

Inhaled Combination Therapy With Long-Acting β_2 -Agonists and Corticosteroids in Stable COPD*

Mario Cazzola, MD, FCCP; and Ronald Dahl, MD

Long-acting β_2 -agonists (LABAs) have been shown to be effective first-line bronchodilators in the treatment of COPD patients, and inhaled corticosteroids (ICSs) have been shown to reduce the frequency and/or severity of exacerbations in COPD patients. The concomitant use of a LABA and an ICS can influence both airway obstruction (*ie*, smooth muscle contraction, increased cholinergic tone, and loss of elastic recoil), and airway inflammation (*ie*, increased numbers of neutrophils, macrophages, and CD8+ lymphocytes, elevated interleukin-8 and tumor necrosis factor- α levels, and protease/antiprotease imbalance). They are also able to reduce the total number of bacteria adhering to the respiratory mucosa in a concentration-dependent manner without altering the bacterial tropism for mucosa, and to preserve ciliated cells. Several clinical trials support the concept of inhaled combination therapy with LABAs and corticosteroids in stable COPD patients. This type of therapy not only improves airflow obstruction but also provides clinical benefits, as manifested by sustained reduction in overall symptoms, improvements in health-related quality of life, and reductions in exacerbations. All of these effects are very important because, despite recent advances in our understanding of COPD and its treatment, therapy remains suboptimal for a considerable number of patients.

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Key words: inhaled corticosteroids; long-acting β_2 -agonists

Abbreviations: cAMP = cyclic adenosine 3'5'-monophosphate; C/EBP = CCAAT-enhancer binding protein; EUROSCOP = European Respiratory Society Study on COPD; GR = glucocorticoid receptor; ICS = inhaled corticosteroid; IL = interleukin; LABA = long-acting β_2 -agonist; NF- κ B = nuclear factor- κ B; PEF = peak expiratory flow; QOL = quality of life; SABA = short-acting β_2 -agonist; SEK = Swedish kronor; SGRQ = St. George Respiratory Questionnaire; TNF = tumor necrosis factor; TRISTAN = Trial of Inhaled Steroids and Long-Acting β_2 -Agonists

COPD is a multicomponent disease that includes airway inflammation, airflow limitation, mucociliary dysfunction, and airway structural changes¹ (Fig 1). In particular, obstruction is associated with an airway inflammatory profile consisting mainly of an increased number of T lymphocytes (predominantly CD8+ cells), macrophages, and neutrophils.^{2,3} Airway inflammation also involves inflammatory mediators such as leukotriene B₄, interleukin (IL)-8 and tumor necrosis factor (TNF)- α ,⁴ which

are generally considered to be important mediators in neutrophil recruitment. Oxidative stress and an imbalance between proteases and antiproteases in the lung also have been implicated in the pathophysiology of the condition.⁴

All COPD components contribute to a complex of lung function changes, symptoms, and exacerbations, which affect the health status and, ultimately, the survival of the patient.¹ It is obvious, therefore, that a correct therapeutic approach must try to interfere with these components. The recent evidence-based Global Initiative for Chronic Obstructive Lung Disease guidelines¹ recommend that the overall approach to managing stable COPD patients should be characterized by a stepwise increase in treatment,

*From the Department of Respiratory Medicine (Dr. Cazzola), Unit of Pneumology and Allergology, Antonio Cardarelli Hospital, Naples, Italy; and the Department of Respiratory Diseases (Dr. Dahl), University Hospital Aarhus, Aarhus, Denmark.

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Correspondence to: Mario Cazzola, MD, FCCP, Via del Parco Margherita 24, 80121 Napoli, Italy; e-mail mcazzola@qubisoft.it

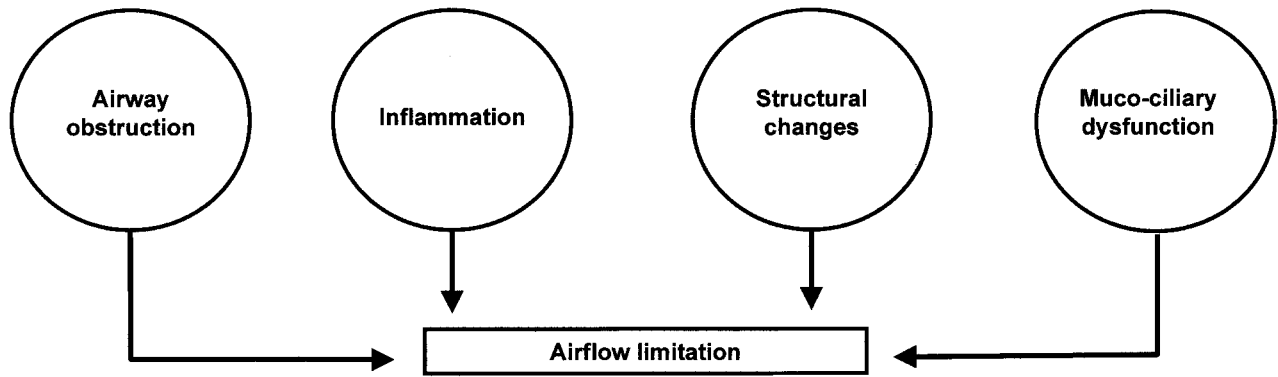


FIGURE 1. Pathophysiologic/clinical features of COPD. The multiple components of COPD combine to produce airflow limitation, which is a key characteristic of the disease.

depending on the severity of the disease. To date, no medication has been shown to alter the rate of decline of FEV₁, but bronchodilator medications are central to symptom management in COPD.⁵ Among the currently available bronchodilators, long-acting inhaled agents are the most convenient.⁶ However, many patients remain symptomatic despite the optimal use of these bronchodilators, and, consequently, it can be necessary to add other drugs to the therapeutic regimen. A body of evidence indicates that combining different bronchodilators may improve efficacy and decrease the risk of side effects compared to increasing the dose of a single agent.^{1,5}

The Global Initiative for Chronic Obstructive Lung Disease guidelines¹ are more cautious in suggesting the use of corticosteroids. They state that regular treatment with inhaled corticosteroids (ICSs) should be prescribed only for symptomatic COPD patients with a documented spirometric response to corticosteroids or in those with an FEV₁ < 50% of predicted values, and repeated exacerbations requiring treatment with antibiotics and/or oral corticosteroids.

USE OF LONG-ACTING β_2 -AGONISTS IN COPD

Long-acting β_2 -agonists (LABAs) have been shown to be elective first-line bronchodilators in the treatment of patients with COPD.^{5,6} They safely attenuate airflow obstruction, decrease the frequency and severity of symptoms by reducing the amount of dynamic hyperinflation, and improve quality of life (QOL). Interestingly, several studies have documented that the LABAs formoterol^{7,8} and salmeterol^{9,10} are more effective than ipratropium bromide for the regular treatment of COPD. This finding is important because for many years ipratropium has been considered to be the first-line therapy

for long-term treatment of stable patients who are symptomatic with COPD.¹¹

LABAs can usefully be prescribed for the combined bronchodilator treatment of COPD.^{12,13} In particular, the addition of a LABA to an anticholinergic compound appears to be more efficacious than adding a short-acting β_2 -agonist (SABA) in stable patients with COPD.¹⁴

In addition to prolonged bronchodilatation, LABAs exert other additive effects that may be of clinical relevance in COPD¹⁵ (Table 1). For example, data indicate that LABAs can increase the level of cyclic adenosine 3'5'-monophosphate (cAMP) in neutrophils, thereby inhibiting neutrophil adhesion, accumulation, and activation, and inducing apoptosis.¹⁵ In particular, salmeterol inhibits neutrophil adhesion to bronchial epithelial cells.¹⁶ Formoterol inhibits chemotaxis to platelet-activating factor¹⁷ over the concentration range 10⁻⁷ to 10⁻⁴ mol/L. Neutrophil activation also was attenuated, as evidenced by reductions in the release of superoxide,¹⁸ IL-8,¹⁹ and bacterial permeability-increasing protein,²⁰ which are not associated with the SABA albuterol. However, in general, these effects were only significant at relatively high concentrations (*ie*, > 10⁻⁶ mol/L) and were not reversed by propranolol, suggesting that they were not mediated by β_2 -adrenoceptors. The end result is a possible reduction in the number and activation status of neutrophils in airway tissue and in the airway lumen²¹

Table 1—LABA Mechanisms of Action in COPD

Bronchodilation
Reduced lung hyperinflation
Increased mucociliary transport
Mucosal cytoprotection
Antineutrophil activity

Table 2—Antineutrophil Effects of Salmeterol²¹

Variables	Placebo	Salmeterol, 50 µg twice daily × 6 wk
Neutrophil numbers		
BAL fluid, %	2.1	1.6*
Bronchial biopsy, cells/mm ²	21.4	14.3†
Neutrophil mediator release		
BAL IL-8, pg/mL	73.0	61.0*
BAL lipocalin, mg/L	14.1	10.3‡
Serum myeloperoxidase, mg/L	302	249*

*p < 0.05.

†p < 0.04.

‡p < 0.03.

(Table 2). LABAs also may decrease the number of neutrophils that adhere to the vascular endothelium at sites of inflammation and may reduce the amount of plasma leakage,²² but the relevance of these findings to COPD patients is unclear. It is noteworthy that glucocorticoids have been shown to increase high-affinity β -agonist binding in human neutrophils.²³ It is also possible that LABAs may increase the peripheral deposition of ICSs, thus enhancing their antiinflammatory activity.²⁴

USE OF CORTICOSTEROIDS IN COPD

The role of corticosteroids in the management of COPD remains controversial. ICSs are commonly prescribed in high doses on the basis that COPD is like poorly responsive asthma, but evidence that they are beneficial in patients with pure COPD is still weak. In effect, although it is well-known that corticosteroids are effective at suppressing airway inflammation, their effect on the inflammation in COPD is still unclear.

Impact of Corticosteroids on Inflammation in COPD

Some studies^{25–28} have indicated that these agents apparently do not exert a major influence on inflammatory cells and mediators in the sputum of stable patients with COPD, but other reports^{29–36} have suggested that treatment with corticosteroids may induce biological responses that may be associated with changes in some clinical outcomes and endoscopic findings in these patients (Table 3).

In vitro corticosteroids attenuate neutrophil recruitment and activation,²⁹ and reduce neutrophil chemotaxis.³⁰ *In vivo*, they attenuate sputum chemotactic activity, increase neutrophil elastase inhibitory capacity,³¹ and induce a decrease in both neutrophils and total cells in induced sputum.^{32,33} Moreover,

they reduce the numbers of mucosal mast cells³⁴ and subepithelial mast cells, and reduce the epithelial CD8/CD4 ratio,³⁵ although they have no effect on the major inflammatory cell types in COPD. ICSs also induce significant reductions in the BAL fluid levels of IL-8 and myeloperoxidase, as well as reductions in cell numbers, the proportion of neutrophils, symptom score, and bronchitis index.³⁶

These findings seem to support the use of ICSs in treating COPD, although long-term treatment with ICSs must be associated with a high risk of adverse systemic effects and involves unnecessary expense.³⁷ Unfortunately, however, clinical trials on the effects of corticosteroids in COPD have reported conflicting results. Consequently, there is considerable controversy concerning the utility of these agents for the long-term treatment of patients with COPD.^{37,38}

Clinical Effects of Corticosteroids in COPD

Negative Findings: Some trials^{39–41} have seemed to indicate that only those patients with an asthmatic component to their disease appear to benefit from corticosteroids. Thus, in the study by Chanez and colleagues,³⁹ 12 of 25 unselected patients in whom COPD had been clinically diagnosed responded to a daily oral dose of prednisolone, 1.5 mg/kg body weight, for 15 days, with an increase in FEV₁ of at least 12% from the baseline value and an absolute value of 200 mL, measured at the end of treatment. By comparison with nonresponders, responders had a significantly larger number of eosinophils and higher levels of eosinophil cationic protein in their BAL fluid, and the responders also had a thicker reticular basement membrane than the nonresponders. Pizzichini and colleagues⁴⁰ reported that an improvement in QOL score and FEV₁ after a short-term course of prednisone therapy in smokers with chronic obstructive bronchitis was paralleled by a significant reduction in eosinophilia and eosinophil activation, as indicated by sputum eosinophil cationic protein levels, but not by changes in neutrophils or neutrophil proteases. Patients without sputum eosinophilia did not show clinical benefit from short-term prednisone therapy. Nishimura and colleagues,⁴¹ in a trial of patients with COPD, found a significant improvement in FEV₁ in a minority of patients (5 of 30 patients) receiving 3,000 µg per day beclomethasone dipropionate over a 4-week treatment period. However, some of these responders showed a positive response to the bronchodilator challenge, as well as an elevated serum IgE level or eosinophil count, which is suggestive of the presence of an asthmatic component to their airflow obstruction.

Besides, four large studies (*ie*, the European Respiratory Society Study on COPD [EURO-

Table 3—Impact of Corticosteroids on Inflammation in COPD*

Study	Type of Patients Enrolled	Treatment, Study Duration	Outcomes
Negative studies			
Thompson et al ²⁵	Current smokers with chronic bronchitis and at least mild obstruction	Beclomethasone, 1 g/d for 6 weeks	Small increase in FEV ₁ , small decrease in macroscopic bronchoscopic index of bronchial inflammation, no reduction in the number of neutrophils in BALF
Keatings et al ²⁶	Patients with severe COPD (mean FEV ₁ , 35% predicted)	Budesonide, 800 µg twice daily for 2 wk	No clinical benefit in either lung function or symptom scores, no significant change in the inflammatory indices as measured by total and differential cell counts and concentrations of TNF-α, eosinophil activation markers, eosinophilic cationic protein, and eosinophil peroxidase, and neutrophil activation markers myeloperoxidase and human neutrophil lipocalin
Culpitt et al ²⁷	Patients with stable COPD	Oral prednisolone, 30 mg daily for 2 weeks Fluticasone, 500 µg twice daily for 4 wk	Sputum eosinophil number, eosinophilic cationic protein, and eosinophil peroxidase not modified No clinical benefit in terms of lung function or symptom scores, no change in induced sputum inflammatory cells, percentage of neutrophils, IL-8 levels, supernatant elastase activity, MMP-1, MMP-9, and the antiproteases secretory leukoprotease inhibitor and tissue inhibitor of MMP-1 levels
Loppow et al ²⁸	Patients with chronic bronchitis (mean FEV ₁ , 83.4% predicted)	Fluticasone, 500 µg twice daily for 4 wk	No improvement in lung function or inflammatory parameters, such as the concentration of exhaled nitric oxide, differential cell counts in induced sputum, and the number of cells positive for inducible nitric oxide synthase, as well as the levels of lactate dehydrogenase, eosinophilic cationic protein, neutrophil elastase and IL-8 in sputum supernatants
Positive studies			
Llewellyn-Jones et al ³¹	Patients with clinically stable, smoking-related chronic bronchitis and emphysema (mean FEV ₁ , 0.71 L)	Fluticasone, 1.5 mg/d for 8 wk	No effect on peripheral neutrophils or on sputum albumin and myeloperoxidase concentrations, but reduction in the neutrophil chemotactic activity of sputum and beneficial effect on the proteinase/antiproteinase balance
Confalonieri et al ³²	Patients with stable COPD (mean FEV ₁ , 60.2% predicted)	Beclomethasone, 500 µg three times daily for 2 mo	Reduction in both neutrophils and total cells in induced sputum, no change in spirometry and blood gases
Yildiz et al ³³	Clinically stable COPD patients	Fluticasone, 1,500 µg/d for 2 mo	No significant changes in the number of peripheral blood neutrophils, blood gases, and spirometry, but decrease in the total cell number and the number of neutrophils in induced sputum
Gizycki et al ³⁴	Patients with mild-to-severe COPD (FEV ₁ , 25–80% predicted)	Fluticasone, 500 µg twice daily for 3 mo	Significant decrease in the numbers of mucosal mast cells, improvement in symptoms
Hattotuwa et al ³⁵	Patients with mild-to-severe stable COPD (mean FEV ₁ , 25–80% predicted)	Fluticasone, 500 µg twice daily for 3 mo	No effect on the major inflammatory cell types in COPD, but reduced epithelial CD8/CD4 ratio and subepithelial mast cell number
Balbi et al ³⁶	Stable COPD patients with mild disease	Beclomethasone, 1.5 mg/d for 6 wk	Reductions in the lavage levels of IL-8 and myeloperoxidase, in cell numbers, neutrophil proportion, symptom score, and bronchitis index

*BALF = BAL fluid; MMP = matrix metalloproteinase.

SCOP]⁴²; the Copenhagen City Lung Study⁴³; the Inhaled Steroids in Obstructive Lung Disease trial⁴⁴; and the American Lung Health-2⁴⁵) have demonstrated that these agents did not have any worthwhile effect on the rate of decline in FEV₁ when asthma was rigorously excluded (Table 4), indicating that there is no effect of ICSs on the progressive inflammatory disease process.³⁷

In effect, there are three principal arguments against the use of ICSs in COPD, as follows: neutrophilic inflammation (which is characteristic in COPD) is generally resistant to corticosteroids; corticosteroids prolong the survival of neutrophils by inhibiting apoptosis; and corticosteroid therapy fails to suppress cytokines such as TNF-α and IL-8, which are elevated in patients with COPD.³⁷ More-

Table 4—Long-term Effect of ICSs in COPD*

Study	Patients Enrolled, No./Study Duration	Rate of FEV ₁ Decline vs Placebo	Health Outcomes
EUROSCOP ⁴²	1,277 patients with mild COPD (mean FEV ₁ , 77% predicted)/F/U at 36 mo	No change with budesonide, 400 µg twice daily	Not evaluated
Copenhagen City Lung Study ⁴³	290 patients with mild to moderate COPD (mean FEV ₁ 86% of predicted value)/F/U at 30 mo	No change with budesonide, 800 µg plus 400 µg daily for 6 mo followed by 400 µg twice daily for 30 mo	No change in exacerbations
ISOLDE ⁴⁴	750 patients, with moderate to severe COPD (mean FEV ₁ 50% of predicted value)/F/U at 36 mo	No change with fluticasone, 500 µg twice daily	Decreased exacerbations; reduced rate in decline of the disease-specific SGRQ
Health Lung Study-2 ⁴⁵	1,116 patients with mild-to-moderate COPD (mean FEV ₁ , 64% predicted)/F/U at 40 mo	No change with triamcinolone, 600 µg twice daily	Less airway reactivity; reduced respiratory symptoms; slightly reduced hospitalizations; loss of bone mineral density; increased skin bruising

*F/U = follow up.

over, a population-based cohort study⁴⁶ documented that ICS use after hospitalization for COPD was not found to reduce mortality and morbidity, but this study had only 979 persons and could not estimate the benefit with great precision.

Positive Findings: Notwithstanding these negative findings, evidence is accumulating to support the therapeutic use of corticosteroids, at least in patients with more advanced COPD.⁴⁷ For example, it was reported that treatment with an ICS for > 3 months improved prebronchodilator airflow obstruction and oxygenation while decreasing dyspnea in patients with moderate-to-severe COPD.⁴⁸ Reductions in exacerbation severity were seen in another study⁴⁹ of patients with moderately severe disease who were treated for 6 months with an inhaled glucocorticoid. The Inhaled Steroids in Obstructive Lung Disease study⁴⁴ reported that, in patients with moderate-to-severe-disease, 3 years of regular treatment with an ICS resulted in fewer exacerbations, a reduced rate of decline in health status, and higher FEV₁ values than did placebo treatment. A recent systematic review⁵⁰ of randomized placebo-controlled trials has demonstrated a beneficial effect of ICSs in reducing rates of COPD exacerbations. These agents also can extend survival in patients with COPD.⁵¹ Furthermore, the withdrawal of ICS therapy has been shown to lead to deterioration in ventilatory function and to increased exercise-induced dyspnea in patients with severe irreversible airway obstruction.⁵²

All these findings lend support to the use of ICSs in stable patients with COPD, and a convincing demonstration of the effectiveness of these agents in COPD patients has come from the COPE study (an

investigation of COPD in the Department of Pulmonology, Enschede, the Netherlands).⁵³ This study showed that the discontinuation of therapy with ICSs was associated with a more rapid onset and a higher risk of recurrence of exacerbations, and with a significant deterioration in aspects of health-related QOL, in the majority of patients.⁵³ However, 40% of subjects in the COPE study experienced no untoward effect from the withdrawal of ICSs.⁵³ This finding indicates that there is an urgent need to identify which subgroups of COPD patients respond well to prolonged inhaled glucocorticoid therapy.

This is not an easy task, since COPD comprises various diseases, which may differ in their pathologic, clinical, and functional features, and in the biological phenomena that cause and maintain airway inflammation. There also may be differences between the baseline disease state (*ie*, in the absence of a current exacerbation) and the disease state during an acute exacerbation, and differences according to the degree of airway obstruction or history of cigarette smoking. Nevertheless, since very few therapies offer significant benefits to patients with COPD, and since ICSs are potentially beneficial in the treatment of this disease (Table 5), the use of these agents remains a possible therapeutic approach to stable patients with COPD.

Table 5—Clinical Efficacy of ICSs in COPD

Increased lung function
Decreased symptoms
Decreased No./severity of exacerbations
Decreased morbidity/mortality
Increased health status

Therefore, until a test is developed that can distinguish potential corticosteroid responders from nonresponders, it is worthwhile giving all patients with COPD a trial (3 to 6 months) of an ICS to determine whether or not they respond.⁵⁴

PHARMACOLOGIC RATIONALE FOR COMBINING A LABA AND AN ICS IN COPD

It must always be emphasized that the use of ICSs should not leave the use of a LABA out of consideration. The concomitant use of a LABA and an ICS can influence airway obstruction (eg, smooth muscle contraction, increased cholinergic tone, and, perhaps, bronchial hyperreactivity), mucociliary dysfunction (eg, reduced mucociliary transport and mucosal damage), and airway inflammation (eg, increased numbers of neutrophils, macrophages, and CD8+ lymphocytes, elevated levels of IL-8, TNF- α , and leukotrieneB₄, protease/antiprotease imbalance, and mucosal edema), as well as structural changes (eg, glandular hypertrophy and goblet cell hyperplasia) [Fig 2]. Furthermore, when a LABA is added to an ICS, it has the potential for countering some of the possible negative effects of the corticosteroid.

There is an exciting possibility that the observed benefit from combining these two classes of drugs might be due to a synergistic interaction, with the resulting synergetic effect being greater than the sum of responses achieved from each drug alone. However, the basic molecular mechanism of such an interaction is still to be fully identified (Fig 3).

Effect of Corticosteroids on LABAs

Corticosteroids can prevent, at least partially, homologous down-regulation of β_2 -adrenoceptor num-

bers and can induce an increase in the rate of receptor synthesis through a process of extended β_2 -adrenoceptor gene transcription.⁵⁵ Although airway smooth muscle is among the tissues least susceptible to homologous down-regulation, long-term treatment with a LABA may result in tolerance to its bronchodilator effects.⁵⁶ Thus, the effects of corticosteroids on β_2 -adrenoceptor expression have the potential for enhancing the airway relaxant response to β -adrenergic stimulation. An *in vitro* experimental finding suggests that, at least in the rabbit, this effect is correlated with increased β -adrenoceptor expression in the tissue.⁵⁷ Moreover, the efficiency of coupling between the β_2 -adrenoceptor and Gs (the G protein that mediates the stimulation of adenylyl cyclase) also has been reported to be modulated by glucocorticoids.⁵⁸ As a result, β_2 -adrenoceptor-stimulated adenylyl cyclase activity and cAMP accumulation increase after glucocorticoid treatment.

Effect of Long-Acting β -Agonists on Corticosteroids

In addition to the beneficial effects of corticosteroids on LABA activity, LABAs also may enhance the effects of corticosteroids. It has been observed *in vitro*, using primary human lung fibroblasts and vascular smooth muscle cells, that LABAs induce glucocorticoid receptor (GR) translocation from the cell cytosol to the nucleus and enhance GR-glucocorticoid response element binding in the absence of ligand.⁵⁹ Preliminary reports *in vivo* have confirmed this finding.⁶⁰ It seems likely, therefore, that LABAs may be able to induce GR nuclear translocation and that this may prime the receptor to be more responsive to a concomitant or subsequent challenge with glucocorticoids (Fig 3). LABAs also may increase the sensitivity of the molecular pathways that are utilized by corticosteroids to suppress

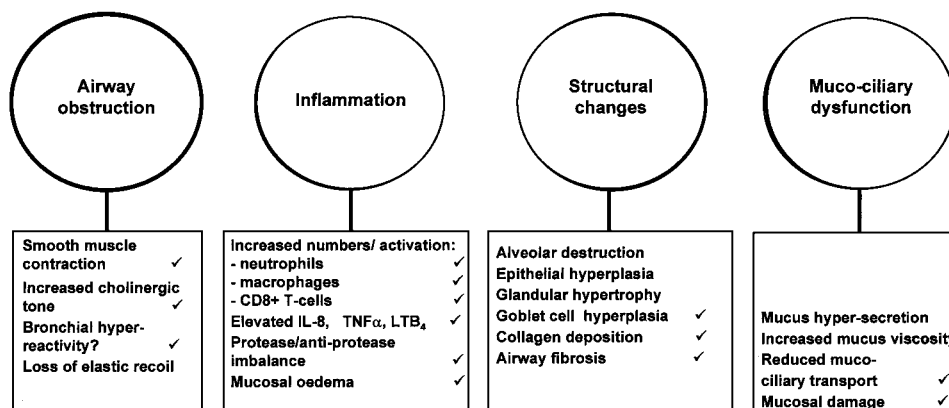


FIGURE 2. Effects of LABA/ICS combination therapy on pathophysiology of COPD. The LABA/ICS combination has additive effects on the pathophysiology of COPD affecting airway obstruction, inflammation, structural changes, and mucociliary dysfunction. It addresses the multicomponent nature of COPD more than does LABA or ICS treatment alone.

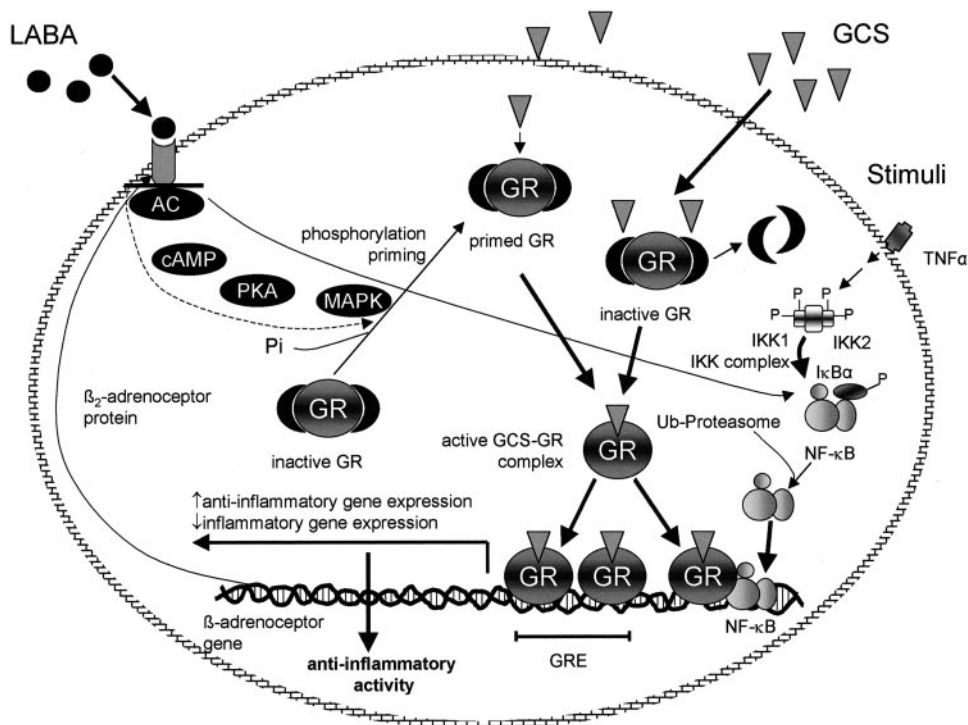


FIGURE 3. The basis for synergy between LABAs and ICSs. Glucocorticoids (GCS) freely diffuse from the circulation across cell membranes into cells, where they activate the GR. On ligand binding, the receptor is activated, dissociates from chaperone proteins, and translocates to the nucleus, where it can bind as a dimer to specific DNA sequences (glucocorticoid response elements [GRE]) upstream of the start site of transcription. The activated GCS-GR also binds to the β_2 -adrenoceptor gene, leading to an increase in the number of β_2 -adrenoceptors in the cell membrane. On the other side, LABA stimulates β_2 -adrenoceptors, leading to the priming of the GR and increasing translocation of the receptor into the nucleus of the cell. The overall response is increased anti-inflammatory activity from a given steroid dose. The primed GR also may have enhanced actions against other transcription factors such as NF- κ B. Besides, LABAs may inhibit NF- κ B by inducing an increase in I κ B- α levels. MAPK = mitogen-activated protein kinase; PKA = protein kinase A; AC = adenylate cyclase; IKK = I κ B kinase coupler; Ub = ubiquitin; Pi = phosphatidyl inositol.

inflammation, including their action on histone acetylation and deacetylation,^{61,62} or through effects on the activation of transcription factors, such as nuclear factor (NF)- κ B.⁶³ Recently, it has been documented⁶⁴ that the combination of glucocorticoids and LABAs synchronizes the activation of the GR and CCAAT-enhancer binding protein (C/EBP)- α , which belongs to a family of transcription factors that are involved in the differentiation process of numerous tissues. This action results in a faster and more prolonged activation of p21(Waf1/Cip1) compared with each drug given alone. In particular, when administered alone, formoterol and budesonide activate the two transcription factors with different potency and kinetics. The combined administration of the two drugs leads to a simultaneous activation of the GR and C/EBP- α , resulting in a synergistic stimulatory effect on p21(Waf1/Cip1) promoter activity. Furthermore, when administered together, the drugs are effective at concentrations that are

ineffective when either drug is administered alone. These data lend support to the idea that the combined application of the two drugs has a beneficial effect that is greater than that of increasing the dose of either drug alone, at least in asthma.^{65,66}

Role of LACA/ICS Interactions in COPD

At present, the role of these interactions in COPD patients cannot be clearly defined. GR does not interact only with C/EBP- α , but also affects the action of other transcription factors, such as activating protein 1, cAMP responsive element-binding protein, and the inhibitor of NF- κ B.⁶⁷⁻⁷⁰ A direct interaction with the GR also has been shown for the signal transducer and activator of transcription 3 and 5.^{71,72} These findings suggest that we must also investigate the possibility of an interaction between glucocorticoids and LABAs at these levels, and evaluate the potential impact on COPD management.

In Vitro Interactions of LABA and ICS Useful in COPD

Examples of interaction between these two classes of drugs that might be useful in COPD patients include the synergistic inhibition by glucocorticoids and LABAs of TNF- α -induced IL-8 release from cultured human airway smooth muscle cells⁶³ and alveolar macrophages in patients with COPD,⁷³ and the capacity of corticosteroids to counteract the enhancement of LABAs on TNF- α -induced IL-8 production in cultured human bronchial epithelial cells.⁷⁴ These effects are mediated through the β_2 -adrenoceptor into a signal transduction pathway that does not involve the GR, but other interactions between glucocorticoid and β_2 -agonist cannot be excluded.⁷⁴ The inhibition of transcription factors, such as NF- κ B, by β -agonists may occur as a result of an increase in the levels of the inhibitor of NF- κ B. One study⁷⁵ of monocytic THP-1 cells stimulated with lipopolysaccharide showed that β -agonists could inhibit TNF- α and IL-8 production, and that the effect was related to increased cytoplasmic concentrations of I κ B- α , possibly through its decreased degradation. Elevated cAMP levels can inhibit NF- κ B-mediated gene transcription in human monocyte and endothelial cells.⁷⁶ cAMP regulates cytokine gene expression by induction of the cAMP-mediated transcriptional repressor in human thymocytes.⁷⁷ The inhibitory effects mediated by induction of the cAMP-mediated transcriptional repressor may be

related to its ability to bind (*ie*, mask) a wide range of cyclic adenosine monophosphate response element and activating protein-1 motifs and/or its ability to inactivate certain transcription complexes via protein-protein interactions.⁷⁶ In any case, IL-8 is a potent chemoattractant and an activator for neutrophils, and this may result in a persistent inflammatory cycle by establishing a positive feedback loop. Reductions in neutrophil number and function could reduce the severity of disease and the degree of airflow obstruction in patients with COPD.⁶³

Another important finding is the capacity of both ICSs and LABAs to reduce the total number of bacteria adhering to the respiratory mucosa in a concentration-dependent manner without altering the bacterial tropism for mucosa, and to preserve ciliated cells.⁷⁸ ICSs and LABAs, when administered together at low concentrations, exhibited a synergistic effect with respect to the preservation of ciliated cells, showing a trend toward reduced damage and a significant preservation of the number of ciliated cells compared to either agent alone at the same concentrations. This result may have clinical significance as it is thought that ciliated cells are the most sensitive to damage by bacterial infection.⁷⁹ It is well-known that airway colonization and chronic infection contribute to progressive pulmonary damage in COPD patients via the action of proinflammatory substances in what is known as the "vicious circle theory"⁸⁰ (Fig 4). Recently, the synergistic

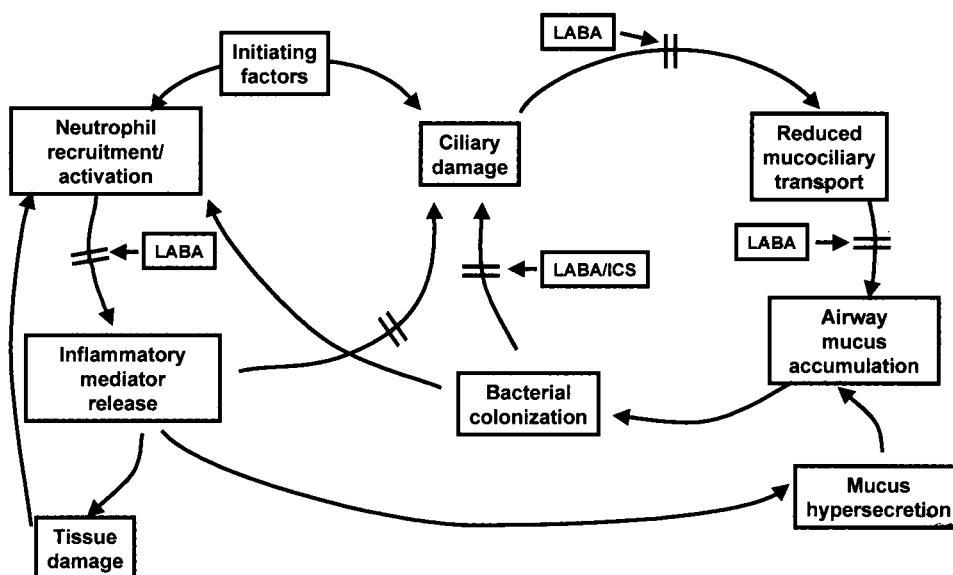


FIGURE 4. The LABA/ICS combination breaks the COPD vicious cycle. LABAs can break the COPD vicious cycle by increasing ciliary beat frequency, and therefore enhancing mucus clearance, by preventing bacterial damage to the epithelium, and by suppressing neutrophil activation and mediator release. Besides, ICSs and LABAs when administered together at low concentrations exhibited a synergistic effect with respect to the preservation of ciliated cells, showing a trend toward reduced damage and a significant preservation of the number of ciliated cells compared to either agent alone at the same concentrations.

effects of salmeterol and fluticasone in human rhinovirus-induced proinflammatory cytokine production have been documented.⁸¹ Rhinoviruses are implicated in many acute exacerbations of COPD, perhaps by inducing proinflammatory cytokines.⁸²

INHALED COMBINATION THERAPY WITH LABAS AND CORTICOSTEROIDS IN STABLE COPD PATIENTS

Several clinical trials support the concept of inhaled combination therapy with LABAs and corticosteroids in stable COPD patients, although some reports have not been published yet. Nonetheless, the currently available information has recently induced the European Agency for the Evaluation of Medicinal Products to issue a positive opinion⁸³ on the use of combination therapy with LABAs and ICSs in the maintenance treatment of patients with severe COPD and a history of exacerbations.

Clinical Effects in Adding an ICS to a LABA

The results of a recent small study⁸⁴ have indicated that the addition of budesonide amplifies the fast onset of action of formoterol, but does not induce systemic effects, in patients with COPD. It has been shown that glucocorticoids act on steroid receptors to increase β_2 -adrenoceptor expression by augmenting the rate of β_2 -adrenoceptor gene transcription.⁸⁵ However, the glucocorticoid-induced increase in the accumulation of β_2 -adrenoceptor messenger RNA could be detected at 15 min, the maximal accumulation occurred at 2 h, and the glucocorticoid-induced increase in β_2 -adrenoceptor messenger RNA returned to the control level by 17 h. In contrast, the increase in the β_2 -adrenoceptor values was slower, reaching a maximum between 17 and 24 h. These findings indicate that another type of interaction may underlie the rapid onset of action of combination therapy with formoterol and budesonide. It is likely that a nongenomic action of budesonide might explain this effect. Thus, it is possible that corticosteroids might induce a fall in intracellular Ca^{2+} that might result, in part, from the inhibition of Ca^{2+} entry through voltage-gated ion channels.⁸⁶

The capacity of corticosteroids to prevent homologous down-regulation of β_2 -adrenoceptor values and to induce an increase in the rate of synthesis of these receptors may be important when a patient is receiving regular treatment with LABAs. The addition of an ICS to a LABA was initially studied in a 3-month trial that enrolled 80 COPD patients. The association therapy progressively improved lung function over the 3-month period compared to long-

acting bronchodilator treatment alone, although the difference was not statistically significant.⁸⁷ However, the association of therapy with salmeterol (50 μg twice daily) with that with fluticasone (250 or 500 μg twice daily) allowed a significantly greater improvement in lung function after salbutamol therapy alone than salmeterol therapy (50 μg twice daily) alone. It is likely that this effect was due to the prevention of the homologous down-regulation of β_2 -adrenoceptors and the induction of an increase in the rate of synthesis of receptors through a process of increased β_2 -adrenoceptor gene transcription induced by fluticasone. This may be considered clinically important, because when airway obstruction becomes more severe the preferred therapeutic option is to add a fast-acting inhaled β_2 -agonist as rescue medication to produce rapid relief of bronchospasm.

Combination Therapy With Salmeterol/Fluticasone

The value of regular combination therapy with LABAs and corticosteroids delivered via a single inhaler to COPD patients has been documented repeatedly (Table 6). Since the overall goals for improving clinical outcomes are to reduce symptoms, especially dyspnea, to improve exercise capacity, to reduce exacerbations and the possible need for hospitalization, and to enhance health status,¹ the different trials have not only evaluated the efficacy of combination therapy on lung function, but also its impact on these other clinical outcomes.

A 24-week study with the combination therapy of salmeterol, 50 μg twice daily, and fluticasone propionate, 500 μg twice daily, explored the potential for increasing airflow, reducing symptoms (including dyspnea), and improving health status, compared with the individual components and placebo.⁸⁸ The results showed that the salmeterol/fluticasone propionate combination not only improved airflow obstruction but also provided clinical benefits, as manifested by reduced severity of dyspnea, reduced use of rescue salbutamol, and improved health status.

A 52-week multicenter, randomized, double-blind, placebo-controlled trial (Trial of Inhaled Steroids and Long-Acting β_2 -Agonists [TRISTAN])⁸⁹ compared the safety and efficacy of the salmeterol, 50 μg twice daily/fluticasone propionate, 500 μg twice daily, combination with that of the individual drugs alone in 1,465 patients with COPD (mean FEV₁, 45% predicted). Following a year of treatment with the salmeterol/fluticasone propionate combination, patients with COPD experienced significant and clinically meaningful improvements in health status, as measured by the St. George Respiratory Questionnaire (SGRQ), compared with pla-

Table 6—Long-term Effect of Combination Therapy in COPD*

Study	Patients Enrolled, No./Study Duration	Changes in FEV ₁ Over Baseline at the End of Treatment	Health Outcomes
S/FP			
Mahler et al ⁸⁸	691 patients with moderate-to-severe COPD (FEV ₁ , > 65% predicted)/F/U at 24 wk	Pretreatment FEV ₁ : +156 mL after S, 50 µg/FP, 500 µg twice daily, +107 mL after S, 50 µg twice daily; +104 after FP, 500 µg twice daily; - 4 mL after placebo	Greater improvements in the transition dyspnea index with S/FP compared with FP, S, and placebo; greater increase in chronic respiratory disease questionnaire score with S/FP compared with FP and placebo, but not with S
Calverley et al ⁸⁹	1,465 patients, with moderate-to-severe COPD (mean FEV ₁ , 45% predicted)/F/U at 52 wk	+10% after S, 50 µg/FP 500, µg twice daily; +2% after S, 50 µg twice daily; +2% after FP, 500 µg twice daily; -3% after placebo	Significantly greater reduced rate in decline of the disease-specific SGRQ after S/FP than after FP and placebo; No. of exacerbations per patient per year reduced by 25% after S/FP, 20% after S, and 19% after FP, all vs placebo, but 30% reduction with S/FP vs placebo when baseline FEV ₁ < 50% predicted, and 10% reduction when baseline FEV ₁ > 50% predicted
Dal Negro et al ⁹⁰	18 patients with moderate COPD (mean FEV ₁ , 49.1% predicted)/F/U at 52 wk	+6.6% after S, 50 µg/FP 250 µg twice daily; +0.3% after S 50 µg twice daily; -2.6% after placebo	Decrease in exacerbations only after S/FP
F/B			
Szafranski et al ⁹¹	812 patients with moderate-to-severe COPD (mean FEV ₁ , 36% of predicted)/F/U at 52 wk	+15% after F, 6 µg/B, 200 µg twice daily vs placebo; +9% after F, 6 µg/B, 200 µg twice daily vs B, 200 µg twice daily; +1% after F, 6 µg/B, 200 µg twice daily vs F, 6 µg twice daily	Significantly greater reduced rate in decline of the disease-specific SGRQ after F/B than after placebo; No. of severe exacerbations per patient per year reduced after F/B by 24% vs placebo, 23% vs F, and 11% vs B
Calverley and Olsson, ⁹² Jones and Ståhl, ⁹³ and Calverley and Peterson ⁹⁴	1,022 patients with moderate-to-severe COPD (mean FEV ₁ , 36% predicted)/F/U at 52 wk	+14% after F, 12 µg/B, 400 µg twice daily vs placebo; +11% after F, 12 µg/B, 400 µg twice daily vs B, 400 µg twice daily; +5% after F, 12 µg/B, 400 µg twice daily vs F, 12 µg twice daily	Significantly greater reduced rate in decline of the disease-specific SGRQ after F/B than after F, B, and placebo; No. of exacerbations per patient per year reduced by 24% after F/B, 11.6% after B, and 3% after FP, all vs placebo

*S = salmeterol; FP = fluticasone propionate; F = formoterol; B = budesonide. See Table 4 for abbreviation not used in the text.

cebo or fluticasone alone therapy. In addition, patients treated with combination therapy had greater reductions in symptom scores compared with all other treatments, and greater reductions in activity (limitation) scores compared with placebo and fluticasone therapy alone. These findings could have a real impact on the management of COPD. Patients with COPD have significant and measurable decreases in health status, which worsen with disease progression. The fatigue and emotional distress resulting from COPD symptoms such as dyspnea, cough, and phlegm production reduce the ability of patients to work or carry out their normal activities. As there is currently no cure for COPD, the goals of therapy are to prevent symptoms and exacerbations, to preserve lung function, and to maintain health status.

The prevention of exacerbations is a key goal for the effective management of COPD,¹ since exacerbations are known to reduce QOL and are cost

drivers in the treatment of COPD patients. Therefore, controlling exacerbations is important from both economic and patients' perspectives. Subgroup analyses of the TRISTAN trial⁸⁹ have been performed to determine the relative efficacy of the salmeterol, 50 µg twice daily/fluticasone, 500 µg twice daily, combination therapy in reducing exacerbations in COPD patients with FEV₁ values of < 50% predicted at baseline (*ie*, the more severe subgroup) or FEV₁ values of ≥ 50% predicted at baseline (*ie*, the less severe subgroup). Exacerbations were defined *a priori* as a worsening of COPD symptoms that required treatment with antibiotics, oral corticosteroids, or both. Episodes that required corticosteroid treatment or hospital admission were noted separately. A total of 949 patients (65%) had a prebronchodilator FEV₁ of < 50% predicted and were classified into the more severe subgroup, and 513 patients (35%) had FEV₁ values of ≥ 50% predicted and were classified into the less severe

subgroup. During the study, there was a higher incidence of exacerbations in the more severe subgroup compared with the less severe subgroup (567 patients [60%] vs 226 patients [44%], respectively). In the total population, the salmeterol/fluticasone combination treatment produced a significant reduction in the exacerbation rate of 25%, compared with placebo treatment. This reduction was 30% in the more severe subgroup, compared with a 10% reduction in the less severe subgroup. These effects of combination therapy were more pronounced when considering exacerbations requiring oral corticosteroids, which were reduced by 39% in the total population, 43% in the more severe subgroup, and 24% in the less severe subgroup. Salmeterol/fluticasone combination treatment also reduced the number of exacerbations requiring therapy with oral corticosteroids by 19%, compared with therapy with salmeterol alone, in the more severe subgroup. Although twice-daily therapy with salmeterol/fluticasone was effective in significantly reducing the number of exacerbations across both categories of disease severity, there was a trend for a larger reduction in exacerbations and exacerbations requiring oral corticosteroids in the more severe subgroup.

Recently, Dal Negro et al⁹⁰ have compared therapy with salmeterol, 50 µg twice daily/fluticasone, 250 µg twice daily, via a single inhaler with therapy with salmeterol, 50 µg twice daily, alone and placebo in 52-week study that has enrolled a small group of ICS-naive patients with moderate COPD who had already been treated with theophylline, 400 mg per day, and SABAs on demand. The mean (\pm SD) number of exacerbations per year decreased from 3.5 ± 0.8 to 1.16 ± 0.75 in the salmeterol/fluticasone group ($p < 0.001$), from 3.0 ± 0.89 to 2.3 ± 0.81 in the salmeterol group (difference not significant), and from 3.16 ± 1.16 to 4.16 ± 0.75 in the placebo group (difference not significant). Patients receiving salmeterol/fluticasone therapy showed the highest mean improvement in FEV₁ (mean increase, $6.6 \pm 2.4\%$) over the baseline pretreatment value ($p < 0.001$), with FEV₁ remaining unchanged after 52 weeks of treatment in the salmeterol group (mean increase, $0.3 \pm 0.9\%$) and with a substantial decrease following placebo therapy (mean decrease, 2.6 ± 0.5 ; $p < 0.001$).

Combination Therapy With Formoterol/Budesonide

The effects of formoterol/budesonide combination treatment in COPD patients also have been investigated. In a 12-month, randomized, double-blind, placebo-controlled, parallel-group, multicenter study,⁹¹ 812 adults with moderate-to-severe COPD (mean age, 64 years; mean FEV₁, 36% predicted;

median time since diagnosis, 5 years) received two inhalations twice daily of formoterol (6 µg)/budesonide (200 µg), budesonide (200 µg) alone, formoterol (6 µg) alone, or placebo. Formoterol/budesonide treatment increased FEV₁ by 15% vs placebo, 9% vs budesonide alone, and 1% vs formoterol alone. Significant improvements in morning and evening peak expiratory flow (PEF) values were seen during the 12 months of therapy with formoterol/budesonide compared with all other treatment groups (adjusted mean change from run-in: morning PEF, 24, 16, and 12 L/min vs placebo, budesonide alone, and formoterol alone, respectively; evening PEF, 20, 15, and 11 L/min vs placebo, budesonide alone, and formoterol alone, respectively; all $p < 0.001$). These lung function improvements were maintained throughout the 12-month study. The improvement in lung function observed after formoterol/budesonide treatment must be considered to be important, but the greater capacity of formoterol/budesonide to decrease the mean total symptom score ($p < 0.001$ vs placebo; $p < 0.001$ vs budesonide alone; $p = 0.103$ vs formoterol alone), to increase the number of days free from shortness of breath by 12% vs placebo ($p < 0.001$), and to increase the number of awakening-free nights by 14% vs placebo ($p < 0.001$), each of which is equivalent to approximately 1 extra day/night per week, is likely to have a more substantial impact on health status. Formoterol/budesonide also significantly reduced the use of reliever medication by 1.3 and 0.7 inhalations per 24 h vs placebo and budesonide alone, respectively (both $p < 0.001$).

The sustained reduction in overall symptoms, including the number of sleep-disturbed nights, in patients with moderate-to-severe COPD was reflected in improvements in health-related QOL. Mean reductions from baseline were -3.9 , -1.9 , -3.6 and -0.03 , respectively, after therapy with formoterol/budesonide, budesonide, formoterol, and placebo.⁹¹ Compared with treatment with placebo, treatment with formoterol/budesonide significantly improved the SGRQ total score ($p = 0.009$), and the symptom domain score ($p < 0.001$) and impact domain score ($p = 0.006$). Greater improvements were seen with formoterol/budesonide compared with the other active treatments, but these did not achieve statistical significance.

Formoterol/budesonide therapy also produced clinically significant reductions in the number of exacerbations in patients with moderate-to-severe COPD.⁹¹ It reduced the number of severe exacerbations per patient per year by 24% vs placebo therapy ($p = 0.035$), by 23% vs formoterol therapy ($p = 0.043$), and by 11% vs budesonide therapy ($p = 0.385$). Moreover, budesonide/formoterol therapy also reduced the number of mild exacerbations

compared with therapy with placebo (62%; $p < 0.001$), budesonide alone (35%; $p = 0.022$); and formoterol alone (15%; $p = 0.403$).

In another randomized, double-blind, active-controlled study, 1,022 patients (mean age, 64 years; FEV₁, 0.99 L and 36% predicted) with moderate-to-severe COPD and a history of exacerbations had their condition optimized with therapy with formoterol, 12 µg twice daily, and oral prednisolone prior to being given formoterol (12 µg twice daily)/budesonide (400 µg twice daily), budesonide alone (400 µg twice daily), formoterol alone (12 µg twice daily), or placebo for 1 year.⁹²⁻⁹⁴ The study, which has not been published yet, was designed to determine whether these treatments prevented COPD exacerbations. Formoterol/budesonide therapy was significantly better than that with long-acting β₂-agonists alone, corticosteroids alone, or placebo in maintaining the lung function improvement achieved after optimization.⁹² Mean FEV₁ in the budesonide/formoterol group remained 14% above the value in the placebo group ($p < 0.001$), 11% above the value in the budesonide group ($p < 0.001$), and 5% above the value in the formoterol group ($p < 0.01$). Morning PEF in the budesonide/formoterol group was 18 L/min above the value in the placebo group ($p < 0.001$), 15 L/min above the value in the budesonide group ($p < 0.001$), and 7 L/min above the value in the formoterol group ($p < 0.01$). The value for evening PEF in the budesonide/formoterol group was 14 L/min above the value in the placebo group ($p < 0.001$), 12 L/min above the value in the budesonide group ($p < 0.001$), and 5 L/min above the value in the formoterol group ($p = 0.056$). For FVC, the value in the formoterol/budesonide group was 9% above that in the placebo group ($p < 0.001$), 8% above that in the budesonide group ($p < 0.001$), and 2% above that in the formoterol group. These changes, which occurred soon after treatment began, showed no sign of tachyphylaxis over the 12-month study period. Formoterol/budesonide therapy also improved SGRQ total score.⁹³ In particular, it improved all domain scores vs placebo ($p < 0.01$), activity score by 3.6 vs budesonide therapy ($p < 0.05$) and 3.5 vs formoterol therapy ($p < 0.05$), and impact score by 5.7 vs budesonide ($p < 0.001$) and 3.7 vs formoterol ($p < 0.05$).

Interestingly, formoterol/budesonide therapy provided significantly better protection against exacerbations than either LABA alone, corticosteroid steroid alone, or placebo treatment.⁹⁴ It prolonged the time to the first exacerbation requiring medical intervention (*ie*, hospitalization and/or the use of oral steroids/antibiotics) compared with therapy with budesonide alone ($p < 0.05$), formoterol alone ($p < 0.01$), or placebo ($p < 0.05$). The median dura-

tions to the first exacerbation were as follows: formoterol/budesonide, 254 days; budesonide alone, 178 days; formoterol alone, 154 days; and placebo, 96 days. Formoterol/budesonide therapy also reduced the risk of a first exacerbation by 29% vs placebo ($p < 0.01$), 30% vs formoterol alone ($p < 0.01$), and 23% vs budesonide alone ($p < 0.05$). Fewer exacerbations per patient per year were recorded in the formoterol/budesonide group (1.38) than in the budesonide alone group (1.60), the formoterol-alone group (1.85), or the placebo group (1.80). The reduction in the mean exacerbation rate, compared with placebo, was 11.6% in the budesonide-alone group (difference not significant), 3.0% in the formoterol-alone group (difference not significant), and 24% in the formoterol/budesonide group ($p < 0.05$). The numerically larger reduction in the formoterol/budesonide group suggests that synergy may occur. Furthermore, formoterol/budesonide treatment significantly reduced the mean number of oral steroid courses by 45% vs placebo ($p < 0.001$), 28% vs budesonide alone, and 30% vs formoterol alone (both $p < 0.05$). Budesonide therapy alone reduced the mean number of oral steroid courses by 23% vs placebo ($p < 0.05$).

Comparison Between Combination Therapy With Salmeterol/Fluticasone and Formoterol/Budesonide

Apparently, there is no substantial difference between salmeterol/fluticasone and formoterol/budesonide in patients with COPD when these combinations are prescribed at the dosages recommended for this pathology, at least after acute administration.⁹⁵

SYSTEMIC ADVERSE EFFECTS USING COMBINATION THERAPY WITH LABAS AND CORTICOSTEROIDS

Consideration of the risk/benefit ratio is important when using inhaled combination therapy with LABAs and ICSs in patients with COPD, mainly because of the potential systemic adverse effects induced by ICSs.

Side Effects of Long-term Treatments With High-Dose ICSs

It is well-known that long-term treatments with high-dose ICSs have the potential to produce systemic adverse effects. Patients with COPD may be particularly vulnerable to these systemic effects as they are often elderly, immobile, and have poor nutrition, thus increasing the risk of osteoporosis.³⁷ However, the results of different trials have been variable. In the Lung Health Study,⁴⁵ a significantly

lower bone density in the lumbar spine and femur was observed, whereas the EUROSCOP study⁴² reported no difference between study arms. A recent Cochrane review⁹⁶ of the effects on bone metabolism in COPD patients reported that there is no evidence of an effect of ICS treatment on bone mineral density or vertebral fracture when given at conventional doses for 2 or 3 years. Higher doses have been associated with biochemical markers of increased bone turnover, but data on bone mineral density and the incidence of fractures at these doses are not available. There is a need for further, even longer term, prospective studies of conventional and high doses of ICSs.

Elderly patients also may have an increased risk of developing cataracts, glaucoma, and diabetes.³⁷ However, no differences in the rates of cataract were seen in the most important long-term trials.^{42–45,49} In the EUROSCOP study,⁴² 10% of patients developed skin bruising compared to 4% in the control group. It is generally accepted that any discussion of the use of high-dose ICSs in patients with COPD must weigh the real risk of systemic side effects against the minimal clinical value provided by this treatment.³⁷

Side Effects of Combination Therapy With Salmeterol/Fluticasone

Although there is clear evidence that the concentration of ICSs can be reduced when combined with β_2 -agonists, thus minimizing the risk of side effects,⁶⁴ there is a real need to establish whether any side effects induced by combination therapy with LABAs and ICSs are outweighed by the clinical advantages.

The combined use of salmeterol and fluticasone propionate in a single formulation provides additive benefit in the treatment of COPD but with comparable safety to the individual components used alone.^{88,89} The safety profile of salmeterol/fluticasone combination treatment was consistent with that observed with the administration of salmeterol plus fluticasone, and was not different from that for the monocomponents alone after a 12-week treatment period.⁸⁷ The overall adverse event profile of the combination therapy showed no new or unexpected adverse events. There was no evidence that combination treatment with the salmeterol/fluticasone combination was associated with any increased risk of clinically relevant hypothalamic-pituitary-adrenal axis suppression (as assessed by cosyntropin stimulation testing) compared with treatment with fluticasone, salmeterol, or placebo alone. No unexpected cardiovascular effects, as assessed by Holter monitoring and routine ECG, were observed in those patients receiving salmeterol/fluticasone combina-

tion treatment compared with patients receiving salmeterol or placebo. In the TRISTAN study,⁸⁹ all treatments were well-tolerated, although the percentage of patients with oropharyngeal candidiasis was higher in groups receiving fluticasone-containing treatment (placebo treatment, 2%; salmeterol treatment alone, 2%; fluticasone treatment alone, 7%; salmeterol/fluticasone combination treatment, 8%). The majority of patients (96%) had serum cortisol levels that were within the reference range or not significantly changed from baseline. The incidence of skin bruising was low and was comparable among treatment groups (placebo group, 6%; salmeterol alone group, 6%; fluticasone alone group, 7%; salmeterol/fluticasone combination group, 8%). ECG findings were unchanged by therapy.

Side Effects of Combination Therapy With Formoterol/Budesonide

Formoterol/budesonide therapy also was shown to be well-tolerated and to have a safety profile similar to that for therapy with placebo and the monocomponents in patients with moderate-to-severe COPD during 12 months of treatment.⁹¹ Most adverse events were reported for respiratory system disorders, particularly COPD (formoterol/budesonide group, 17%; placebo group, 26%; budesonide-alone group, 13%; and formoterol-alone group, 19%). No treatment-related patterns were discernible regarding death (26 deaths) or serious adverse events (number of serious adverse events per 1,000 treatment days: formoterol/budesonide group, 0.8; placebo group, 0.9; budesonide-alone group, 0.7; formoterol-alone group, 0.7). Discontinuations due to disease deterioration were highest in the placebo group (placebo group, 21%; formoterol/budesonide group, 10%; budesonide-alone group, 12%; formoterol-alone group, 14%), while the frequency of discontinuations due to other adverse events was similar in all groups (6 to 8%). No clinically important between-group differences were identified for laboratory or ECG measurements (including QTc prolongation).

Effect of LABA/ICS Combination Therapy on β_2 -Adrenoceptor Tolerance

These clinical findings regarding combination treatment with both salmeterol/fluticasone and formoterol/budesonide are important. Tolerance generally develops to systemic β_2 -mediated adverse effects, but it has been reported⁹⁷ that concomitant therapy with inhaled budesonide resensitized the response of cardiac β_2 -adrenoceptors to salbutamol in subjects who were receiving regular twice-daily formoterol therapy, which could suggest an in-

creased propensity for the development of systemic β_2 -mediated adverse effects. However, a previous study⁹⁸ has demonstrated that the addition of the recommended dose of formoterol to ICS therapy did not induce significant nonpulmonary consequences, except tremor, which may sometimes be a limiting effect.

NEED FOR FURTHER STUDIES

First of all, there is a need to assess the antiinflammatory effects of long-term inhaled combination therapy with LABAs and ICSs. There is little knowledge at present about the effects of regular treatment with LABAs and/or ICSs on airway inflammation in COPD patients. Studies⁹⁹ using bronchial biopsy specimens obtained during fiberoptic bronchoscopy in patients with COPD have yielded valuable information about the inflammatory process in the large airways of patients with this disease. Bronchoscopic biopsy provides specimens of airway wall tissue from which tissue morphology can be assessed, inflammatory cells can be quantified, and gene products can be identified. Consequently, the immunopathology of bronchial biopsy specimens could be used to evaluate the antiinflammatory effects of combination therapy. Obviously, less invasive methods also can be used.¹⁰⁰

Another important point is the need to relate the short-term response to either ICSs or LABAs to a long-term benefit with inhaled combination therapy. This information is extremely important when we consider that only a positive response to a 2-week course of oral prednisolone (*eg*, 30 mg per day) or a 6-week course of ICSs (*eg*, beclomethasone, 500 μg twice daily, or equivalent) would justify the prescription of regular inhaled steroids to COPD patients, and that a limitation on the use of LABAs to patients with a demonstrable response to SABAs also has been recommended.¹⁰¹ It should be noted that formoterol/budesonide combination treatment was effective even in patients who had not been optimized with oral corticosteroid therapy.⁹³

There is also a clear need for a study to examine whether adding a LABA to ICS therapy produces greater improvements in lung function and symptom control than increasing the ICS dose. On the other hand, we also need to know whether adding an ICS to a regular LABA treatment produces greater improvements in lung function and symptom control than increasing the dose of LABA alone. In other words, we need to know whether more integrated medical treatment can be more effective and safer than more aggressive therapy with the individual agent alone. Obviously, it is also important to inves-

tigate whether such integrated therapy can change the natural history of COPD.

Information on the economic impact of the various treatment options in COPD is also required. To date, few studies have examined the clinical and economic impact of treatments in COPD patients. One study¹⁰² has examined the use of a LABA in COPD patients, and has demonstrated that significant improvements in health and QOL may be obtained at a moderate cost. In another study,¹⁰³ early intervention with an ICS resulted in significant health gains at a relatively low cost. We do not know whether the higher medication cost of combination therapy with a LABA and ICS, compared with monotherapy, is really justified from a pharmacoeconomic point of view for COPD patients. Looking at the results of studies that have been published in the literature or have been presented as abstracts to relevant international meetings,⁸⁸⁻⁹⁴ it is likely that the higher cost of medication is almost completely offset by savings in other costs, such as consultations and hospital admissions for treating exacerbations. Exacerbations are particularly important as they are major cost drivers, the costs varying considerably with the severity of the exacerbation. One study¹⁰⁴ found that β_2 -agonist therapy in conjunction with corticosteroid therapy resulted in improved health outcomes at a small increase in cost compared to β_2 -agonist therapy alone. The addition of an ICS to a β_2 -agonist resulted in net additional health-care costs of \$201 per patient-year compared to therapy with a β_2 -agonist alone, but more than half of the additional cost of adding the ICS was offset by a reduction in the costs of other health-care services. A recent health economic analysis¹⁰⁵ comparing formoterol/budesonide therapy (12/400 μg , respectively, bid) with therapy with formoterol (12 μg bid), budesonide (400 μg bid), and placebo was performed on data from a 1-year multinational, prospective, randomized, parallel-group clinical study among 1,022 patients with COPD. The difference in total health-care costs (*eg*, hospitalizations, emergency department visits, general practitioner visits, study drugs, and concomitant medication use) associated with the different treatments was analyzed using Swedish unit costs. Health-care costs were numerically, but not statistically, significantly lower for formoterol/budesonide therapy (22,893 Swedish kronor [SEK] per year) compared with therapy with formoterol (33,211 SEK per year), budesonide (28,134 SEK per year), and placebo (29,207 SEK per year). The results were robust, as this pattern remained the same when applying UK unit costs (formoterol/budesonide therapy, £1,595 per year; formoterol, £2,577 per year; budesonide, £2,748 per year; placebo, £2,044 per year), and when analyzing sub-

groups of severe patients ($FEV_1 < 40\%$ predicted) and European patients separately.

CONCLUSIONS

There is an increasing volume of evidence showing that combination treatment with both salmeterol/fluticasone and formoterol/budesonide can provide an effective treatment option for COPD patients. However, it must always be considered that the trials that explored the effect of these therapies recruited a select group of patients with COPD, and the results may not be generalizable to patients with extremely advanced or mild forms of the disorder.¹⁰⁶ Nevertheless, since the goals of COPD therapy are to control symptoms, to prevent exacerbations, and to improve lung function and health status, the efficacy of combination therapy in inducing long-lasting bronchodilation, improving QOL, and preventing exacerbations must be considered a real advancement in a disease for which treatment remains suboptimal for a considerable number of patients.

The capacity of combination treatment to reduce exacerbations is particularly important. As correctly stressed by Calverley and coauthors,⁸⁹ the low rate of acute episodes might be attributable to regression to the mean in the number of exacerbations or an effect of improved care associated with clinical trials, but suggests that a study of longer duration and with a larger number of participants would be needed to show a difference. Such long-term studies are underway. They will allow us to better evaluate the effect of such combination therapy on disease progression and mortality, and to determine the long-term tolerability of combination therapy in patients with COPD.

REFERENCES

- 1 Pauwels RA, Buist AS, Calverley PMA, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) workshop summary. *Am J Respir Crit Care Med* 2001; 163:1256–1276
- 2 Saetta M, Turato G, Maestrelli P, et al. Cellular and structural bases of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 163:1304–1309
- 3 Fabbri LM, Romagnoli M, Corbetta L, et al. Differences in airway inflammation in patients with fixed airflow obstruction due to asthma or chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2003; 167:418–424
- 4 Barnes PJ. Chronic obstructive pulmonary disease. *N Engl J Med* 2000; 343:269–280
- 5 Cazzola M, Spina D, Matera MG. The use of bronchodilators in stable chronic obstructive pulmonary disease. *Pulm Pharmacol Ther* 1997; 10:129–144
- 6 Cazzola M, Donner CF. Long-acting β_2 agonists in the management of stable chronic obstructive pulmonary disease. *Drugs* 2000; 60:307–320
- 7 Dahl R, Greefhorst LAPM, Nowak D, et al. Inhaled formoterol dry powder versus ipratropium bromide in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 164:778–784
- 8 Wadbo M, Lofdahl CG, Larsson K, et al. Effects of formoterol and ipratropium bromide in COPD: a 3-month placebo-controlled study. *Eur Respir J* 2002; 20:1138–1146
- 9 Mahler DA, Donohue JF, Barbee RA, et al. Efficacy of salmeterol xinafoate in the treatment of COPD. *Chest* 1999; 115:957–965
- 10 Rennard SI, Anderson W, ZuWallack R, et al. Use of a long-acting inhaled β_2 -adrenergic agonist, salmeterol xinafoate, in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 163:1087–1092
- 11 Sifakas NM, Vermeire P, Pride NB, et al. Optimal assessment and management of chronic obstructive pulmonary disease (COPD): a consensus statement of the European Respiratory Society. *Eur Respir J* 1995; 8:1398–1420
- 12 van Noord JA, de Munck DR, Bantje TA, et al. Long-term treatment of chronic obstructive pulmonary disease with salmeterol and the additive effect of ipratropium. *Eur Respir J* 2000; 15:878–885
- 13 ZuWallack RL, Mahler DA, Reilly D, et al. Salmeterol plus theophylline combination therapy in the treatment of COPD. *Chest* 2001; 119:1661–1670
- 14 D'Urzo AD, De Salvo MC, Ramirez-Rivera A, et al. In patients with COPD, treatment with a combination of formoterol and ipratropium is more effective than a combination of salbutamol and ipratropium: a 3-week, randomized, double-blind, within-patient, multicenter study. *Chest* 2001; 119:1347–1356
- 15 Johnson M, Rennard S. Alternative mechanisms for long-acting β_2 -adrenergic agonists in COPD. *Chest* 2001; 120: 258–270
- 16 Bloeman PGM, Van den Tweek MC, Henricks PAJ, et al. Increased cAMP levels in stimulated neutrophils inhibit their adhesion to human bronchial epithelial cells. *Am J Physiol* 1997; 272:L580–L587
- 17 Eda R, Tanimoto Y, Marou H, et al. Comparison of effect of formoterol and salbutamol on human neutrophil function *in vitro*. *Allergy* 1997; 91:301
- 18 Ottonello L, Morone P, Dapino P, et al. Inhibitory effect of salmeterol on the respiratory burst of adherent human neutrophils. *Clin Exp Immunol* 1996; 106:97–102
- 19 Ward C, Li X, Wang N, et al. Salmeterol reduces BAL IL-8 levels in asthmatics on low dose inhaled corticosteroids [abstract]. *Eur Respir J* 1998; 12:380S
- 20 Dentener MA, Buurman WA, Wouters EFM. Theophylline and the β -agonists salmeterol and salbutamol potentially block neutrophil activation, as indicated by a reduced release of bacterial/permeability-increasing protein [abstract]. *Am J Respir Crit Care Med* 1998; 157:A602
- 21 Johnson M. Effects of β_2 -agonists on resident and infiltrating inflammatory cells. *J Allergy Clin Immunol* 2002; 110: S282–S290
- 22 Bowden JJ, Sulakvelidze I, McDonald DM. Inhibition of neutrophil and eosinophil adhesion to venules of rat trachea by β_2 -adrenergic agonist formoterol. *J Appl Physiol* 1994; 77:397–405
- 23 Davies A, Lefkowitz R. Agonist-promoted high affinity state of β -adrenergic receptor in human neutrophils: modulation by corticosteroids. *J Clin Endocrinol Metab* 1981; 53: 703–708

- 24 Saari SM, Vidgren MT, Herrala J, et al. Possibilities of formoterol to enhance the peripheral lung deposition of the inhaled liposome corticosteroids. *Respir Med* 2002; 96:999–1005
- 25 Thompson AB, Mueller MB, Heires AJ, et al. Aerosolized beclomethasone in chronic bronchitis: improved pulmonary function and diminished airway inflammation. *Am Rev Respir Dis* 1992; 146:389–395
- 26 Keatings VM, Jatakanon A, Worsdell YM, et al. Effects of inhaled and oral glucocorticoids on inflammatory indices in asthma and COPD. *Am J Respir Crit Care Med* 1997; 155:542–548
- 27 Culpitt SV, Maziak W, Loukidis S, et al. Effect of high dose inhaled steroid on cells, cytokines, and proteases in induced sputum in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1999; 160:1635–1639
- 28 Loppow D, Schleiss MB, Kanness F, et al. In patients with chronic bronchitis a four week trial with inhaled steroids does not attenuate airway inflammation. *Respir Med* 2001; 95:115–121
- 29 Lomas DA, Ip M, Chamba A, et al. The effect of *in vitro* and *in vivo* dexamethasone on human neutrophil function. *Agents Actions* 1991; 33:279–285
- 30 Llewellyn-Jones CG, Hill SL, Stockley RA. Effect of fluticasone propionate on neutrophil chemotaxis, superoxide generation, and extracellular proteolytic activity *in vitro*. *Thorax* 1994; 49:207–212
- 31 Llewellyn-Jones CG, Harris TA, Stockley RA. Effect of fluticasone propionate on sputum of patients with chronic bronchitis and emphysema. *Am J Respir Crit Care Med* 1996; 153:616–621
- 32 Confalonieri M, Mainardi E, Della Porta R, et al. Inhaled corticosteroids reduce neutrophilic bronchial inflammation in patients with chronic obstructive pulmonary disease. *Thorax* 1998; 53:583–585
- 33 Yildiz F, Kaur AC, Ilgazli A, et al. Inhaled corticosteroids may reduce neutrophilic inflammation in patients with stable chronic obstructive pulmonary disease. *Respiration* 2000; 67:71–76
- 34 Gizycki MJ, Hattotuwa KL, Barnes N, et al. Effects of fluticasone propionate on inflammatory cells in COPD: an ultrastructural examination of endobronchial biopsy tissue. *Thorax* 2002; 57:799–803
- 35 Hattotuwa KL, Gizycki MJ, Ansari TW, et al. The effects of inhaled fluticasone on airway inflammation in chronic obstructive pulmonary disease: a double-blind, placebo-controlled biopsy study. *Am J Respir Crit Care Med* 2002; 165:1592–1596
- 36 Balbi B, Majori M, Bertacco S, et al. Inhaled corticosteroids in stable COPD patients: do they have effects on cells and molecular mediators of airway inflammation? *Chest* 2000; 117:1633–1637
- 37 Barnes PJ. Inhaled corticosteroids are not beneficial in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000; 161:342–344
- 38 Calverley PM. Inhaled corticosteroids are beneficial in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000; 161:341–342
- 39 Chanez P, Vignola AM, O'Shaughnessy T, et al. Corticosteroid reversibility in COPD is related to features of asthma. *Am J Respir Crit Care Med* 1997; 155:1529–1534
- 40 Pizzichini E, Pizzichini MM, Gibson P, et al. Sputum eosinophilia predicts benefit from prednisone in smokers with chronic obstructive bronchitis. *Am J Respir Crit Care Med* 1998; 158:1511–1517
- 41 Nishimura K, Koyama H, Ikeda A, et al. The effect of high-dose inhaled beclomethasone dipropionate in patients with stable COPD. *Chest* 1999; 115:31–37
- 42 Pauwels RA, Löfdahl CG, Laitinen LA, et al. Long-term treatment with inhaled budesonide in persons with mild chronic obstructive pulmonary disease who continue smoking: European Respiratory Society Study on Chronic Obstructive Pulmonary Disease. *N Engl J Med* 1999; 340:1948–1953
- 43 Vestbo J, Sorensen T, Lange P, et al. Long-term effect of inhaled budesonide in mild and moderate chronic obstructive pulmonary disease: a randomized controlled trial. *Lancet* 1999; 353:1819–1823
- 44 Burge PS, Calverley PM, Jones PW, et al. Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. *BMJ* 2000; 320:1297–1303
- 45 Lung Health Study Research Group. Effect of inhaled triamcinolone on the decline in pulmonary function in chronic obstructive pulmonary disease. *N Engl J Med* 2000; 343:1902–1909
- 46 Suissa S. Effectiveness of inhaled corticosteroids in chronic obstructive pulmonary disease: immortal time bias in observational studies. *Am J Respir Crit Care Med* 2003; 168:49–53
- 47 Pauwels R. Inhaled glucocorticosteroids and chronic obstructive pulmonary disease: how full is the glass? *Am J Respir Crit Care Med* 2002; 165:1579–1580
- 48 Thompson WH, Carvalho P, Souza JP, et al. Controlled trial of inhaled fluticasone propionate in moderate to severe COPD. *Lung* 2002; 180:191–201
- 49 Paggiaro PL, Dahle R, Bakran I, et al. Multicentre randomised placebo-controlled trial of inhaled fluticasone propionate in patients with chronic obstructive pulmonary disease. *Lancet* 1998; 351:773–780
- 50 Alsaeedi A, Sin DD, McAlister FA. The effects of inhaled corticosteroids in chronic obstructive pulmonary disease: a systematic review of randomized placebo-controlled trials. *Am J Med* 2002; 113:59–65
- 51 Sin DD, Tu JV. Inhaled corticosteroids and the risk of mortality and readmission in elderly patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 164:580–584
- 52 O'Brien A, Russo-Magno P, Karki A, et al. Effects of withdrawal of inhaled steroids in men with severe irreversible airflow obstruction. *Am J Respir Crit Care Med* 2001; 164:365–371
- 53 van der Valk P, Monninkhof E, van der Palen J, et al. Effect of discontinuation of inhaled corticosteroids in patients with chronic obstructive pulmonary disease: the COPE Study. *Am J Respir Crit Care Med* 2002; 166:1358–1363
- 54 O'Brien A, Ward NS. Steroid therapy in chronic obstructive pulmonary disease. *Med Health R I* 2002; 85:52–55
- 55 Barnes PJ. Efficacy of inhaled corticosteroids in asthma. *J Allergy Clin Immunol* 1998; 102:531–538
- 56 Lipworth BJ. Airway subsensitivity with LABAs: is there cause for concern? *Drug Saf* 1997; 16:295–308
- 57 Schramm CM. β -Adrenergic relaxation of rabbit tracheal smooth muscle: a receptor deficit that improves with corticosteroid administration. *J Pharmacol Exp Ther* 2000; 292:280–287
- 58 Mak JCW, Nishikawa M, Shirasaki H, et al. Protective effects of a glucocorticoid on downregulation of pulmonary β_2 -adrenergic receptors *in vivo*. *J Clin Invest* 1995; 96:99–106

- 59 Eickelberg O, Roth M, Lox R, et al. Ligand-independent activation of the glucocorticoid receptor by β_2 -adrenergic receptor agonists in primary human lung fibroblasts and vascular smooth muscle cells. *J Biol Chem* 1999; 274:1005–1010
- 60 Adcock IM, Maneechotesuwan K, Usmani O. Molecular interactions between glucocorticoids and long-acting β_2 -agonists. *J Allergy Clin Immunol* 2002; 110(suppl):S261–S268
- 61 Ito K, Barnes PJ, Adcock IM. Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits IL-1 β -induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol* 2000; 20:6891–6903
- 62 Ito K, Jazwari E, Cosio B, et al. p65-activated histone acetyltransferase activity is repressed by glucocorticoids: mifepristone fails to recruit HDAC2 to the p65/HAT complex. *J Biol Chem* 2001; 276:30208–30215
- 63 Pang L, Knox AJ. Synergistic inhibition by β_2 -agonists and corticosteroids on tumor necrosis factor α induced interleukin-8 release from cultured human airway smooth muscle cells. *Am J Respir Cell Mol Biol* 2000; 23:79–85
- 64 Roth M, Johnson PR, Rudiger JJ, et al. Interaction between glucocorticoids and β_2 agonists on bronchial airway smooth muscle cells through synchronised cellular signalling. *Lancet* 2002; 360:1293–1299
- 65 Woolcock A, Lundback B, Ringdahl N, et al. Comparison of addition of salmeterol to inhaled steroids with doubling of the dose of inhaled steroids. *Am J Respir Crit Care Med* 1996; 153:1481–1488
- 66 Pauwels RA, Lofdahl CG, Postma DS, et al. Effect of inhaled formoterol and budesonide on exacerbations of asthma. *N Engl J Med* 1997; 337:1405–1411
- 67 Jonat C, Rahmsdorf HJ, Park KK, et al. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. *Cell* 1990; 62:1189–1204
- 68 Heck S, Bender K, Kullmann M, et al. I κ B α -independent down regulation of NF- κ B activity by glucocorticoid receptor. *EMBO J* 1997; 16:4698–4707
- 69 Sheppard KA, Phelps KM, Williams AJ, et al. Nuclear integration of glucocorticoid receptor and nuclear factor- κ B signaling by CREB-binding protein and steroid receptor coactivator-1. *J Biol Chem* 1998; 273:29291–29294
- 70 Strahle U, Schmid W, Schutz G. Synergistic action of the glucocorticoid receptor with transcription factors. *EMBO J* 1988; 7:3389–3395
- 71 Zhang Z, Jones S, Hagood JS, et al. STAT3 as a co-activator of glucocorticoid receptor signaling. *J Biol Chem* 1997; 272:30607–30610
- 72 Stocklin E, Wissler M, Gouilleux F, et al. Functional interactions between Stat5 and the glucocorticoid receptor. *Nature* 1996; 383:726–728
- 73 Seeto LJ, Burgess JK, Johnson PR, et al. Effect of fluticasone and salmeterol on human alveolar macrophage cytokine production in patients with chronic obstructive pulmonary disease (COPD) [abstract]. *Am J Respir Crit Care Med* 2003; 167:A318
- 74 Korn SH, Jerre A, Brattsand R. Effects of formoterol and budesonide on GM-CSF and IL-8 secretion by triggered human bronchial epithelial cells. *Eur Respir J* 2001; 17:1070–1077
- 75 Farmer P, Pugin J. β -adrenergic agonists exert their anti-inflammatory effects in monocytic cells through the I κ B/NF- κ B pathway. *Am J Physiol* 2000; 279:L675–L682
- 76 Ollivier V, Parry GCN, Cobb RR, et al. Elevated cyclic AMP inhibits NF- κ B-mediated transcription in human monocytic cells and endothelial cells. *J Biol Chem* 1996; 271:20828–20835
- 77 Bodor J, Habener JF. Role of transcriptional repressor ICER in cyclic AMP-mediated attenuation of cytokine gene expression in human thymocytes. *J Biol Chem* 1998; 273:9544–9551
- 78 Dowling RB, Johnson M, Cole PJ, et al. Effect of fluticasone propionate and salmeterol on *Pseudomonas aeruginosa* infection of the respiratory mucosa *in vitro*. *Eur Respir J* 1999; 14:363–369
- 79 Tsang KW, Rutman A, Tanaka E, et al. Interaction of *Pseudomonas aeruginosa* with human respiratory mucosa *in vitro*. *Eur Respir J* 1994; 7:1746–1753
- 80 Wilson R. Infections of the airways. *Curr Opin Infect Dis* 1991; 4:166–177
- 81 Edwards MR, Johnson M, Johnston SL. Synergistic effects of salmeterol and fluticasone in human rhinovirus induced pro-inflammatory cytokine production [abstract]. *Am J Respir Crit Care Med* 2003; 167:A399
- 82 Seemungal T, Harper-Owen R, Bhowmik A, et al. Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001; 164:1618–1623
- 83 European Committee for Proprietary Medicinal Products. Press release: European Agency for the Evaluation of Medicinal Products; Committee for Proprietary Medicinal Products meeting of 21 to 23 January 2003. Available at: <http://www.emea.eu.int/pdfs/human/press/pr/029403en.pdf>. Accessed June 14, 2004
- 84 Cazzola M, Santus P, Di Marco F, et al. Onset of action of budesonide/formoterol in single inhaler vs. formoterol in patients with COPD [abstract]. *Am J Respir Crit Care Med* 2003; 167:A318
- 85 Mak JC, Nishikawa M, Barnes PJ. Glucocorticosteroids increase β_2 -adrenergic receptor transcription in human lung. *Am J Physiol* 1995; 12:L41–L46
- 86 Borski RJ. Nongenomic membrane actions of glucocorticoids in vertebrates. *Trends Endocrinol Metab* 2000; 11:427–436
- 87 Cazzola M, Di Lorenzo G, Di Perna F, et al. Additive effects of salmeterol and fluticasone or theophylline in COPD. *Chest* 2000; 118:1576–1581
- 88 Mahler DA, Wire P, Horstman D, et al. Effectiveness of fluticasone propionate and salmeterol combination delivered via the Diskus device in the treatment of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2002; 166:1084–1091
- 89 Calverley P, Pauwels R, Vestbo J, et al. Combined salmeterol and fluticasone in the treatment of chronic obstructive pulmonary disease: a randomised controlled trial. *Lancet* 2003; 361:449–456
- 90 Dal Negro RW, Pomari C, Tognella S, et al. Salmeterol & fluticasone 50 μ g/250 μ g bid in combination provides a better long-term control than salmeterol 50 μ g bid alone and placebo in COPD patients already treated with theophylline. *Pulm Pharmacol Ther* 2003; 16:241–246
- 91 Szafarski W, Cukier A, Ramirez A, et al. Efficacy and safety of budesonide/formoterol in the management of chronic obstructive pulmonary disease. *Eur Respir J* 2003; 21:74–81
- 92 Calverley PMA, Olsson H. Budesonide/formoterol in a single inhaler sustains improvements in lung function over 12 months compared with monocomponents and placebo in patients with COPD [abstract]. *Am J Respir Crit Care Med* 2003; 167:A319
- 93 Jones PW, Ståhl E. Budesonide/formoterol in a single inhaler improves health status in patients with COPD [abstract]. *Am J Respir Crit Care Med* 2003; 167:A320

- 94 Calverley PMA, Peterson S. Combining budesonide/formoterol in a single inhaler reduces exacerbation frequency in COPD [abstract]. *Am J Respir Crit Care Med* 2003; 167:A948
- 95 Cazzola M, Santus P, di Marco F, et al. Combination therapy with salmeterol + fluticasone and formoterol + budesonide in patients with COPD. *Respir Med* 2003; 97:453–457
- 96 Jones A, Fay JK, Burr M, et al. Inhaled corticosteroid effects on bone metabolism in asthma and mild chronic obstructive pulmonary disease. *Cochrane Database Syst Rev* 2002; 1:CD003537
- 97 Aziz I, McFarlane LC, Lipworth BJ. Concomitant inhaled corticosteroid resensitises cardiac β_2 -adrenoceptors in the presence of long-acting β_2 -agonist therapy. *Eur J Clin Pharmacol* 1998; 54:377–381
- 98 Centanni S, Carlucci P, Santus P, et al. Non-pulmonary effects induced by the addition of formoterol to budesonide therapy in patients with mild or moderate persistent asthma. *Respiration* 2000; 67:60–64
- 99 Hattotuwa K, Gamble EA, O'Shaughnessy T, et al. Safety of bronchoscopy, biopsy, and BAL in research patients with COPD. *Chest* 2002; 122:1909–1912
- 100 Rytala PH, Laitinen A, Lindqvist A, et al. Comparison between bronchial biopsy and induced sputum in patients with chronic bronchitis and chronic obstructive pulmonary disease [abstract]. *Am J Respir Crit Care Med* 1999; 159:A515
- 101 COPD Guidelines Group of the Standards of Care Committee of the BTS. BTS guidelines for the management of chronic obstructive pulmonary disease. *Thorax* 1997; 52(suppl):S1–S28
- 102 van den Boom G, Rutten-van Molken MP, Molema J, et al. The cost effectiveness of early treatment with fluticasone propionate 250 μ g twice a day in subjects with obstructive airway disease: results of the DIMCA program. *Am J Respir Crit Care Med* 2001; 164:2057–2066
- 103 Jones PW, Wilson K, Sondhi S. Cost-effectiveness of salmeterol in patients with chronic obstructive pulmonary disease: an economic evaluation. *Respir Med* 2003; 97: 20–26
- 104 Rutten-Van Molken MP, Van Doorslaer EK, Jansen MC, et al. Cost and effects of inhaled corticosteroids and bronchodilators in asthma and chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1995; 151:975–982
- 105 Löfdahl C-G, Andreasson E, Svensson K, et al. Budesonide/formoterol in a single inhaler improves overall health status in patients with COPD without increasing healthcare costs [abstract]. *Am J Respir Crit Care Med* 2003; 167:A89
- 106 Hackam DG. Treatment of chronic obstructive pulmonary disease: combination or component therapy? *Can Med Assoc J* 2003; 168:1296–1297