinical studies provide evidence of additive, compensatory, complementary and synergistic effects of ICS and LABA in the control of inflammation and airway and lung remodelling. These effects may contribute to the improved efficacy seen when treating asthma and COPD with ICS/LABA combinations in clinical studies.

Two ICS/LABA combination products are available: budesonide/formoterol (Symbicort®) and salmeterol/fluticasone propionate (Seretide™). An ICS/LABA combination in a single inhaler represent safe, effective and convenient treatment options for the management of patients with asthma and COPD. Clinical results also suggest that adjustable dosing with budesonide/formoterol provides better asthma control than fixed dosing. Further elucidation of the underlying mechanisms responsible for this superior disease control is needed.

Key Words: Asthma, chronic obstructive pulmonary disease (COPD), budesonide, fluticasone propionate, formoterol, salmeterol, Seretide™, Symbicort®

INTRODUCTION

The management of asthma has evolved over the last 20 years from the treatment of bronchoconstriction with short-acting β_2-agonists, *via* the management of airway inflammation with inhaled corticosteroids (ICS), to the current trend for combination therapy with an ICS and a long-acting β_2-agonist (LABA) (Fig. **1**) [1].

In patients with asthma, ICS and LABA complement each other by acting on two different key components of the disease: inflammation and bronchoconstriction. Contrary to earlier fears that LABA may mask inflammation and possibly exacerbate the underlying disease process, superior control of both asthma and chronic obstructive pulmonary disease (COPD) by combination ICS/LABA therapy has been shown in many clinical studies over the last decade. ICS/LABA therapy leads to greater improvement in lung function, better symptom control and lower incidence of exacerbations compared with treatment with ICS alone — even at much higher ICS doses. It is conceivable that, besides the obvious complementary effects of ICS and LABA, the superior disease control achieved by combination therapy is associated with improved β_2-adrenoceptor and glucocorticoid receptor (GR) function, as well as with a reduction in inflammation and, potentially, remodelling in the airways and lung. In this review, we discuss clinical studies investigating the efficacy of ICS/LABA combination therapy in patients with asthma or COPD and also address possible mechanisms responsible for the superior clinical efficacy of this type of therapy.

Two combination ICS/LABA products are currently available: salmeterol/fluticasone propionate (Seretide™) and budesonide/formoterol (Symbicort®). Although both combinations contain an ICS and an LABA, there are significant differences in their pharmacological properties, which impact on how they are used. These differences are discussed in detail in this review.

CLINICAL EFFICACY OF ICS/LABA COMBINATION THERAPY IN ASTHMA

Current clinical practice guidelines state that ICS should be the first-line maintenance treatment for patients with persistent asthma [2]. Therefore, studies evaluating the efficacy of ICS/LABA in patients with persistent asthma use equivalent or higher doses of the ICS alone as the comparator regimen rather than LABA monotherapy.

ICS and LABA Delivered *via* Separate Inhalers

The first study to look at the effects of treating patients with an ICS plus an LABA was a 6-month trial evaluating

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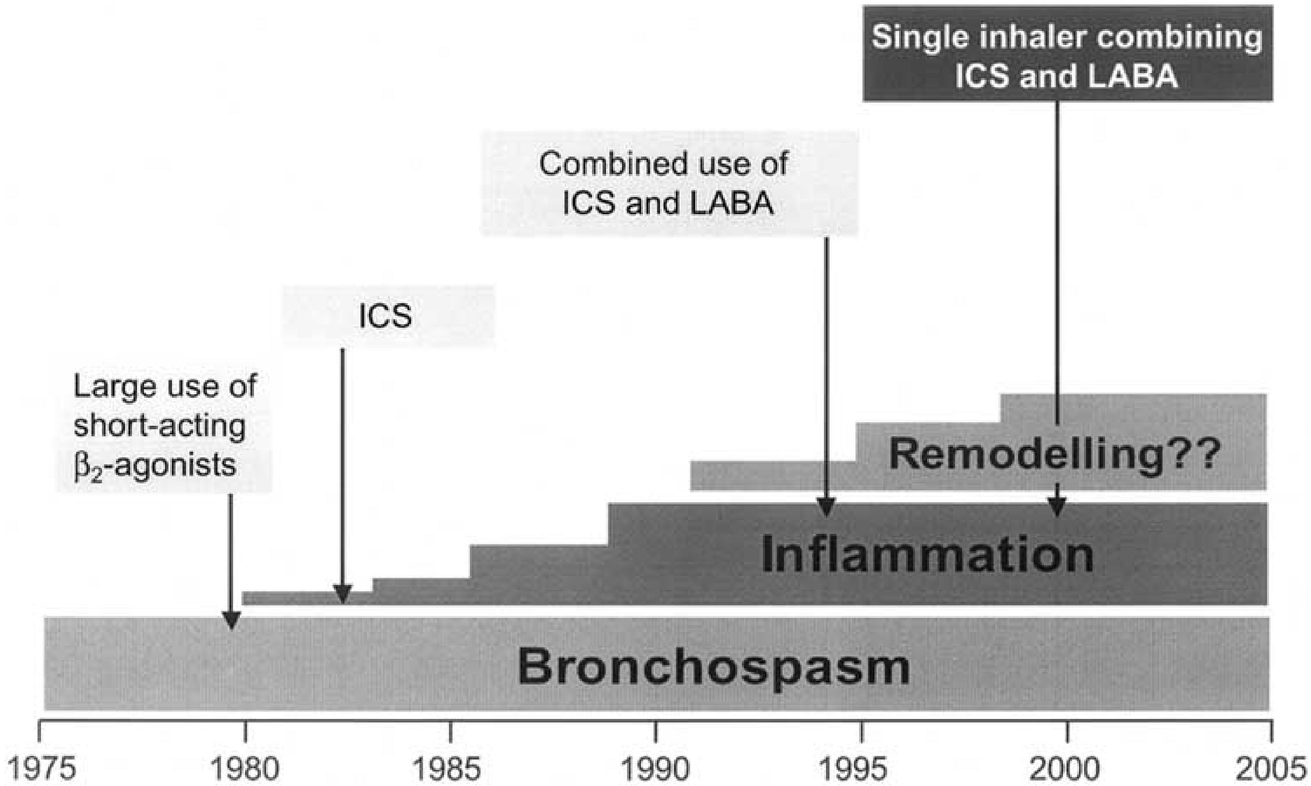

Fig. (1). Evolving therapy of asthma (modified from Bousquet 2000 [1], with permission). Use of combined ICS and LABA began with the study by Greening *et al.* [3].

the efficacy of adding salmeterol 50 μg twice daily (bid) to a standard dose of beclomethasone dipropionate 200 μg bid, compared with a higher dose of beclomethasone dipropionate (500 μg bid) [3]. Significantly better airway function, fewer asthma symptoms and a reduced need for reliever medication were seen in patients treated with the combination compared with beclomethasone dipropionate alone. No differences in exacerbation rate or safety profile were reported. A similar study in patients with more severe asthma compared beclomethasone dipropionate 500 μg in combination with salmeterol 50 or 100 μg bid against beclomethasone dipropionate 1000 μg bid [4]. As in the previous study, combination therapy was significantly more effective than high-dose beclomethasone dipropionate in improving lung function, asthma symptoms and in reducing the use of reliever medication. No difference in efficacy was found between the two doses of salmeterol, but the prevalence of tremor was 3-fold higher in the higher-dose group than in the lower-dose group (p=0.012). In another study, Ind *et al*. [5] confirmed that adding salmeterol to fluticasone propionate was superior to doubling the dose of fluticasone propionate to 500 μg bid.

The landmark FACET study was the first to use exacerbation rate as the primary efficacy variable [6]. In this study, Pauwels and colleagues evaluated the efficacy of adding formoterol 9 μg bid or placebo to low-dose (100 μg bid) or high-dose (400 μg bid) budesonide in patients with moderately severe asthma. The higher budesonide dose resulted in significantly fewer mild and severe asthma exacerbations than low-dose budesonide. However, the addition of formoterol to both doses of budesonide significantly reduced the rate of both severe (Fig. **2**) and mild exacerbations. A separate analysis showed no difference in the type and duration of exacerbations between the four treatment groups [7]. In another 12-month study, which included just two treatment groups but was otherwise identical in design to the FACET study, inflammatory markers were analysed in patients treated with high-dose budesonide compared with low-dose budesonide plus formoterol [8]. This study showed that the addition of formoterol did not appear to mask underlying inflammation. The addition of formoterol to low-dose budesonide did not result in any safety concerns. Collectively, these studies clearly document the safety and efficacy of combination therapy in patients with moderate persistent asthma.

Despite this body of evidence in patients with moderate to severe asthma, the question remained whether the FACET study results were applicable to patients with mild persistent asthma. This question was answered by O'Byrne and colleagues in the OPTIMA study [9], in which formoterol 4.5 μg bid or placebo was added to budesonide 100 or 200 μg bid. In patients not previously treated with ICS, treatment with budesonide alone was as good as treatment with the combination of budesonide plus formoterol. However, in patients who were already using ICS but who remained symptomatic, the addition of formoterol resulted in reduced exacerbation rates (Fig. **2**), similar to the reductions observed in the FACET study. No safety concerns were identified in this study.

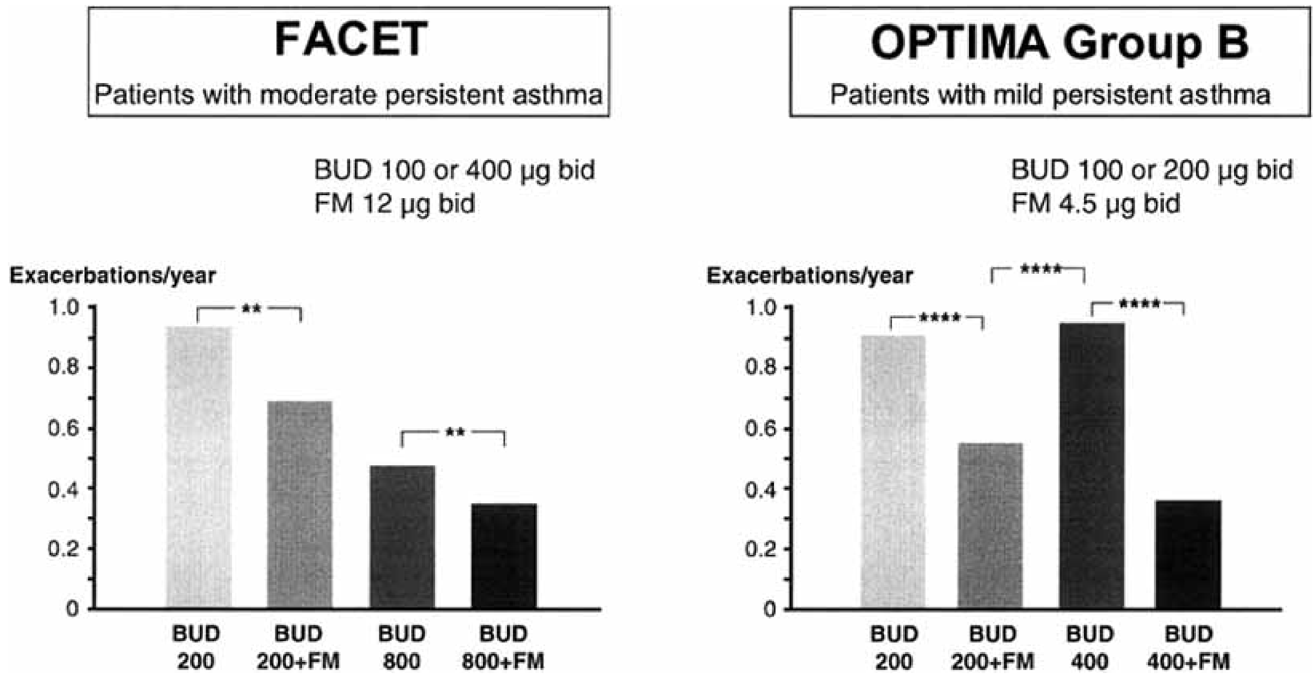

Fig. (2). Decrease in the frequency of severe exacerbations in patients with asthma treated twice daily (bid) with inhaled budesonide (BUD) alone, or in combination with inhaled formoterol (FM) in two separate studies: FACET [6] and OPTIMA (patient group B, i.e. those whose symptoms remained uncontrolled despite treatment with ICS) [9]. In both studies, treatment with BUD plus FM resulted in significantly fewer exacerbations/year than treatment with BUD alone (** = $p<0.01$; **** = $p<0.0001$). In mild persistent asthma (OPTIMA study), combination treatment with FM and lower-dose BUD had a significantly greater effect than treatment with a 2-fold higher dose of BUD alone. Adapted from data in Pauwels *et al*. (N Engl J Med 1997; 337: 1405-1411, with permission) and O'Byrne *et al*. (AJRCCM 2001; 164: 1392-1397, with permission).

Results from several controlled studies evaluating the efficacy and safety of salmeterol together with an ICS compared with a higher dose of ICS have been summarised in two meta-analyses [10, 11]. Results from the first meta-analysis, which evaluated the efficacy of salmeterol plus low-dose fluticasone propionate compared with a higher dose of fluticasone propionate [10], were in agreement with both the FACET and OPTIMA studies. Improved asthma control and lung function, fewer asthma symptoms and lower use of reliever medication were observed in patients treated with the combination compared with those receiving a higher dose of ICS. Both meta-analyses reported fewer exacerbations in patients treated with the ICS plus LABA combination, even though the individual studies had not found a statistically significant difference in asthma exacerbation rates between the two treatment groups. It appears that the individual studies may not have been adequately powered to compare differences in exacerbation rates.

ICS Plus LABA Delivered in a Single Inhaler

Once the safety and efficacy of the combination of ICS plus LABA had been confirmed in patients with asthma, the next logical step was to combine the two agents in a single inhaler. Two products have been developed to date: Seretide™ (salmeterol/fluticasone propionate) and Symbicort® (budesonide/formoterol).

Salmeterol/Fluticasone Propionate

The first study investigating this combination compared salmeterol/fluticasone propionate 50/100 μg bid in a single inhaler with the monocomponents delivered *via* separate inhalers [12]. No differences between the two methods of delivery were found for any asthma variable. Later studies evaluated the efficacy of salmeterol/fluticasone propionate 50/250 μg and 50/500 μg. The combination inhaler showed better efficacy than the same dose of either component alone [13, 14] and better efficacy than a higher dose of the ICS (budesonide or fluticasone propionate) alone [15-17]. All treatments were well tolerated. A slightly higher frequency of localised effects (throat irritation, candidiasis and hoarseness) was noted in the patients treated with salmeterol/fluticasone or fluticasone alone, compared with either salmeterol alone or placebo [13, 16].

Four studies have compared salmeterol/fluticasone propionate in a single inhaler with the same doses delivered *via* separate inhalers [12, 18-20]. Similar efficacy and safety profiles were demonstrated, irrespective of whether one or two inhalers were used. However, in a meta-analysis of the same four studies [21], the authors claimed that single inhaler delivery had improved efficacy based on the odds of achieving an improvement of >15 or >30 L/min in morning peak expiratory flow (PEF) values. However, the primary meta-analysis did not demonstrate a clinically relevant difference in morning PEF or any other parameters for delivery *via* a single versus separate inhalers. Based on general recommendations and practice accepted for bioequivalence studies (including all studies referred to in this meta-analysis), two inhalation products are considered equivalent if the mean difference for morning PEF and its 95% confidence interval lie entirely within the ±15 L/min range. The difference obtained in the meta-analysis, however, was only 5.4 L/min (95% confidence interval 1.5-9.2) thus lying entirely within the accepted range of equivalence. Furthermore, the mean difference for all asthma control measures numerically favoured inhalation by separate inhalers, suggesting that the efficacy of treatment with salmeterol and fluticasone was comparable or better in patients given separate inhalers.

Budesonide/Formoterol

Like salmeterol/fluticasone propionate, budesonide/formoterol in a single inhaler has been studied extensively in patients with asthma. The first of these studies compared the efficacy and safety of budesonide/formoterol (160/4.5 µg, 2 inhalations bid) with the same doses delivered *via* separate inhalers and with the same dose of budesonide alone over a 12-week period [22]. Treatment with budesonide/formoterol, whether delivered by single or separate inhalers, improved airway function and reduced asthma symptoms and reliever medication use compared with budesonide alone. All treatments were well tolerated and the adverse event profiles were similar in the three groups. Interestingly, over the first 40-50 days of the study, budesonide/formoterol in a single inhaler was slightly more effective than the monocomponents delivered *via* separate inhalers (Figs. **3A** and **3B**). The reason for this difference may be a function of improved patient compliance. It could also indicate, as postulated by Nelson and colleagues [21], that a difference in time between inhalation of the bronchodilator and the anti-inflammatory medication – whatever that difference might be – is important. The rapid improvement in symptom control seen in patients treated with budesonide/formoterol in a single inhaler (Fig. **3B**) [22] provides stronger evidence of a synergistic effect with a single inhaler than the salmeterol/fluticasone studies described above, meta-analysis of which showed no symptomatic improvement for the combination product compared with the monocomponents.

A 6-month double-blind study in adults with moderate persistent asthma who were already using ICS and LABA showed no differences in efficacy and safety when budesonide (160 µg 2 inhalations bid) and formoterol (4.5 µg 2 inhalations bid) were administered *via* one or two inhalers [23]. This study had a 6-month open extension period, during which good tolerability and safety were documented with combination therapy, irrespective of the number of inhalers used [24]; however, more patients using two inhalers withdrew from the study.

Results of subsequent studies have shown that budesonide/formoterol is more effective than a higher dose of ICS alone [25, 26]. Treatment with budesonide/formoterol in a single inhaler consistently resulted in improved airway function, fewer symptoms and less use of reliever medication, compared with a 2-fold higher dose of budesonide. In adult patients with moderate to severe asthma, budesonide/formoterol (320/9 µg, 2 inhalations bid) was also superior to the same dose of budesonide alone [27]. Similar results were seen in children with asthma: a study by Tal *et al.* demonstrated that budesonide/formoterol 80/4.5 µg had greater clinical efficacy in children than the same dose of budesonide alone [28].

In a 3-month study in patients with moderate persistent asthma not fully controlled with ICS (400–1000 µg/day; n=523), budesonide/formoterol (160/4.5 µg), administered either as 2 inhalations once daily (od) in the evening or as 1 inhalation bid, was compared with budesonide 400 µg od [29]. Budesonide/formoterol, both once and twice daily, improved airway function, asthma symptoms and exacerbations, compared with budesonide alone. No significant differences were observed between the two budesonide/formoterol groups.

Adjustable Dosing with Budesonide/Formoterol in a Single Inhaler

Double-blind controlled studies in steroid-naïve patients with asthma [30] and in patients already using ICS [31] have documented statistically significant dose-response relationships for budesonide, even if the dose-response curve is relatively flat. Improvements in airway function seen with budesonide also occur rapidly, within hours rather than days of administration [32]. In addition, a double-blind study showed that asthma could be controlled equally well by a low maintenance dose of budesonide (100 µg bid) as by a higher dose (400 µg bid), provided that patients received additional doses of budesonide as soon as a deterioration in asthma control became apparent [33]. Inhaled formoterol also exhibits a clear dose-response relationship in patients with asthma [34].

As both components of the budesonide/formoterol combination show a dose-response relationship and additional doses taken as needed can provide further efficacy [33, 35], a logical progression was to investigate the effect of adjusting the dose of budesonide/formoterol according to the patient's needs. The concept of adjustable maintenance treatment with budesonide/formoterol has recently been reviewed in detail by Buhl [36]. Briefly, adjustable dosing with budesonide/formoterol means that asthma patients who are well controlled (according to predefined criteria) on 2 inhalations of budesonide/formoterol bid can decrease their dosage to a low maintenance dose (1 inhalation bid). If the patient's clinical status deteriorates, the dose is increased from 1 to 4 inhalations bid. Once asthma control is regained, the dose can be reduced once again to 1 inhalation bid.

This adjustable dosing regimen was first tested in 1034 patients with moderately severe asthma using ICS (between 400 and 1000 µg per day) [37]. During a 1-month run-in period, patients received budesonide/formoterol 80/4.5 µg or 160/4.5 µg, 2 inhalations bid; the doses given were determined by their previous ICS doses, ≤500 µg per day or >500 µg per day. Thereafter, patients were randomised to treatment with the same daily doses of budesonide/formoterol as during the run-in phase, but used either the fixed (2 inhalations bid) or adjustable dosing schedules described above for 6 months. Patients in the adjustable maintenance dosing group increased their medication when their asthma control deteriorated, as assessed by use of reliever medication, night-

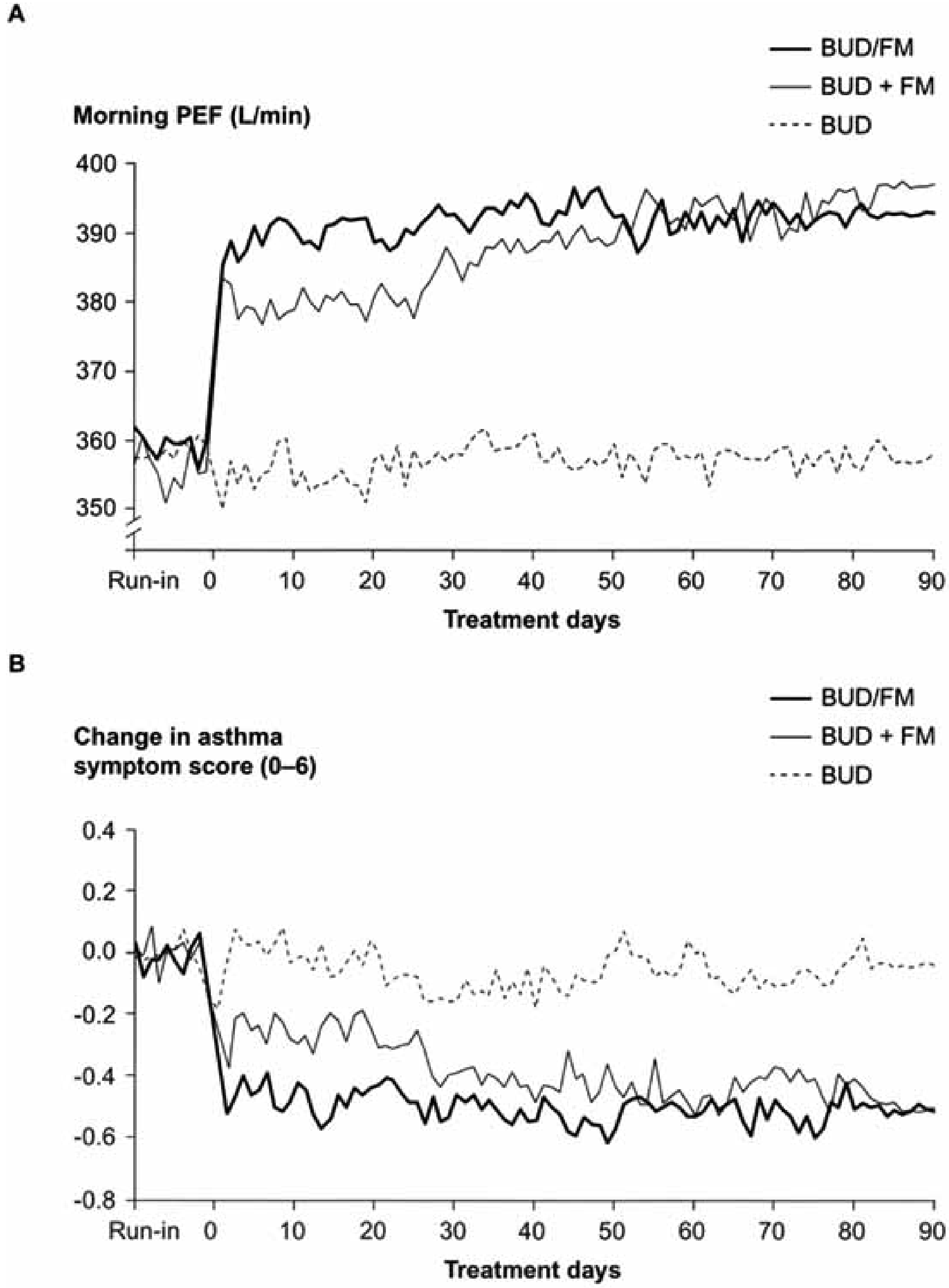

Fig. (3). Improvements in A) morning peak expiratory flow (PEF) and B) symptom score in a 12-week study after treatment with budesonide and formoterol administered in a single inhaler (BUD/FM; Symbicort®; 160/4.5 μg, 2 inhalations bid) or two inhalers (BUD + FM) compared with budesonide (BUD) alone (200 μg metered dose, corresponds to a delivered dose of 160 μg; 2 inhalations bid) [22] Permission granted by: European Respiratory Society Journals Ltd.

time awakenings and/or a PEF value <85% of the mean baseline value (two of these three criteria were required). All patients used a short-acting inhaled β_2-agonist, as needed, for relief from symptoms. The primary efficacy variable was the rate of severe asthma exacerbations.

Despite the fact that patients in the adjustable maintenance dosing group used 40% less study medication than those receiving fixed doses (2.35 vs 3.95 doses per day; $p<0.001$), there were significantly fewer exacerbations (6.2% vs 9.5% of patients with one or more exacerbations; $p<0.05$) in the adjustable maintenance dosing group than in the control group (Fig. **4**, Study A). Thus, better asthma control was achieved with lower doses of ICS when patients adjusted their medication levels according to their asthma control. Few adverse events were recorded and the serious adverse events reported (in 3% and 2% of patients in the adjustable and fixed dosing groups, respectively) were not considered to be related to treatment. In addition, adjustable dosing was found to be highly cost-effective [37].

Similar results were reported in a study by Leuppi *et al*. [38] and in a 5-month study in 995 patients by FitzGerald *et al*. [39]. In the latter trial, patients were randomised to budesonide/formoterol 80/4.5 μg or 160/4.5 μg, two inhalations bid, depending on their previous use of ICS (250-400 μg per day or 500-1000 μg per day, respectively). The rate of severe asthma exacerbations was reduced by 57% in patients in the adjustable maintenance dosing group compared with fixed dosing (p=0.001). A total of 8.9% of patients in the fixed dosing group experienced at least one exacerbation compared with 4.0% of patients in the adjustable treatment group (p=0.002) (Fig. **4**, Study B). A further 3-month study included 3297 adult patients with mild to moderate asthma previously treated with ICS doses ≤1000 μg per day [40]. Despite a >30% lower mean dose of study medication (2.6 vs 3.8 inhalations per day; p<0.001), adjustable dosing and fixed dosing were equally effective in improving the primary efficacy variable, i.e. changes in a standardised asthma quality of life questionnaire. Few adverse events were reported; these were equally distributed between the treatment groups [38-40].

In a recent study, Aalbers *et al*. [41] compared a fixed dose of budesonide/formoterol (160/4.5 μg 2 inhalations bid) with a fixed dose of salmeterol/fluticasone propionate (50/250 μg bid) during a 1-month double-blind period. Patients receiving budesonide/formoterol were then randomised to either continue with fixed dosing or to adjustable dosing for 6 months in an open-label period. Because the dose of salmeterol is always fixed, an adjustable regimen of salmeterol/fluticasone propionate could not be tested without the use of additional inhalers (salmeterol/fluticasone propionate 50/125, 50/250 or 50/500 μg), which is impractical and likely to be confusing for the patient.

In this study, there were no statistically significant differences between the two fixed dosing regimens in the 1-month double-blind period. During the 6-month open study period, however, adjustable dosing with budesonide/formoterol significantly reduced the rate of severe asthma exacerbations by 40% compared with salmeterol/fluticasone propionate [41] (Fig. **5**). The difference in exacerbation rates for the fixed and adjustable dosing budesonide/formoterol groups did not reach statistical significance in this study (p=0.08). The number of well-controlled asthma weeks (defined as a week with no night-time awakenings, no exacerbations and no change in asthma medication, plus at least two of the following: asthma symptoms on ≤2 days, ≤2 days of reliever medication use and morning PEF ≥80% predicted normal every day) was similar with all treatments. All three regimens were well tolerated and no safety issues were raised. To date, this is the only study directly comparing the efficacy and safety of budesonide/formoterol and salmeterol/fluticasone propionate in patients with asthma. It is noteworthy that in all studies using Turbuhaler® delivery of ICS, with or without formoterol, the incidence of local side effects has been low (<1% to 3%) [37-40].

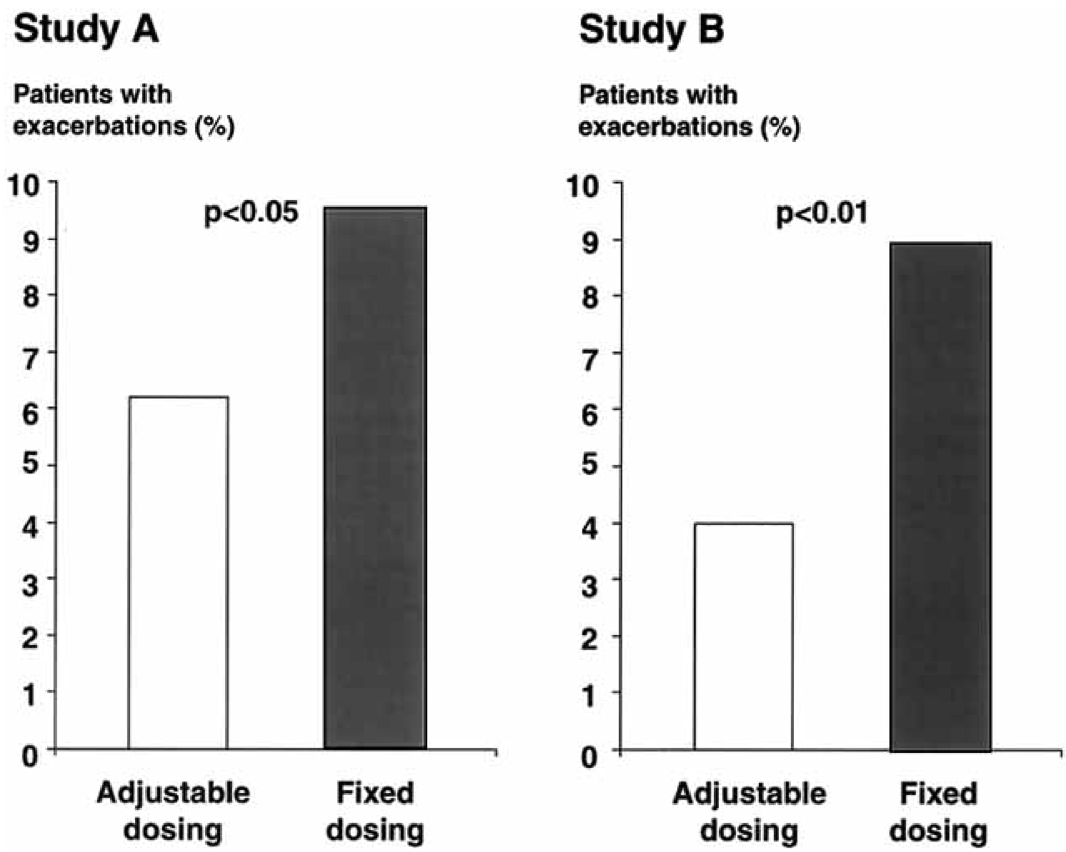

Fig. (4). Percent of patients with asthma experiencing exacerbations in two studies comparing fixed and adjustable dosing of budesonide/formoterol (BUD/FM; Symbicort®; 80/4.5 μg 2 inhalations bid in both studies, depending on previous ICS dose. For dose adjustments, see text). Based on Ställberg *et al.* [37] (Study A) and FitzGerald *et al.* [39] (Study B, reproduced with permission Can Respir J 2003;10(8):427-434.

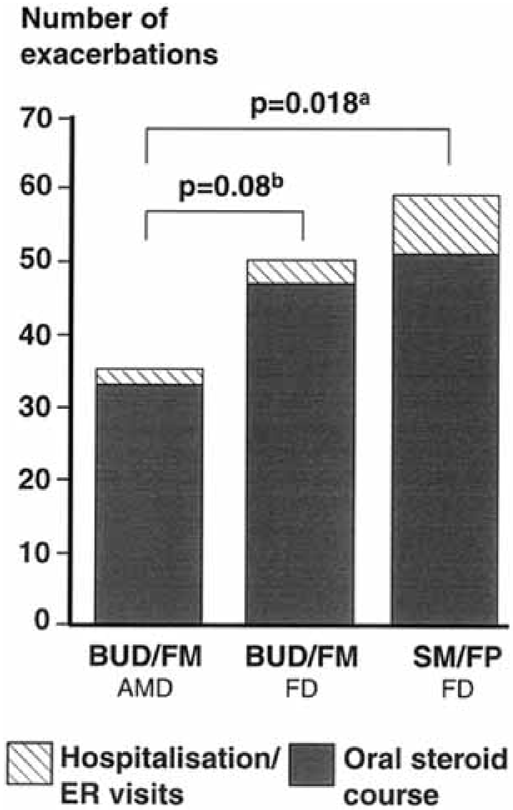

Fig. (5). Total number of exacerbations (sum of hospitalisations/ emergency room (ER) visits and oral steroid courses in a study comparing fixed dosing (FD) with budesonide/formoterol (BUD/ FM; Symbicort®; 160/4.5 µg, 2 inhalations bid) and salmeterol/ fluticasone propionate (SM/FP; Seretide™; 50/250 µg 1 inhalation bid) and adjustable dosing (AMD; see text for dose adjustments) with BUD/FM in patients with asthma [41]. [a]39.7% reduction in rate of total exacerbations in BUD/FM AMD versus SM/FP FD (p=0.018); [b]32.0% reduction in rate of total exacerbations in BUD/FM AMD versus FD (p=0.08). Based on Aalbers *et al.* 2004 [41]. Reproduced with permission.

Single Inhaler Therapy with Budesonide/Formoterol

Because of the rapid onset of bronchodilation achieved with formoterol, in contrast to salmeterol, the onset of effect of budesonide/formoterol is faster than that of salmeterol/fluticasone propionate [42]. Budesonide/formoterol has also been shown to rapidly reverse methacholine-induced moderate to severe bronchoconstriction; the effect of budesonide/formoterol was significantly faster than that of salmeterol/fluticasone [43]. These properties, combined with the success of adjustable dosing of budesonide/formoterol in a single inhaler, suggested that budesonide/formoterol might be suitable for use not only as adjustable maintenance dosing but also on an as-needed basis, Single inhaler Therapy. With such a regimen, patients would need only one inhaler for both maintenance and as-needed use, potentially impacting on adherence to treatment.

Current asthma guidelines state that a temporary increase in asthma symptoms should be treated with extra doses of a rapid-acting bronchodilator. However, both inflammation and bronchoconstriction may be involved in the progression from an increase in asthma symptoms to a clear-cut clinical exacerbation [44]. Therefore, it may be useful to simultaneously administer additional doses of anti-inflammatory medication when additional doses of bronchodilator are needed.

A prerequisite for the use of budesonide/formoterol for both maintenance and as-needed use is that both components have a good safety profile, even at high doses. Formoterol, administered *via* Turbuhaler®, has been demonstrated to be safe and well tolerated at doses of 90 µg per day given over a few hours in patients with severe acute asthma [45] and over 3 days in patients with stable asthma [46]. Furthermore, in a double-blind tolerability study in 10 patients using budesonide/formoterol 1920/45 µg per day for 3 days, no clinically relevant differences versus placebo were seen in serum potassium levels, pulse rate, blood pressure, QTc interval, blood glucose and plasma lactate levels [47].

Results of controlled Single inhaler Therapy studies with budesonide/formoterol have now been reported [156-158]. They show that compared with higher fixed doses of budesonide and terbutaline used as needed, budesonide/formoterol as both maintenance and as-needed medication improved asthma control, reduced the risk of asthma exacerbations but with less total use of medication and at a lower cost.

CLINICAL EFFICACY OF ICS/LABA COMBINATION THERAPY IN COPD

Together with inhaled anticholinergic drugs, LABA represent the first-line treatment for patients with COPD who require maintenance treatment for daily symptom relief [48] and several studies have documented their usefulness [49-57]. Both formoterol (used as either maintenance therapy or maintenance plus as-needed therapy [56, 57]) and salmeterol (50 µg, but not 100 µg) have been shown to significantly improve health-related quality of life in this patient group [58, 59].

In a crossover study in 16 patients with partly reversible airway obstruction (reversibility 11-26%), no difference was detected in the 12-hour time-response curve for forced expiratory volume in 1 second (FEV_1) (AUC_{0-12h}) between salmeterol/fluticasone propionate (50/250 µg) and budesonide/formoterol (320/9 µg) single-dose treatments. Effects on inspiratory capacity were also similar [60]. Airway function was measured at 15 and 30 minutes, but results at these timepoints were not described. More interestingly, a study in 20 patients with stable COPD showed a greater increase in FEV_1 following a single dose of budesonide/formoterol (160/ 4.5 µg 2 inhalations) compared with formoterol alone (4.5 µg 2 inhalations) [61]. Also the FEV_1 $AUC_{0-60min}$ was larger after inhalation of budesonide/formoterol than after formoterol alone. These clinical results indicate that the addition of budesonide amplifies the fast onset of action of formoterol.

The role of ICS in COPD remains controversial since four 3-year placebo-controlled, parallel group studies failed to show any disease-modifying effect on the annual rate of decline in FEV_1 [62-65]. A recent meta-analysis, however, has documented a significant effect on the decline in FEV_1 [66]. Other beneficial effects have been documented, including improved airway function during the first months of treatment, improved exercise capacity, improved quality of life and reduced symptoms and exacerbations [64, 67, 68]. Retrospective cohort analyses have also suggested that ICS may reduce the risk of re-hospitalisation and mortality in COPD [69-71].

Salmeterol/Fluticasone Propionate

The salmeterol/fluticasone propionate combination has been extensively tested in patients with COPD; a detailed review on the use of this combination in this patient population has been recently published [72]. Four placebo-controlled studies have been reported; two 6-month studies [73, 74] and two 12-month studies [75, 76]. Overall, more than 3500 patients have been included in these studies, with baseline FEV_1 values of ≤65% [73, 74], ≤70% [75] or ≤80% predicted normal [76]. Two studies each compared salmeterol/fluticasone propionate (50/250 μg [74, 76] and 50/500 μg [73, 75]) with salmeterol or fluticasone propionate alone.

In the low-dose 6-month study by Hanania and colleagues, combination treatment with salmeterol/fluticasone propionate substantially improved morning lung function compared with salmeterol or fluticasone propionate alone; these improvements were sustained for the duration of the study [74]. The transitional dyspnoea index decreased significantly in the combination and salmeterol groups compared with placebo, but not for the combination versus the monocomponents. Health status also improved significantly in the combination group compared with placebo, but not compared with the monocomponents. In a 1-year parallel-group trial in COPD patients treated with theophylline [76], 18 patients were treated with either salmeterol/fluticasone propionate, salmeterol or placebo. Morning airway function, COPD symptom scores and reliever medication use improved to a greater extent in the salmeterol/fluticasone propionate group than in the other two groups. The mean numbers of exacerbations per year fell from 3.5 during the year prior to the study to 1.2 in the combination group ($p<0.001$) and from 3.0 to 2.3 in the salmeterol group (not significant), but increased from 3.2 to 4.2 in the placebo group (not significant).

The results of the higher-dose studies showed consistently and significantly better improvements in airway function and health-related quality of life with salmeterol/fluticasone propionate than with salmeterol or fluticasone propionate alone [73, 75]. Combination treatment also significantly reduced dyspnoea and use of reliever medication compared with placebo, salmeterol and fluticasone propionate. The exacerbation rate was studied in the 12-month study [75] and was significantly reduced in patients treated with combination therapy compared with placebo, but not when compared with the individual agents; the exacerbation rate fell by 25% in the combination group and by 20% and 19% in the salmeterol and fluticasone propionate groups, respectively, compared with placebo. The treatment effect was more pronounced in patients with a baseline FEV_1 <50% of predicted normal, but significant differences in exacerbation rates were not evident between the patients treated with the combination and the monocomponents. The overall adverse-event profile of combination therapy showed no new or unexpected adverse events and all treatments were generally well tolerated [73, 75]. In the study by Mahler *et al*. however, the frequency of candidiasis was 7% and 10% with salmeterol/fluticasone combination and fluticasone, respectively, compared with <1% for placebo and salmeterol alone [73]. The frequency of candidiasis in the study by Calverley and colleagues was 6% in patients treated with either the combination or fluticasone alone and 1% in patients treated with placebo or salmeterol, respectively [75].

Budesonide/Formoterol

The efficacy of budesonide/formoterol has been investigated in two studies in a total of 1834 COPD patients with a baseline FEV_1 ≤50% predicted normal [77, 78]. The studies differed in design with respect to the run-in period: in the study by Szafranski *et al*. all medications were withdrawn except for terbutaline [77], whereas in the study by Calverley *et al*. patients received prednisolone 30 mg od and inhaled formoterol 9 μg bid during the run-in period in order to optimise their clinical status [78]. This difference in design was reflected in the withdrawal rates (Fig. **6**). Without optimisation, the withdrawal rate was high in the placebo group but there were no differences between the three active treatments (Fig. **6**, Study A; [77]). With optimisation, treatment with budesonide/formoterol resulted in a significantly lower withdrawal rate than the three other treatments (Fig. **6**, Study B; [78]).

In both studies, budesonide/formoterol significantly improved airway function and health-related quality of life compared with placebo (Fig. **7**), as well as reducing the rate of severe exacerbations, COPD symptoms and use of reliever medication [77, 78]. In the study by Szafranski and colleagues, the rate of severe exacerbations (defined as events requiring hospitalisation, antibiotics or a course of oral prednisolone) was also significantly reduced in the budesonide/formoterol group compared with the formoterol-alone group [77]. In the study by Calverley and colleagues, a significantly prolonged time to the first severe exacerbation was observed for patients treated with budesonide/formoterol compared with patients treated with formoterol or placebo [78]. Combination therapy resulted in significantly greater improvements in most of the secondary variables studied, especially when compared with budesonide. The tolerability of all treatments was good [77, 78]. The mean number of adverse events experienced with budesonide/formoterol was similar to placebo (5, 5, 6 and 5 adverse events per 1,000 treatment days for the budesonide/formoterol combination, budesonide, formoterol and placebo groups, respectively) [78]. The frequency of local side effects, including hoarseness and oropharyngeal candidiasis, was low and reported by <1% to 2% of patients in all treatment groups [77, 78].

The difference in results for budesonide/formoterol and salmeterol/fluticasone propionate studies in patients with COPD (statistically significant differences in the rate of severe exacerbations [77] or time to first severe exacerbation [78] with budesonide/formoterol versus formoterol, but not with salmeterol/fluticasone versus salmeterol alone [75]) may, at least in part, be due to differences in inclusion criteria, as the budesonide/formoterol studies recruited patients with more severe disease.

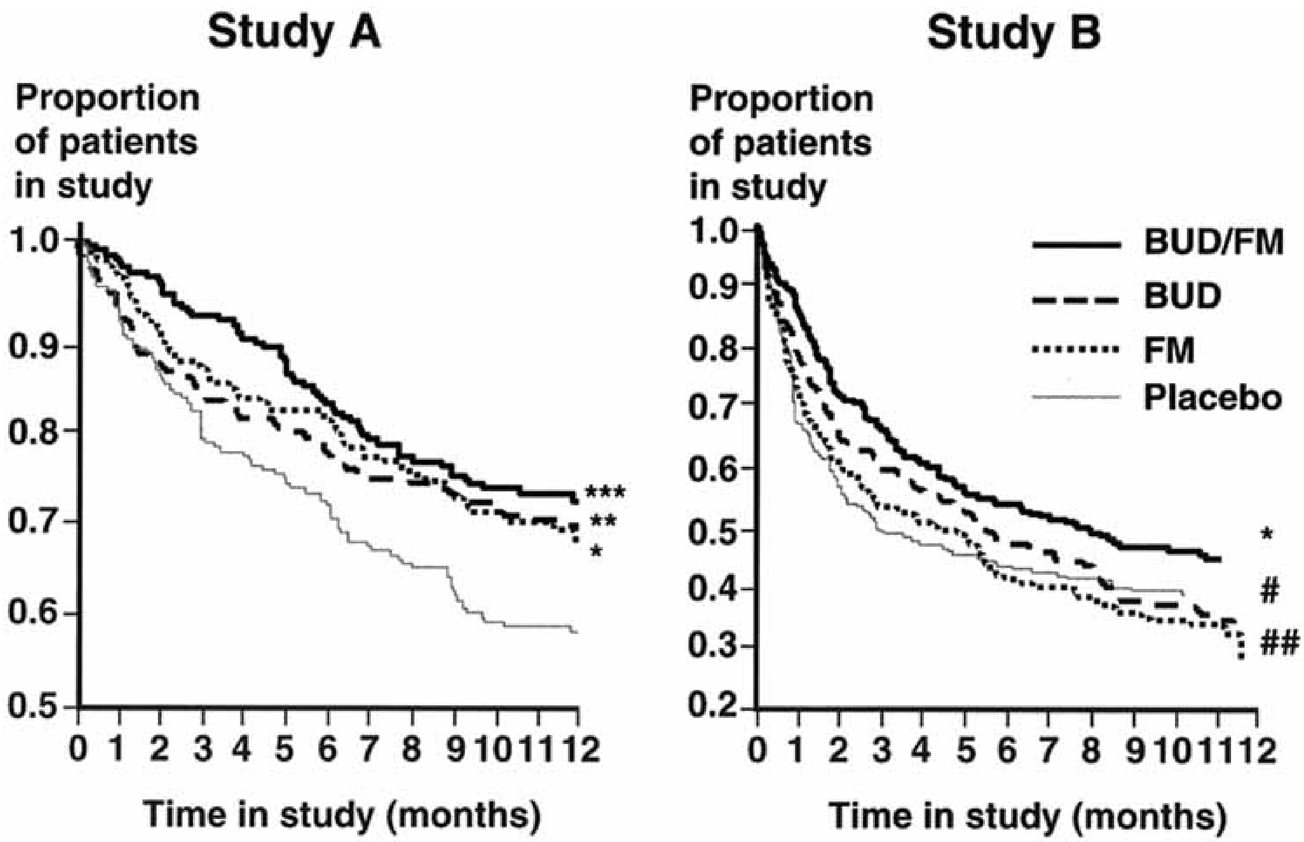

Fig. (6). Withdrawal rates (total withdrawals in Study A and withdrawals as a result of exacerbations in Study B) in two 12-month COPD studies with different treatments during run-in. Patients were treated with budesonide/formoterol (BUD/FM; Symbicort®; 160/4.5 μg 2 inhalations bid), budesonide (BUD; 200 μg metered dose, corresponds to a delivered dose of 160 μg; 2 inhalations bid), formoterol (FM; 4.5 μg 2 inhalations bid) or placebo. During the 2-week run-in phase, previous treatments were withdrawn (Study A) [77] or treatment was optimised with oral prednisolone and inhaled FM (Study B) [78]. In Study A: *** = p<0.001 for BUD/FM vs placebo, ** = p=0.01 for FM vs placebo, * = p<0.05 for BUD vs placebo. In Study B: * = p<0.05 for BUD/FM vs placebo, # = p<0.05 for BUD vs BUD/FM, ## = p=0.002 for FM vs BUD/FM. Based on Szafranski *et al.* [77] (Study A, permission granted by: European Respiratory Society Journals Ltd) and Calverley *et al.* [78] (Study B, permission granted by: European Respiratory Society Journals Ltd).

PROPOSED MECHANISMS FOR THE EFFICACY OF ICS/LABA COMBINATION THERAPY: PRECLINICAL DATA

From the studies discussed above, it is clear that the efficacies of ICS and LABA complement each other in a clinically meaningful way in both asthma [79] and COPD [80, 81]. For many of the variables studied, combination therapy with the two drugs results in additive effects. Some of the results described, however, indicate that the effects are more than just additive, especially in more severe forms of asthma and COPD. This suggests that ICS and LABA may enhance the effects of each other, leading to improved lung function, bronchoprotection and anti-inflammatory activity. In particular, the reduction in disease exacerbations may be a result of a decrease in airway and lung inflammation. Therefore, questions arise regarding the mechanisms leading to these effects and whether, compared with ICS monotherapy, combination therapy with ICS/LABA is associated with improved β_2-adrenoceptor and GR function as well as enhanced anti-inflammatory activity, which potentially leads to greater control over airway remodelling processes. Many preclinical studies have attempted to answer these questions.

Anti-Inflammatory Activity

In recent years, evidence from preclinical studies has emerged suggesting that at clinically relevant concentrations, glucocorticosteroid (GCS)/LABA combinations exert greater anti-inflammatory effects and, in some cases, greater anti-remodelling effects, than GCS alone.

This was first demonstrated *in vitro* in airway structural cells where additive and even synergistic effects were demonstrated. In cultured human lung fibroblasts, GCS and LABA were shown to additively decrease the pro-inflammatory cytokine-induced expression of adhesion molecules regulating infiltration of inflammatory cells into the airways and lung. Fluticasone propionate plus salmeterol decreased tumour necrosis factor-α (TNFα)-induced intercellular adhesion molecule-1 (ICAM-1) expression [82], whereas budesonide plus formoterol additively decreased interleukin (IL)-1β -induced expression of both ICAM-1 and vascular adhesion molecule-1 (VCAM-1) [83]. In these cells, the combination of budesonide plus formoterol also additively decreased IL-1β-stimulated production of granulocyte-macrophage colony-stimulating factor (GM-CSF), a cytokine important for the activation and survival of eosinophils [83]. However, the

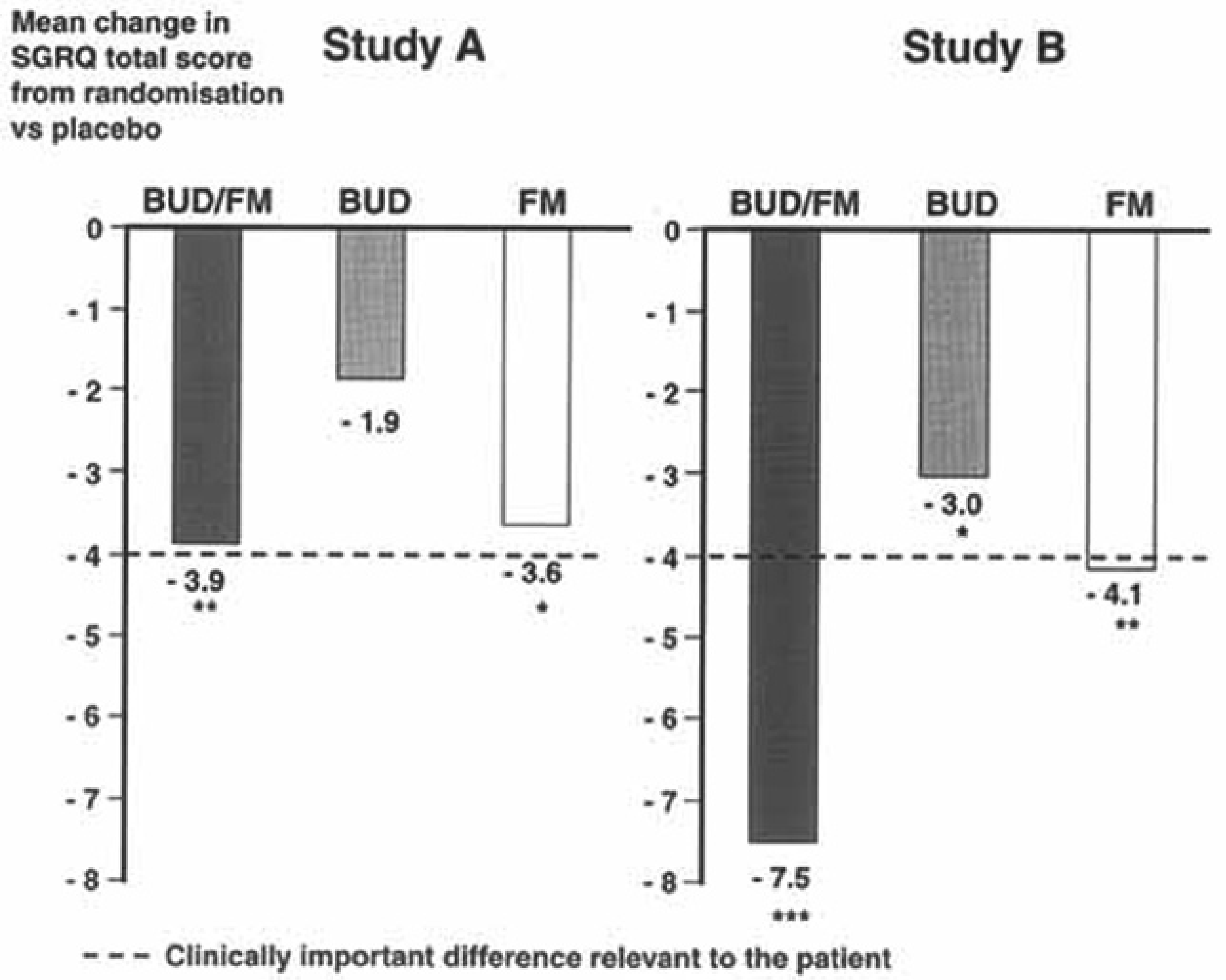

Fig. (7). Health-related quality of life scores in two 12-month placebo-controlled studies in patients with COPD comparing budesonide/formoterol (BUD/FM; Symbicort®), budesonide (BUD) and formoterol (FM) (Study A [77] and Study B [78]; daily doses as in Fig 6). Health-related quality of life was assessed using the St George's Respiratory Questionnaire (SGRQ). In both studies: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$ vs placebo. In Study B: $p=0.001$ for BUD vs BUD/FM and $p=0.014$ for FM vs BUD/FM. Based on Szafranski *et al.* [77] (Study A, permission granted by: European Respiratory Society Journals Ltd) and Calverley *et al.* [78] (Study B, permission granted by: European Respiratory Society Journals Ltd).

production of IL-8, which regulates the activation and infiltration of neutrophils, was decreased by budesonide plus formoterol to a similar extent as by budesonide alone.

Similarly, budesonide and formoterol additively decreased (to basal levels) the secretion of GM-CSF in human bronchial epithelial cells stimulated by TNFα [84]. In contrast, formoterol alone increased the secretion of IL-8; the decrease in IL-8 achieved with budesonide and formoterol in combination did not differ from the effect achieved with budesonide alone.

In cultured human airway smooth muscle cells, fluticasone propionate or dexamethasone, in combination with either salmeterol or the short-acting β_2-agonist salbutamol, additively decreased TNFα-induced release of eotaxin, a chemokine involved in increasing activation and infiltration of eosinophils, basophils and T-helper-2 (Th2) -like lymphocytes [85]. Furthermore, salmeterol and salbutamol synergistically potentiated the inhibitory effect of fluticasone propionate and dexamethasone on IL-8 release from these cells, although on their own salmeterol and salbutamol had no effect [86]. In addition, in both studies, combinations of fluticasone propionate or dexamethasone with other agents that increase intracellular levels of cyclic adenosine monophosphate (cAMP), such as forskolin, were more effective than GCS alone [85, 86].

Experimental support for the inhibitory effects of LABA, both alone and in combination with GCS, is less consistent for inflammatory cells than for airway and lung structural cells. Formoterol, and to a lesser extent salbutamol (but not salmeterol), were shown to inhibit superoxide production and elastase release in human neutrophils [87]. In human eosinophils stimulated by conditioned medium from bronchial epithelial cells, formoterol alone and in combination with budesonide decreased superoxide generation [88]. In contrast, Nielson and Hadjokas [89] showed that salmeterol and salbutamol blocked the inhibition of superoxide generation induced by dexamethasone in eosinophils. On the other hand, the combination of dexamethasone with salmeterol had enhanced inhibitory effects on human monocyte proliferation and cytokine release [90]. Recently, the combination of fluticasone propionate and salmeterol was shown to be more effective than fluticasone propionate alone on *in vitro* inhibition of lipopolysaccharide-stimulated IL-8 and TNFα release from alveolar macrophages taken from COPD patients [91].

To date, few *in vivo* studies have addressed the possible potentiation of the anti-inflammatory efficacy of GCS by LABA. Wiesenberg and colleagues [92] showed that the addition of formoterol to budesonide potentiated the inhibitory effect of budesonide on lung infiltration of eosinophils in the mouse ovalbumin model and lowered the threshold for the effective budesonide dose. In rats, a single low dose of budesonide plus formoterol significantly reduced (by 40-50%) Sephadex-induced lung oedema and lung concentrations of IL-1β whereas budesonide or formoterol alone had no effect at these low doses [93, 94]. In these studies, the inhibitory effects of a combination of low-dose budesonide and formoterol were equal to the effects of much higher doses of budesonide or formoterol alone. Both *in vivo* and in multicellular-like systems mimicking the *in vivo* situation [95, 96], GCS and LABA may have complementary actions involving different cells and mediators, working directly on inflammatory cells and indirectly *via* inhibition of pro-inflammatory mediators released from structural cells. Thus, some of the synergistic effects of GCS/LABA observed *in vivo* could be explained by the complementary actions of these drugs.

In addition to their complementary activity, compensatory effects may also contribute to the activity of the GCS/LABA combination: one drug may compensate for the lack of anti-inflammatory activity of the other drug or even reverse the potentiation of some inflammatory responses. For example, ICS reverse delayed eosinophil apoptosis in asthma, whereas salmeterol and salbutamol prolong eosinophil survival [97]. On the other hand, LABA may compensate for the known relative resistance of neutrophils to GCS. The mutual compensatory effects of ICS and LABA are illustrated by the recent study of Reid *et al*. [98] where fluticasone propionate increased neutrophil numbers while salmeterol decreased IL-8 concentration in bronchoalveolar lavage fluid from patients with asthma.

Anti-Remodelling Activity

Airway remodelling is an inherent component of asthma. Remodelling activity includes increased numbers of airway smooth muscle cells, increased subepithelial airway vascularity, epithelial shedding, goblet cell hyperplasia and fibrotic rearrangement of the airway extracellular matrix. The ability of ICS to influence airway remodelling is still under debate and there is a question as to whether or not ICS/LABA in combination result in greater control over airway remodelling than ICS alone. For example, Orsida *et al*. [99] demonstrated that inhaled salmeterol, but not fluticasone propionate, decreased the number of vessels in the subepithelial lamina propria of patients with asthma; the effects of both agents in combination were not investigated in this study.

In recent years, the effect of combination therapy on airway remodelling has been investigated in several preclinical studies. It is known that proliferation of airway smooth muscle cells is increased in asthma [100] and that β2-agonists have potential to decrease the enhanced proliferation of airway smooth muscle cells *via* β_2-adrenoceptor-dependent mechanism [101]. Recently, Roth and colleagues [102] demonstrated that even very low concentrations of formoterol (10^{-12}-10^{-8} M) effectively inhibited serum-stimulated proliferation of airway smooth muscle cells *in vitro*, an effect that was significantly increased by the addition of budesonide (Fig. **8**). In this study, the combination of budesonide plus formoterol also had a synergistic effect on the increased promoter activity of *p21*$^{(WAF1/CIP1)}$, a cell kinase inhibitor that downregulates cell proliferation [102]. Most recently, budesonide and formoterol in combination synergistically and completely inhibited the increased proliferation of airway smooth muscle cells in the ovalbumin mouse model [103]. In this study, proliferation of endothelial cells was also decreased by both budesonide and formoterol. Goncharova *et al*. [104] showed that salmeterol at very high concentrations (10^{-5} M) had no inhibitory effect on platelet-derived growth factor (PDGF)-stimulated airway smooth muscle cell migration *in vitro* but it slightly potentiated the inhibitory effects of fluticasone propionate and dexamethasone. Similar effects were observed for combinations of fluticasone propionate or dexamethasone with other agents increasing cAMP levels (such as forskolin or prostaglandin E_2 [PGE_2]). Unlike salmeterol, however, forskolin and PGE_2 were effective inhibitors in their own right of PDGF-stimulated migration.

Activation of lung fibroblasts is thought to play a key role in the fibrotic reorganisation of the extracellular matrix in asthmatic airways. Increased synthesis of specific collagens [105] and proteoglycans is a part of this process [106]. Recently, Goulet and colleagues demonstrated in cultures of lung fibroblasts and vascular smooth muscle cells that both GCS and β_2-agonists were able to decrease expression of some matrix metalloproteinases — key enzymes involved in remodelling of the extracellular matrix in asthma and COPD [107]. Furthermore, Todorova *et al*. [108] demonstrated the synergistic inhibition of serum-stimulated proteoglycan production by budesonide and formoterol in human lung fibroblasts. Formoterol potentiated the effect of budesonide on proteoglycans, which are characteristic of early fibrotic processes. Moreover, the combination of budesonide plus formoterol, but not budesonide or formoterol alone, decreased the production of decorin, a proteoglycan present in the later stages of fibrosis. In contrast, fibrotic processes, which were assessed by the amount of fibronectin and collagen in the airway wall of allergic rats, were increased by the addition of salmeterol to fluticasone propionate [109]; however, combining salmeterol and fluticasone propionate counteracted goblet cell hyperplasia in this model.

Interactions between GCS and LABA

The precise molecular mechanisms responsible for the superior control of asthma and COPD by ICS/LABA combi-

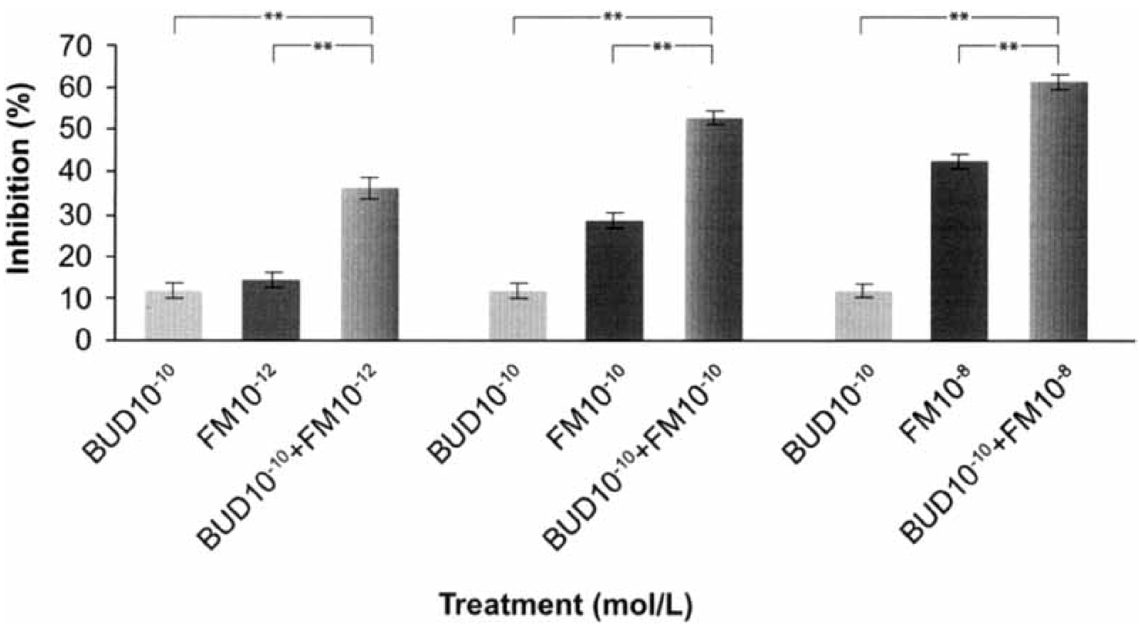

Fig. (8). Inhibitory effects of budesonide (BUD) and formoterol (FM) alone and in combination on 5% serum-induced proliferation of human airway smooth muscle cells *in vitro* (mean values ± SEM). All treatment groups showed significant inhibition ($p<0.01$) vs control (control = Proliferation at 5% serum - Negative control at 0.5% serum). The effects of treatment with BUD plus FM were significantly different vs BUD and FM alone (** = $p<0.01$ for both comparisons). The inhibition exerted by combinations of BUD with FM (10^{-12} M and 10^{-10} M) was significantly different ($p<0.05$; *t*-test) from the additive inhibition (arithmetic sum) of each drug alone. Modified from Roth *et al.* 2002 [102]. Reproduced with permission.

nation therapy are under intensive investigation. There is an increasing body of evidence suggesting that GCS and LABA may potentiate each other's effects *via* interactions at the receptor level and *via* interwoven signal transduction pathways.

The responsiveness of β_2-adrenoceptors in asthma may be decreased by chronic use of β_2-agonists and by inflammatory cytokines present in the airways (for a review see [110]). GCS have been shown to counteract and reverse the downregulation [111, 112] and desensitisation [113, 114] of β_2-adrenoceptors *via* several mechanisms. These include increased expression of β_2-adrenoceptors [115, 116] and G_s-protein [117] and decreased expression and activity of G-protein coupled receptor kinase-2, which by phosphorylation uncouples β_2-adrenoceptors from the G_s-protein [114]. Correspondingly, LABA have the potential to enhance GR expression and activation, as it has been shown that an increase in cAMP levels leads to increased expression of GR (*via* mRNA stabilisation [118]) and to increased GR activity [119].

The GR is a cytoplasmic receptor that, upon ligand binding, translocates to the cell nucleus where it influences the transcription of multiple genes by binding to DNA or transcription factors such as NF-κB and AP-1. Recently, Eickelberg and colleagues [120] demonstrated that β_2-agonists are able to translocate GR to the cell nucleus, a prerequisite for GCS activity. This ligand-independent translocation has been observed *in vitro* with salmeterol, formoterol and salbutamol in various human cells, namely lung fibroblasts [120], vascular smooth muscle cells [120], airway smooth muscle cells [102] (Fig. **9**) and mononuclear cells [121], but it does not seem to occur in cultured human bronchial epithelial cells [121-123]. GR translocation has also been demonstrated *in vivo* in blood leucocytes after inhalation of formoterol [124] and in sputum epithelial cells and macrophages after inhalation of salmeterol [125]. The ligand-independent modulation of GR is a novel finding, although it is well documented for other steroid receptors activated by cAMP, growth factors, cyclins and other stimuli (reviewed by Weigel [126] and Cenni [127]). It was previously believed that these stimuli could only modulate glucocorticoid-activated GR (reviewed by Weigel [126]).

An increase in GR nuclear translocation by β_2-agonists may be an important mechanism responsible for, or a contributing factor to, the superior effects of ICS/LABA combinations in controlling asthma exacerbations, severe asthma and COPD. Bellettato *et al.* [128] have recently shown that nuclear translocation of the active isoform-α of GR is reduced in airway epithelial cells infected by rhinovirus. Rhinovirus infections are among the most common triggers of asthma exacerbations [129] and are the most common viral infections in COPD patients [130]. Furthermore, nuclear localisation of GR seems to be impaired in a group of steroid-resistant and oral steroid-dependent patients [131]. Recently, Ito and colleagues have shown that dexamethasone-induced nuclear translocation of GR is impaired in peripheral blood mononuclear cells isolated from steroid-resistant asthma patients, compared with healthy subjects [132]. In both groups, incubation of cells with formoterol enhanced dexamethasone-induced GR translocation however, cells from steroid-resistant asthma patients were more sensitive to formoterol than those of healthy volunteers. After treatment of cells with formoterol plus dexamethasone, there was no

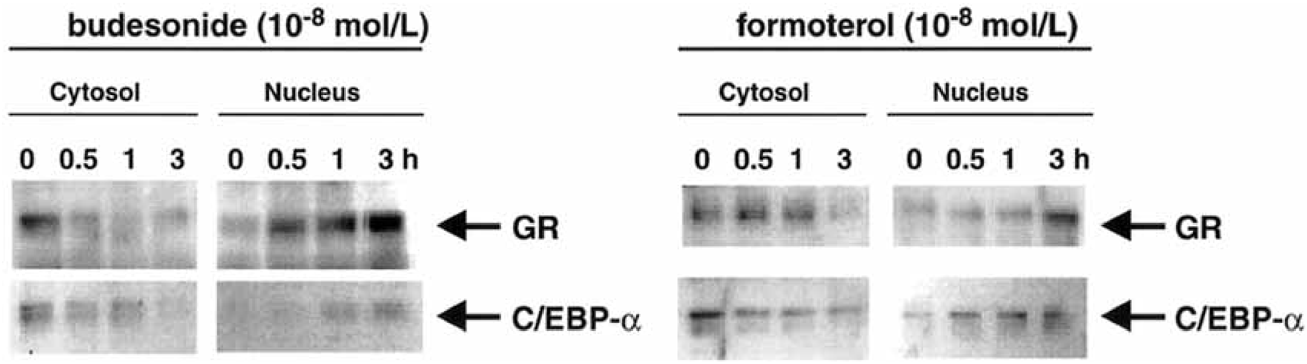

Fig. (9). Drug-induced translocation of the glucocorticoid receptor (GR) and CCAAT-enhancer binding protein-α (C/EBP-α) from the cytosol into the nucleus as shown by Western blot in human bronchial airway smooth muscle cells incubated with budesonide or formoterol for various time periods. Reproduced with permission from Roth *et al.* 2002 [102].

difference between healthy subjects and steroid-resistant asthma patients in nuclear localisation of GR in these cells. Thus, formoterol completely reversed the impairment of steroid-induced GR translocation in the peripheral blood mononuclear cells from steroid-resistant asthma patients. Several recent reports indicate that impairment of GR activation may be associated with GR phosphorylation at specific sites by mitogen-activated protein (MAP) kinases such as JNK, p38 and ERK [133-135]. Phosphorylation of GR by MAP-kinases may also play role in relative steroid resistance in COPD, as reactive oxygen species are potent activators of these kinases [136] and oxidative stress is a key pathological factor in COPD. Future studies are warranted to elucidate whether LABA improve impaired GR nuclear translocation by interfering with pathways of MAP-kinases.

Translocation of GR by β_2-agonists is mediated *via* G-protein-coupled β_2-adrenoceptors and appears to involve cAMP and protein kinase A (PKA) pathway as shown for salmeterol in cultured lung fibroblasts [120]. On the other hand, inhibition of TNFα-induced eotaxin by salbutamol in combination with dexamethasone was not dependent on PKA in airway smooth muscle cells [85]. Schmidt *et al*. [137] demonstrated that activation of β_2-adrenoceptors can enhance ligand-dependent GR transactivation *via* stimulation of phosphoinositide 3-kinase and that this occurs independently of PKA. These data suggest that signalling pathways for β_2-adrenoceptor-mediated activation of GR may be different for GCS-independent versus GCS-dependent GR activation. Signalling pathways may also be cell- and response-specific and may differ for GR-mediated repression (transrepression; not dependent on GR-GRE interactions) versus activation (transactivation; mediated *via* GR-GRE binding) of gene transcription.

The enhanced anti-inflammatory efficacy of GCS/LABA combination in preclinical studies described above suggest that LABA may potentiate the anti-inflammatory activity of GCS *via* enhanced gene transrepression, such as decreased NF-κB- or AP-1-dependent expression of pro-inflammatory cytokines, which is believed to be the major mechanism of GCS anti-inflammatory action. LABA may also repress pro-inflammatory genes through GR-independent pathways, as shown in human bronchial epithelial cells by Lovén *et al.* [122] who demonstrated that formoterol reduced AP-1-dependent activity without an involvement of GR. In Lovén *et al*. study, formoterol did not affect GRE-regulated activity. On the other hand, other studies showed that LABA may increase the anti-inflammatory effects of GCS by increased gene transactivation because GR translocated by β_2-agonists binds to GRE (Fig. **10**) and induces gene transcription, as shown for *p21*$^{(WAF1/CIP1)}$ [102, 120]. Thus, LABA may increase the GCS-induced expression of anti-inflammatory proteins, such as IL-10, as shown in monocyte/macrophage cell line (U937) for formoterol, but not for salmeterol [138]. Recently, Ito *et al.* [123] have demonstrated in a human bronchial epithelial cell line (BEAS2B) that, although neither formoterol nor salmeterol affected GR translocation, both drugs significantly enhanced dexamethasone-induced GR binding to GRE. Furthermore, both formoterol and salmeterol significantly enhanced dexamethasone-induced production of secretory leucocyte protease inhibitor. Both formoterol and salmeterol also partially inhibited IL-1β-induced IL-8 production but did not affect the ability of dexamethasone to induce inhibition of IL-8 nor did they affect the binding of GR to NF-κB. These results suggest that LABA enhance GCS-induced transactivation in these cells rather than GCS-induced repression of NFκB-dependent transcription of pro-inflammatory genes. GCS-induced gene transactivation is believed to be involved in the side effects of GCS. However, nearly 10 years' experience of asthma treatment with combined ICS/LABA suggest that increased GR activation by LABA enhances the anti-asthmatic efficacy of ICS without adversely affecting the safety profiles of these agents.

Recently, Roth and colleagues [102] demonstrated that the enhanced anti-proliferative effects of budesonide and formoterol in combination in airway smooth muscle cells (Fig. **8**) may be explained by the action of both drugs on nuclear translocation and activity of GR and CCAAT-enhancer binding protein-α (C/EBPα) (Fig. **9**), which acting synergistically enhance activation of the *p21*$^{(WAF1/CIP1)}$ gene promoter. It is conceivable that similar synergistic interactions between GCS and LABA *via* GR and C/EBP transcription factors operate in lung epithelial cells, as C/EBPβ and C/EBPδ were shown to play a role in GR signalling in these

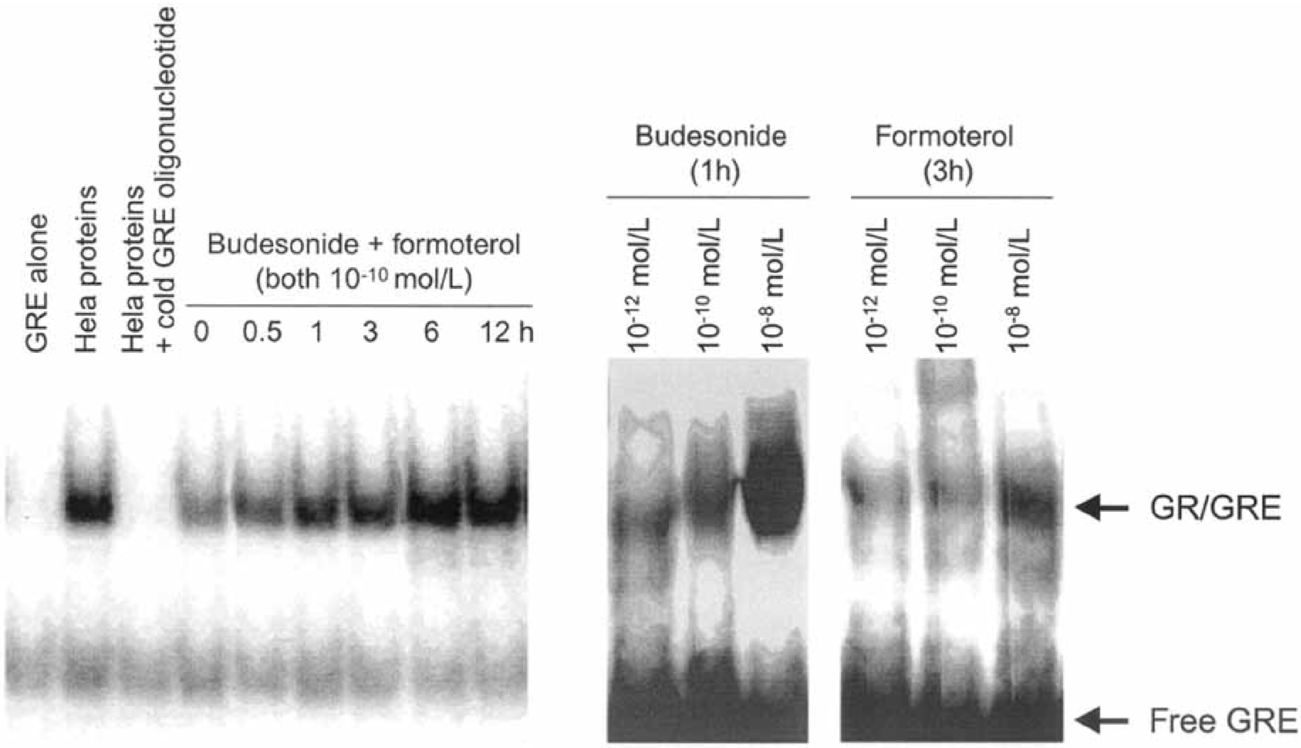

Fig. (10). Electrophoretic mobility shift assay of nuclear protein extracts showing binding of glucocorticoid receptor (GR) to DNA glucocorticoid response elements (GRE) after incubation of airway smooth muscle cells with budesonide alone (for 1 hour), formoterol alone (for 3 hours) or budesonide plus formoterol in combination (for 0-12 hours). Reproduced with permission from Roth *et al.* 2002 [102].

cells [139]. Recently, it has been demonstrated that asthmatic airway smooth muscle cells lack C/EBPα [140]. This may explain the impaired anti-proliferative action of budesonide and other GCS in asthmatic smooth muscle cells [140-142]. Importantly, this impairment was overcome upon addition of formoterol [142], which inhibits proliferation of both non-asthmatic and asthmatic cells [141, 142]. GCS and LABA may also affect the activity of other transcription factors, as recently shown for GATA-3, which upregulates expression of Th2 genes [143-145]. Nuclear localisation of GATA-3 was decreased in asthmatic patients' peripheral blood mononuclear cells after inhalation of salmeterol/fluticasone propionate in combination; this decrease was greater than that seen after inhalation of fluticasone propionate alone [146].

The relative resistance of severe asthma and COPD to ICS may be related to oxidative stress resulting in impaired GR function (as discussed above), and also in an imbalance between histone acetylation and deacetylation [147] interfering with GCS effects on gene transcription. The transrepressive, anti-inflammatory activity of GCS involves the recruitment *via* activated GR of corepressor proteins with histone deacetylase (HDAC) activity; these proteins inhibit the acetylation of core histones necessary for inflammatory gene transcription [148]. Ito *et al*. [149] showed that HDAC activity is decreased in asthmatic airways whereas activity of histone acetyltransferases (HAT) is increased, possibly reflecting increased expression of multiple inflammatory genes. These changes were reduced for HDAC and abolished for HAT in patients with asthma treated with inhaled budesonide [149]. Recently, enhanced HAT and reduced HDAC activity have also been demonstrated in alveolar macrophages of patients with asthma [150]. The altered histone acetylation status may also contribute to the increased expression of inflammatory genes and decrease of GCS function in COPD, possibly due to cigarette smoke-induced oxidative stress [151-153]. Accordingly, HDAC expression and activity are reduced in alveolar macrophages of smokers and COPD patients [151, 154]; this correlates significantly with the reduced inhibitory effect of dexamethasone on the expression of pro-inflammatory cytokines in alveolar macrophages *ex vivo*, as seen in smokers [151]. Further studies are warranted to investigate whether LABA may influence the anti-inflammatory activity of ICS in the ICS/LABA combination *via* interference with GCS-induced modulation of histone acetylation status.

Whether the molecular interactions between GCS and LABA discussed above are different for full versus partial β_2-agonists remains to be elucidated. In contrast to salmeterol, which is a partial receptor agonist, formoterol is a full agonist, which ensures high efficacy and a dose-response relationship [155]. The full agonist activity of formoterol may be especially important in cells with limited numbers of β_2-adrenoceptors, such as mast cells and other inflammatory cells.

CONCLUSIONS

Superior control of asthma and COPD by ICS/LABA combination therapy has been demonstrated in many clinical studies. The additive and potentially synergistic effects of these combined drugs have been seen on bronchodilation, symptom reduction, health-related quality of life and use of reliever medication, as well as on prevention of exacerbations. Importantly, no safety-related issues have been identified with ICS/LABA combination therapy in patients with asthma or COPD. The enhanced efficacy of ICS/LABA depends on many factors, including additive and complementary effects, as well as the possible drug interactions at the receptor level and on signalling pathways discussed above, which may contribute to the observed steroid-sparing effects. Other contributing factors may include the properties of the drugs, the inhalation device used, the treatment strategy and patient compliance. While preclinical studies continue to elucidate the mechanisms responsible for the beneficial effects seen in clinical studies, the combination of ICS and LABA in a single inhaler will continue to play an important part in the treatment of asthma and COPD.

ABBREVIATIONS

AP-1	=	Activator protein-1
AUC	=	Area under curve
bid	=	Twice daily
COPD	=	Chronic obstructive pulmonary disease
cAMP	=	Cyclic adenosine monophosphate
cEBP	=	CCAAT-enhancer binding protein
DNA	=	Deoxyribonucleic acid
ERK	=	Extracellular signal-regulated kinase
FEV_1	=	Forced expiratory volume in 1 second
GATA-3	=	(A/T)GATA(A/G) DNA-sequence-recognizing transcription factor-3
GCS	=	Glucocorticosteroid
GM-CSF	=	Granulocyte macrophage colony stimulating factor
GR	=	Glucocorticoid receptor
GRE	=	Glucocorticoid-response elements
HAT	=	Histone acetyltransferase
HDAC	=	Histone deacetylase
ICAM-1	=	Intercellular adhesion molecule-1
ICS	=	Inhaled corticosteroids
IL	=	Interleukin
JNK	=	c-Jun N-terminal kinase
LABA	=	Long-acting β_2-agonist
MAP-kinase	=	Mitogen activated protein kinase
mRNA	=	Messenger ribonucleic acid
NFκB	=	Nuclear factor kappa B
od	=	Once daily
PDGF	=	Platelet-derived growth factor
PEF	=	Peak expiratory flow
PGE_2	=	Prostaglandin E_2
PKA	=	Protein kinase A
TNFα	=	Tumour necrosis factor α
Th2	=	T-helper-type 2 lymphocytes
QTc	=	Electrocardiographic heart rate adjusted QT interval
VCAM-1	=	Vascular adhesion molecule-1

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