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Benefit-Risk Assessment of Long-Acting β2-Agonists in Asthma

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Abstract

The use of a regular long-acting β2-adrenoceptor agonists (β2-agonists; LABA) is now established in asthma guidelines as the preferred option for second-line controller therapy in addition to inhaled corticosteroids. This has been driven by data showing beneficial effects of LABAs on exacerbation rates, in turn suggesting a putative corticosteroid-sparing effect. As LABAs are devoid of any clinically meaningful anti-inflammatory activity *in vivo*, their effects on exacerbations are presumably due to a diurnal stabilising effect on airway smooth muscle.

LABAs have marked effects on symptoms and lung function, and this may make it difficult to assess anti-inflammatory control with inhaled corticosteroids when used in a combination inhaler such as fluticasone propionate/salmeterol or budesonide/formoterol. The use of fixed-dose combination inhalers is in many respects counter-intuitive to conventional teaching regarding flexible dosage titration with inhaled corticosteroids. It would therefore seem prudent first to gain optimal control of inflammation with inhaled corticosteroids before considering adding a LABA. Increasing the dosage of inhaled corticosteroids will have a relatively greater effect on exacerbations than on symptoms and lung function, whereas the converse applies when adding a LABA. Another option is to add a leukotriene receptor antagonist, which confers additional anti-inflammatory activity and is as effective on exacerbations as adding a LABA.

Despite *in vitro* and *ex vivo* data showing a ligand-independent effect of LABAs on glucocorticoid receptor activation, clinical data do not indicate any relevant synergy between LABAs and inhaled corticosteroids when used together in the same inhaler. In particular, there is no evidence of potentiation by LABAs of the *in vivo* anti-inflammatory activity of inhaled corticosteroids that would suggest any genuine corticosteroid-sparing activity. Nonetheless, the data support the additive effects of inhaled corticosteroids and LABAs when used together due to their separate effects on inflammation and smooth muscle, respectively.

Tolerance with LABAs is a predictable pharmacological phenomenon that occurs despite concomitant therapy with inhaled corticosteroids. Moreover, cross-tolerance also develops to short-acting β_2-agonists used for protection against bronchoconstrictor stimuli as a result of LABA-induced down-regulation, desensitisation and prolonged occupancy of β_2-adrenoceptors. The exact role of β_2-adrenoceptor polymorphism in determining tolerance with LABAs requires further prospective clinical studies evaluating long-term effects on outcomes such as exacerbations in patients with relevant genotypes and haplotypes.

The next decade will provide challenging issues for clinicians with respect to defining further the role of LABAs as add-on controller therapy, particularly in evaluating the long-term effects of combination inhalers on inflammatory outcomes and airway remodelling.

The asthmatic condition comprises airway inflammation, airway hyperreactivity and reversible airway obstruction. The inflammatory component is predominated by eosinophilia, which is usually responsive to therapy with inhaled corticosteroids. Untreated asthmatic inflammation may subsequently result in remodelling, with associated irreversible airway fibrosis. The importance of the underlying inflammatory process is recognised in current guidelines for asthma management, which emphasise the role of inhaled corticosteroids as first-line anti-inflammatory therapy for persistent asthma.[1] Although there is good evidence to show a dose-related effect of inhaled corticosteroids on asthma morbidity and mortality, there has also been an increasing awareness of the propensity for inhaled corticosteroids to produce concomitant dose-related systemic adverse effects.[2-4] In general terms, the therapeutic ratio for inhaled corticosteroids begins to decline above a threshold dosage of approximately 800 μg/day of beclometasone dipropionate (or its equivalent) in adults, and above 400 μg/day in children.[4] This has resulted in a trend to using lower dosages of inhaled corticosteroids in conjunction with second-line controller therapy as 'corticosteroid-sparing agents'.

Inhaled β_2-adrenoceptor agonists (β_2-agonists) have been used since the 1960s and are the most effective bronchodilator agents used for the treatment of asthma. Salbutamol (albuterol) is the most β_2-selective short-acting β_2-agonist (SABA), and is used as acute reliever therapy. It is therefore interesting to note that the use of inhaled β_2-agonists in asthma has turned full circle over the past decade. Increasing evidence began to emerge in the 1970s and 1980s to indicate that regular exposure to SABAs could worsen asthmatic disease control.[5,6] However, epidemiological case-control studies at that time were difficult to interpret because of confounding by severity, in that patients with more severe asthma had greater reliever requirements for SABAs, which did not necessarily equate with worsening asthma being due to more frequent SABA use. Various theories were postulated to explain the putative link between SABA use and worsening asthma control, including a possible end-of-dose rebound increase in airway hyperreactivity and loss of protection against bronchoconstrictor stimuli caused by the development of tolerance.[7-9] However, it is also worth pointing out that other prospective studies subsequently failed to demon-

strate the adverse effects of regular salbutamol on asthma control.[10,11]

The potential concerns regarding the use of SABAs were then incorporated into guidelines, which recommended that SABAs should not be used on a regular basis, but restricted for use on demand as reliever therapy during acute episodes of bronchoconstriction.[1] Consequently, the patient's requirement for SABAs was recognised as a sensitive marker of asthmatic disease control and is conventionally used as an endpoint for titrating the optimal dosage of inhaled corticosteroids in the clinical setting.

In the early 1990s, the introduction of long-acting β2-agonists (LABAs) such as salmeterol and formoterol was followed by an altered perception of the use of β2-agonists for the treatment of persistent asthma. Initial studies reported superior diurnal control of asthma with regular LABAs administered twice daily compared with regular SABAs four times daily.[12,13] This was followed by a series of key trials that showed that regular LABAs taken twice daily in conjunction with inhaled corticosteroids produced better control of symptoms and lung function, along with a similar reduction in exacerbations, compared with increasing the dosage of inhaled corticosteroids.[14-17] These data were the driving force behind current management guidelines for the treatment of persistent asthma, which advocate LABAs as the preferred second-line controller drug to be used as add-on therapy to low- or medium-dosage inhaled corticosteroids.[1]

Subsequently, fixed-dose combination inhalers were developed containing inhaled corticosteroids and LABAs (e.g. fluticasone propionate/salmeterol and budesonide/formoterol). The use of these combination inhalers has greatly increased, principally because they are more convenient to use than two separate inhalers, along with the perceived benefits of improvement in compliance. Furthermore, the imminent availability of generic formulations of budesonide and fluticasone propionate has resulted in aggressive promotion of the potential benefits of these combination inhalers over inhaled corticosteroids alone.

In this promotion of the benefits of combination therapy, relatively little attention has been given to the potential problems associated with LABAs in the treatment of asthma. The purpose of this review article is therefore to provide a critical and balanced appraisal of the relative benefits and risks of LABA therapy. Particular attention will be given to the effects of LABAs on the inflammatory process and on exacerbations, the development of tolerance with LABAs and interaction with the response to SABAs, and the potential relevance of β2-adrenoceptor genotype.

This article is not intended to be a systematic review of the use of LABAs in asthma, but rather to highlight some of the more relevant and contentious issues with respect to the benefit-risk equation of LABA therapy. It is not within the scope of this article to go into any detail with regard to other options for add-on controller therapy, such as leukotriene receptor antagonists, as this has recently been reviewed in detail elsewhere.[18] The methodology for literature search in this review included the use of Pubmed and Embase as well as the identification of recent relevant abstracts from key international meetings such as the American Thoracic Society, the American Academy of Allergy, Asthma and Immunology and the European Respiratory Society, using appropriate key terms such as 'asthma', 'long-acting β2-agonists', 'salmeterol', 'formoterol', 'tolerance' and 'inflammation'.

1. Effects of Long-Acting β2-Agonists

1.1 Airway Inflammation

Airway inflammation may be assessed invasively with bronchoscopy using bronchial biopsy or bronchoalveolar lavage to evaluate the effects of treatment on the cell profile in asthma. Noninvasive evaluation of airway eosinophilia may be performed by induced sputum, which is now considered to be an established and validated research tool. Another validated surrogate marker of airway inflammation in asthma is the measurement of exhaled breath nitric oxide. Systemic inflammatory markers such as peripheral blood eosinophilia and serum eosinophilic cationic protein may reflect changes in the bone marrow more than in the airway, although they are commonly used in clinical trials.

Studies using bronchial biopsy and bronchoalveolar lavage have revealed conflicting results. For

example, the use of salmeterol compared with placebo in patients with asthma, whether previously treated or untreated with inhaled corticosteroids, revealed no effects on inflammatory cells.[19,20] In another study, regular treatment with salmeterol produced a small but significant fall in the number of activated (EG_1-positive) eosinophils in bronchial biopsy but no effect on submucosal mast cells, lymphocytes or macrophages, as compared with treatment with placebo.[21] In the same study, fluticasone propionate was used as a positive control, but had no significant effect on inflammatory cells, which casts doubt on the small changes seen within the salmeterol group. Neither fluticasone propionate nor salmeterol had any significant effect on the cell profile obtained from bronchoalveolar lavage.

Treatment with formoterol was found to produce a significant reduction in the number of submucosal mast cells and eosinophils, but not T cells, when comparing the within-group response pre- and post-treatment, but not when comparing the response between groups versus placebo.[22] In the same study, the positive control with budesonide caused a significant reduction in mast cells, eosinophils and T cells when compared with the placebo group. In a separate study, it was shown that the use of formoterol or budesonide resulted in a significant reduction of activated (EG_2-positive) submucosal eosinophils in conjunction with reduced epithelial expression of activated nuclear factor-κB (a transcription factor), as compared with the placebo group.[23] However, the changes observed with formoterol were not associated with any commensurate reduction in immunoreactivity for cytokines or adhesion molecules. The same group also compared fluticasone propionate alone 200 or 500μg twice daily versus fluticasone propionate 200μg plus salmeterol 50μg twice daily in corticosteroid-treated patients with mild to moderate asthma, and found a small but significant reduction in submucosal mast cells when comparing pre- versus post-treatment within the combination group, but there was no reduction within the fluticasone propionate alone groups and no significant difference between groups.[24] In a study using segmental bronchial allergen challenge, regular salmeterol had no effect on the bronchoalveolar lavage cell profile after challenge despite improvements in lung function.[25]

Medium-term data with formoterol were evaluated by Kips et al. using induced sputum eosinophils as the primary outcome.[26] Following an initial run-in period with budesonide 800μg twice daily, patients were subsequently randomised over 12 months to step down to either budesonide 400μg twice daily alone or budesonide 100μg twice daily plus formoterol 12μg twice daily. There were non-significant changes in sputum eosinophils when comparing pre- and post-treatment in the group receiving the medium dosage of budesonide (from 0.88% to 1.74%) or in the group receiving low-dose budesonide plus formoterol (0.6% to 3.41%). No significant differences were observed in the proportion of activated (EG_2-positive) eosinophils for budesonide alone (0.75% to 2.63%) or budesonide plus formoterol (1.5% to 3.09%). Due to the inherent variability in measuring sputum eosinophils, it is possible that the study may have been underpowered, as there appeared to be a numerical trend towards increased eosinophils in patients taking the lower dosage of budesonide with formoterol. The addition of formoterol, as expected, improved lung function as measured by peak flow.

In vivo data from ovalbumin-sensitised rats have shown that combining salmeterol with fluticasone propionate counteracted the inhibitory effect of fluticasone propionate alone on allergen-induced fibronectin and collagen deposition in the airway wall, but without increasing airway hyperreactivity.[27] Other *in vitro* data have shown an additive anti-inflammatory effect of formoterol and budesonide on interleukin-1β-stimulated human lung fibroblasts.[28] Formoterol also exhibits an anti-inflammatory action in healthy human airways, in terms of attenuation of histamine-induced plasma exudation of α_2-macroglobulin.[29]

A study from Green et al. measured sputum eosinophils in 49 symptomatic patients with persistent asthma taking budesonide 100μg twice daily who were randomised in double-blind four-way crossover fashion to receive 1 month of budesonide 400μg twice daily alone, or budesonide 100μg twice daily with the addition of placebo, formoterol 12μg twice daily or montelukast 10mg once daily.[30] Increasing the dose of budesonide alone from 100μg to 400μg twice daily produced a significant 1.6-fold reduction in sputum eosinophils, whereas adding

formoterol to the lower dosage of budesonide produced a significant 1.7-fold increase. Adding placebo or montelukast to the lower dosage of budesonide had no significant impact. Measurements of exhaled nitric oxide were also performed, showing a small but significant 1.3-fold reduction after increasing the dosage of budesonide alone compared with a significant 1.4-fold increase when adding formoterol to the lower dosage of budesonide. There was also a significant 1.4-fold increase in exhaled nitric oxide when adding placebo to low-dosage budesonide, and no significant change when adding montelukast. Although adding formoterol caused a significant worsening of inflammatory markers, it produced a small but significant improvement of 16 L/min in peak flow. However, it is unclear whether these short-term observations on noninvasive surrogate inflammatory markers with formoterol are of any real clinical relevance in terms of the propensity for longer-term airway remodelling changes.

Bronchial challenge testing with adenosine monophosphate (AMP) may also be used as a surrogate inflammatory marker, as AMP acts indirectly on airway smooth muscle via priming of airway mast cells to release inflammatory mediators. In a randomised cross-over study comparing fluticasone propionate 250μg/salmeterol 50μg twice daily combination inhaler versus fluticasone propionate 500μg twice daily alone, significantly greater improvements were observed with twice the dosage of fluticasone propionate on both AMP hyperreactivity and exhaled nitric oxide (figure 1).[31] This is consistent with the observation by Green et al. that increasing the dosage of budesonide alone resulted in greater anti-inflammatory activity (effects on sputum eosinophils and nitric oxide) compared with adding formoterol to a lower dosage of budesonide.[30]

The importance of targeting therapy against airway inflammation was also clearly seen in a prospective 1-year study where patients were randomised to have their inhaled corticosteroid dosage titrated either against conventional outcomes, such as symptoms, lung function and reliever use, or against sputum eosinophils.[32] The main finding was that titrating inhaled corticosteroid dosage against sputum eosinophils resulted in significantly fewer severe exacerbations over the 12-month period (109 vs 35 exacerbations). The reduction in sputum eosinophils was associated with a commensurate fall in exhaled nitric oxide and methacholine-induced hyperreactivity when comparing the two groups, whereas symptoms, quality of life, lung function and reliever use were not significantly different. Leuppi and colleagues also showed that exacerbation during

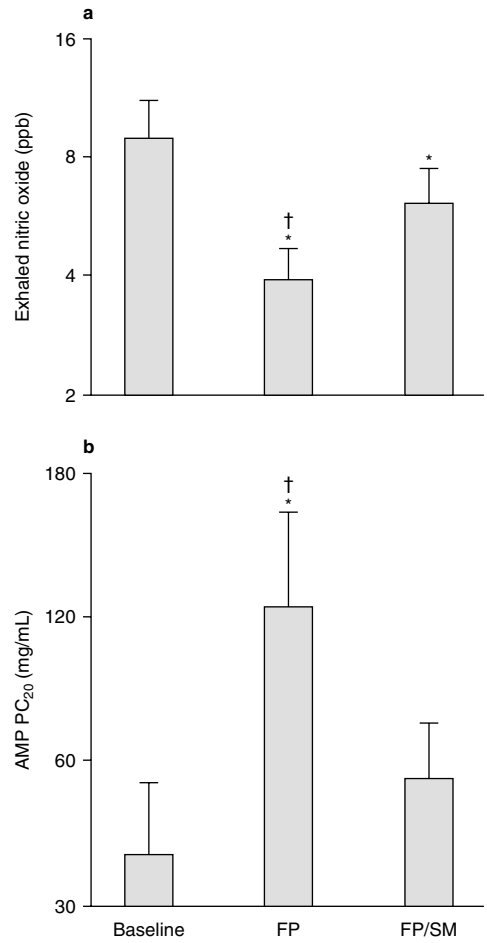

Fig. 1. Effects on (**a**) exhaled nitric oxide, and (**b**) adenosine monophosphate (AMP) challenge in patients with asthma receiving either fluticasone propionate 250μg/salmeterol 50μg twice daily combination (FP/SM) or fluticasone propionate 500μg twice daily alone (FP). Baseline measurements were made on the patient's usual inhaled corticosteroid therapy, a mean dosage of beclometasone 470 μg/day. Values are shown as means and SEM. * indicates a significant difference versus baseline; † indicates a significant difference between the two randomised treatments (reproduced from Currie et al.,[31] with permission from Springer-Verlag). **PC_{20}** = provocative concentration producing a 20% fall in forced expiratory volume; **ppb** = parts per billion.

tapered step-down of inhaled corticosteroids was predicted by airway hyperresponsiveness to mannitol and sputum eosinophilia, but not by forced expiratory volume in 1 second (FEV_1).[33] This points to the disconnect between the underlying inflammatory process and the conventional outcomes of asthma control, particularly lung function. Parameters such as lung function are much more responsive to effects on airway smooth muscle, which explains why LABAs may improve FEV_1 and peak flow without having any effects on inflammatory outcomes.

Taken together, these studies suggest that adding LABAs may have an apparent 'corticosteroid-sparing' effect when monitoring lung function, which is improved, but at the same time inflammation may be less well controlled compared with increasing the dosage of inhaled corticosteroid. Thus, when stepping down the dosage of inhaled corticosteroid in conjunction with a LABA, it appears that measurements of lung function may be dissociated from the underlying inflammatory process.

Two separate studies have evaluated step-down therapy with inhaled corticosteroids, revealing interesting findings regarding the disconnect between inflammation and lung function when adding a LABA. In a study of patients with severe asthma receiving high-dosage inhaled corticosteroids, rapid tapering of inhaled corticosteroids was performed on a weekly basis, with patients being randomised to receive concomitant therapy with either placebo or salmeterol, until they experienced an exacerbation.[34] The results showed that adding salmeterol allowed a lower dosage of inhaled corticosteroids after tapering, although there was a concomitant increase in induced sputum eosinophils. At the time of exacerbation, the mean daily dosage of inhaled corticosteroid was 227 μg/day and 612 μg/day in the salmeterol and placebo groups, respectively, whereas in the week prior to exacerbation sputum eosinophil counts were 20% and 9%, respectively. It is also pertinent that the salmeterol-treated patients had no warning before an exacerbation, as lung function and symptoms and reliever use were maintained, suggesting that salmeterol may have been masking the worsening underlying inflammatory process during the tapering of the inhaled corticosteroid. One could argue that in real life such rapid tapering would not be employed and that patients would not experience exacerbations during more gradual tapering.

A study was also performed in patients with moderate persistent asthma who had an initial 1 month run-in on beclometasone 1000μg twice daily, followed by parallel-group randomisation to step down to 2 months of either extra fine particle hydrofluoroalkane beclometasone 200μg twice daily (equivalent to fluticasone propionate 200μg twice daily) or fluticasone propionate 100μg/salmeterol 50μg twice daily combination.[35] The results showed that for the primary outcome of methacholine hyperreactivity there was an initial improvement on high-dosage beclometasone, which was maintained after subsequent step-down to the lower dosage of beclometasone alone, and a further improvement after step-down to fluticasone propionate/salmeterol. These effects were mirrored by changes in FEV_1 and peak flow, suggesting that the effects of the combination inhaler were probably due to the action of salmeterol on airway smooth muscle, producing functional antagonism against methacholine challenge. This hypothesis is supported by effects on inflammatory markers, including exhaled nitric oxide and serum eosinophilic protein, where a deterioration was seen after step-down to the combination inhaler but not to beclometasone alone.

In a meta-analysis of 596 patients taking regular LABAs or placebo as add-on to inhaled corticosteroids, for the primary outcome of airway hyperreactivity the overall residual estimated protection after the last dose amounted to a 0.8 (0.6–1.0) doubling dose shift. This residual degree of bronchoprotection may be taken as a surrogate for airway smooth muscle stabilisation and possibly explain the effects of LABAs on exacerbations.[36]

To summarise this section, the available data on inflammatory markers point to the importance of first establishing optimal control of inflammation before considering adding a LABA. For patients who are not controlled on inhaled corticosteroids alone, when switching to a combination inhaler it would therefore seem prudent to keep the inhaled corticosteroid dosage the same rather than using the LABA as a corticosteroid-sparing agent.

1.2 Asthma Exacerbations

There have been numerous studies of the effects of addition of LABAs on exacerbations in patients who were not optimally controlled on inhaled corticosteroids. Two studies with formoterol are worth discussing in more detail because they were prospectively powered on exacerbations over a 12-month period.

In a study from O'Byrne and collaborators, two separate groups of patients with mild persistent asthma were evaluated over a period of 12 months.[37] In inhaled corticosteroid-naive patients, exacerbations were significantly reduced by monotherapy with budesonide 100μg twice daily compared with placebo, whereas the combination of budesonide with formoterol 4.5μg twice daily was no better than budesonide alone. In the group of patients previously treated with inhaled corticosteroids (up to 400 μg/day), no significant difference in exacerbations was observed between budesonide 100 and 200μg twice daily as monotherapy, whereas in this group adding formoterol conferred a significant further small reduction in exacerbations. However, it is worth pointing out that there was no placebo comparator group, and so it is not possible to ascertain whether the patients were already close to the plateau of the inhaled corticosteroid dose-response curve prior to enrolment in order to explain the lack of difference between budesonide 100 and 200μg twice daily.

In the study of Pauwels et al., patients with moderate persistent asthma, previously treated with inhaled corticosteroids, were initially treated for 1 month with budesonide 800μg twice daily, followed by a randomised step-down to budesonide 100 or 400μg twice daily alone, or in combination with formoterol 9μg twice daily, for a period of 12 months.[38] In comparison with budesonide 100μg twice daily alone, adding formoterol resulted in a 26% reduction in severe exacerbations, compared with a 49% reduction with budesonide 400μg twice daily alone and a 63% reduction with budesonide 400μg twice daily plus formoterol. Thus, significant additive effects of formoterol were observed on top of either a low or medium dosage of budesonide alone, although the improvement from increasing the dosage of budesonide from 100 to 400μg twice daily was greater than that from adding formoterol to budesonide 100μg twice daily. It was noticeable that, although adding formoterol to budesonide 100μg twice daily fared worse on exacerbations compared with increasing the dosage of budesonide, the former was better on lung function outcomes such as FEV_1 and peak flow. In an analysis of 425 severe exacerbations over 12 months from the same study, the pattern of exacerbations was not influenced by the dosage of inhaled corticosteroid or concomitant therapy with formoterol.[39]

The studies of O'Byrne et al.[37] and Pauwels et al.[38] illustrate some important points on clinical management. Therapy with inhaled corticosteroids alone will be sufficient for a large proportion of patients with mild to moderate persistent asthma. Adding a LABA will have a relatively greater effect on lung function than on exacerbations. The observed effects of LABAs will always be greater for smooth muscle-responsive outcome measures such as lung function, which may be misleading when following patients in the short or medium term in the clinic situation. Nonetheless, the improvements in airway calibre seen with LABAs, especially when used in combination inhalers, might conceivably reinforce patients' perception of improved control, especially if they are monitoring their own peak flow, thereby improving compliance in the longer term. However, there are no long-term data available to show that this is the case with combination inhalers compared with inhaled corticosteroids alone.

Several trials have also evaluated adding salmeterol in patients taking inhaled corticosteroids. In a randomised controlled trial, patients who were found to be suboptimally controlled on inhaled triamcinolone 400μg twice daily subsequently had the dosage of inhaled corticosteroid reduced by half for the first 2 months followed by cessation for the next 2 months, in conjunction with adding either salmeterol or placebo in two groups, with a third group receiving an unchanged dosage of triamcinolone throughout the 4 months in conjunction with salmeterol.[40] There was found to be no overall difference over the 4 months when comparing the placebo and salmeterol groups in terms of the primary endpoint of percentage treatment failures during the reduction and elimination of inhaled corticosteroid (47.4% vs 43.2%, respectively), in contrast with

the group who received an unchanged dosage of inhaled corticosteroid plus salmeterol, where there were significantly fewer treatment failures (12.2%). The main criticism of this study is that there was no comparator arm where the inhaled corticosteroid dosage was unchanged without concomitant salmeterol. Nonetheless, this trial showed that salmeterol did not confer any overall additional anti-inflammatory protection during tapering or withdrawal of inhaled corticosteroid. In another part of the same trial, the patients who were initially controlled on the run-in period with triamcinolone 400μg twice daily were subsequently switched in random fashion to receive salmeterol alone or placebo over 4 months, with a third group where patients continued on the same dosage of inhaled corticosteroid alone.[41] The results showed a similar deterioration of asthma control in patients who switched to placebo or salmeterol, which was not observed in patients who continued with the same dosage of inhaled corticosteroid. It is particularly relevant that the deterioration that occurred when switching from inhaled corticosteroid to salmeterol was associated with a concomitant worsening of surrogate inflammatory outcome variables such as sputum eosinophilic cationic protein and tryptase (a mast cell marker), exhaled nitric oxide and methacholine hyperreactivity. This observation again indicates that salmeterol confers no meaningful anti-inflammatory activity.

A meta-analysis of nine trials compared increasing the dosage of inhaled corticosteroid alone with adding salmeterol, showing a significant difference in the pooled estimate for moderate to severe exacerbations, amounting to a 2.42% difference in favour of adding salmeterol (95% CI 0.24–4.6%).[42] To prevent an exacerbation in one additional patient, 40 patients would need to be treated with salmeterol. However, it is important to note that none of the individual studies were powered on exacerbations and none showed any difference for effects on exacerbations. The same meta-analysis also showed, as expected, that salmeterol improved smooth muscle-responsive outcomes, such as lung function, symptom control and reliever use.

It is important for clinicians to be aware of the potential pitfalls of being over-reliant on lung function outcomes as the key marker of asthma control when adding a smooth muscle agent. The main problem at present is that there are no reliable surrogate inflammatory markers that can be used in everyday clinical practice to dissect out what is happening to the underlying inflammatory process in asthma in the presence of improved lung function when adding LABA therapy. It is hardly surprising to find that patients taking a bronchodilator 24 hours a day in the form of a LABA will have a much reduced reliever requirement for SABAs. Thus, regular use of LABAs will mask the requirement for reliever therapy, monitoring of which is recommended in guidelines as an important marker of disease activity.

1.3 Asthma Mortality

In July 1996, GlaxoSmithKline initiated the Salmeterol Multicentre Asthma Research Trial (SMART), which was a 28-week prospective safety study comparing salmeterol and placebo in the treatment of asthma.[43] This study was designed in agreement with the US FDA to evaluate the safety of salmeterol following concerns regarding the safety of regular SABA and LABA use in the management of asthma. The primary outcome variable of the SMART study was the combined number of respiratory-related deaths and respiratory-related life-threatening experiences, such as intubations or mechanical ventilation. Other outcomes were asthma-related events and asthma-related deaths.

Patients continued with their regular asthma therapy, but were randomised to receive either salmeterol twice a day or placebo twice a day as additional therapy. The study protocol included a planned interim analysis once half of the patients were recruited, which occurred towards the end of 2002 using all of the available data on 25 858 patients at that time. These preliminary findings were reported to healthcare professionals in the form of a non-peer-reviewed letter. For the primary outcome there were no significant differences, but there was a non-significant trend towards a higher number of asthma-related life-threatening events including deaths in patients treated with salmeterol. However, subgroup analysis revealed some interesting observations. In patients of African-American origin, which comprised 17% of the study population, there was a significantly greater number of primary events and

asthma-related events, including deaths, in those randomised to receive salmeterol compared with placebo, although <1% of all African-Americans who were enrolled experienced such events during the trial. There were no significant differences in Caucasian patients, who comprised 71% of the study population.

One possible explanation for the ethnic differences was that the African-Americans had more severe asthma than Caucasians at baseline, in terms of symptoms, peak flow, prior intubations, emergency room visits and hospitalisations. Furthermore, there was a rather low level of use of inhaled corticosteroids (47%) for all patients in the trial, with 50% of Caucasians and 38% of African-Americans using inhaled corticosteroids at baseline. It was also found that for the total patient population receiving inhaled corticosteroids at baseline, no significant differences were seen in primary events or asthma-related events including death, whereas in those not taking inhaled corticosteroids there was a significantly greater number of asthma-related deaths in all salmeterol-treated patients compared with placebo. As the SMART study was not designed to evaluate subgroup effects, it was decided to discontinue the study. GlaxoSmithKline collaborated with the FDA to review potential changes in salmeterol labelling, which resulted in 'black box' warnings in the prescribing information for salmeterol and fluticasone propionate/salmeterol.

In essence, the SMART study reinforces the need to take inhaled corticosteroids concomitantly in addition to salmeterol, and reinforces the guidelines regarding the key role of anti-inflammatory therapy for asthma. The SMART findings are therefore in keeping with the previous studies from Lemanske et al.[40] and Lazarus et al.,[41] which show that salmeterol does not confer any clinically meaningful anti-inflammatory activity. An alternative interpretation of the SMART study is that patients who need therapy with both an inhaled corticosteroid and a LABA would be advised to use a combination inhaler, which would result in patients always having to take their inhaled corticosteroid at the same time as their LABA.

There are other data evaluating the safety of salmeterol. Castle et al. reported on 16 787 patients treated with salmeterol or salbutamol over 16 weeks and found 54 deaths from all causes (0.32%), including 12 deaths from asthma (0.07%).[44] In a prescription-event monitoring study in a UK cohort of 15 407 patients treated with salmeterol and observed for at least 1 year, there were 1022 deaths (6.6% of the cohort), with 73 of the deaths (7.1% of the total deaths) being due to asthma, but only 39 of these 73 patients were taking salmeterol in the last month of life.[45] Moreover, 37 of the 39 patients who died of asthma and who were taking salmeterol were receiving regular inhaled corticosteroid or oral corticosteroid or both before death. The study suggested that advanced age and severity of disease were the most likely factors contributing to asthma mortality in the population studied, and found no evidence that salmeterol contributed to death in any of the patients studied.

2. Cardiovascular Safety of Long-Acting β2-Agonists

The main adverse effects of LABAs are related to their systemic activity, for example due to stimulation of β2-adrenoceptors in the heart and peripheral vasculature, as well as in skeletal muscle producing hypokalaemia.[46] There are important pharmacological differences between salmeterol and formoterol, in that formoterol has a much higher degree of intrinsic agonist efficacy than salmeterol, with formoterol being almost a full agonist compared with isoprenaline (isoproterenol) and salmeterol a partial agonist.[47-49] Moreover, formoterol is more lipophilic than salmeterol, which may influence its ability to cross the lung-blood barrier.

In a dose-ranging comparison of formoterol and salmeterol in healthy subjects, both drugs produced dose-related effects on systemic measures, including heart rate, systolic and diastolic blood pressure, heart rate-corrected QT interval and plasma potassium.[50] However, there were differences between the drugs in that formoterol had a more rapid onset of action whereas salmeterol had a more prolonged action. The relative potencies of the two drugs were similar to the 4-fold difference in recommended doses (i.e. formoterol 6μg = salmeterol 25μg). However, it was noticeable from the study that at the lowest administered dose of formoterol (24μg) and salmeterol (100μg), there were no clinical relevant adverse effects, and one could argue that patients

would normally take formoterol in a dose of 12μg or salmeterol in a dose of 50μg, so there would appear to be a relatively wide therapeutic ratio for both drugs.

In a study of healthy volunteers and asthmatic patients, an open-label evaluation was made of salmeterol 50μg twice daily for 3 days followed by a further 3 days of 100μg twice daily, although there was no placebo control.[51] In this study, salmeterol did not produce any significant change in mean heart rate, supraventricular or ventricular premature complexes, or in blood pressure, in either group of subjects. In a well-controlled multicentre parallel-group study, 352 patients with mild persistent asthma received salmeterol 50μg twice daily or placebo for 52 weeks, showing no clinically significant differences between the groups in heart rate, QT interval, supraventricular or ventricular ectopic events, or in arterial blood pressure.[52]

However, it may also be relevant to consider the potential interaction between LABAs and hypoxaemia, which may occur in acute asthma. This subject has been reviewed in detail elsewhere.[53] In a small study of 12 patients with chronic obstructive pulmonary disease (COPD) who had pre-existing cardiac arrhythmias and hypoxaemia, a randomised single-blind placebo-controlled study was performed to compare the cardiac effects of single doses of formoterol (12 and 24μg) and salmeterol (50μg).[54] The results from 24-hour ECG monitoring showed a higher heart rate after formoterol 24μg versus 12μg or versus salmeterol 50μg, with more frequent supraventricular and ventricular premature beats with the higher dose of formoterol. Furthermore, formoterol 24μg significantly reduced plasma potassium for 9 hours when compared with placebo, whereas plasma potassium with formoterol 12μg was different after 2 hours and with salmeterol 50μg from 4 hours to 6 hours.

In a meta-analysis where 1443 patients received placebo and 1410 received salmeterol 50μg twice daily, there was no increased risk of cardiovascular adverse events compared with placebo (relative risk 1.03, 95% CI 0.8–1.3).[55] Both groups had a similar incidence of cardiovascular events (8%), including cardiovascular deaths.

In summary, therefore, it would appear that conventional recommended doses of salmeterol and formoterol are not associated with clinically relevant cardiovascular effects, although this does not exclude the possibility that for patients who might inadvertently take excessive doses of LABAs, particularly in the setting of an acute asthma exacerbation, where there is concomitant hypoxaemia, this could result in susceptibility to cardiac arrhythmias. Another factor to take into the equation here is that although tolerance to the systemic effects of LABAs occurs with long-term administration, both oral and inhaled corticosteroids reverse LABA-induced down-regulation and tolerance and therefore resensitise cardiac β_2 receptors to the adverse effects of LABAs.[56,57]

3. Tolerance with Long-Acting β_2-Agonists

Tolerance (or subsensitivity of response) develops to the effects of regular LABA exposure as a result of down-regulation of β_2-adrenoceptors and associated desensitisation of response. *In vitro* studies with human lung β_2-adrenoceptors showed a significant reduction in density of β_2-adrenoceptors within 24 hours of acute exposure to salmeterol and formoterol at a concentration of 1 μmol/L, in conjunction with a significant reduction of β_2-adrenoceptor mRNA expression within 4 hours.[58] The clinical manifestation of tolerance with LABAs will depend on the degree of functional antagonism exhibited by the prevailing bronchomotor tone.[59] Thus, in considering the bronchodilator response to LABAs there is a relatively weak degree of functional antagonism conferred by a low level of bronchomotor tone, which manifests in a minimal degree of subsensitivity of response. The opposite applies when there is a stronger degree of functional antagonism in the presence of a high bronchomotor tone, resulting in a maximal degree of subsensitivity of response. An analogy is lifting against a heavy weight to uncover a mild degree of muscle weakness. As corticosteroids may have facilitatory effects on β_2-adrenoceptor regulation and responsiveness, it is important to consider studies that have evaluated effects of LABAs in patients who are taking concomitant inhaled corticosteroids.

3.1 Bronchodilator Effects

Bronchodilator tolerance to the LABA itself is difficult to demonstrate, because this occurs in the presence of resting bronchomotor tone where there is only a relatively weak degree of functional antagonism to overcome. The best example is from a large multicentre study is from Pauwels et al.,[38] where in the first 2 weeks of initiating treatment with formoterol twice daily in conjunction with budesonide, either 100 or 400μg twice daily, there was a progressive reduction in peak flow response, with approximately 50% loss of bronchodilator efficacy within the first 2 weeks. Subsequently, the peak flow response remained at a lower level over the ensuing 12 months and remained significantly higher than baseline values during the run-in period. However, in a study by Zetterstrom et al.,[60] no such diminution in peak flow response was seen using budesonide and formoterol via separate inhalers or as a single combination inhaler. Likewise, in a study by Mahler et al.[61] with fluticasone propionate and salmeterol in combination, the improvement in peak flow response was maintained during the first 3 weeks of treatment.

Other studies have looked at the dose-response relationship for formoterol in more detail in patients with moderate persistent asthma receiving concomitant inhaled corticosteroids. In two similar studies from Newnham et al., following an initial run-in period free of β_2-agonist, patients were subsequently randomised to receive cross-over treatment with either formoterol or placebo twice daily, followed by a dose-response study to cumulative repeated doubling doses of formoterol performed 12–24 hours after the last dose of each randomised treatment.[62,63] In addition, *ex vivo* peripheral blood lymphocyte β_2-adrenoceptor function was evaluated, and showed a persistent degree of down-regulation and desensitisation in response to isoprenaline stimulation. These receptor changes were accompanied by a rightward shift in the bronchodilator dose-response curve to cumulative doses of formoterol. It was also evident that the degree of bronchodilator subsensitivity was greater at 6 hours after the last cumulative dose of formoterol compared with the peak response.

However, it should be noted that most multicentre studies with LABAs have shown that bronchodilator tolerance is not a significant clinical problem.[12-17,60]

3.2 Protection Against Bronchoconstrictor Stimuli

An acute bronchoconstrictor challenge may be performed under controlled conditions in the laboratory with direct stimuli that act on airway smooth muscle, such as methacholine or histamine, or with indirect stimuli that act via airway inflammatory cells, such as AMP or allergen, or by neuronal mechanisms such as exercise or cold air. In real life, bronchoconstrictor stimuli tend to be mediated by indirect mechanisms, and these challenges are generally considered to be more clinically relevant.

In some respects, the protective effects of LABAs against such bronchoconstrictor stimuli could be considered as a surrogate for what might happen during acute asthma, where there is a high degree of functional antagonism to overcome. Tolerance to bronchoprotection occurs following regular exposure to SABAs in corticosteroid-naive patients with mild asthma, with the loss of protection being more evident with an indirect stimulus rather than a direct stimulus.[64] Thus, one might expect that with more prolonged receptor occupancy associated with LABA therapy, bronchoprotective tolerance would, if anything, be more pronounced. Several studies using methacholine challenge have demonstrated loss of protection between the first and last doses of LABAs when given alone or with concomitant inhaled corticosteroids.[65-70] Tolerance occurs quickly, and in one study was demonstrated within 24 hours of starting salmeterol.[71] Tolerance to protection against allergen- or exercise-induced bronchoconstriction has also been reported with regular salmeterol.[72-76] It has been demonstrated with formoterol that, for dosage intervals of 12 or 24 hours, there is a similar degree of tolerance to protection against methacholine or adenosine challenge in patients treated with inhaled corticosteroids.[77,78] Likewise, Simons et al. have shown tolerance to protection against exercise-induced bronchoprotection with once-daily salmeterol in children.[79]

There are other agents used as add-on therapy with inhaled corticosteroids, such as leukotriene re-

ceptor antagonists, that confer bronchoprotective properties and have been compared with LABAs in the same study. For exercise protection, tolerance has been shown to occur with salmeterol, but not with montelukast.[75,76] Using AMP challenge in inhaled corticosteroid-treated patients, add-on therapy with montelukast 10mg once daily for 2 weeks resulted in sustained protection compared with placebo at 24 hours after the first and last dose, whereas for salmeterol 50μg twice daily significant protection seen after the first, but not the last, dose.[80] In another study of inhaled corticosteroid-treated patients comparing added montelukast 10mg or formoterol 9μg twice daily, the findings were similar in terms of sustained protection with montelukast but not formoterol.[81] Indeed the results for add-on therapy with montelukast were remarkably reproducible in both of these studies,[80,81] with there being a one doubling dilution shift in AMP hyperreactivity after the first and last doses. Moreover, in both studies, the effects of montelukast on AMP hyperreactivity were accompanied by suppression of blood eosinophils, suggesting a corticosteroid-independent anti-inflammatory effect complementing that of inhaled corticosteroids.

3.3 Interaction Between Long-Acting and Short-Acting β_2-Agonists

In terms of the clinical relevance of tolerance with LABAs, it is probably most important to consider whether there is cross-tolerance to SABAs such as salbutamol, as this would mimic what happens during an acute episode of bronchoconstriction when used as reliever therapy. The first study that attempted to evaluate the potential interaction was performed in patients with moderately severe asthma receiving concomitant inhaled corticosteroids, who, when randomised after an initial 2-week run-in period without β_2-agonists, received cross-over double-blind add-on therapy with either salmeterol 50μg twice daily or placebo.[82] *Ex vivo* peripheral blood lymphocyte β_2-adrenoceptor function was also evaluated at 36 hours after taking the last dose of each randomised treatment, at the same time as performing a dose-response study with cumulative doses of salbutamol. The results showed that salmeterol produces a significant reduction in β_2-receptor numbers compared with placebo, while at the same timepoint baseline lung function (i.e. prior to salbutamol administration) was not significantly different. This indicates a prolonged degree of receptor occupancy by salmeterol. The salbutamol dose-response showed a rightward shift after salmeterol versus placebo for FEV_1 and for peak flow; regression analysis indicated that a 2.5-fold higher dose of salbutamol was required to produce a 0.5L improvement in FEV_1 after salmeterol versus placebo, and a 4-fold higher dose of salbutamol was required to produce an 80 L/min improvement in peak flow. However, it is important to note that the final value after salbutamol for percentage predicted FEV_1 was not significantly altered by salmeterol compared with placebo.

Fuglsang et al. reported almost complete blunting of the acute bronchodilator response to terbutaline in asthmatic children pretreated with salmeterol compared with placebo.[83]

Other studies have not shown any evidence of cross-tolerance to the salbutamol bronchodilator response after salmeterol, although interpretation of these studies is confounded by a washout period without salmeterol prior to the salbutamol dose-response curve. Consequently, a salmeterol carry-over effect resulted in there being little room for subsequent improvement with salbutamol administration. This is illustrated by one of the studies by Nelson and coworkers,[84] whereby after treatment with salmeterol for 4 weeks, the improvement in FEV_1 was only 0.2L between 180 and 2000μg of salbutamol,[85] while in a study from Wilding et al. there was only a 0.1L improvement in FEV_1 between 90 and 720μg of salbutamol.[86] In the presence of a flat dose-response curve to salbutamol, it is not possible to make any proper conclusions regarding cross tolerance to salbutamol. Korosec et al. reported on patients who were admitted with acute severe asthma, and found that those taking salmeterol showed an equal improvement in peak-flow response to high-dose nebulised salbutamol compared with controls who were not taking salmeterol.[87] Peters et al. showed an improvement in function conferred by adding salmeterol to conventional therapy during the first 2 days of an acute exacerbation of asthma,[88] although there were no data on the acute response to salbutamol during the first few hours of admission.[89]

In view of the methodological problems with evaluating the bronchodilator response to salbutamol in the presence of weak functional antagonism, it is probably more meaningful to look at studies that have been designed using bronchoconstrictor stimuli where the bronchomotor tone is greatly increased. In a study by Yates et al., methacholine challenge was performed 15 minutes after inhalation of salbutamol, before and after 1 week of treatment with salmeterol given concomitantly with either placebo or budesonide.[90] Salmeterol was found to induce cross-tolerance to the protection afforded by salbutamol, with the loss in protection being similar in the presence of concomitant placebo or budesonide, the only difference being that budesonide-treated patients started from and fell to a higher value compared with placebo.

Other studies have shown salmeterol-induced cross-tolerance for the effects of salbutamol administered prior to methacholine-induced bronchoconstriction.[69,70] These studies all used a standard 200μg dose of salbutamol and so the question arises as to whether a higher dose of salbutamol may overcome the salmeterol-induced cross-tolerance. This hypothesis was investigated in a study of inhaled corticosteroid-treated patients who randomly received in cross-over fashion treatment with placebo, salmeterol or formoterol twice daily for 9 days following an initial 1-week run-in period free of β_2-agonist.[91] A methacholine challenge was performed 1 hour after a single bolus dose of salbutamol 1600μg, with the previous dose of randomised treatment being taken 12 hours previously. The protective effect of salbutamol after placebo was 2.7-fold higher than after salmeterol and 3.6-fold higher than after formoterol. As this was a long-term study, it was not possible to deduce whether the salbutamol interaction was due to cross-tolerance alone or whether there may have been a component due to prolonged receptor occupancy by the LABAs. Thus, a further study was performed, also in inhaled corticosteroid-treated asthmatic patients, whereby single doses of inhaled placebo, formoterol or salmeterol were administered in a cross-over fashion, followed by a single dose of salbutamol 400 or 1600μg 12 hours later, a methacholine challenge being performed 1 hour after salbutamol.[92] The results showed significant dose-related salbutamol protection after pretreatment with placebo, but not after formoterol and salmeterol. This study therefore showed that salmeterol and formoterol exhibited a significant degree of *in vivo* antagonism against the effects of salbutamol in methacholine-contracted bronchi, which could be due to prolonged β_2-adrenoceptor occupancy by LABAs, or perhaps development of early tolerance after exposure to a single dose.[71]

It is probably more clinically relevant to look at what happens when salbutamol is administered as acute rescue therapy after methacholine-induced bronchoconstriction, since this more closely reflects what happens in real life, as opposed to evaluating the effects of salbutamol prior to challenge. In one such study, Van der Woude et al. administered salbutamol at the end of a methacholine challenge and demonstrated a blunting of response to salbutamol 50μg in patients treated with inhaled corticosteroid who were receiving concomitant salmeterol or formoterol compared with those receiving concomitant placebo.[93] In the first 3 minutes after salbutamol administration, there was a 20% increase in FEV_1 after placebo, which was significantly greater than the 14% seen after formoterol or 12% after salmeterol.

In patients with moderate persistent asthma, Lee et al. evaluated the recovery to salbutamol 200μg given at the end of a methacholine challenge in a randomised cross-over study comparing fluticasone propionate 250μg/salmeterol 50μg twice daily versus fluticasone propionate 250μg alone twice daily, as well as budesonide 400μg/formoterol 9μg twice daily versus budesonide 400μg alone twice daily.[94] The salbutamol recovery time profile measured over 30 minutes following methacholine challenge was significantly blunted by 73% when comparing budesonide/formoterol with budesonide alone, and by 50% when comparing fluticasone propionate/salmeterol with fluticasone propionate alone. This was also evident when comparing recovery at 30 minutes after taking salbutamol in individuals receiving budesonide/formoterol or budesonide alone (figure 2). There was no significant difference in recovery when comparing the respective combination inhalers or corticosteroid inhalers.

In a study by van Veen et al., the recovery to fenoterol administered after methacholine challenge

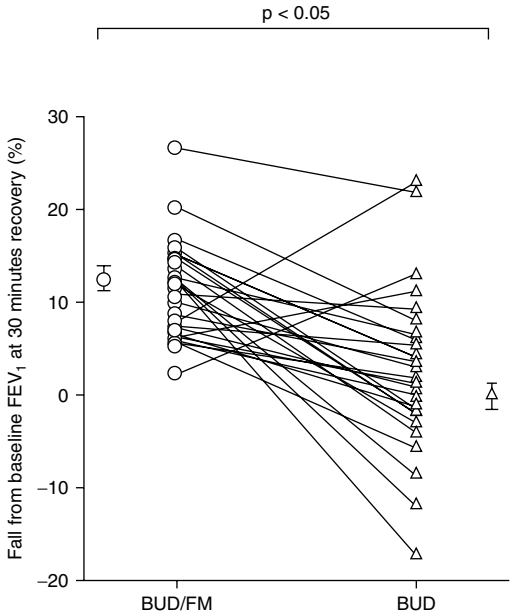

Fig. 2. Recovery at 30 minutes after inhalation of salbutamol 200μg given as acute rescue therapy following acute bronchoconstriction with methacholine. Individual data points are shown for salbutamol recovery after treatment with either budesonide 400μg/formoterol 9μg twice daily combination (BUD/FM) or budesonide 400μg twice daily alone (BUD). Data points for the same patient with each treatment are joined by a line. Mean and SEM values for each treatment (isolated data points) are also depicted.[94] **FEV_1** = forced expiratory volume in 1 second.

was evaluated after 2 weeks of prior treatment with placebo, salmeterol or formoterol as add-on therapy to the patient's usual inhaled corticosteroid.[95] The results showed significant blunting of the response to fenoterol after prior treatment with either salmeterol or formoterol compared with placebo, but there was no difference between the two LABAs in terms of the percentage predicted FEV_1 response to fenoterol. For the fenoterol dose-response curve, there was a significantly greater rightward shift in methacholine hyperresponsiveness induced by salmeterol than formoterol, but only at the highest dose of fenoterol, amounting to a 0.66 doubling dose difference.

A study using exercise challenge made a comparison of fluticasone propionate 100μg twice daily alone, fluticasone propionate with salmeterol or fluticasone propionate with montelukast.[96] Salbutamol 100μg was administered after exercise challenge, revealing a delay in recovery over 30 minutes in patients who were treated with fluticasone propionate plus salmeterol, but not with fluticasone propionate alone or fluticasone propionate plus montelukast.

There are data to show that acute administration of a high dose of systemic or inhaled corticosteroids may either fully or partially reverse the tolerance or receptor down-regulation induced by LABAs.[97-99] This might imply that during an acute asthma exacerbation, patients might be advised to self-administer a high dose of inhaled corticosteroid in addition to their reliever SABAs.

To summarise, in the presence of a strong degree of functional antagonism during an acute episode of bronchoconstriction, there appears to be a clinically relevant interaction in terms of cross-tolerance between a LABA and the response to a SABA. This is a pharmacologically predictable and reproducible phenomenon, is not prevented by concomitant administration of inhaled corticosteroid with the LABA, and does not appear to be overcome by administering a higher dose of SABA.

4. Influence of β_2-Adrenoceptor Genotype

Single nucleotide polymorphisms of the β_2-adrenoceptor at positions 16 and 27 may influence the response to treatment with β_2-agonists. Cell lines transfected with the glycine-16 variant of the receptor are more prone to agonist-induced down-regulation and desensitisation in comparison with the arginine-16 variant, whereas the glutamic acid variant confers relative protection against down-regulation and desensitisation compared with the glutamine-27 variant.[100,101] There is linkage disequilibrium in terms of the haplotype combination, with homozygous glutamic acid at position 27 always being associated with homozygous glycine at position 16, although the latter may occur in combination with any other genotype at position 27. The homozygous arginine-16 genotype occurs in approximately 15% of individuals compared with 40% of individuals who have the homozygous glycine-16 genotype, the remainder being heterozygous glycine/arginine.[102] The situation for a given individual is further complicated by the occurrence of complex haplotypes occurring at other loci apart from 16 and 27.[103]

Although these complex haplotypes may be associated with altered β_2-adrenoceptor responsiveness, they are relatively rare and are therefore only relevant to a few individual patients, but probably not to larger populations involved in clinical trials.

To date, most of the literature has focused on retrospective comparisons of the effects of β_2-agonists in individuals with various genotypes at position 16. On the basis of the *in vitro* data from transfected cell lines, it was assumed that the homozygous glycine-16 genotype would exhibit a diminished response to β_2-agonists, in terms of being more susceptible to the development of tolerance, with consequent subsensitivity of response after long-term exposure to regular β_2-agonist therapy. Most of these early studies were performed using regular exposure to SABAs. For example, in a study of healthy volunteers who only expressed the homozygous glycine-16 genotype, short-term exposure to high doses of metaproterenol produced a marked reduction in the number of β_2-adrenoceptors on alveolar macrophages and bronchial epithelial cells, although there was no control group with the homozygous arginine-16 genotype.[104] Lima and colleagues found that patients with the homozygous or heterozygous glycine-16 genotype had a significantly reduced acute bronchodilator response to oral salbutamol in comparison with patients with the homozygous arginine-16 genotype.[105] In a retrospective analysis, patients with the homozygous glycine-16 genotype were less likely to respond to a single dose of salbutamol 200μg compared with those with the homozygous arginine-16 genotype.[106] This is supported by other prospective data in patients with asthma (with a 1-week run-in period with no β_2-agonist exposure) showing that the acute systemic β_2-adrenoceptor response to inhaled salbutamol is blunted in patients with the homozygous glycine-16/glutamic acid-27 haplotype compared with the homozygous arginine-16/glutamine-16 haplotype.[107]

In a retrospective analysis of inhaled corticosteroid-treated patients with asthma who were randomised to receive formoterol or placebo for 4 weeks, those with the homozygous glycine-16 genotype exhibited a greater degree of bronchodilator tolerance compared with those with the homozygous arginine-16 genotype, and those with the heterozygous genotype had an intermediate response.[108]

A prospective evaluation was made of inhaled corticosteroid-treated patients who only expressed the homozygous glycine-16 genotype, who were randomised in a cross-over fashion to receive placebo, formoterol or salmeterol, with a methacholine challenge performed at 12 hours after the first and last doses.[109] The results showed significant improvements in FEV_1 after the first, but not the last, dose of formoterol and salmeterol compared with the placebo response. There was significant protection against methacholine challenge with both LABAs after the first and last doses compared with placebo, which amounted to <1 doubling dose shift in methacholine hyperreactivity. This study was difficult to interpret because there was no difference between first- and last-dose methacholine protection, which may have been due to the measurements being made at trough. Intuitively, one might expect a greater degree of agonist-induced down-regulation and desensitisation with a full agonist such as formoterol compared with a partial agonist such as salmeterol, although it is possible that differences in agonist efficacy might become more evident at peak receptor occupancy than at trough. Moreover, this study did not have a comparator group of patients with the homozygous arginine-16 genotype. Another possible interpretation of these data is that down-regulation in genetically susceptible patients occurred rapidly after the first dose of LABAs, such that it was not possible to detect any loss of protection between the first and last dose.

In another retrospective genotype analysis in inhaled corticosteroid-treated patients, it was shown that tolerance to methacholine protection occurred readily following exposure to regular doses of terbutaline or formoterol (for peak response), which was irrespective of polymorphisms at position 16 and 27.[110] However, the study was not properly powered to compare the responses in different genotypes.

In an evaluation of 60 patients with asthma with no recent SABA or LABA therapy, *ex vivo* measurements were made of the cyclic AMP response to isoprenaline stimulation of peripheral blood lymphocyte β_2-adrenoceptors as well as *in vivo* measurements of the acute protection conferred by a

single dose of formoterol against methacholine challenge.[111] The *post hoc* analysis of β_2-adrenoceptor genotype showed that it did not determine the binding density or stimulated coupling of lymphocyte β_2-adrenoceptors, or the degree of acute protection conferred by inhaled formoterol. As this study involved administering a single dose of LABAs, it did not look at the development of subsensitivity during long-term administration.

Against the background of these studies, which focused on the glycine form of the receptor, other retrospective studies have suggested that it may be the arginine form of the receptor that is more important in determining the response to β_2-agonist therapy. For example, Israel and colleagues found that the homozygous arginine-16 genotype was associated with a significantly lower peak flow in patients who used salbutamol on a regular basis compared with those with the same genotype who used it on demand.[112] Moreover, in patients who took regular salbutamol, the decline in peak flow associated with the homozygous arginine-16 genotype was not evident in the homozygous glycine-16 genotype. In another retrospective genotype analysis, the use of regular salbutamol, but not salmeterol, was found to be associated with increased asthma exacerbations in patients with the homozygous arginine genotype.[113] There are also data from healthy volunteers in whom the acute forearm venodilator response to isoprenaline was assessed prospectively showing that the haplotype homozygous for arginine-16 and glutamine-27 was associated with enhanced subsensitivity of response compared with the two haplotypes homozygous for glycine-16.[114]

In order to understand this apparent paradox, it is important to consider the possible role of endogenous catecholamines on basal β_2-adrenoceptor regulation prior to exposure to exogenous agonists. Liggett postulated that, according to a dynamic model of receptor kinetics, the glycine form of the receptor would be relatively susceptible to down-regulation by endogenous catecholamines compared with the arginine form of the receptor.[102] Thus, the basal state of the glycine form of the receptor would already be down-regulated by endogenous catecholamines, so that subsequent exposure to exogenous β_2-agonists would result in no further down-regulation. According to the same dynamic model, the arginine form of the receptor would therefore be relatively up-regulated in its basal state, due to its resistance against endogenous catecholamines, such that subsequent exposure to exogenous β_2-agonists would be expected to result in tolerance and associated subsensitivity of response. In the alternative static model proposed by Liggett,[102] endogenous catecholamines would have little or no impact on basal β_2-adrenoceptor expression, such that the glycine form of the receptor would, as predicted from the *in vitro* model, be relatively more susceptible to down-regulation by exogenous β_2-agonist exposure, whereas the arginine form would be less susceptible.

In order to attempt to resolve which of these two models is applicable in patients, we performed a retrospective analysis of data from six placebo-controlled randomised cross-over studies in which asthma patients treated with inhaled corticosteroids were given placebo or LABAs (either formoterol or salmeterol) as add-on medication, the primary outcome variable in all studies being protection against either methacholine or AMP challenge following the first and last doses.[115] For the composite endpoint of protection against both challenges after the last dose, patients who had homozygous or heterozygous genotypes containing the arginine-16 polymorphism (i.e. arginine-arginine or arginine-glycine) exhibited 1.5 (95% CI 0.5–2.5) doubling dose less protection than those with the homozygous glycine genotype (i.e. glycine-glycine) [figure 3]. For the same genotype comparison on methacholine challenge alone after the last dose, the arginine polymorphism conferred 2.5 (95% CI 1.1–4.0) mean doubling dose less protection. When analysing the various haplotypes at positions 16 and 27 together, the greatest degree of subsensitivity was associated with the combined homozygous arginine-16 and homozygous glutamine-27 genotypes. Moreover, with the arginine polymorphism, the magnitude of protection after the last dose was less with formoterol than with salmeterol, amounting to 3.00 (95% CI 1.01–4.99) doubling dose difference in protection for the composite endpoint. These findings show that the arginine-16 polymorphism is associated with subsensitivity of response for the bronchoprotection afforded by LABAs as add-on to inhaled corticosteroids, with the degree of subsensitivity being greater for formoterol than salmeterol, prob-

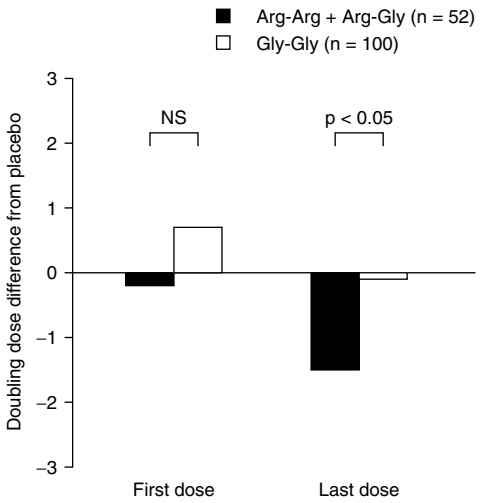

Fig. 3. Bronchoprotection afforded by long-acting β_2-agonist, shown as mean doubling dose shift from placebo, after first and last dose of treatment, for the composite endpoint of methacholine or adenosine monophosphate challenge. A comparison is shown between polymorphisms at position 16 of the β_2-adrenoceptor for homozygous and heterozygous arginine genotypes (i.e. Arg-Arg or Arg-Gly) versus homozygous glycine genotypes (i.e. Gly-Gly). The degree of protection loss between first versus last dose (i.e. degree of tolerance) was significantly greater with the arginine-16 polymorphism than with the homozygous glycine-16 polymorphism, whereas there was significantly less protection afforded by the arginine-16 polymorphism after the last but not the first dose. A change in a positive direction indicates that long-acting β_2-agonist faired better than placebo, and a change in a negative direction indicates that long-acting β_2-agonist faired worse than placebo, for protection again bronchoconstriction.[115] **NS** = not significant.

ably because of its higher β_2-agonist intrinsic efficacy, which is presumably associated with a greater degree of down-regulation and subsensitivity in genetically susceptible patients.

However, these findings should be replicated in a prospective study of β_2-adrenoceptor regulation and *in vivo* responsiveness to regular LABA exposure. It would be particularly relevant to know whether putative differences in genotype-dependent response are associated with any consequences for the more long-term protective effects of LABAs against exacerbations. Such a study would require a haplotype or genotype-enriched sample from a screened population database, followed by a 6- to 12-month prospective follow-up study looking at the effect of LABA or placebo on exacerbation rates in patients with moderate to severe asthma, where exacerbations would be more frequent.

5. Synergy Between Inhaled Corticosteroids and Long-Acting β_2-Agonists

5.1 Putative Mechanisms

From a pharmacological perspective, the term synergy refers to the combined effects of inhaled corticosteroids and LABAs in combination exceeding the sum of the individual effects taken as separate inhalers. In other words, is there a unique pharmacological interaction by having inhaled corticosteroids and LABAs in a single combination inhaler, or are the effects merely additive because of the predictable pharmacological properties of each drug on inflammation and smooth muscle pathways? Synergy between inhaled corticosteroids and LABAs can occur in two different ways. Inhaled corticosteroids may potentiate the effect of the LABAs, or the LABAs may potentiate the effect of inhaled corticosteroids.

The former mechanism of synergy is well documented, whereby corticosteroids are known to potentiate the effect of β_2-agonists via augmentation of β_2-adrenoceptor function. This is a known class effect between corticosteroids and β_2-agonists and is therefore not specific to an interaction between inhaled corticosteroids and LABAs as such. For example, previous data have shown that a single bolus dose of systemic corticosteroid produces acute facilitatory effects on β_2-adrenoceptor function in healthy and asthmatic subjects following desensitisation by SABAs.[116,117] Clinical studies have also demonstrated that, in healthy and asthmatic subjects, a single bolus dose of systemic corticosteroid can rapidly restore peripheral blood lymphocyte β_2-adrenoceptor binding density previously down-regulated by SABAs.[118-120] In this respect, corticosteroids may reverse β_2-agonist-induced tolerance by reversing β_2-adrenoceptor internalisation, promoting the high-affinity state of the receptor and increasing β_2-adrenoceptor gene transcription.[121-124]

Other clinical studies have shown that a bolus of systemic or inhaled corticosteroid may acutely reverse, partially or completely, agonist-induced down-regulation and associated subsensitivity of response caused by prolonged exposure to formoter-

ol in asthmatic patients.[97-99] Studies in healthy volunteers exposed to regular doses of formoterol have shown that concomitant administration of low-dose prednisolone or high-dose budesonide may protect against agonist-induced subsensitivity of response.[56,57] However, despite these observations showing reversal of down-regulation and associated subsensitivity by a high dose of corticosteroid, clinical studies where inhaled corticosteroids and LABAs are given concomitantly have shown that LABA-induced tolerance and cross-tolerance to SABAs is not prevented by concomitant administration of inhaled corticosteroids.[62,63,69,77,78,80-82,91]

More recently, some interesting *in vitro* studies have suggested that LABAs may potentiate the response to inhaled corticosteroids. The study from Eickelberg et al. showed increased nuclear translocation of the glucocorticoid receptor and altered downstream gene transcription, which was evident with both salmeterol and salbutamol, as seen on Western blotting or immunohistochemical analysis of cytosol and nuclear extracts.[125] This suggests that the ligand-independent activation of glucocorticoid receptors by SABAs and LABAs is a class effect. However, one could argue that the *in vitro* experiments give a false impression because of the prolonged incubation with SABAs, which would not perhaps be seen *in vivo*. Other *in vitro* data from Roth et al. showed that formoterol may also exhibit ligand-independent activation of glucocorticoid receptors via increased nuclear glucocorticoid receptor translocation and associated alteration in gene transcription.[126] It was found that, in combination, budesonide and formoterol led to simultaneous activation of the glucocorticoid receptor and associated transcription factors, resulting in synergistic inhibitory effects on proliferation of cultured human airway smooth muscle cells. However, there was no control experiment with SABAs to compare with formoterol.

More recent, although preliminary, *ex vivo* data were obtained from a study in seven inhaled corticosteroid-naive subjects with asthma evaluating the effects of single inhaled doses of fluticasone propionate 100 and 500μg alone, salmeterol 50μg alone and fluticasone propionate 100μg/salmeterol 50μg combination on glucocorticoid receptor activation measured in induced sputum.[127] Dose-dependent glucocorticoid receptor activation was observed following fluticasone propionate 100μg alone (47% glucocorticoid receptor translocation) and fluticasone propionate 500μg alone (61% translocation) compared with the effect of placebo (31% translocation). Moreover, salmeterol 50μg alone achieved 43% translocation, but combination therapy with fluticasone propionate 100μg/salmeterol 50μg produced 54% translocation (figure 4). Only the effects of fluticasone propionate 500μg alone and the fluticasone propionate 100μg/salmeterol 50μg combination were significantly different from placebo, probably reflecting the lack of power due to the small sample size. The difference in glucocorticoid receptor activation between fluticasone propionate 100μg alone versus fluticasone propionate 100μg/salmeterol 50μg combination amounted to 7%, compared with a difference of 14% versus fluticasone propionate 500μg alone. This suggests that increasing the dosage of inhaled corticosteroid would be a more effective strategy for optimising anti-inflammatory therapy. These *ex vivo* data would tend to support the proposition that glucocorticoid nuclear translocation may partly explain the complementary effects

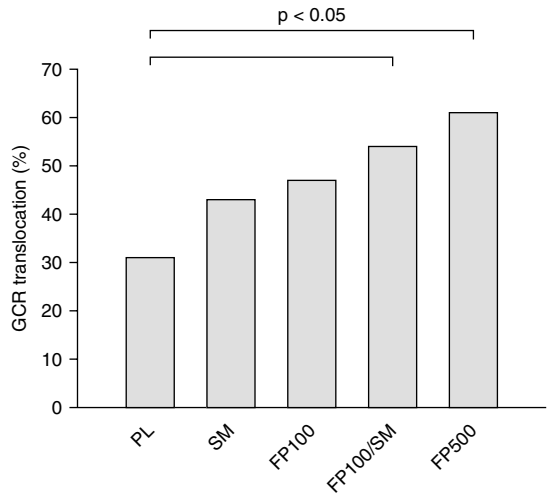

Fig. 4. Glucocorticoid receptor (GCR) activation measured in induced sputum from corticosteroid-naive patients with asthma randomised to receive single doses of either placebo (PL), salmeterol (SM) 50μg, fluticasone propionate (FP) 100μg alone, FP 500μg alone or FP 100μg/SM 50μg combination inhaler. The effects of FP 500μg alone or FP 100μg/SM 50μg combination were significantly different from PL. Values are shown as means for GCR percentage translocation.[127]

of inhaled corticosteroids and LABAs given in combination. The actual signal transduction mechanisms remain unknown, but it is possible that LABAs may prime the inactive glucocorticoid receptor through phosphorylation and subsequently the glucocorticoid receptor may require less inhaled corticosteroid for nuclear translocation.

Other preliminary data are from an *in vitro* study in which U937 cells were incubated with fluticasone propionate, budesonide, formoterol and salmeterol alone and in combination for 1 hour prior to lipopolysaccharide stimulation, followed by quantification of secreted cytokines.[128] The addition of formoterol or salmeterol gave greater suppression of lipopolysaccharide-induced release of granulocyte-macrophage colony-stimulating factor and tumour necrosis factor α compared with the effect of budesonide or fluticasone propionate alone, although neither altered the corticosteroid concentration-response curve. Furthermore, both LABAs prevented corticosteroid-induced repression of interleukin (IL)-1 release. The actions of the LABAs differed with respect to IL-10, where formoterol in combination with fluticasone propionate enhanced production compared with fluticasone propionate alone, whereas salmeterol did not. The data were interpreted as showing that the added benefit of formoterol may relate to the anti-inflammatory gene-inducing action of corticosteroids, rather than enhancing the repressive functions of corticosteroids towards inflammatory cytokines.

5.2 Clinical Studies

A number of clinical studies have evaluated the potential for synergy *in vivo*. In a study by Zetterstrom et al., 362 patients were randomised over 3 months to receive budesonide 320μg plus formoterol 9μg twice daily in the same inhaler, or corresponding treatment with budesonide plus formoterol concomitantly via separate inhalers, or budesonide alone.[60] The results showed a significantly greater increase in the primary outcome of peak flow with the combination of formoterol and budesonide given as single or separate inhalers compared with budesonide alone. Similar significant differences were observed with the combination versus budesonide alone for other outcomes, including symptoms and use of rescue medication. However, there was no difference in response to combination therapy for any of the outcomes comparing the single and separate inhalers. Thus, there was no evidence of synergy of response by combining both drugs in the same inhaler compared with separate concomitant inhalers.

In a study by Lee et al.[94] comparing budesonide 400μg plus formoterol 9μg twice daily in a single combination inhaler versus budesonide 400μg alone twice daily, and fluticasone propionate 250μg plus salmeterol 50μg twice daily in a single combination inhaler versus fluticasone propionate 250μg twice daily alone, neither of the combination inhalers were significantly superior to the respective inhaled corticosteroids alone for effects on surrogate inflammatory markers, including exhaled nitric oxide (figure 5) and serum eosinophilic cationic protein. This study therefore demonstrated no evidence of potentiation of the budesonide response by combining with formoterol in the same inhaler, or of the fluticasone propionate response by combining with salmeterol in the same inhaler. However, in the same study the combination inhalers were superior to the respective inhaled corticosteroids alone for effects on FEV_1 and peak flow, which is a predict-

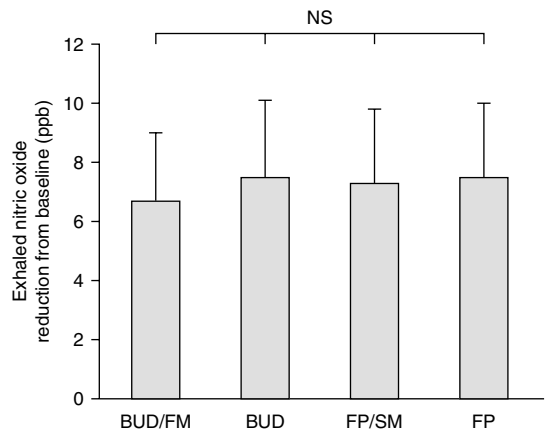

Fig. 5. Fall in exhaled nitric oxide from baseline in response to randomised treatments with either budesonide (BUD) 400μg/formoterol (FM) 9μg twice daily combination, BUD 400μg twice daily alone, fluticasone propionate (FP) 250μg/salmeterol (SM) 50μg twice daily combination or FP 250μg twice daily alone. Values are shown as means and SEM. All four treatments produced a significant reduction in exhaled nitric oxide as change from baseline, but there was no potentiation of anti-inflammatory activity when comparing each combination inhaler with their respective inhaled corticosteroid alone.[94] **NS** = not significant; **ppb** = parts per billion.

able observation due to the known effects of inhaled corticosteroids and LABAs, with the LABAs producing additional bronchodilator effects.

In a study by Aziz et al., a randomised double-blind experimental design was used to administer formoterol alone, budesonide alone and budesonide and formoterol together via separate inhalers.[129] The primary outcome variable for the study was AMP hyperreactivity. The results showed no evidence of potentiation of the budesonide response by administering concomitant formoterol therapy. The same was seen for effects on exhaled nitric oxide, another surrogate inflammatory marker. This study indicated no synergy between budesonide and formoterol for anti-inflammatory activity of the former. However, the study did not evaluate the effects of budesonide and formoterol given together in the same inhaler.

A series of pivotal trials also evaluated the effects of fluticasone propionate and salmeterol given in a single inhaler. In a study of subjects with mild to moderate asthma, Kavuru et al. evaluated the primary outcome of area under the 12-hour FEV_1 response curve, comparing the combination of fluticasone propionate 100μg/salmeterol 50μg twice daily versus the separate components given alone, or placebo.[130] The combination product was significantly superior to each of the separate components at week 12, but there was evidence of additivity, not of synergism, with the combination. The secondary outcome was the survival curve for the probability of remaining in the study over 12 weeks, for which the combination product was significantly superior to each separate component, but once again there was evidence of additivity but not synergism. However, due to the relatively mild nature of the asthma in these patients, even the group randomised to treatment with fluticasone propionate alone showed very few patients dropping out of the study due to poor control, so this study is not discriminating for showing superiority with the combination inhaler in patients with milder forms of asthma.

In a study from Shapiro et al. in subjects with moderate to severe asthma, again using the same primary outcome variable of area under the 12-hour FEV_1 response curve, a comparison was made between fluticasone propionate 250μg/salmeterol 50μg twice daily versus the separate components alone, or placebo, over 12 weeks.[131] The results showed clear evidence of additivity of response, but not synergism, of the two separate components compared with the response to the combination product, although additivity as such was not formally statistically tested as part of the protocol analysis. In the same study, looking at the secondary outcome of the survival curve for the probability of remaining in the study over the 12-week period, the combination product was significantly superior to either drug alone, although compared with placebo the two separate drugs appeared to exhibit additivity, but no evidence of synergy, when compared with the combination. Due to the more severe nature of the asthma in the patients in Shapiro et al.[131] compared with those in Kavuru et al.,[130] the former study was more discriminatory in terms of showing additivity. Nonetheless, the overall conclusion from these two studies is that there was no unique interaction between the components of LABA and inhaled corticosteroid in the same inhaler, but rather evidence of pharmacologically predictable additivity, with both drugs working through independent inflammatory and smooth muscle pathways.

In a study from Aubier et al. in patients with severe corticosteroid-dependent asthma, fluticasone propionate 500μg/salmeterol 50μg twice daily was compared with the separate components given concurrently as single inhalers, and also compared with monotherapy with fluticasone propionate 500μg twice daily alone, over 7 months.[132] Because of the severity of the asthma in the patients in this study, no placebo arm was felt to be ethically justified. For effects on morning peak flow, the combination group and the concurrent therapy group were significantly better than the group receiving fluticasone propionate alone, but there was no difference between the combination group and the concurrent group, again pointing to the lack of any significant synergism by having the two separate components together in the same inhaler. These observations are similar to those of Zetterstrom et al.[60] with budesonide and formoterol as a single combination or concurrent separate inhalers.

In a study by Pearlman et al.,[133] a comparison was made over 4 weeks of placebo, fluticasone propionate 88μg or 220μg twice daily alone, salmeterol 42μg twice daily alone, or the combinations of

salmeterol 42μg plus fluticasone propionate 88μg twice daily and salmeterol 42μg plus fluticasone propionate 220μg twice daily. For the primary outcome of area under the 12-hour FEV_1 response curve after 12 weeks, there was evidence of additivity when comparing the effects of the respective monotherapies with each dose of fluticasone propionate and salmeterol compared with the same dose of fluticasone propionate and salmeterol in combination. However, again, there was no evidence of synergism. This difference (5.4 L/min) is clinically irrelevant given that most peak expiratory flow meters are only calibrated to within 10 L/min, while in each of four individual studies, the 95% CI for the difference in peak expiratory flow included zero, indicating a lack of a significant effect. Moreover, for the secondary outcome of FEV_1 the 95% CI in all four individual studies included zero, while the 95% CI for the pooled estimate also included zero – a mean difference of 40mL (95% CI 0–80). The authors interpreted these findings as being indicative of synergy by combining both drugs in the same inhaler.[134,135]

As part of a randomised cross-over study in patients with mild to moderate persistent asthma, a comparison was made of fluticasone propionate 250μg twice daily alone or fluticasone propionate 250μg/salmeterol 50μg twice daily as a single combination inhaler, showing no differences for effects on surrogate inflammatory markers including AMP challenge, exhaled nitric oxide (figure 6) and peripheral blood eosinophils.[136] In the same study, the leukotriene receptor antagonist montelukast was found to improve all inflammatory outcomes when added to fluticasone propionate 250μg twice daily alone or fluticasone propionate 250μg/salmeterol 50μg twice daily. Thus, there was clearly room for the additional anti-inflammatory efficacy conferred by adding montelukast, which was not evident when adding salmeterol to the same dosage of fluticasone propionate. Indeed, the combination of fluticasone propionate plus montelukast was superior to fluticasone propionate plus salmeterol for anti-inflammatory effects, despite the latter combination being superior on lung function.

These data on inflammatory markers are supported by findings from a global multicentre study of 1490 patients with moderate persistent asthma

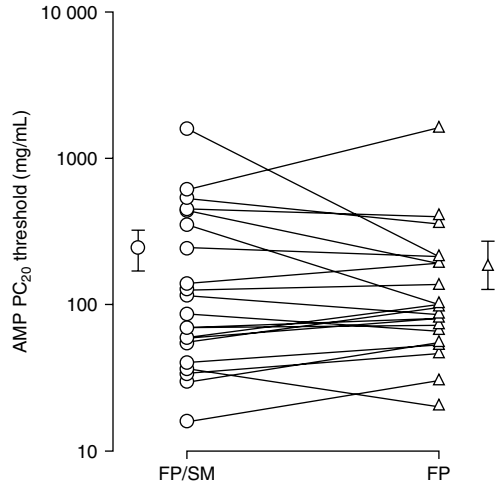

Fig. 6. Comparative effects on adenosine monophosphate (AMP) threshold in individual patients for fluticasone propionate (FP) 250μg twice daily alone or FP 250μg/salmeterol (SM) 50μg twice daily combination. Data points for the same patient with each treatment are joined by a line. Means and SEM (isolated data points) are also shown. There was no significant difference between the two treatments, showing that salmeterol does not potentiate the anti-inflammatory effects of fluticasone propionate *in vivo*.[136] **PC_{20}** = provocative concentration producing a 20% fall in forced expiratory volume.

who, after an initial run-in period of 2 weeks on fluticasone propionate 100μg twice daily, were subsequently randomised in double-blind fashion to receive add-on therapy with either salmeterol 50μg twice daily or montelukast 10mg once daily for 12 months, with the primary outcome being the number of exacerbations.[137] After 12 months, 20.1% of patients had experienced an exacerbation with fluticasone propionate plus montelukast versus 19.1% with fluticasone propionate plus salmeterol, with no significant difference between the randomised treatments. Montelukast and salmeterol were equally effective in reducing nocturnal awakenings and improving quality of life. Montelukast, but not salmeterol, significantly reduced blood and sputum eosinophil counts, whereas salmeterol was more effective than montelukast on peak flow and pre-β_2-agonist FEV_1, but both were similar on post-β_2-agonist FEV_1. Thus, both treatments were equally effective in improving asthma control in the medium term, with montelukast conferring anti-inflammatory activity complementary to that of in-

haled corticosteroids, whereas salmeterol was a more effective bronchodilator. This study illustrates a key point, in that exacerbations can be reduced to the same degree either by targeting smooth muscle (i.e. with salmeterol) or by targeting the component of inflammation that is not responsive to corticosteroids (i.e. with montelukast). It is unclear whether this complementary anti-inflammatory activity of montelukast might attenuate airway remodelling in the longer term.

Other data by Nelson et al.[138] have shown superior effects on lung function conferred by adding salmeterol, rather than montelukast, to fluticasone propionate 100μg twice daily. However, this study included patients who had marked reversibility to a SABA (salbutamol), and so it is perhaps not entirely surprising that the results also showed a marked response to a LABA (salmeterol), given that both drugs act on the same receptor. In everyday clinical practice a large proportion of patients with mild to moderate asthma do not exhibit such marked β_2-agonist reversibility, which questions the clinical relevance of such studies in real life.

5.3 Overview

There is *in vitro* and *ex vivo* evidence to suggest that LABAs may exhibit ligand-independent activation of the glucocorticoid receptor, which would be expected to enhance the response to inhaled corticosteroids. However, such observations at a molecular level are not substantiated by clinical data to show any evidence of true synergism when combining inhaled corticosteroids and LABAs in the same inhaler. The studies of Zetterstrom et al.[60] and Aubier et al.,[132] in particular, provide evidence of additivity but not synergism when comparing the combination inhalers with the individual components, LABAs and inhaled corticosteroids, given concurrently via separate inhalers, for effects on lung function and exacerbations. A pooled analysis of studies by Nelson et al.[134] showed a statistically but clinically irrelevant mean difference of 5.4 L/min in peak expiratory flow when comparing fluticasone propionate and salmeterol combination versus their separate components as concurrent inhalers. Moreover, when combination inhalers are compared with their respective inhaled corticosteroids alone there is no evidence of potentiation of anti-inflammatory activity. Thus, it would appear that combination inhalers show additivity of response, resulting from their predictable effects on independent inflammatory and smooth muscle pathways. Adding a leukotriene receptor antagonist to inhaled corticosteroids confers complementary non-corticosteroid-dependent anti-inflammatory activity and reduces exacerbations to a similar degree as adding a LABA, although the latter exhibits better bronchodilator efficacy.

6. Conclusions and the Way Forward

LABAs are now established in asthma management guidelines at step 3 as the preferred option for use as additional controller therapy in patients treated with inhaled corticosteroids. This recommendation has been driven by data showing beneficial effects of LABAs on exacerbation rates, which from a safety perspective are reassuring. As LABAs do not exhibit any clinically meaningful anti-inflammatory activity *in vivo*, their effects on asthma exacerbations are more likely to be due to diurnal stabilisation of airway smooth muscle resulting from their 24-hour bronchodilator activity associated with twice-daily regular administration.

For patients with mild to moderate asthma not controlled by a low dosage of inhaled corticosteroid alone, increasing the corticosteroid dosage has a greater effect on exacerbations than does adding a LABA, which produces a greater improvement in lung function and symptoms, but a relatively smaller effect on exacerbations. This in turn would suggest that combination inhaled corticosteroid/LABA inhalers should be considered for patients with more severe asthma who have impaired lung function and diurnal variability, where sustained bronchodilation and associated airway smooth muscle stabilisation would be beneficial. Further long-term data are required to establish whether combination inhalers confer potential benefits on compliance.

Recent concerns arising from the SMART study[43] showing an association between regular salmeterol use and mortality can probably be explained by the use of salmeterol in the absence of concomitant inhaled corticosteroid therapy. This in turn reinforces the importance of proper treatment of the inflammatory process in asthma with inhaled corticosteroids before considering adding a LABA.

For patients who require both agents, it would appear logical to combine them both in the same inhaler for ease of use and possibly improved compliance.

Recent *in vitro* and *ex vivo* data have shown a ligand-independent effect of LABAs on glucocorticoid receptor translocation, which in theory might be expected to potentiate the response to a given dose of inhaled corticosteroid therapy. This so-called synergy between LABAs and inhaled corticosteroids has been proposed to support the earlier use of inhaled corticosteroid/LABA combination inhalers in the treatment of mild to moderate persistent asthma, with the LABA moiety conferring corticosteroid-sparing effects. However, the available data on inflammatory markers do not show any *in vivo* evidence of potentiation of inhaled corticosteroids by concomitant LABAs. There are no clinical data to support true synergism between LABAs and inhaled corticosteroids in the same inhaler compared with using the two agents concomitantly via separate inhalers. The data merely point to a predictable additivity of response due to the effects of inhaled corticosteroids and LABAs on separate components of the inflammatory and smooth muscle pathways.

Step-down studies apparently showing a corticosteroid-sparing action of combination inhalers are invariably biased towards adding a LABA on smooth muscle-sensitive outcome measures, which are used to define exacerbations.[139,140] Moreover, *in vivo* data show that adding a LABA to a lower dosage of inhaled corticosteroid does not potentiate anti-inflammatory activity compared with a higher dosage of inhaled corticosteroid alone.[31] The data from published trials on exacerbations in patients with mild to moderate asthma all show that adequate control can usually be achieved with a low to medium dosage of inhaled corticosteroid alone. It is also worth bearing in mind, in terms of direct drug costs, that combination inhalers are approximately 50% more expensive than inhalers containing twice the dosage of inhaled corticosteroid alone. This in turn will have major long-term implications for managed care, particularly with the imminent availability of cheaper generic formulations of fluticasone propionate and budesonide.

The use of LABAs as corticosteroid-sparing therapy has aroused safety concerns in terms of the potential for masking underlying inflammation when using a suboptimal dosage of inhaled corticosteroid in a combination inhaler. This is because LABAs are particularly effective at improving smooth muscle outcomes such as lung function and symptoms, and their airway stabilising effects may therefore mask any worsening inflammation in the longer term, especially if this is not manifested as an overt exacerbation. Despite medium-term data showing additive beneficial effects of LABAs on exacerbations, the more long-term concern for patients who may be undertreated with inhaled corticosteroids is that they may develop consequences of burnt-out inflammation in terms of airway fibrosis and associated remodelling. Longer-term studies (over several years) looking at surrogates for airway remodelling in patients using combination inhalers compared with a higher dosage of inhaled corticosteroid alone are required to explore this hypothesis further. This is a current dilemma at step 3 of the guidelines, because there are now alternative agents for add-on controller therapy such as leukotriene receptor antagonists, which reduce exacerbations and improve symptoms by conferring complementary non-corticosteroid-dependent anti-inflammatory activity, but are less effective as bronchodilator agents compared with LABAs.

The other main concern with LABAs is their propensity for producing β_2-adrenoceptor down-regulation and associated subsensitivity of response, which is a predictable and reproducible pharmacological phenomenon occurring despite concomitant therapy with inhaled corticosteroids. LABA-induced tolerance is more apparent for bronchoprotective than for bronchodilator effects. LABA-induced tolerance is evident in terms of subsensitivity of response to the LABA itself, as well as cross-tolerance by inducing subsensitivity to the acute reliever response to SABAs, which may not be overcome by using a higher dose. Cross-tolerance may be relevant in the presence of increased bronchomotor tone where there is a high degree of functional antagonism, as might occur during an acute episode of bronchoconstriction during an asthma exacerbation. It appears that LABA-induced tolerance is at least partly determined by β_2-adrenoceptor polymorphism at position 16, although long-term prospective data comparing relevant genotypes and

haplotypes are required to characterise further the clinical importance of these polymorphisms in terms of asthma control parameters, including exacerbations.

The asthmatic disease process is characterised by chronic inflammation followed by a variable degree of bronchoconstriction. This is especially the case during an exacerbation, where a higher dosage of inhaled corticosteroid may be required temporarily to damp down the inflammatory process. Combining the two separate components in the same fixed-dose combination inhaler is counter-intuitive to conventional teaching regarding flexible titration of the inhaled corticosteroid dosage. The problem with fixed-dose combination inhalers is that during exacerbations increasing the dosage of inhaled corticosteroid will invariably be associated with increasing the dosage of LABA, which will further aggravate agonist-induced β_2-adrenoceptor down-regulation and associated subsensitivity. A possible solution is to provide patients with an additional corticosteroid-only inhaler to cover the worsening inflammation during an exacerbation on a temporary basis, although it is unlikely that compliance with such a complicated regimen would be satisfactory. For this reason, it might make more sense for patients with more severe asthma to keep the two components separate, in order to allow the greatest flexibility for dosage titration of the inhaled corticosteroid. If a LABA is to be used in the same way as a SABA for on-demand relief, then it would be logical to choose formoterol rather than salmeterol, as it has a faster onset of action. Although the flexible on-demand indication for formoterol is currently licensed by the manufacturer AstraZeneca, it is not yet recommended for use in this way in current asthma management guidelines.

The next decade will require clinicians to ask questions about the appropriate use of LABAs, to address challenging issues with respect to the long-term treatment of asthma, and to move the focus away from outcome measures such as lung function that are rather distant from the inflammatory process.

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