Effects of Once-Daily Formoterol and Budesonide Given Alone or in Combination on Surrogate Inflammatory Markers in Asthmatic Adults*

Imran Aziz, MBChB; Andrew M. Wilson, MBChB; and Brian J. Lipworth, MD

Objectives: We wished to evaluate the effects of once-daily combination therapy on surrogate inflammatory markers.

Methods: Fifteen patients with atopic persistent asthma were evaluated (mean age, 32.4 years; FEV₁, 75.2% predicted) in a randomized, double-blind, double-dummy, placebo-controlled crossover study with a 1-week placebo washout period, comparing the following once-daily nighttime treatments: (1) formoterol (FM), 12 μg, for 2 weeks and FM, 24 μg, for 2 weeks; or (2) budesonide (BUD), 400 μg, for 2 weeks and BUD, 800 μg, for 2 weeks; or (3) FM, 12 μg, plus BUD, 400 μg, for 2 weeks and FM, 24 μg, plus BUD, 800 μg, for 2 weeks. Adenosine monophosphate (AMP) bronchial challenge, exhaled nitric oxide (NO), and serum eosinophilic cationic protein (ECP) were evaluated at 12 h postdosing after administration of each placebo and after 2 and 4 weeks of each treatment.

Results: The results of AMP challenge (provocative concentration causing a 20% fall in FEV₁) at 4 weeks showed significant (p < 0.05) improvements after patients had received all active treatments compared to placebo (20 mg/mL), with FM plus BUD, 261 mg/mL, being superior (p < 0.05) to FM alone, 82 mg/mL, but not to BUD, 201 mg/mL. NO and ECP showed significant (p < 0.05) reductions compared to placebo with FM plus BUD or BUD alone but not with FM alone. Combination therapy was associated with optimal patient preference (rank order, FM plus BUD > FM > BUD; p < 0.0005), highest domiciliary peak expiratory flow, and lowest rescue inhaler usage. All three treatments produced equivalent improvements in spirometry.

Conclusions: Patients preferred once-daily combination therapy, but this had no greater effect on inflammatory markers than therapy with BUD alone. FM alone had no anti-inflammatory activity but exhibited bronchoprotection. This emphasizes the importance of first optimizing anti-inflammatory control with inhaled corticosteroids before considering adding a regular long-acting β₂-agonist.

Key words: adenosine monophosphate; bronchial hyperresponsiveness; budesonide; exhaled nitric oxide; formoterol; inflammation

Abbreviations: AMP = adenosine monophosphate; BUD = budesonide; CI = confidence interval; ECP = eosinophilic cationic protein; FEF₂₅₋₇₅ = midexpiratory phase of forced expiratory flow; FM = formoterol; NO = nitric oxide; PC₂₀ = provocative concentration causing a 20% fall in FEV₁

Long-acting β₂-agonists such as salmeterol and formoterol (FM) are used on a regular twice-daily basis as a second-line controller therapy in addition to twice-daily administration of inhaled corticosteroids in order to improve symptomatic control of asthma. The use of regular therapy with FM twice daily has been shown to improve diurnal asthma control and to decrease exacerbation rates when given in addition to inhaled budesonide (BUD). Similarly, the addition of salmeterol therapy...
twice daily to inhaled beclomethasone has been shown to be as effective as doubling the dose of inhaled beclomethasone.2,3 Consequently, asthma management guidelines have recommended an earlier introduction of long-acting β₂-agonists as additional therapy to low-dose inhaled corticosteroids instead of monotherapy with high-dose corticosteroids.4,5 There are, however, emerging concerns that the regular use of long-acting β₂-agonists may potentially mask an increase in the underlying inflammatory process in patients who have been able to step down to a lower maintenance dose of inhaled corticosteroids.6

BUD and FM are currently licensed for regular once-daily evening administration in patients with mild-to-moderate persistent asthma. Therefore, we decided to evaluate the effects of once-daily low and high doses of FM and BUD given alone or in combination on lung function and surrogate inflammatory markers, the latter assessed by adenosine monophosphate (AMP) challenge, exhaled nitric oxide (NO) levels, and eosinophilic cationic protein (ECP).7–9 At the same time, we wished to evaluate whether the patient’s preference for monotherapy or combination therapy was mirrored by the effects of those therapies on inflammatory markers or lung function. We selected patients with stable mild-to-moderate asthma who were already receiving regular inhaled corticosteroids in line with the accepted recommendations on the additive use of long-acting β₂-agonists.4,5

**Materials and Methods**

**Patients**

Fifteen atopic asthmatic patients in stable condition (8 men and 7 women; mean [± SE] age, 32.4 ± 3 years) who were all taking inhaled corticosteroids (mean dose, 473 ± 57 µg/d) were randomized (Table 1). The asthma patients had been in stable condition, according to the American Thoracic Society10 criteria, for at least 3 months, and none of them had used oral corticosteroids or antibiotics during this period. The subjects had mild-to-moderate persistent asthma and were using inhaled corticosteroids (beclomethasone dipropionate, 9 patients; BUD, 6 patients) in doses of < 1,000 µg/d.

At recruitment, the results of spirometry showed a mean FEV₁ of 2.74 ± 0.20 L (75.2 ± 2.8% predicted) and a mean midexpiratory phase of forced expiratory flow (FEF 25–75) of 2.29 ± 0.23 L/s (52.4 ± 4.0% predicted). On testing (UniCAP Phadiotop; Pharmacia & Upjohn Ltd; Milton Keynes, UK), all patients were required to be atopic to a batch of common inhaled allergens and to be responsive to AMP with a geometric mean provocative concentration causing a 20% fall in FEV₁ (PC₂₀) of 29.1 ± 10.1 mg/mL. At screening, one subject was using oral theophylline therapy, and one was receiving long-acting β₂-agonist therapy with FM; treatment with these was subsequently stopped. All patients gave written informed consent prior to randomization in the study, which was approved by the Tayside Committee on Medical Research Ethics.

**Protocol**

The study had a placebo-controlled, double-blind, double-dummy, crossover design. The subjects attended the laboratory for the initial screening visit in the morning between 9 am and 11 am, and all the subsequent visits were performed within the same 2-h window. An AMP bronchial challenge test was performed. The patients who had a PC₂₀ of < 200 mg/mL entered the run-in phase. From the start of the run-in until the end of the

**Table 1—Demographic Data at Recruitment**

<table>
<thead>
<tr>
<th>Patient No./Age yr/Gender</th>
<th>FEV₁, L</th>
<th>FEV₁, % predicted</th>
<th>FEF₂₅–₇₅ L/s</th>
<th>FEF₂₅–₇₅ % predicted</th>
<th>Dose, µg/d Steroid</th>
<th>AMP PC₂₀, mg/mL</th>
<th>Atopic</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/23/F</td>
<td>2.58</td>
<td>77</td>
<td>2.4</td>
<td>58</td>
<td>400/BDP</td>
<td>33</td>
<td>Yes</td>
</tr>
<tr>
<td>2/29/F</td>
<td>3.00</td>
<td>94</td>
<td>2.53</td>
<td>63</td>
<td>400/BDP</td>
<td>33</td>
<td>Yes</td>
</tr>
<tr>
<td>3/26/F</td>
<td>2.87</td>
<td>91</td>
<td>3.01</td>
<td>74</td>
<td>800/BUD</td>
<td>93</td>
<td>Yes</td>
</tr>
<tr>
<td>4/21/F</td>
<td>3.11</td>
<td>81</td>
<td>2.94</td>
<td>68</td>
<td>200/BDP</td>
<td>0.4</td>
<td>Yes</td>
</tr>
<tr>
<td>5/19/M</td>
<td>3.72</td>
<td>80</td>
<td>3.05</td>
<td>59</td>
<td>400/BDP</td>
<td>102</td>
<td>Yes</td>
</tr>
<tr>
<td>6/46/F</td>
<td>1.32</td>
<td>59</td>
<td>0.82</td>
<td>25</td>
<td>400/BUD</td>
<td>92</td>
<td>Yes</td>
</tr>
<tr>
<td>7/36/M</td>
<td>2.8</td>
<td>70</td>
<td>1.99</td>
<td>44</td>
<td>800/BUD</td>
<td>93</td>
<td>Yes</td>
</tr>
<tr>
<td>8/49/M</td>
<td>2.45</td>
<td>77</td>
<td>1.98</td>
<td>52</td>
<td>400/BUD</td>
<td>32</td>
<td>Yes</td>
</tr>
<tr>
<td>9/20/M</td>
<td>3.77</td>
<td>74</td>
<td>3.15</td>
<td>59</td>
<td>200/BUD</td>
<td>97</td>
<td>Yes</td>
</tr>
<tr>
<td>10/24/M</td>
<td>4.02</td>
<td>86</td>
<td>4.13</td>
<td>80</td>
<td>200/BDP</td>
<td>103</td>
<td>Yes</td>
</tr>
<tr>
<td>11/35/M</td>
<td>2.28</td>
<td>58</td>
<td>1.35</td>
<td>30</td>
<td>800/BUD</td>
<td>40</td>
<td>Yes</td>
</tr>
<tr>
<td>12/34/F</td>
<td>2.02</td>
<td>71</td>
<td>1.72</td>
<td>46</td>
<td>800/BUD</td>
<td>3</td>
<td>Yes</td>
</tr>
<tr>
<td>13/47/F</td>
<td>1.60</td>
<td>59</td>
<td>1.33</td>
<td>39</td>
<td>400/BUD</td>
<td>9</td>
<td>Yes</td>
</tr>
<tr>
<td>14/51/M</td>
<td>2.18</td>
<td>75</td>
<td>1.43</td>
<td>40</td>
<td>500/BDP</td>
<td>26</td>
<td>Yes</td>
</tr>
<tr>
<td>15/20/M</td>
<td>3.43</td>
<td>76</td>
<td>2.49</td>
<td>49</td>
<td>400/BDP</td>
<td>28</td>
<td>Yes</td>
</tr>
<tr>
<td>Mean† 32.4</td>
<td>2.74</td>
<td>75.2</td>
<td>2.29</td>
<td>52.4</td>
<td>473</td>
<td>29.1</td>
<td></td>
</tr>
<tr>
<td>SEM 3.02</td>
<td>0.20</td>
<td>2.8</td>
<td>0.23</td>
<td>4.0</td>
<td>37</td>
<td>10.1</td>
<td></td>
</tr>
</tbody>
</table>

*BPD = beclomethasone dipropionate; F = Female; M = Male.
†All values given as arithmetic mean except PC₂₀, which is given as geometric mean.

1050 Clinical Investigations
trial, all therapy with theophylline, long-acting β2-agonists, and inhaled corticosteroids was stopped. Inhaled ipratropium bromide (Atrovent Forte; Boehringer Ingelheim; Bracknell, UK), 2 puffs and 40 μg/puff, was used by subjects as required for symptomatic relief purposes as a first-line rescue therapy, with inhaled salbutamol used as second-line rescue therapy. During subsequent visits, the subjects did not use either ipratropium bromide or salbutamol for at least 12 h as well.

At the start of the initial 1-week placebo run-in period, the patients were given placebo BUD via an inhaler (Turbohaler; AstraZeneca; Kings Langley, UK) along with placebo FM via a Turbohaler on a regular basis once daily at 8 PM. The patients were taught to perform the correct technique for using the inhaler, making sure that they were able to generate an inspiratory flow of at least 60 L/min using a Turbohaler training device (AstraZeneca; Lund, Sweden).

The subjects made the next visit to the laboratory after the 7-day placebo run-in period, having taken the last dose of the placebo at 8 PM the previous evening. The patients collected their urine overnight for a 10-h cortisol collection test from 10 PM until 8 AM prior to making the visit. At this first study visit, the patients had 5 mL of blood taken for the measurement of ECP followed by measurement of exhaled NO. After that, the subjects underwent baseline spirometry testing followed by an AMP challenge. The subjects then were given their randomized treatments with one of the following: (1) inhaled FM (efomoterol fumarate, 12 μg/puff; Oxis Turbohaler; AstraZeneca), 1 puff once daily, with a placebo, 1 puff; (2) inhaled BUD (400 μg/puff; Pulmicort Turbohaler, AstraZeneca), 1 puff once daily, with a placebo, 1 puff; or (3) inhaled FM, 1 puff of 12 μg once daily, with inhaled BUD, 1 puff of 400 μg once daily. One puff of both inhalers was taken at 8 PM each night for the first 2 weeks. The subjects also were instructed to record the best of three values for their morning and evening peak flows, the latter measured using a peak expiratory flowmeter (Mini-Wright; Clement Clarke Ltd; Harlow, UK) prior to taking the evening medication and recorded on a diary card. The subjects also recorded the total number of puffs of ipratropium bromide or salbutamol rescue therapy taken each day.

The subjects made the next visit to the laboratory after 2 weeks of each randomized treatment, 12 h after taking the 14th dose of their randomized treatment, and bringing along their overnight urinary cortisol collection and diary cards. The same process was repeated for the measurement of NO, for spirometry, and for AMP challenge except that a sample for ECP was not collected at this occasion. The patients then were instructed to start taking 2 puffs from the same randomized inhalers each evening for a further 2 weeks and to record their peak flows and rescue inhaler use on a new diary card. The subjects then returned to the laboratory after another 2 weeks, 12 h after taking their 28th dose (the 14th dose of the 2 puff/sol period). At this third visit, a sample for the measurement of ECP levels was collected, and measurement of exhaled NO, spirometry, and AMP challenge were performed.

The subjects then entered a 7-day washout period in which they again used the two placebo inhalers once daily, at night. The same process was repeated with the next randomized treatment once daily, for 2 weeks (1 puff) and 4 weeks (2 puffs). This process was again followed by another 7-day placebo washout period and a subsequent randomized 4-week treatment block. Patient preference was evaluated at the second and third sequences after 2 and 4 weeks of treatment.

Measurements

Spirometry: Spirometry was performed according to American Thoracic Society criteria using a compact spirometer (Vitalograph Ltd; Buckinghamshire, UK) with a pneumotachograph head and pressure transducer, and an on-line computer-assisted determination of FEV1 and FEF_{25-75}.

AMP Bronchial Challenge: An AMP bronchial challenge test was performed as previously described.12 The test was continued until the FEV1 had dropped by > 20% from the baseline level or until the maximum concentration of 800 mg/mL had been administered. The PC_{20} was calculated using a computer-assisted curve-fitting package (Biolab Assistant, version 1.1; University of Dundee; Dundee, UK). If the FEV1 did not show a 20% fall after the maximum concentration had been given, or if the curve fitting revealed an extrapolated value > 1,000 mg/mL, a censored PC_{20} value of 1,600 mg/mL (double of the maximum concentration) was assigned for that test for the purpose of statistical analysis.13

ECP: The samples for measuring ECP levels were collected in silicone gel-containing tubes (Vacutainer Hemogard SST; Becton Dickinson Vacutainer Systems Europe; France). After collection, the samples were kept at room temperature for 90 min before being spun at 3,000 rotations/min for 10 min. The supernatant was collected in a separate aliquot bottle and was frozen until analysis at the end of the study. The ECP was measured using a radioimmunoassay kit (Pharmacia & Upjohn Diagnostics AB; Uppsala, Sweden). The interassay coefficient of variation was 6.3%.

Exhaled NO: Patients had a measurement of exhaled NO using an integrated, clinical, real-time NO gas analyzer (model LB2000; Logan Research; Rochester, UK) with an accuracy of ±2% and a precision of ±1% at 1000 parts per billion NO. ECP was measured using a radioimmunoassay kit (Pharmacia & Upjohn Diagnostics AB; Uppsala, Sweden). The interassay coefficient of variation was 7.9%, and 12.1% for between assays.

Urinary Free Cortisol: The urinary cortisol was extracted from urine using dichlormethane prior to analysis. Urinary cortisol level was measured using a commercial radioimmunoassay kit (Incatar Ltd., Wokingham, Berkshire UK) that has no cross-reactivity for BUD. For urine-free cortisol excretion, the within-assay coefficient of variation was 7.9%, and 12.1% for between the assays.

Statistical Analysis

The study was powered at 80% to detect a one doubling-dose (twofold) difference in AMP PC_{20} (the primary end point). The data for PC_{20}, exhaled NO levels, and ECP levels were all log-transformed to normalize their distribution prior to analysis. The parameters were analyzed as geometric mean values and as fold differences (with 95% confidence intervals [CIs]). The first visits for all three randomized treatments (ie, after the placebo run-in period or the placebo washout period) were compared according to their order in sequence. In addition, the first visits for all three treatments were compared irrespective of sequence (ie, prior to receiving FM alone, BUD alone, and FM plus BUD, respectively). A pooled placebo was then calculated and used for comparison with active treatments.

The statistical analysis was performed for within-treatment effects (ie, comparing each active treatment at 2 and 4 weeks to pooled placebo) and between-treatment effects (ie, comparing the three active treatments at 2 weeks and 4 weeks). The statistical analysis was performed by multifactorial analysis of variance using subject, treatment, visit, and sequence as factors. This was followed by Bonferroni multiple-range testing (set at 95% CI) to obviate multiple pairwise comparisons, in order not to confound the overall α error (p < 0.05; two-tailed test). Hence,
all parameters are described as being significant \( p < 0.05 \) or not according to the Bonferroni test. The patient preference was analyzed using the Friedman rank test. All data analysis was performed using a statistical software package (Statgraphics; STSC Software Publishing Group; Rockville, MD).

**Results**

All subjects completed the study. Two subjects complained of hoarseness, both during the steroid arms of the study. Subjects were reminded to wash their mouths after inhalation, and they did not complain of the symptoms during the subsequent arms of the study. The urine-free cortisol measurements did not show any significant differences between pooled placebo and BUD (placebo, 24.6 ± 2.6 nmol; BUD \[400 \text{ mg}, 29.4 ± 6.2 nmol; BUD \[800 \text{ mg}, 22.6 ± 5.1 nmol).

**Domiciliary Diary Cards**

The domiciliary peak flow recordings showed significant improvements after subjects received BUD plus FM compared to results of receiving FM or BUD alone (Fig 1). The following results were obtained for morning peak flow measurements (reported as the mean over 4 weeks): FM plus BUD, 473 L/min; vs FM alone, 460 L/min (95% CI for difference, 2 to 22); vs BUD alone, 453 L/min (95% CI for difference, 9 to 29). Similarly, the following results were obtained for evening peak flow measurement: FM plus BUD, 486 L/min; vs FM alone, 472 L/min (95% CI for difference, 5 to 22); vs BUD alone, 476 L/min (95% CI for difference, 1 to 19). The level of salbutamol use for rescue was much less than that for ipratropium bromide. The inhaler use for ipratropium bromide rescue therapy was significantly lower after therapy with FM plus BUD compared to FM alone, but not compared to therapy with BUD alone, while salbutamol usage was significantly lower after therapy with FM plus BUD compared to FM alone, but not compared to therapy with FM alone.

**Placebo Visits**

Values for AMP PC20, ECP levels, and NO levels did not show any significant differences when comparing the visit 1 values after placebo according to sequence, irrespective of the sequence of treatments (ie, placebo prior to FM, placebo prior to BUD, or placebo prior to FM plus BUD) (data not shown).

**Prechallenge Spirometry**

The prechallenge spirometry results showed significant improvements in FEV1 and FEF25–75 with all active treatments compared to the pooled placebo, but there were no significant differences between treatments (Fig 2).

**AMP Bronchial Challenge**

The AMP bronchial challenge test (Fig 3) showed that all active treatments afforded significantly better protection compared to pooled placebo (Table 2). There was a dose-response effect in terms of a significant difference between low and high doses of FM plus BUD for visit 2 vs visit 3 (ie, FM, 12 mg, plus BUD, 400 mg vs FM, 24 mg, plus BUD, 800 mg, yielded a 2.4-fold difference [95% CI, 1.0- to 5.5-fold]).

There were no significant differences between the three low-dose treatments at visit 2 after 2 weeks. For high-dose treatment after 4 weeks, there was significantly better bronchoprotection with both BUD regimens compared to FM alone (ie, FM, 24 mg, vs BUD, 800 mg, yielded a 2.5-fold difference [95% CI, 1.1- to 5.4-fold]; FM, 24 mg, vs FM, 24 mg, plus BUD, 800 mg, yielded a 3.2-fold difference [95% CI, 1.5- to 7-fold]). There was no significant difference between BUD alone and BUD plus FM at either 2 or 4 weeks. Rank order for effects on AMP PC20 showed significant \( p = 0.004 \) differences with both BUD-containing arms compared to FM alone.

**Exhaled NO**

For FM alone, there were no significant differences at either dose compared to pooled placebo. Doses of both BUD alone and combination therapy yielded significantly lower NO levels (Fig 3) compared to placebo (Table 2). For therapy with BUD alone at both doses and for combination therapy at high dose, there were significant reductions in exhaled NO compared to NO levels after therapy with FM alone (ie, FM, 12 mg, vs BUD, 400 mg, yielded a 1.7-fold difference [95% CI, 1.2- to 2.4-fold]; FM, 24 mg, vs BUD, 800 mg, yielded a 2.1-fold difference [95% CI, 1.1- to 3.4-fold]; and FM, 24 mg, vs FM, 24 mg, plus BUD, 500 mg, yielded a 1.8-fold difference [95% CI, 1.1- to 2.9-fold]). Rank order for effects on exhaled NO levels showed significant \( p = 0.004 \) differences with both BUD-containing arms compared to FM alone.

**ECP**

There were significant reductions in ECP levels after therapy with both BUD-containing arms of the study. There was no significant reduction in ECP levels (measured only at visit 3) after FM treatment.
Figure 1. Top: rescue inhaler usage of ipratropium bromide (first-line rescue therapy) and salbutamol (second-line rescue therapy) over the 4-week treatment period. Bottom: domiciliary peak expiratory flow rate for morning and evening recordings. The values are the overall mean for the 4-week treatment period. Values are expressed as the arithmetic mean ± SEM. FM = FM alone; BUD = BUD alone; FM + BUD = FM plus BUD; PEF = peak expiratory flow.
Figure 2. Pre-AMP challenge spirometry results given as (top) FEF_{25-75} and (bottom) FEV_{1} for the active treatments compared to pooled placebo. The values are given as the arithmetic mean ± SEM. PL = placebo. See Figure 1 for abbreviations not used in the text.
Figure 3. Top: exhaled NO levels for active treatments compared to pooled placebo. Bottom: AMP bronchial challenge for active treatments compared to pooled placebo. The values are given as the geometric mean ± SEM. Values are plotted on a Log2 scale. See Figures 1 and 2 for abbreviations not used in the text.
compared with pooled placebo (the 95% CI for fold difference included unity due to the wider intrasubject variance (Table 2)).

**Patient Preference**

The patients preferred the combined treatment as their first choice followed by therapy with FM alone and then BUD alone in rank order (p < 0.0005)

**DISCUSSION**

The main findings of our study were as follows. (1) Patients preferred combined once-daily treatment with FM and BUD to therapy with BUD alone, which was associated with improvements in domiciliary peak expiratory flow and use of rescue medication. (2) Despite patient preference and improved peak flow, combined therapy was not associated with better anti-inflammatory control (measurements of exhaled NO, serum ECP levels, and AMP challenge results) compared to BUD monotherapy. (3) FM monotherapy had no significant anti-inflammatory effects on exhaled NO and exhibited antagonism against AMP challenge to a lesser degree than BUD monotherapy. (4) BUD exhibited a numerical dose-related effect on the AMP challenge when given as monotherapy (nonsignificant) or combined therapy (significant) in keeping with its anti-inflammatory activity. (5) All three treatments exhibited equivalent improvements in FEV$_1$ and FEF$_{25-75}$.

In the study of Pauwels et al,\(^1\) adding therapy with twice-daily administration of FM to that with BUD produced improvements in peak expiratory flow and reductions in exacerbation rates. Our data showed that once-daily FM administration conferred additive effects on peak expiratory flow but exhibited no significant anti-inflammatory activity on its own. In a subgroup analysis of the study by Pauwels et al,\(^1\) therapy with the combination of FM and low-dose BUD showed no significant difference in the levels of induced sputum eosinophils after 1 year compared to the effects of high-dose BUD as monotherapy.\(^1\)

The use of salmeterol on its own or in addition to inhaled corticosteroids has been shown to have no significant effect on inflammatory cell profiles or activation markers derived from bronchial biopsy specimens or BAL fluid.\(^17\),\(^18\) However, some data have suggested that twice-daily monotherapy with FM may reduce the number of mast cells and eosinophils in bronchial biopsy specimens taken from patients with mild atopic asthma.\(^19\)

This begs the question as to the underlying mechanism for the reduction in exacerbation rates with therapy with FM plus BUD. The study of Pauwels et al,\(^1\) showed that there was a proportionally greater reduction in exacerbation rates by increasing the dose of BUD as monotherapy (from 200 to 800 µg/d) compared to the additive effects of therapy with FM.

This is in keeping with our own data, in that doubling the dose of BUD exhibited further protection against AMP challenge irrespective of the addition of FM. Our results, showing greater suppression of exhaled NO and bronchial hyperreactivity (as in the AMP challenge) with therapy with BUD alone compared to therapy with FM alone, are similar to the observations of Verberne et al,\(^20\) and Simons,\(^21\) in which beclomethasone dipropionate was superior to salmeterol for effects on bronchial hyperreactivity (as in a methacholine challenge) and exacerbation rates.

Studies have shown that adding salmeterol to inhaled corticosteroids is as effective as doubling the corticosteroid dose in controlling exacerbation rates.\(^2\),\(^2\) However, adding salmeterol has been shown to potentially mask the underlying airway inflammation in terms of increased induced sputum eosinophils with unchanged symptoms and lung function prior to an exacerbation, during the step-down with inhaled beclomethasone.\(^6\) This emphasizes the importance of optimizing anti-inflammatory control with inhaled corticosteroids before considering the addition of regular long-acting β$_2$-agonist treatment.

We observed a patient preference for the combination treatment compared to treatment with the
corticosteroid alone, which was associated with increased peak expiratory flow rates and lower rescue requirements. This preference may reflect patients’ perceptions of a rapid onset of bronchodilator response with FM as compared to the more gradual onset of action with the corticosteroid. Combining long-acting $\beta_2$-agonists and inhaled corticosteroids in the same inhaler formulation (eg, fluticasone plus salmeterol [Seretide; Glaxo Wellcome; Oxbridge, UK]) might conceivably result in improved compliance, at least when initiating therapy.

We accept the limitations of our study in that we used a relatively small sample of patients who had mild-to-moderate asthma. We chose patients with mild-to-moderate asthma as we intended to evaluate the comparative effects of BUD and FM, and, consequently with the washout period, there was a potential 6-week period when the patients might conceivably not be taking any inhaled corticosteroids. In order to make sure that the patients did not develop short-term worsening of their disease control, it was decided to evaluate those patients who were using < 1,000 $\mu$g/d of inhaled corticosteroids. Interestingly, the mean PC$_{20}$ value (29 mg/mL) at recruitment prior to the initial placebo run-in was similar to the value after the run-in (22 mg/mL), suggesting that patients may not have been complying with their usual inhaled corticosteroid therapy at the time of recruitment. This is supported by the subsequent improvement in mean PC$_{20}$ values to 109 mg/mL after 2 weeks of therapy with BUD, 400 $\mu$g/d, compared to the average corticosteroid dose of 473 $\mu$g/d at recruitment.

We used the BUD Turbohaler once daily, as it is licensed for use in the United Kingdom, up to 800 $\mu$g/d in patients with mild-to-moderate asthma. In the present study, at 12 h after the administration of BUD, 400 $\mu$g once daily for 2 weeks, there was a 2.5-doubling dilution shift in AMP PC$_{20}$. This compares to a 2.7-doubling dilution shift in AMP PC$_{20}$ in a previous study at 24 h after the administration of the BUD inhaler, 400 $\mu$g, once daily for 2 weeks. Furthermore, we also have shown that at 12 h after the administration of the BUD inhaler, 200 $\mu$g, twice daily for 3 weeks, there was a 2.5-doubling dilution shift in AMP PC$_{20}$. Taken together, the results of these trials suggest that the results of the present study with once-daily treatment can probably be extrapolated to what happens with twice-daily therapy.

We elected to use AMP challenge, ECP levels, and exhaled NO levels together as surrogate end points of airway inflammation, as it is possible that a given marker may not correlate with active inflammation in different patients. In previous studies, the effects of BUD were proportionately greater for AMP challenge than for methacholine challenge. This reflects the indirect nature of AMP challenge in terms of mast cell priming and release of inflammatory mediators in contrast to the direct effect of methacholine on bronchial smooth muscle. It has been postulated that AMP is a more appropriate marker of bronchial inflammation in the evaluation of corticosteroid responses. The effects of FM on AMP challenge are thought to be partly due to the direct inhibition of mast cell $\beta_2$-adrenoceptors and partly due to the stimulation of bronchial smooth muscle $\beta_2$-adrenoceptors.

Exhaled NO has a positive correlation with sputum eosinophil count and responsiveness to methacholine bronchial challenge. We found no further suppression of exhaled NO by increasing the dose of BUD from 400 to 800 $\mu$g/d. This is in keeping with other data from the study of BUD by Jatakanon et al in which dose-related reductions in exhaled NO and sputum eosinophil levels showed a plateau effect at a dose of 400 $\mu$g/d from the BUD Turbohaler. We also have previously performed a dose-ranging study with the BUD Turbohaler in which a plateau in response to exhaled NO was seen at a dose of 400 $\mu$g/d of BUD, in contrast to dose-related improvements in AMP and methacholine reactivity up to 1,600 $\mu$g/d in patients with mild-to-moderate atopic asthma. Serum ECP level may be used as a surrogate inflammatory marker in atopic asthmatics and has been shown to correlate with ECP levels in BAL fluid, with eosinophil numbers in bronchial biopsy specimens, and with asthma exacerbations. Thus, we thought that by choosing serum ECP levels, exhaled NO levels, and AMP challenge reactions, we were broadly assessing the underlying inflammatory process.

In summary, we have found that optimal patient preference, improved peak flow rates, and reduced rescue medication with combined once-daily BUD and FM therapy were not associated with significantly better anti-inflammatory control than the same dose of BUD as monotherapy, while all three treatments produced equivalent improvements in spirometry. FM as monotherapy did not appear to exhibit significant anti-inflammatory effects and produced less bronchoprotection than BUD as monotherapy. Our results support optimizing the anti-inflammatory efficacy of inhaled corticosteroid therapy first before considering the addition of long-acting $\beta_2$-agonists.

ACKNOWLEDGMENT: The authors acknowledge the technical assistance of Erika Sims, Wendy Coutie, and Michelle Paterson; the secretarial assistance of Anne Muirhead; and the statistical advice of Dr. R. A. Brown (University of Dundee, Department of Mathematical Sciences).

CHEST / 118 / 4 / OCTOBER, 2000 1057
REFERENCES


8. 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; 155:1273–1277


20. Nakanishi Y. Relationship between activated eosinophils of the bronchial mucosa and serum eosinophilic cationic protein in atopic asthma. Int Arch Allergy Immunol 1997; 112:59–64