Evaluation of Salmeterol or Montelukast as Second-Line Therapy for Asthma Not Controlled With Inhaled Corticosteroids*

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Objective: To assess the addition of a leukotriene receptor antagonist and a long-acting β₂-agonist as second-line therapy in asthma.

Design: Placebo-controlled, double-dummy, crossover study.

Setting: Outpatient clinic.

Patients: Twenty patients with persistent asthma not controlled with inhaled corticosteroid therapy.

Interventions: Montelukast, 10 mg once daily, or salmeterol, 50 µg bid, each for 2 weeks with 1-week run-in and washout placebo periods.

Measurements and results: Adenosine monophosphate (AMP) bronchial challenge, blood eosinophil count (EOS), exhaled nitric oxide, and lung function after both placebo periods and after the first and last doses of each active treatment. Patients recorded their domiciliary peak expiratory flow (PEF), asthma symptoms, and rescue bronchodilator requirement (RES) twice daily throughout the study. For the primary end point of the provocative concentration of AMP causing a 20% fall in FEV₁, compared to placebo (47.5 ± 13.0 mg/mL), there were significant differences with the first (114.1 ± 36.9 mg/mL) and last (94.2 ± 30.4 mg/mL) doses of montelukast as well as the first (160.1 ± 64.5 mg/mL) but not the last (70.1 ± 23.7 mg/mL) dose of salmeterol. Only montelukast produced significant suppression of the EOS. Neither drug affected exhaled nitric oxide levels. There were significant improvements with the first doses of salmeterol for all parameters of lung function. After 2 weeks of treatment, there were significant improvements with both drugs for RES and morning PEF. There were no significant differences between drugs for any end points except EOS.

Conclusions: Montelukast and salmeterol exhibited significant improvements in asthma control when given as second-line therapy. Montelukast also produced significant effects on AMP challenge and EOS suggesting anti-inflammatory activity.

Key words: adenosine monophosphate; inhaled corticosteroids; montelukast; nitric oxide; salbutamol; symptoms

Abbreviations: AMP = adenosine monophosphate; CI = confidence interval; EOS = blood eosinophil count; FEF₂₅–₇₅% = forced expiratory flow, midexpiratory phase; PC₂₀ = provocation concentration of AMP causing a 20% fall in FEV₁; PEF = peak expiratory flow; ppb = parts per billion; RES = rescue bronchodilator requirement; sGAW = specific airways conductance

Asthma is a chronic inflammatory condition of the small airways, with inhaled corticosteroids being the first-line therapy.¹ However, many patients do not have adequate symptom control while receiving low-dose inhaled corticosteroid treatment and additional second-line therapy is necessary.

Combining a long-acting β₂-agonist with low-dose inhaled corticosteroid has been shown to provide equivalent or superior asthma control than using higher doses of inhaled corticosteroid alone,² as is reflected in current asthma management guidelines. However, as long-acting β₂-agonists are bronchodilators and act mainly at the bottom of the inflammatory cascade, there are concerns that they may mask underlying inflammation.³ Also, when given on a regular basis, long-acting β₂-agonists have been shown to exhibit tolerance to their bronchoprotect-
tive and bronchodilator effects as a result of β2-adrenoceptor downregulation.4–7

Leukotriene receptor antagonists are a novel class of therapy available in the management of asthma. They have both anti-inflammatory and bronchodilator activity, although not as great as that of inhaled corticosteroids or long-acting β2-agonists, respectively.8 They have been shown to have additive clinical effects in patients with severe asthma during tapering step-down with inhaled corticosteroid therapy.9,10 In a multicenter trial11 of patients with persistent asthma, 80% of whom were receiving concomitant inhaled corticosteroids, treatment with salmeterol produced significantly greater improvements than zafirlukast in overall asthma control as assessed by peak flow, asthma symptoms, and albuterol use. This study, however, did not evaluate the effects on bronchial hyperresponsiveness or airway inflammation.

We have previously documented that single doses of montelukast and salmeterol are bronchoprotective against adenosine monophosphate (AMP) bronchial challenge.12 We therefore decided to evaluate the long-term dosing effects of salmeterol and montelukast as second-line treatment in patients whose asthma was not adequately controlled with inhaled corticosteroid as monotherapy. The primary end point was to assess the effects on AMP bronchial challenge, which causes bronchoconstriction indirectly by release of inflammatory mediators from primed mast cells.13 AMP bronchial challenge has been shown to be more sensitive in detecting anti-inflammatory effects than a direct bronchial challenge, such as methacholine, and is probably more clinically relevant.14 We also assessed subgroup inflammatory markers, including exhaled nitric oxide and blood eosinophil count (EOS),15,16 as well as daily symptom control, rescue bronchodilator requirements (RESs), peak expiratory flow (PEF), and lung function.

**Materials and Methods**

**Patients**

Twenty patients with moderate persistent asthma (11 male patients; mean [SE] age, 32.5 [2.2] years; FEV1, 79.1 [3.9] L/min; forced expiratory flow, midexpiratory phase [FEF25–75%], 51.5% [4.5%]) predicted were recruited into the study. All patients had asthma according to defined criteria17 and were required to have their conditions suboptimally controlled despite receiving > 400 μg/d of inhaled corticosteroids as monotherapy (median dose, 800 μg/d; interquartile range, 400 to 1000 μg/d); budesonide (n = 5), beclomethasone (n = 14), fluticasone (n = 1). Patients were eligible for inclusion if they had persistent asthma symptoms (day or night), required at least two puffs per day of reliever therapy with their usual short-acting β2-agonist, and had at least 10% diurnal variability between their morning and evening PEF rates. All patients were required to be responsive to AMP challenge testing with a provocation concentration of AMP causing 20% fall in FEV1 (PC20) of < 200 mg/mL (geometric mean, 32.9 ± 9.5 mg/mL) before the run-in period. Approval for the study was obtained from the Tayside Medical Ethics Committee, and all patients gave their written informed consent.

**Methods**

The study was of a randomized, placebo-controlled, single-blind, double-dummy, crossover design. Unfortunately, we were unable to obtain identical placebo tablets; therefore, the study was of a single-blind design. Patients continued with their usual maintenance dose of inhaled corticosteroid treatment throughout the study. In addition, patients were randomized to receive (1) inhaled salmeterol 50 μg twice daily, plus placebo tablet once daily, or (2) oral montelukast, 10 mg once daily, plus placebo inhaler twice daily. Each active-treatment phase was for a duration of 2 weeks. Before each treatment and at crossover, patients had a 1-week treatment period with placebo inhaler (one inhalation twice daily) and placebo tablets once daily while continuing with their inhaled corticosteroids. All tablets were taken at 8 AM, and inhaled medications were taken at 8 AM and 8 PM. Two puffs of inhaled ipratropium bromide, 40 μg per puff, were used as required for symptomatic relief purposes as first-line rescue, with inhaled salbutamol as second-line rescue.

The inhalers and tablets were masked and sealed in envelopes by a pharmacist along with instruction sheets at the beginning of the trial. Before the study and at each visit, subjects were given detailed instruction by a third party in how to use their inhalers. Each subject received a written instruction sheet to follow while taking his or her medication at home, based on the recommended advice of the manufacturers, and a simple tick chart was used as an aide to compliance. Data from patients with > 90% compliance over the study were considered to be evaluable.

**Measurements**

All laboratory measurements were performed at 8 AM. Patients attended at the end of the 1-week run-in and crossover washout placebo periods, and after each 2-week active-treatment period. Patients also attended after the first dose of active therapy, ie, 12 h after the first dose of inhaled salmeterol and 24 h after the first dose of oral montelukast.

AMP challenge testing was performed as previously described,18 between 8 AM and 10 AM, after patients had withheld treatment with their reliever medication for 12 h. In brief, AMP that had been prepared fresh daily was administered in doubling cumulative doses given at 5-min intervals until a fall in FEV1 ≥ 20% was recorded. The PC20 was calculated using a computer-assisted curve-fitting package (Biolab Assistant 1.1; University of Dundee; Dundee, Scotland, UK) and interpolation of the steep part of the log dose-response curve. A value of 1,600 mg/mL was assigned if the FEV1 did not fall below 20% of the baseline value.

Patients underwent measurement of exhaled nitric oxide using an integrated, clinical, real-time, nitric oxide gas analyzer (model LR2000; Logan Research; Rochester, UK) using the procedures described by Kharitonov et al.19 Three measures of nitric oxide were taken, and the results were analyzed as the mean of the three values. Spirometry was performed using a Vitalograph compact spirometer (Vitalograph Ltd; Buckinghamshire, UK).

Airways resistance was measured in a constant-volume, pressure-compensated, whole-body plethysmograph (PK Morgan; Gillingham, Kent, UK). Patients also provided a blood sample for measurement of EOS at 8 AM on arrival to the laboratory on each occasion. EOS was measured using an SE-9000 Hematology Analyzer (Sysmex UK Ltd; Bucks, UK).
Throughout the study, at 8 AM and 10 PM, patients recorded the highest of three measurements of PEF using a Mini-Wright peak flowmeter (Clement Clarke; Essex, UK), with their symptoms of asthma according to a 4-point scale with zero indicating no symptoms and 3 indicating severe symptoms, and their requirement for rescue bronchodilator therapy.

Statistical Analysis

The study was designed with at least 80% power to detect a 1.0 doubling-dose difference (twofold) in PC20 (the primary end point) for active treatment vs placebo, with the α error set at 0.05 (two tailed). The data for PC20 were log transformed in order to normalize their distribution before analysis. All PC20 data are therefore presented as geometric mean with the geometric SEM in parenthesis. For all domiciliary diary data, mean values for the 7-day run-in and washout placebo periods and for the 2 weeks of each active-treatment period were analyzed. Overall comparisons for active treatments vs placebo treatments were made by multifactorial analysis of variance using subject, treatment, period, and duration of treatment (first dose/last dose) as factors. This was followed by Bonferroni multiple-range testing (set at 95% confidence interval [CI]) in order to obviate multiple pairwise comparisons. Consequently, comparisons are only denoted as being significant (p < 0.05) or not significant in order to not confound the α error. The analysis was performed using a statistical software package (Statgraphics; STSC Software Publishing Group; Rockville, MD).

RESULTS

There were no significant carryover effects between the first and second placebo values in sequence with any of the measurements (Table 1). Consequently, a pooled placebo value was used for the purposes of analysis.

AMP Challenge, EOS, and Exhaled Nitric Oxide

For PC20, compared to placebo (47.5 ± 13.0 mg/mL), there were significant (p < 0.05) differences with first (114.1 ± 36.9 mg/mL) and last (94.2 ± 30.4 mg/mL) doses of montelukast as well as the first (160.1 ± 64.5 mg/mL) but not the last (70.1 ± 23.7 mg/mL) dose of salmeterol (Fig 1). Salmeterol, but not montelukast, was shown to exhibit significant (p < 0.05) tolerance between the first-dose and last-dose protection against AMP bronchial challenge. This amounted to 1.19 doubling doses (95% CI, 0.60 to 1.78) for salmeterol and 0.28 doubling doses (95% CI, 0.60 to 0.85) for montelukast. For EOS after 2 weeks, there was a significant (p < 0.05) difference between montelukast vs placebo, and montelukast vs salmeterol (placebo, 0.40 [0.06] × 10⁹/L; montelukast, 0.31 [0.04] × 10⁹/L; salmeterol, 0.44 [0.07] × 10⁹/L). There was no significant difference between either of the treatments and placebo in terms of exhaled nitric oxide after 2 weeks of treatment (placebo, 10.5 [1.3] parts per billion [ppb]; montelukast, 10.2 [1.5] ppb; salmeterol, 9.3 [1.6] ppb).

For FEV₁, FEF25–75%, and specific airways conductance (sGaw), there was a significant (p < 0.05) difference between placebo and the first, but not the last, dose of salmeterol. For montelukast, there was no significant improvement except with the first dose in terms of FEF25–75% (Fig 2).

Salmeterol showed significant (p < 0.05) improvements in terms of daytime and nighttime asthma symptom scoring and RES as well as morning PEF rate (Table 2). Montelukast showed significant (p < 0.05) improvement in terms of nocturnal and daytime RES and morning PEF rate (Fig 3). There were no significant differences between the two drugs for any end point apart from effects on EOS.

Table 1—Data for First (Run-in) and Second (Crossover) Placebo Washout Periods

<table>
<thead>
<tr>
<th>Variables</th>
<th>First Placebo</th>
<th>Second Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC20, mg/mL</td>
<td>44.6 (12.4)</td>
<td>50.7 (15.8)</td>
</tr>
<tr>
<td>NO, ppb</td>
<td>10.3 (1.9)</td>
<td>10.8 (1.6)</td>
</tr>
<tr>
<td>EOS, × 10⁹</td>
<td>0.42 (0.06)</td>
<td>0.39 (0.06)</td>
</tr>
<tr>
<td>sGaw, % predicted</td>
<td>50.8 (6.9)</td>
<td>53.7 (7.6)</td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>75.0 (3.4)</td>
<td>74.5 (3.4)</td>
</tr>
<tr>
<td>FEF25–75%, % predicted</td>
<td>45.4 (3.6)</td>
<td>45.5 (3.6)</td>
</tr>
<tr>
<td>PEFAM, L/min</td>
<td>423.1 (17.5)</td>
<td>419.4 (15.5)</td>
</tr>
<tr>
<td>PEFPM, L/min</td>
<td>470.9 (18.3)</td>
<td>457.5 (17.4)</td>
</tr>
<tr>
<td>RESAM, puffs/12 h</td>
<td>2.5 (0.4)</td>
<td>2.4 (0.4)</td>
</tr>
<tr>
<td>RESPm, puffs/12 h</td>
<td>1.2 (0.3)</td>
<td>1.1 (0.3)</td>
</tr>
<tr>
<td>SYMAM, U/12 h</td>
<td>0.7 (0.1)</td>
<td>0.9 (0.2)</td>
</tr>
<tr>
<td>SYMPM, U/12 h</td>
<td>0.6 (0.1)</td>
<td>0.6 (0.1)</td>
</tr>
</tbody>
</table>

*Data are presented as mean (SEM). NO = exhaled nitric oxide; PEFAM = morning PEF; PEFPM = evening PEF; RESAM = daytime RES; RESPm = nighttime RES; SYMAM = daytime symptom score; SYMPm = nighttime symptom score. There were no significant differences for any points.
Discussion

This is the first study (to our knowledge) to evaluate the long-term dosing effects of second-line therapy with a long-acting β₂-agonist or a leukotriene receptor antagonist, in patients with asthma not adequately controlled with inhaled corticosteroid treatment, in terms of AMP bronchial challenge. We have shown a significant bronchoprotective effect vs placebo after 2 weeks with the addition of once-daily montelukast treatment but not with twice-daily salmeterol treatment. This is in keeping with two studies in which after 4 weeks of treatment, protection against exercise-induced bronchoconstriction was significantly greater with montelukast than salmeterol.20,21 Regarding diurnal asthma control, we found salmeterol to have a greater or equal effect to that of montelukast, which is in agreement with another study11 comparing salmeterol and zafirlukast. We have also shown salmeterol to be a better bronchodilator than montelukast in terms of improvements in lung function after the first dose compared with placebo, although there was no significant difference for either drug after 2 weeks of therapy compared to placebo.

In the present study, there was no loss of beneficial effects in diurnal asthma control over the 2-week period (Fig 3), in contrast to marked loss in bronchoprotection against bronchial challenge (Fig 1). This apparent discrepancy between the comparative effects on bronchial challenge or lung function and diurnal asthma control may be explained by their relative degrees of tolerance, although proper evaluation of bronchodilator tolerance would have required assessment of a salbutamol dose-response curve. Tolerance with long-acting β₂-agonists is recognized to be more pronounced with their bronchoprotective than bronchodilator effects.22

Table 2—Domiciliary Diary Card Data After 1 Week of Placebo Treatment (Pooled) and After 2 Weeks of Treatment With Montelukast and Salmeterol

<table>
<thead>
<tr>
<th>Variables</th>
<th>Placebo</th>
<th>Montelukast</th>
<th>Salmeterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>PEFAM, L/min</td>
<td>426.3 (17.2)</td>
<td>433.5† (16.6)</td>
<td>450.0† (20.3)</td>
</tr>
<tr>
<td>PEFPM, L/min</td>
<td>469.5 (18.5)</td>
<td>466.3 (17.8)</td>
<td>480.4 (19.9)</td>
</tr>
<tr>
<td>RESAM, puffs/12 h</td>
<td>2.41 (0.37)</td>
<td>1.54† (0.31)</td>
<td>1.42† (0.40)</td>
</tr>
<tr>
<td>RESPM, puffs/12 h</td>
<td>1.14 (0.30)</td>
<td>0.76† (0.21)</td>
<td>0.64† (0.20)</td>
</tr>
<tr>
<td>SYMAM, U/12 h</td>
<td>0.81 (0.12)</td>
<td>0.61 (0.13)</td>
<td>0.47† (0.11)</td>
</tr>
<tr>
<td>SYMPM, U/12 h</td>
<td>0.58 (0.11)</td>
<td>0.46 (0.10)</td>
<td>0.41† (0.11)</td>
</tr>
</tbody>
</table>

*Data are presented as mean (SEM). See Table 1 for abbreviations.
†Significant difference between placebo and active treatments.
Lipworth\textsuperscript{5} showed in a study comparing the effects of salmeterol, 50 \( \mu \)g bid, or placebo in patients receiving inhaled corticosteroids, that although there were improvements in PEF and reductions in rescue bronchodilator therapy, there was only a 0.7-doubling-dose residual protection against histamine challenge after 4 weeks. In a study with regular formoterol, 24 \( \mu \)g bid for 2 weeks, in addition to inhaled corticosteroid therapy, there was 0.5-doubling-dose protection against methacholine after 2 weeks with sustained improvement in peak flows.\textsuperscript{4} Similarly, with AMP bronchial challenge, there was an 0.8-fold protection after 1 week of regular formoterol, 24 \( \mu \)g bid.\textsuperscript{7} The mechanism for this tolerance is thought to occur as a result of \( \beta_2 \)-adrenoceptor uncoupling from the stimulatory G protein, internalization of surface receptors, and reduced transcription of receptor messenger RNA.\textsuperscript{23,24}

Our results are consistent with previous studies\textsuperscript{4,5} with salmeterol and formoterol, where there was a residual degree of trough bronchoprotection with AMP and methacholine between 0.5 doubling doses and 0.8 doubling doses. We deliberately elected to measure trough measurements with both drugs at the end of their usual dosing interval, as this reflects the period when the airways are most susceptible to bronchoconstrictor stimuli.

Leukotriene receptor antagonists have been shown to have bronchoprotective properties with allergen, exercise, and other challenges,\textsuperscript{8} although there are no previous data regarding their effects on AMP bronchial challenge that is a useful marker of inflammation.\textsuperscript{13} The bronchoprotective properties of long-acting \( \beta_2 \)-agonists on AMP bronchial challenge are partly because of functional antagonism of smooth muscle \( \beta_2 \)-adrenoceptors, although there may be a component because of direct inhibition of the mast cell \( \beta_2 \)-adrenoceptors.\textsuperscript{25}

There are conflicting data on the effects of leukotriene receptor antagonist, administered as mono-therapy, on bronchial hyperreactivity. In a dose-response study for 12 weeks, montelukast had no significant effect on methacholine challenge, compared to placebo.\textsuperscript{26} In a randomized, double-blind, crossover study, zafirlukast, 20 mg bid, and fluticasone propionate, 100 \( \mu \)g bid, for 2 weeks exhibited 1.7-fold and 2.8-fold protection, respectively, against histamine challenge.\textsuperscript{27} In a subgroup analysis of a randomized crossover trial, there was 2.4-fold protection against methacholine hyperreactivity after 2 weeks of zafirlukast, 20 mg bid, compared to placebo.\textsuperscript{28} Two studies with pranlukast have also shown protection against methacholine challenge, which in one study was accompanied by biopsy specimen changes of reduced airway inflammatory cells.\textsuperscript{29,30}

It is possible that the relatively small effect observed in the present study with montelukast may have been because of the relatively short duration of treatment and that it would take longer to achieve a maximal response. It should also be pointed out that underlying bronchial hyperreactivity would have already been blunted by the preexisting inhaled corticosteroid therapy. The importance of assessing bronchial hyperreactivity is reinforced by a study from Sont et al.,\textsuperscript{31} in which it was found that altering steroid therapy according to bronchial hyperreactivity in addition to symptom control and lung function resulted in better asthma control and a reduction in airway inflammation on bronchial biopsy specimens. This, in turn, suggests that using two drugs that have additive effects on airway inflammation (ie, inhaled corticosteroids and leukotriene receptor antagonists) may have beneficial effects on asthma disease activity. This is supported by two separate studies,\textsuperscript{9,10} in which adding-in montelukast facilitated a lower maintenance dose of inhaled corticosteroids during tapered step-down.

We also assessed asthmatic inflammation by measuring effects on peripheral EOSs and exhaled nitric oxide. The blood eosinophil concentration is considered to be a surrogate marker of asthmatic disease activity,\textsuperscript{16} with an increase in number because of recruitment from the bone marrow during asthmatic inflammation.\textsuperscript{32} We found significant reduction in blood eosinophil numbers with montelukast but not with salmeterol, which is in keeping with previous studies.\textsuperscript{33,34} It may be unfair, however, to compare the effects of a topical drug such as salmeterol and a systemic drug such as montelukast on a systemic marker of disease activity. In this respect, recent data\textsuperscript{35} have shown that treatment with regular formoterol as monotherapy induced significant reductions in eosinophil numbers in bronchial biopsy specimens. Exhaled nitric oxide is a sensitive marker of airway inflammation, and it therefore may be surprising to find no difference with either treatment when compared to placebo. However, recent data\textsuperscript{36} have shown that the dose-response curve for exhaled nitric oxide becomes flat after 400 \( \mu \)g/d of inhaled budesonide. As all of our patients were receiving inhaled corticosteroids at a dose > 400 \( \mu \)g/d before the run-in period, exhaled nitric oxide levels would not be expected to significantly change with the addition of second-line treatment.

In conclusion, although we have shown that salmeterol and montelukast are both useful adjunctive therapies for asthma not adequately controlled with inhaled corticosteroids, the addition of montelukast may confer anti-inflammatory effects. Longer-term studies are required to evaluate the effects of salmeterol and montelukast on asthma exacerbation rates when administered as second-line therapy in addition to inhaled corticosteroids.
REFERENCES

1 Lipworth BJ. Modern drug treatment of chronic asthma. BMJ 1999; 318:380–384
5 Grove A, Lipworth BJ. Bronchodilator subsensitivity to inhaled salbutamol after twice daily salmeterol in asthmatic patients. Lancet 1995; 346:201–216
8 Lipworth BJ. Leukotriene-receptor antagonists. Lancet 1999; 353:57–62
15 Dinh-Xuan AT, Texereau J. Measuring exhaled nitric oxide: not only a matter of how - but also why - should we do it? Eur Respir J 1998; 12:1005–1007
17 American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. Am Rev Respir Dis 1987; 136:225–244
18 Tan KS, McFarlane LC, Lipworth BJ. Loss of normal cyclical β₂-adrenoceptor regulation and increased premenstrual responsiveness to adenosine monophosphate in stable female asthmatics. Thorax 1997; 52:608–611
22 Lipworth BJ. Airway subsensitivity with long-acting β₂-agonists: is there cause for concern? Drug Safety 1997; 16:295–308
25 Nightingale JA, Rogers DF, Barnes PJ. Differential effects of formoterol on adenosine monophosphate and histamine reactivity in asthma. Am J Respir Crit Care Med 1999; 159:1786–1790
27 Westbrock J, Psma HR. The effect of inhaled fluticasone propionate (FP) 100 μg bd compared with oral zafirlukast 20 mg bd on bronchial hyperresponsiveness in mild to moderate asthmatics [abstract]. Eur Respir J 1997; 16(suppl 25):P1554