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ORIGINAL ARTICLE

Effects of Salmeterol and Fluticasone Propionate Combination versus Fluticasone Propionate on Airway Function and Eosinophilic Inflammation in Mild Asthma

Makoto Hoshino[1,2], Hiroshi Handa[1] and Teruomi Miyazawa[1]

ABSTRACT

Background: Salmeterol and fluticasone propionate combination (SFC) provides better asthma control than fluticasone propionate (FP) alone, however, little is known on the effects of differential treatments on airway function and inflammation in patients with mild asthma.

Methods: We randomized 27 mild persistent asthma patients treated with the equivalent of 400 μg beclomethasone dipropionate to receive SFC (50/100 μg, 13 patients) or FP (100 μg, 14 patients) twice daily for 8 weeks. We compared the effects of SFC and FP on pulmonary function assessed by spirometry and impulse oscillometry (IOS), eosinophil percentage of induced sputum and serum, and with asthma symptoms and control after each treatment.

Results: We observed that SFC significantly improved forced expiratory volume in one second ($p < 0.05$), IOS measurements of total resistance R5 ($p < 0.01$), central resistance R20 ($p < 0.05$), and distal reactance X5 ($p < 0.01$) compared with FP. The percentage of eosinophils in sputum, but not in serum, decreased significantly more in the SFC group than in the FP group ($p < 0.05$). There was also a significant improvement in symptom control in the SFC group ($p < 0.05$).

Conclusions: These findings suggest that SFC is more useful than FP in mild asthma cases. The clinical benefit of SFC provides evidence that IOS and induced sputum allows for the detection of changes in airway function and inflammation.

KEY WORDS

asthma, fluticasone propionate, impulse oscillometry, salmeterol and fluticasone propionate, sputum eosinophils

INTRODUCTION

Bronchial asthma is characterized by variable airflow obstruction in association with chronic inflammation of the airways. Anti-inflammatory treatments with inhaled corticosteroids (ICS) are recommended as first line therapy.[1] For patients whose asthma is poorly controlled on ICS therapy alone, the addition of a long acting β_2 agonist (LABA) yields more effective treatment. Several clinical studies have shown that the salmeterol and fluticasone propionate combination (SFC) could improve lung function and control symptoms more effectively than equal or double doses of fluticasone propionate (FP) in patients with asthma.[2-7]

Conventional spirometric measurements have been widely used, however the data is often insensitive to physiological changes in response to bron-

[1]Division of Respiratory and Infectious Diseases, Department of Internal Medicine, St. Marianna University School of Medicine, Kanagawa and [2]Present address: Department of Respiratory Medicine, Atami Hospital, International University of Health and Welfare, Shizuoka, Japan.
Correspondence: Makoto Hoshino, MD, PhD, Department of Respiratory Medicine, Atami Hospital, International University of Health and Welfare, 13–1 Higashikaigan-cho, Atami, Shizuoka 413–0012, Japan.
Email: hoshino@iuhw.ac.jp
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chodilators, such as in patients with mild asthma.[8] Impulse oscillometry (IOS) involves the application of small pressure oscillations at the mouth during spontaneous breathing to obtain a measure of respiratory resistance and reactance. The IOS technique is sensitive in detecting physiologic changes and is useful in evaluating both central and distal lung function.[9] Furthermore there is little information on the effects of salmeterol on eosinophilic airway inflammation in mild asthma. Induced sputum is a reliable and responsible method to safely obtain airway secretions[10] and collected sputum has been reported to be useful for evaluating airway inflammation.[11] Therefore, the clinical benefits of combination therapy with SFC in patients with mild asthma are apparent if pulmonary function and airway inflammation are used, and SFC may be more useful than FP.

We sought to compare the effects of SFC and FP on lung function assessed by spirometry and IOS, airway inflammation by sputum and serum, and to relate these effects on symptoms and control in patients with mild asthma.

METHODS

SUBJECTS

All patients had a clear history of relevant symptoms for asthma as defined by the American Thoracic Society criteria with a bronchodilator response defined as an increase in forced expiratory volume in 1 second (FEV_1) by more than 12% after administration of inhaled β_2 agonists. All patients attending our hospital had step 2 severity asthma,[1] with stable symptoms, and had been receiving a daily dose of ICS equivalent to 400 μg beclomethasone dipropionate for at least 3 months. A diagnosis of atopy was based on the presence of one or more specific serum IgE antibodies against common inhalant allergens. The study was approved by the ethics committee of our institution, and informed written consent to the study protocol was obtained from all subjects.

STUDY DESIGN

All patients were treated with FP Diskus (Flutide Diskus, 100 μg, GlaxoSmithKline, Tokyo, Japan) twice daily during an 8-week run-in period. At the end of the run-in period, all patients were randomized to receive either SFC Diskus (Adoair Diskus, 50/100 μg, GlaxoSmithKline) twice daily or FP 100 μg twice daily for an 8-week treatment period. No other asthma medications were permitted except for inhaled short acting β_2 agonists only when necessary. Pulmonary function and induced sputum were performed, and blood samples were taken for measurement of the serum eosinophil percentage before and after the 8 weeks of treatment. Patients were given diary cards to record morning peak expiratory flow (PEF) measurements. Asthma symptoms and control were evaluated before and after the 8-week period with the asthma control test (ACT) developed by QualityMetric Incorporated, RI, USA.[12] All patients showed good compliance and adherence to treatment with either SFC or FP. This study was carried out in accordance with the principles of the Helsinki Declaration of 1995 (revised in Edinburgh, 2000).

Table 1 Subjects characteristics

	SFC group	FP group
Subjects (*n*)	13	14
Gender (Male/Female)	5/8	4/10
Age (years)	49.7 ± 14.3	42.8 ± 14.0
Disease duration (years)	10.0 ± 2.9	12.8 ± 5.1
Ex-smoker/non-smoker	3/10	2/12
Atopy/non-atopy	9/4	11/3

Data are presented as mean ± SD.

PULMONARY FUNCTION MEASUREMENTS

Pulmonary function tests were repeated in the same order; IOS followed by spirometry. For IOS, MasterScreen IOS (Jaeger, Wurzburg, Germany) was performed using the recommended techniques of the manufacturer.[13] Among IOS parameters, the total respiratory resistance at 5 Hz (R5), central resistance at 20 Hz (R20), and the reactance at 5 Hz (X5) representing the elastic properties which are indirect indicators of peripheral obstruction were recorded. R5 minus R20 (R5 – R20), frequency dependence of resistance, could be used as a marker for distal airway dysfunction.[14,15] IOS data were accepted if the coherence (correlations between oscillatory pressure and flows used to calculate all resistance and reactance) values were >0.8. Spirometry function such as FEV_1, forced vital capacity (FVC), mid forced expiratory flow (FEF25–75), FEF50, and FEF25 was conducted by FUDAK-77 (Fukuda Electronics, Tokyo, Japan). These parameters are expressed as percentages of predicted values according to the prediction equations of the Japanese Society of Chest Disease.[16]

SPUTUM INDUCTION AND PROCESSING

Sputum induction and processing were performed as previously described.[17] After inhalation of 200 μg of salbutamol via a metered dose inhaler, the subjects inhaled 5% hypertonic saline using an ultrasonic nebulizer (NE-U22, Omron, Tokyo, Japan). The subjects were encouraged to cough deeply and sputum was expectorated into sterile containers. Adequate plugs of sputum were separated from saliva. Sputum was transferred in small amounts and finely distributed and smeared onto microscopic slides. Each smear was air dried and stained with May-Grunwald-Gimsa, and differential cell counts were obtained from 400 non-squamous cells by a blinded investigator.

Table 2 Spirometry and PEF before and after treatment with SFC or FP

	SFC group		FP group	
	Before	After	Before	After
FEV_1 (% predicted)	87.4 ± 5.9	94.8 ± 8.9*†	87.0 ± 6.8	88.7 ± 8.1
FVC (% predicted)	97.0 ± 7.4	100.8 ± 9.6	99.1 ± 6.3	100.1 ± 7.0
FEF25−75 (% predicted)	49.5 ± 23.0	53.7 ± 20.1	48.5 ± 22.0	52.5 ± 26.8
FEF50 (% predicted)	47.4 ± 22.4	51.0 ± 18.6	45.4 ± 18.3	48.0 ± 21.6
FEF25 (% predicted)	32.7 ± 15.4	35.8 ± 11.9	30.7 ± 13.9	33.1 ± 17.5
Morning PEF (L/min)	371.8 ± 75.9	392.0 ± 64.4*†	354.5 ± 64.7	359.8 ± 58.9

Data are presented as mean ± SD.

*$P < 0.05$: for comparison between values before and after treatment (within group).

†$P < 0.05$: for comparison between treatment values (before minus after treatment) on SFC *vs* FP.

FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; FEF25−75, mid forced expiratory flow; FEF50, forced expiratory flow at 50%; FEF25, forced expiratory flow at 25%; PEF, peak expiratory flow.

STATISTICAL ANALYSIS

Data are expressed as means ± SD. The mean morning PEF was calculated during the last week of the baseline period and at the end of the treatment period. All statistical analyses were performed using Stat View software (SAS Institute, Cary, NC, USA). The results were analyzed using Student's paired *t*-test for within-group comparisons and the two-sample *t*-test for between-group comparisons. For between-group comparisons, the Mann-Whitney *U* test was performed on the delta values (baseline minus after treatment). A *p*-value less than 0.05 was considered to indicate a statistically significant difference.

RESULTS

Of the 34 patients recruited, 7 had inadequate induced sputum samples. Thirteen patients in the SFC treatment group and 14 patients in the FP treatment group completed the study. There were 22 nonsmokers, and the remaining 5 were ex-smokers, with less than 10 pack-years, who quit smoking at least 1 year before the study. Their characteristics are shown in Table 1. There was no significant difference between the two groups in terms of clinical data.

The values of spirometry and morning PEF measurements at baseline and 8 weeks after treatment are given in Table 2. There was a small but significant improvement in FEV_1% predicted for SFC compared with FP ($p < 0.05$). No significant change was observed in other spiromeric values in either SFC or FP group. The mean morning PEF significantly increased in the SFC group compared with the FP group ($p < 0.05$).

There were significant improvements in IOS measured resistance R5 (0.39 ± 0.09 to 0.34 ± 0.08 kPa/L/s; $p < 0.001$), R20 (0.33 ± 0.07 to 0.30 ± 0.07 kPa/L/s; $p < 0.05$), R5–R20 (0.07 ± 0.04 to 0.04 ± 0.03 kPa/L/s; $p < 0.001$), and reactance X5 (–0.15 ± 0.05 to –0.10 ± 0.05 kPa/L/s; $p < 0.001$) in the SFC group. However, IOS measurements remained unchanged in the FP group. The difference between SFC and FP treatment in these parameters was statistically significant ($p < 0.01$, $p < 0.05$, $p < 0.01$, $p < 0.01$, respectively, Fig. 1).

In the SFC group, sputum eosinophil percentage decreased from 5.9 ± 3.1 to 3.9 ± 1.9% ($p < 0.05$), but there was no significant change in sputum eosinophils in the FP group. A comparison between the two groups showed a significant difference ($p < 0.05$), however, there was no significant change in serum eosinophil percentage for either group (Fig. 2).

The ACT scores for both groups are shown in Figure 3. Total ACT scores in the SFC group significantly increased from 22.4 ± 1.8 to 23.8 ± 1.0 ($p < 0.01$), whereas the patients in the FP group exhibited no change in ACT scores. The difference between the two groups was significant ($p < 0.05$).

DISCUSSION

We demonstrated that treatment with 50/100 μg SFC twice daily can specifically improve parameters reflecting function and inflammation of airways in patients with mild asthma, treated with 100 μg FP twice daily, and lead to better symptom control.

The bronchodilator profiles of salmeterol in asthma is well established and furthermore, may have a potency of small airways.[18] Recently, strong evidence that small airways significantly contribute to total airway resistance have been reported and several studies have confirmed distal airways involvement in asthma.[19,20] Although spirometry is a standard method, the assessment of small airway dysfunction may not be accurate. The IOS technique takes advantage of the changes in airflow when the airways are subjected to impulses. Changes in multiple impulses are measured to calculate resistance and reactance; the total airway resistance (R5), the large airway resistance (R20), and distal capacitive reactance (X5).[21] Frequency-dependent changes in resistance and compliance have been demonstrated in small airway disease.[22] Because of low frequency, elastic components in peripheral airways are dominant and X5 reflects

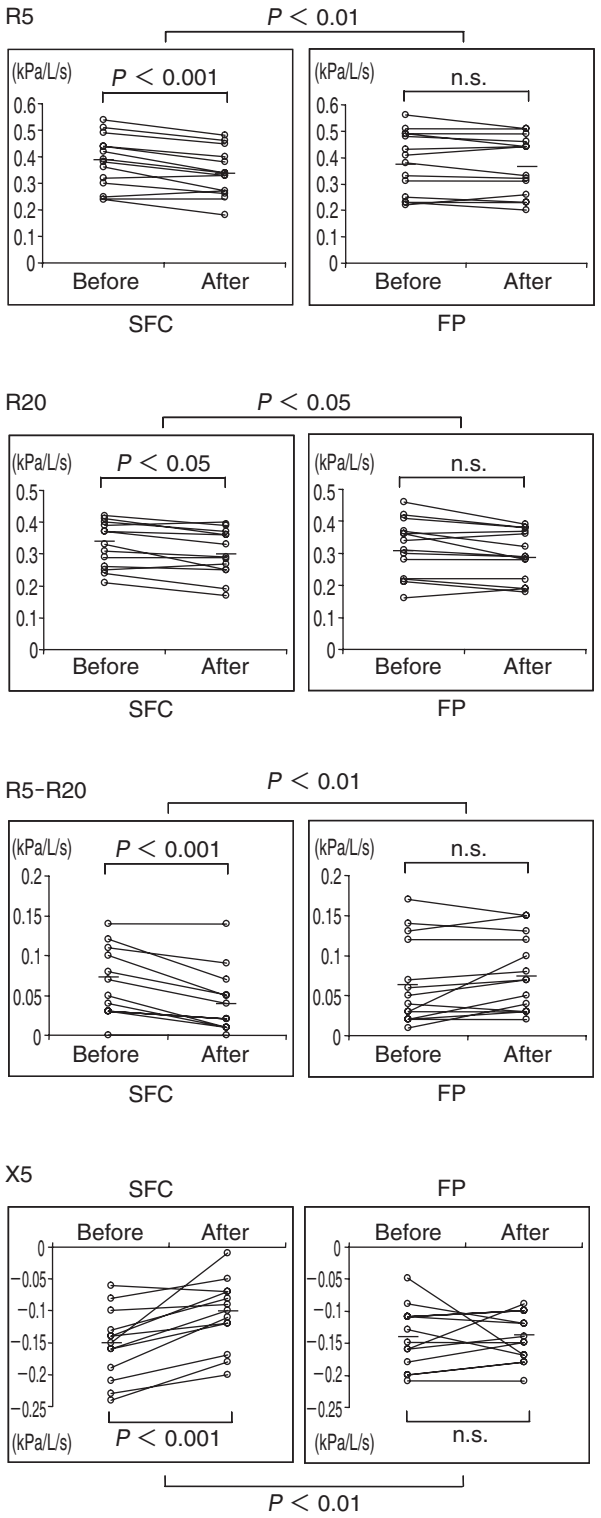

Fig. 1 Changes in impulse oscillometry (IOS) measured resistance of R5, R20, R5 − R20, and reactance X5 in the SFC- and FP-treated group. R5, respiratory resistance at 5 Hz; R20, respiratory resistance at 20 Hz; R5 − R20, respiratory resistance at 5 to 20 Hz; X5, respiratory reactance at 5 Hz. Horizontal bars represent mean values.

small airway dysfunction.[23,24] We also calculated the difference between R5 and R20 (R5–R20) as an index of frequency dependence of resistance, which was reported to significantly correlate with spirometric values of FEV_1 and FEF25–75, and reflect peripheral airway obstruction and dysfunction.[14,15] In this study, SFC improved all measured IOS parameters involving total airway resistance (R5), central airway resistance (R20) and distal airway function (R5–R20, X5). This finding clearly indicates that SFC exerts a bronchodilator effect not only to the central but also the peripheral airways. There was a significant increase in the spirometric measurement of FEV_1 but not in FEF25–75, FEF50, and FEF25, which are commonly used for small airway function. This discrepancy is likely to be due to reduced sensitivity of spirometry and the increased variability of these parameters since spirometry is effort dependent and the deep inspiration required can lead to changes in bronchomotor tone. Inclusion of IOS indices in the clinical study may help in comprehensive assessment of bronchodilator effects that cannot be measured by standard spirometry in patients with asthma.

Sputum eosinophil percentage showed a significant decrease after treatment with SFC compared with FP. However, there was no significant change in serum eosinophil percentage in the SFC group. One reason may be that the anti-inflammatory effect is related to topical administration of SFC, since a previous study showed that SFC does not affect the plasma cortisol level in clinical dose.[25] It has been demonstrated that eosinophil counts of induced sputum significantly correlated with those obtained by bronchial washing and bronchoalveolar lavage, and therefore, induced sputum was useful to evaluate airway inflammation.[26] We used the noninvasive technique of sputum induction to investigate the effect of SFC versus FP. Our results are based on the following molecular mechanisms of interaction between ICS and LABAs: corticosteroids increase the number of β_2-receptors and inhibit down-regulation of β_2-receptors. In contrast, LABAs exert an effect on the glucocorticoid receptor (GR) by priming it for subsequent steroid binding and by enhancing the translocation of GR from the cytoplasm to the nucleus in airway cells.[27] β_2-agonists and corticosteroids, when used in combination, may have additional and/or synergic effects on inflammatory cells.[28] We found that asthma being maintained with FP administration, residual eosinophilic inflammation was ameliorated by switching treatment to SFC. Our findings of reduced sputum eosinophil percentage are in agreement with a study by Li *et al.* suggesting that the addition of salmeterol in asthma patients receiving ICS led to a reduction in the number of submucosal eosinophils using bronchial biopsies.[29] These data suggest that SFC inhibits eosinophil recruitment into the airway and exhibits an anti-inflammatory effect greater than that of FP alone.

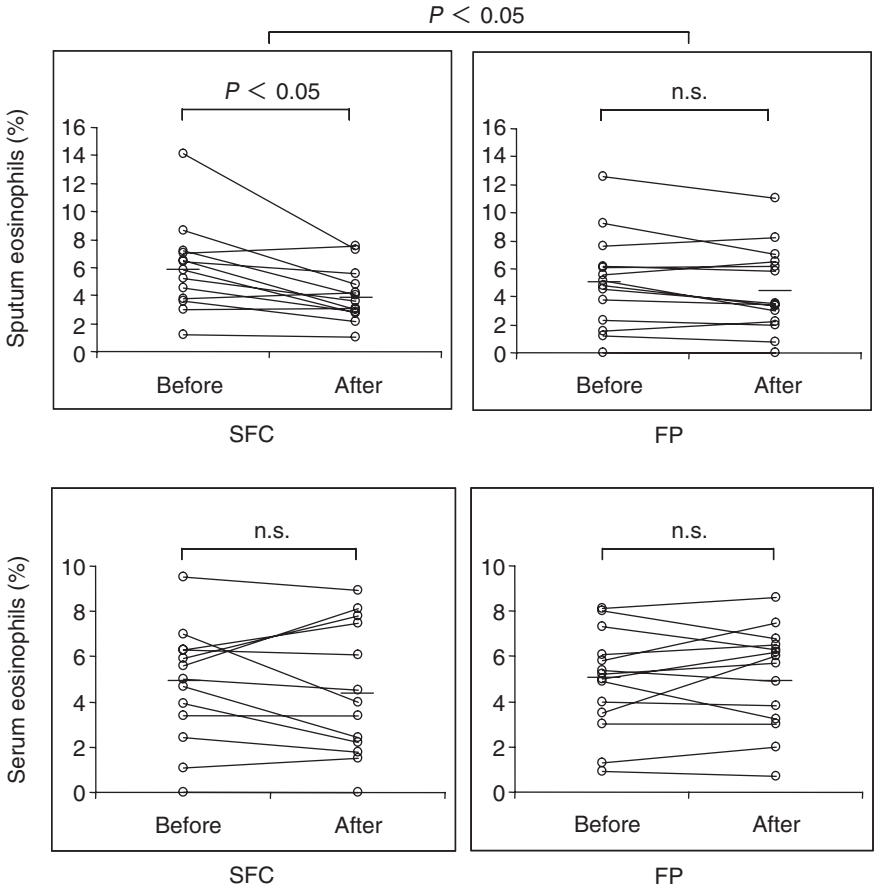

Fig. 2 Changes in the percentage of eosinophil in sputum and serum in the SFC- and FP- treated group. Horizontal bars represent mean values.

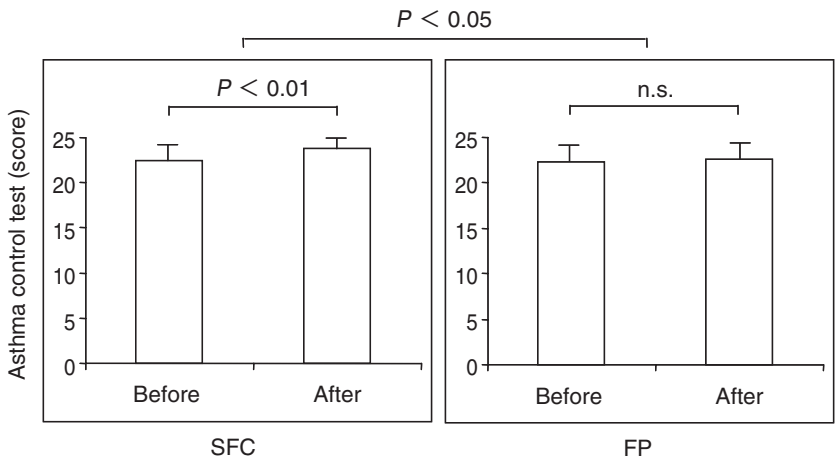

Fig. 3 Changes in asthma control test (ACT) scores in the SFC- and FP-treated group. Columns and vertical bars represent the means ± SD.

We observed a significant increase of ACT scores after switching FP to SFC. This is important because the control of asthma is the main therapeutic goal of asthma guidelines.[1] Asthma control can be assessed using several methods such as questionnaires. The ACT survey is a self-administered questionnaire with

5 items assessing asthma symptoms, use of rescue medications, and the effect of asthma on daily functioning.[12] The ACT score is reliable, valid, and can be useful to evaluate control status. Generally, SFC led to better symptom control and also achieved total and well-controlled asthma more rapidly than for the same dose of FP.[7,30,31] Our findings show that the improvement in symptom control after treatment with SFC contributes to changes in pulmonary function and airway inflammation. It has been a concern that delayed awareness of clinical expression of asthma due to treatment with salmeterol might be associated with masking of eosinophic airway inflammation.[32] This study clearly indicated that, when given in the form of a combination inhaler, there was no evidence of worsening of airway inflammation.

In conclusion, we have demonstrated that SFC improves both airway function and inflammation in patients with mild asthma treated with FP. These beneficial effects were sensitively assessed by IOS and induced sputum. Further and longer duration studies are needed to elucidate the necessity of SFC treatment and its efficacy and safety on pulmonary function and airway inflammation in subjects with mild asthma.

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