

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Efficacy of theophylline plus salmeterol/fluticasone propionate combination therapy in patients with asthma

Hanxiang Nie^{a,*}, Guqin Zhang^{b,c}, Min Liu^a, Xuhong Ding^a, Yi Huang^a, Suping Hu^a

^a Department of Respiratory Medicine, Renmin Hospital of Wuhan University, 238 Jiefang Road, Wuchang District, Wuhan 430060, China

^b Department of Respiratory Medicine, Zhongnan Hospital of Wuhan University, Wuhan 430071, China

Received 16 November 2012; accepted 5 December 2012

KEYWORDS

Theophylline;
Salmeterol/
fluticasone
propionate
combination product;
Small airway
function;
Airway inflammation;
Asthma

Summary

Objective: To compare the efficacy of theophylline plus salmeterol/fluticasone propionate combination product (SFC) with SFC plus placebo in asthmatic patients.

Methods: In this randomized, stratified, parallel-group study, 325 patients were randomized to receive either 200 mg theophylline plus 50/250 µg SFC or placebo tablet plus 50/250 µg SFC twice daily for 24 weeks. Outcome variables included the level of asthma control (assessed by the Asthma Control Test) and the number of patients experiencing ≥ 1 exacerbations during the 24-week treatment period. Testing of lung function as well as measurement of the levels of inflammatory markers in induced sputum was performed.

Results: There were significantly fewer patients with ≥ 1 asthma exacerbation in the theophylline plus SFC group (29.6%) when compared with the SFC plus placebo group (46.9%) ($p = 0.004$). Theophylline plus SFC improved the $FEF_{25-75\%}$ value, which indicates enhanced small airway function, to a greater extent than SFC plus placebo ($66.9 \pm 18.8\%$ and $57.4 \pm 17.6\%$, respectively; $p < 0.001$). A significant decrease in eosinophil count and concentration of eosinophil cationic protein in induced sputum was also seen in the theophylline plus SFC group when compared with the SFC plus placebo group ($4.1 \pm 2.2\%$ and $6.3 \pm 2.7\%$, 63.6 ± 39.5 µg/L and 89.4 ± 45.6 µg/L, respectively; all $p < 0.01$).

Conclusion: The combination of theophylline plus SFC may provide greater protection against asthma exacerbations, and its administration is accompanied by significant improvements in small airway function and airway inflammation.

© 2012 Elsevier Ltd. All rights reserved.

* Corresponding author. Tel.: +86 27 8804 1919x82137; fax: +86 27 8804 2292.

E-mail address: nhxiang@yahoo.cn (H. Nie).

^c These authors contributed equally to this research.

Introduction

Asthma is a chronic disease that is characterized by inflammation, obstruction and hyperresponsiveness of the airways. The aims of asthma management are to achieve and maintain asthma control by treating inflammation and relieving bronchoconstriction and symptoms.¹ Current guidelines for asthma management concur that inhaled corticosteroids (ICS) are the mainstays of anti-inflammatory treatment of asthma.^{1–3} Nevertheless, asthma is often inadequately controlled, even with high doses of ICS, warranting additional therapies. Accordingly, long-acting β_2 agonists (LABAs) are commonly used in combination with ICS therapy.¹ While the addition of LABAs to ICS treatment results in an improvement in symptom scores and reduces exacerbation, recent studies have shown that insufficiently controlled asthma remains a significant problem, even when ICS and LABAs are titrated to the maximal dose.^{4,5} Therefore, alternative therapies that can be added to either ICS or ICS plus LABAs therapy regimens are needed.

Theophylline, a methylxanthine, has been widely used for the treatment of asthma, and is seen to have a role in asthma management guidelines.^{6,7} However, its popularity has declined because theophylline is a less effective bronchodilator agent than β_2 agonists. Furthermore, theophylline has a narrow therapeutic index, and the dose must be carefully titrated with routine blood monitoring to maintain blood levels within the therapeutic range of 10–20 $\mu\text{g/mL}$ and to avoid the occurrence of side effects, which include nausea, vomiting, and cardiac arrhythmias. Despite these concerns, theophylline is widely used in clinical practice in China. Recent studies have shown that theophylline has anti-inflammatory and immunomodulatory effects at lower concentrations ranging from 5 to 10 $\mu\text{g/mL}$.^{8,9} Some studies have shown that theophylline can be used in patients with milder asthma or as an add-on therapy to low doses of inhaled corticosteroids.^{8–11} No serious adverse effects have been reported in these studies. As a result, we hypothesized that a theophylline plus salmeterol/fluticasone propionate combination would be more effective than salmeterol/fluticasone propionate combination alone in patients with asthma. The aim of this study was to compare the efficacy of theophylline (200 mg twice daily) plus inhaled salmeterol/fluticasone propionate combination (SFC, 50/250 μg twice daily) with inhaled salmeterol/fluticasone propionate combination (50/250 μg twice daily) plus placebo.

Methods

Patients

The study was conducted between October 2009 and March 2012 in the Department of Respiratory Medicine of Renmin Hospital of Wuhan University (Wuhan, China), and was approved by the Ethics Committee of Renmin Hospital of Wuhan University. All patients gave written informed consent. Four hundred and seven non-smoking adult asthmatic patients aged between 18 and 68 who fulfilled the diagnostic criteria of the Global Initiative for Asthma were enrolled. All patients who were diagnosed as asthmatics did

not take any anti-asthma medication before the trial. All patients had one or more positive skin prick test reactions and did not experience a lower respiratory tract infection at least 8 weeks prior to the trial. Patients who had experienced an exacerbation of asthma or who had taken theophylline or any other treatment for asthma (except inhaled salmeterol/fluticasone propionate combination or salbutamol inhaler) within four weeks of the run-in period were ineligible. Patients with a history of serious diseases or other lung conditions, as well as current smokers, were not admitted into the study. Pregnant or lactating females were also excluded from study entry.

Study protocol

This randomized, stratified, parallel-group study was conducted in the Department of Respiratory Medicine of Renmin Hospital of Wuhan University (Wuhan, China). A screening visit was followed by a 4-week run-in period, randomization at baseline, and a 24-week treatment period. The patients kept diary cards throughout the study. The patients returned for evaluation every four weeks during the treatment period.

Screening visit

At screening, the patients underwent the following investigations: medical history and routine physical examination; laboratory work-up, including hematology and biochemistry; pulmonary function testing, including forced expiratory volume in 1 s (FEV_1 , expressed as a percentage of the predicted normal value), forced vital capacity (FVC), forced expiratory flow between 25% and 75% of FVC ($\text{FEF}_{25-75\%}$, expressed as a percentage of the predicted normal value), and peak expiratory flow (PEF). Spirometry (Vmax229, United States Sensor-Medics, Yorba Linda) was used to measure FEV_1 , FVC and $\text{FEF}_{25-75\%}$. PEF measurements were made with a Mini-Wright Peak Flow Meter (Shanghai Wanbo Scientific and Technological Ltd, Shanghai, China). Patients performed three measurements each morning and evening before taking their study medications, and recorded the best of the three measurements each time.

Patients were then prescribed a salmeterol/fluticasone propionate combination inhaler (SFC, 50/250 $\mu\text{g/puff}$) and instructed to take 50/250 μg twice daily during the run-in period. A salbutamol inhaler (Glaxo Wellcome, Chongqing, China) was also dispensed, and patients were instructed to use this as needed.

The patients were issued a diary card and asked to make two entries per day describing their salbutamol usage and the severity of their symptoms. Symptoms and salbutamol usage recorded in the morning indicate events from the previous night, and information recorded in the evening refers to events that took place during the day. At the end of 4-week run-in period, patients were asked to complete the five-question Asthma Control Test (ACT), which consists of five questions rated on a scale from 1 to 5 (Table 1).¹² The results of individual questions were added up to a total score, which ranged between 5 (completely uncontrolled asthma) and 25 (completely controlled

Table 1 Individual questions of the Asthma Control Test (ACT).

- 1 In the past 4 weeks, how much of the time did your asthma keep you from getting as much done at work, school or at home as usual?
- 2 During the past 4 weeks, how often have you had shortness of breath?
- 3 During the past 4 weeks, how often did your asthma symptoms (wheezing, coughing, shortness of breath, chest tightness or pain) wake you up at night or earlier than usual in the morning?
- 4 During the past 4 weeks, how often have you used your rescue inhaler or nebulizer medication (such as salbutamol)?
- 5 How would you rate your asthma control during the past 4 weeks?

Score of 1 = worst asthma control for each question; score of 5 = best asthma control for each question.

asthma). To undergo randomization, patients had to have a total ACT score of ≥ 10 at the end of 4-week run-in period. Patients with a total ACT score of < 10 were excluded due to the possibility that the severity of their illness might compel them to drop out of the study for safety reasons. Patients who had a total score of 25 at the end of 4-week run-in period were withdrawn.

Baseline and randomization

For the baseline measurement, pulmonary function was assessed. The diurnal PEF variability from the last week of the run-in period served as the baseline value. Before treatment, sputum induction was performed to measure the levels of inflammatory markers. Patients were then randomized to either 50/250 μg of salmeterol/fluticasone propionate combination plus 200 mg of theophylline or 50/250 μg of salmeterol/fluticasone propionate combination plus matched placebo. All doses were given twice daily. Randomization was stratified to ensure that the severity of symptoms was equal in the two groups. There were two strata of severity, which were defined according to FEV_1 : One group of patients had a FEV_1 less than 60% of the predicted normal value, and the other group had a FEV_1 greater than 60%. Blood samples were taken to measure baseline theophylline levels.

Study visits

During the treatment period, the patients returned to the clinic after 4, 8, 12, 16, 20 and 24 weeks of treatment. Their symptoms were recorded, and all patients were asked to complete the five-question Asthma Control Test (ACT) to assess asthma control. The incidence of exacerbations of asthma was recorded during the 24-week treatment period. Pulmonary function was measured at each visit. At the end of the 24-week treatment period, sputum induction was performed again to measure levels of inflammatory markers.

Assessments

The primary endpoints were asthma control and asthma exacerbations. Asthma control was assessed by scoring the

patients' responses to the ACT, which consists of five questions rated on a scale from 1 to 5 (Table 1). The results of individual questions were added up to yield a total score, which ranged from 5 (completely uncontrolled asthma) to 25 (completely controlled asthma).¹² Patients were placed into the following four categories based on their results: (1) < 16 , uncontrolled; (2) ≥ 16 to ≤ 19 , poorly controlled; (3) ≥ 20 to ≤ 24 , well controlled; and (4) 25, completely controlled. The incidence and severity of asthma exacerbations were recorded. Exacerbations of asthma were classified as mild (a deterioration in asthma symptoms requiring a clinically relevant increase in salbutamol use, which is defined as more than three additional inhalations per 24-h period with respect to baseline for > 2 consecutive days), moderate (requiring oral corticosteroids and/or antibiotics) or severe (requiring hospitalization).¹³ Severity of asthma exacerbations was judged by the investigator. Patients were withdrawn from the study in the event of moderate or severe exacerbations of asthma.

The secondary endpoints were changes of pulmonary function and the levels of inflammatory markers in induced sputum. Pulmonary function testing included measurements of FEV_1 , FVC, $\text{FEF}_{25-75\%}$ and peak expiratory flow (PEF). Spirometry (Vmax229, United States Sensor-Medics, Yorba Linda) was used to measure FEV_1 , FVC and $\text{FEF}_{25-75\%}$. PEF measurements were made with a Mini-Wright Peak Flow Meter (Shanghai Wanbo Scientific and Technological Ltd, Shanghai, China). After being instructed by the investigator, patients made three measurements each morning and evening before taking their study medications, and recorded the best of the three measurements each time. Diurnal variability in PEF was expressed as the mean value during the final week of the run-in period or the treatment period, and was calculated with the following formula: $(\text{highest daily value} - \text{lowest daily value}) / ((\text{highest daily value} + \text{lowest daily value}) / 2)$.

Sputum was induced and processed as previously described.¹⁴ An eosinophil count was performed using Wright's stain. Five hundred non-squamous cells were counted in Wright's-stained slides and the results were expressed as percentage of the non-squamous counts. The concentration of eosinophil cationic protein (ECP) was measured ($\mu\text{g}/\text{ml}$) by a radioimmunoassay kit (Pharmacia Diagnostics AB, Uppsala, Sweden). A skilled observer who was unaware of the clinical characteristics of the patients made cell counts and measurements of ECP concentration in sputum. If sputum induction was not performed at the end of the run-in period or the treatment period, the patient was withdrawn from the study.

Drugs and laboratory analysis

Patients were prescribed theophylline sustained-release tablets (each tablet contained 100 mg anhydrous theophylline, Guangzhou Maite Xinhua Pharmaceutical Factory, Guangzhou, China) or matched placebo. The salmeterol/fluticasone propionate combination product was purchased from Glaxo Wellcome (Chongqing, China). The inhalers used were identical in the two treatment groups.

The serum concentration of theophylline was detected using an automated chemiluminescence system (ACS 180, USA), with a range of sensitivity of 0.3–40 mg/L.

Statistical analysis

All continuous data are expressed as mean $\pm$ standard deviation (SD), and discrete data are expressed as percentages. Data were analyzed within groups by Student's paired *t*-test or between groups by the Mann–Whitney *U* test. Frequency comparisons were analyzed using the χ^2 test. A *p* value of less than 0.05 was considered to indicate a statistically significant difference. All statistical analysis was performed using SPSS14.0 software (Chicago, Illinois, USA).

Results

A total of four hundred seven patients were screened. Eighty-two patients withdrew prior to randomization because they do not meet the inclusion criteria. Three hundred twenty-five patients were randomized to treatment with either theophylline plus SFC (*n* = 162) or SFC plus placebo (*n* = 163). Of the 325 randomized patients, 60 patients withdrew (27 patients from the theophylline plus SFC group and 33 patients from the SFC plus placebo group). The reasons for withdrawal are shown in Table 2. One hundred thirty-five patients in the theophylline plus SFC group and 130 patients in the placebo plus SFC group completed the study according to the protocol and had valid measurements of the primary variables at baseline. Their demographic characteristics and baseline data from the end of the run-in period are outlined in Table 3. The demographic and baseline characteristics of the two groups were generally similar, with no significant differences at baseline in pulmonary function or the mean ACT score.

In the theophylline plus SFC group, the median serum theophylline concentration was 7.8 mg/L (range, 3.6–15.9 mg/L), which was below the reported therapeutic bronchodilator range of 10–20 mg/L.

Table 2 Reasons for withdrawal from study.

	Theophylline plus SFC group	SFC plus placebo group
No. of patients	162	163
No. of withdrawals	27	33
Exacerbations and uncontrolled asthma	11	24
Did not complete sputum induction	7	4
Non-compliance with study guidelines	3	1
Side effects attributed to medication	4	3
Other reasons	2	1

SFC, salmeterol/fluticasone propionate combination.

Table 3 Patient demographic and baseline data.

	Theophylline plus SFC group	SFC plus placebo group
No. of patients	135	130
Males/females (%)	59.3/40.7	53.8/46.2
Age (yr)		
Mean $\pm$ SD ^a	45.3 $\pm$ 12.4	46.1 $\pm$ 14.3
Range	18–65	18–68
Screening pulmonary function ^a		
FEV ₁ /FVC ratio	53.3 $\pm$ 12.7	52.9 $\pm$ 13.3
FEV ₁ (% of Predicted)	56.7 $\pm$ 11.3	55.8 $\pm$ 12.5
Reversibility in FEV ₁ (%)	21.3 $\pm$ 6.4	22.6 $\pm$ 7.1
FEF _{25–75%} (% of Predicted)	38.3 $\pm$ 17.9	39.2 $\pm$ 18.6
Diurnal PEF variability (%)	26.4 $\pm$ 7.8	27.2 $\pm$ 8.4
Baseline pulmonary function (after 4 wk treatment with SFC) ^a		
FEV ₁ /FVC ratio	64.4 $\pm$ 10.7	65.1 $\pm$ 11.8
FEV ₁ (% of Predicted)	68.8 $\pm$ 9.7	70.2 $\pm$ 8.9
Reversibility in FEV ₁ (%)	15.4 $\pm$ 5.9	16.3 $\pm$ 6.1
FEF _{25–75%} (% of Predicted)	44.3 $\pm$ 15.8	45.1 $\pm$ 16.7
Diurnal PEF variability (%)	21.8 $\pm$ 6.7	22.1 $\pm$ 7.1
Baseline ACT score ^a	16.3 $\pm$ 5.1	15.9 $\pm$ 4.6

^a Data are presented as mean $\pm$ standard deviation (SD); FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF_{25–75%}, forced expiratory flow between 25% and 75% of FVC; PEF, peak expiratory flow; SFC, salmeterol/fluticasone propionate combination; ACT, Asthma Control Test.

Sputum induction was successful in patients of both treatment groups who completed the study according to study protocol.

Assessment of asthma control by the ACT

According to the ACT responses, the majority of patients in both treatment groups had their asthma well or completely controlled at the end of the 24-week treatment period. Well-controlled asthma was achieved in 39.3% (53/135) and 39.2% (51/130) of patients in the theophylline plus SFC group and in the SFC plus placebo group, respectively, at the end of the treatment period (*p* = 0.996). Completely controlled asthma was achieved in 42.2% (57/135) and 43.1% (56/130) of patients in the SFC plus theophylline group and in the SFC plus placebo group, respectively, at the end of the treatment period (*p* = 0.888). We also found that 18.5% (25/135) and 17.7% (23/130) of patients had asthma that was insufficiently controlled in the SFC plus theophylline group and in the SFC plus placebo group, respectively, at the conclusion of the treatment period (*p* = 0.861). The two treatment groups had no patients with uncontrolled asthma. The full results are outlined in Table 4.

Table 4 Level of asthma control in the two treatment groups at the end of the 24-week treatment period as assessed by the Asthma Control Test.

	Uncontrolled (<16)	Poorly controlled (≥ 16 to ≤ 19)	Well controlled (≥ 20 to ≤ 24)	Completely controlled (25)
Theophylline plus SFC group	0	25	53	57
SFC plus placebo group	0	23	51	56
<i>p</i> value	—	0.861	0.996	0.888

SFC, salmeterol/fluticasone propionate combination.

There was not a statistically significant difference in the mean ACT score at baseline between the theophylline plus SFC group and the SFC plus placebo group (16.3 ± 5.1 and 15.9 ± 4.6 , respectively; $p > 0.05$). Both treatments resulted in an improvement of the mean ACT score at the end of the treatment period. In the theophylline plus SFC group, the mean ACT score increased from 16.3 ± 5.1 at baseline to 23.6 ± 4.2 at the conclusion of the treatment period ($p < 0.001$). In the SFC plus placebo group, the mean ACT score increased from 15.9 ± 4.6 at baseline to a final value of 22.9 ± 4.3 ($p < 0.001$).

The two treatment groups showed similar improvements in the mean ACT score ($p > 0.05$). These results are shown in Fig. 1.

Asthma exacerbations

Eight of the 162 patients randomized to the theophylline plus SFC group withdrew because of moderate asthma exacerbations, whereas 12 of the 163 patients randomized to the placebo plus SFC group withdrew for this reason; this difference did not reach statistical significance ($p = 0.363$). Further, 2 of 162 patients in the theophylline plus SFC group withdrew because of severe exacerbations

of asthma, whereas 11 patients randomized to the placebo plus SFC group withdrew for this reason, and this difference reached statistical significance ($p = 0.011$).

The total number of patients who had at least one asthma exacerbation was significantly lower in the SFC plus theophylline group than in the SFC plus placebo group (40 of 135 patients versus 61 of 130 patients, respectively; $p = 0.004$) during the 24-week treatment period. The SFC plus theophylline group had significantly fewer asthma exacerbations compared with the SFC plus placebo group (79 and 152 total exacerbations, respectively; $p = 0.023$; Fig. 2) during the 24-week treatment period.

Diurnal PEF variability and spirometry

The spirometry and diurnal PEF variability values observed for the two treatment groups are outlined in Table 5. Baseline FEV₁, FEV₁/FVC ratio and FEF_{25–75%} values were not significantly different between the two treatment groups ($p > 0.05$).

Both treatment regimens resulted in improvements in pulmonary function at the end of the 24-week treatment period. In the theophylline plus SFC group, following observations were noted: the FEV₁ values increased from $68.8 \pm 9.7\%$ at baseline to a final value of $82.4 \pm 10.2\%$ at

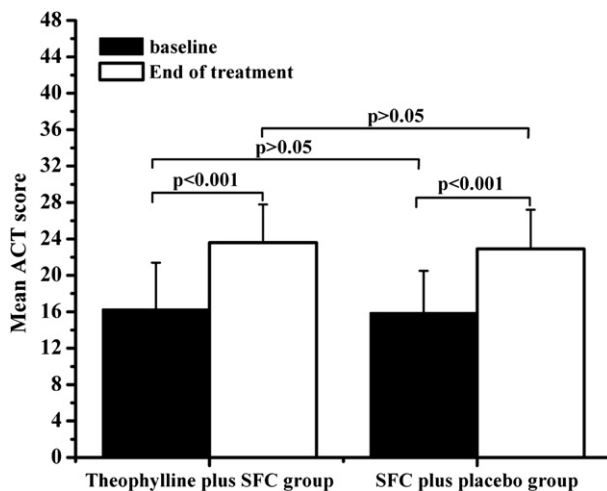


Figure 1 Mean and SD of ACT scores at baseline and the end of 24-week treatment period in the theophylline plus SFC group and the SFC plus placebo group. $p < 0.001$ compared with baseline; $p > 0.05$ compared with the SFC plus placebo group.

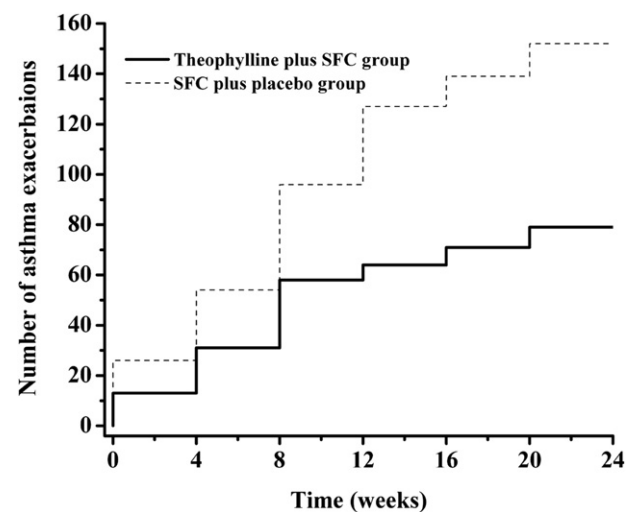


Figure 2 Cumulative asthma exacerbations in the theophylline plus SFC group and the SFC plus placebo group.

Table 5 Pulmonary function in the two treatment groups at baseline and the end of the 24-week treatment period.

	Theophylline plus SFC group	SFC plus placebo group
FEV ₁ (% of Predicted)		
Baseline	68.8 ± 9.7	70.2 ± 8.9
End of treatment	82.4 ± 10.2 ^a	81.3 ± 8.7 ^a
FEV ₁ /FVC ratio (%)		
Baseline	64.4 ± 10.7	65.1 ± 11.8
End of treatment	80.9 ± 8.7 ^a	81.2 ± 9.1 ^a
FEF _{25–75%} (% of Predicted)		
Baseline	44.3 ± 15.6	45.1 ± 16.7
End of treatment	66.9 ± 18.8 ^{a,b}	57.4 ± 17.6 ^a
Diurnal PEF variability (%)		
Baseline	21.8 ± 6.7	22.1 ± 7.1
End of treatment	10.9 ± 3.2 ^a	11.5 ± 3.4 ^a

Data are presented as mean ± standard deviation (SD); FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; FEF_{25–75%}, forced expiratory flow between 25% and 75% of FVC; PEF, peak expiratory flow; SFC, salmeterol/fluticasone propionate combination.

^a $p < 0.001$ compared with baseline.

^b $p < 0.001$ compared with SFC plus placebo group.

the conclusion of the treatment period ($p < 0.001$); the FEV₁/FVC ratio increased from 64.4 ± 10.7% at baseline to a final value of 80.9 ± 8.7% ($p < 0.001$); FEF_{25–75%} increased from a baseline value of 44.3 ± 15.8% to 66.9 ± 18.8% at the end of the treatment period ($p < 0.001$); and diurnal FEF variability decreased from 21.8 ± 6.7% at baseline to 10.9 ± 3.2% at the end of the treatment period ($p < 0.001$). In the SFC plus placebo group, the following improvements were recorded: the FEV₁ values increased from 70.2 ± 8.9% at baseline to a final value of 81.3 ± 8.7% ($p < 0.001$); the FEV₁/FVC ratio rose from 65.1 ± 11.8% at baseline to a final value of 81.2 ± 9.1% ($p < 0.001$); FEF_{25–75%} increased from a baseline value of 45.1 ± 16.7% to 57.4 ± 17.6% at the end of the treatment period ($p < 0.001$); diurnal PEF variability decreased from 22.1 ± 7.1% at baseline to 11.5 ± 3.4% at the end of the treatment period ($p < 0.001$).

Analyses of overall treatment effects of two treatments showed similar improvements in FEV₁, FEV₁/FVC ratio and diurnal PEF variability (all $p > 0.05$). SFC plus theophylline was superior to SFC plus placebo in terms of FEF_{25–75%} values ($p < 0.001$).

Inflammatory markers in induced sputum

The results of assays for inflammatory markers in the induced sputum of patients are outlined in Table 6. The eosinophil counts and ECP concentrations were not significantly different between the two treatment groups at baseline (all $p > 0.05$).

Both treatments resulted in an improvement in the levels of inflammatory markers in induced sputum at the end of 24-week treatment period. In the theophylline plus SFC group, a comparison of the baseline observations to those made at the end of 24-week treatment revealed that there were reductions in both the eosinophil count (13.1 ± 6.2% to 4.1 ± 2.2%) and concentration of ECP

Table 6 Inflammatory markers detected in the induced sputum of the two treatment groups at baseline and at the end of the 24-week treatment period.

	Theophylline plus SFC group	SFC plus placebo group
Eosinophil (%)		
Baseline	13.1 ± 6.2	12.7 ± 5.8
End of treatment	4.1 ± 2.2 ^{a,b}	6.3 ± 2.7 ^a
Macrophage (%)		
Baseline	47.6 ± 11.8	48.1 ± 12.1
End of treatment	61.8 ± 10.7	58.9 ± 12.9
Neutrophil (%)		
Baseline	30.7 ± 11.8	31.9 ± 12.7
End of treatment	25.1 ± 9.2	26.8 ± 11.1
Lymphocyte (%)		
Baseline	3.8 ± 1.7	4.2 ± 1.9
End of treatment	3.5 ± 1.8	4.3 ± 2.4
ECP (μg/L)		
Baseline	176.4 ± 89.4	171.7 ± 93.4
End of treatment	63.6 ± 39.5 ^{a,b}	89.4 ± 45.6 ^a

Data are presented as mean ± standard deviation (SD); ECP, eosinophil cationic protein; SFC, salmeterol/fluticasone propionate combination.

^a $p < 0.001$ compared with baseline.

^b $p < 0.01$ compared with SFC plus placebo group.

(176.4 ± 89.4 to 63.6 ± 39.5 μg/L) in induced sputum (all $p < 0.001$). In the SFC plus placebo group, there were also reductions in eosinophil count (12.7 ± 5.8% to 6.3 ± 2.7%) and ECP concentration (171.7 ± 93.4 to 89.4 ± 45.6 μg/L) in induced sputum when comparing baseline observations to those made at the end of 24-week treatment period (all $p < 0.001$).

Our analyses of the overall impact of both treatment combinations revealed greater improvements in eosinophil counts and ECP concentrations in the induced sputum of patients belonging to the SFC plus theophylline group than in those of the SFC plus placebo group (all $p < 0.01$).

Discussion

The results of our present study clearly demonstrated that both treatments resulted in improvement in the control of asthma, as assessed by the ACT, pulmonary function and inflammatory markers in induced sputum. Both SFC plus theophylline and SFC alone produced similar results with regards to asthma control and pulmonary function, as seen by the changes in the FEV₁, FEV₁/FVC ratio and diurnal FEF variability. However, our study also showed that there was a significant reduction in the number of patients with at least one exacerbation, as well as the cumulative number of asthma exacerbations, when comparing the SFC plus theophylline group to the SFC plus placebo group. Furthermore, improvements in FEF_{25–75%} and control of eosinophilic airway inflammation were greater in the SFC plus theophylline group than in the SFC plus placebo group.

In the majority of patients, asthma can be reasonably well controlled with simple regimens of inhaled drugs. However, some patients suffer from frequent asthma exacerbations, which results in days of absence from work

or school, the need for emergency treatment or admission to hospital. Such exacerbations are associated with morbidity and account for a substantial proportion of the total economic costs of this disease.¹⁵ Therefore, prevention of asthma exacerbations is an important goal in the management of asthma. At the end of 24-week treatment period of our study, the asthma of the majority of patients was well or completely controlled in the two treatment groups according to the ACT. However, the number of patients who had at least one asthma exacerbation, and cumulative number of asthma exacerbations, were significantly lower in the SFC plus theophylline group than in the SFC plus placebo group. Accordingly, for preventing asthma exacerbations, our data suggests that the combination of SFC plus theophylline may be superior to administration of SFC alone.

Asthma is characterized by chronic airway inflammation and associated airway hyperresponsiveness. Inflammation in asthma involves both large and small airways.¹⁶ Evidence suggests that inflammation in small airways, which are <2 mm in diameter, contributes significantly to the pathogenesis of asthma.¹⁷ Small airway involvement in asthma is prominent in severe persistent asthma,¹⁸ and has also been demonstrated to play a role in mild to moderate asthma.¹⁹ Early closure of small airways, perhaps due to uncontrolled inflammation, has been shown to characterize patients with "difficult to control asthma".²⁰ Thus, small airways may be an important therapeutic target.²¹ In order to assess expiratory airflow obstruction in asthma patients, FEV₁ or PEF are generally used as measures of lung function, as they have been demonstrated to have greater reproducibility than other measures; however, they preferentially reflect changes in the large airways. Due to the difficulty of *in vivo* sampling and a lack of pulmonary function tests specific to this site, small airway obstruction in patients with asthma has been largely under-evaluated. The forced expiratory flow between 25% and 75% of forced vital capacity (FEF_{25–75%}) is the spirometric variable most commonly cited as an indicator of small airway obstruction.²² In our study, the two treatment combinations employed showed similar improvements in FEV₁, FEV₁/FVC ratio and diurnal FEF variability; however, the SFC plus theophylline group was superior to SFC alone in terms of FEF_{25–75%} measurements. Our data thus indicates that the addition of theophylline to SFC provided a greater benefit than SFC alone on the function of small airways in patients with asthma.

Ideally, assessment of asthma severity and effectiveness of treatment should be guided directly by the degree of airway inflammation.¹ At present, sputum induction has been proposed as a relatively less invasive method for assessing airway secretion, and provided markers of airway inflammation are in agreement with those from bronchoalveolar lavage and biopsy specimens in asthmatic patients.²³ Sputum induction has evolved to a point where it is repeatable, valid and responsive, and it appears to provide meaningful information about inflammatory events in the lower airways. The use of sputum to investigate airway inflammation also has the great advantage of being non-invasive and thus safe, feasible and suitable for repeated measurement in most patients.²⁴ In our study, eosinophil count and ECP concentration were similar in

both treatment groups at baseline, though greater improvements in eosinophil count and concentration of ECP in induced sputum were observed in the SFC plus theophylline group than in the SFC plus placebo group. Our data suggests that the combination of theophylline plus SFC may have a greater effect on airway inflammation in patients with asthma. Although ICS anti-inflammatory treatment, with or without long-acting β_2 agonists (LABAs), is the cornerstone of asthma management, the most commonly used formulations are deposited in relatively low amounts in the peripheral airways, a reflection of their unfavorable aerodynamic properties.²⁵ Anti-inflammatory treatment needs to be directed to both large and distal airways if maximal suppression of airway inflammation is to be achieved.²⁶ We therefore postulate that the greater improvement in airway inflammation in the SFC plus theophylline group than in the SFC plus placebo group may be attributed, at least in part, to poor delivery of inhaled medications to small airways, leading to insufficient control of inflammation; oral theophylline, on the other hand, would appear to have access to this area of the lung through the circulation. However, specific studies are necessary to confirm the potential action of theophylline on small airway inflammation.

Theophylline is used worldwide for the treatment of asthma. It is a bronchodilator that also has extrapulmonary effects, including anti-inflammatory effects.^{8,9} The bronchodilator effect of theophylline may be related to phosphodiesterase inhibition, which is observed at high serum concentrations (10–20 $\mu\text{g/mL}$), while the anti-inflammatory effect is observed at lower serum concentrations (5–10 $\mu\text{g/mL}$ theophylline).²⁷ In our study, the median serum theophylline concentration was 7.8 $\mu\text{g/mL}$ (range, 3.6–15.9 $\mu\text{g/mL}$) in the theophylline plus SFC group. Therefore, we propose that the superior treatment outcomes seen with the addition of theophylline with regards to the number of asthma exacerbations and FEF_{25–75%} may be related to its anti-inflammatory properties.

In summary, our results show that the addition of theophylline is beneficial in asthma patients who are also being treated with inhaled corticosteroids and long-acting β_2 agonists. The observed effects were achieved at low dose of theophylline, thus minimizing adverse side effects.¹⁰ Improved protection against asthma exacerbations due to improvements in small airway function parameters and reduced airway inflammation may be achieved with theophylline in combination with SFC.

Conflict of interest

The authors state that they have no conflict of interests.

References

1. Global Strategy or Asthma Management and Prevention. Global initiative for asthma (GINA) (revised 2009). Available from: <http://www.ginasthma.org>.
2. Levy ML, Thomas M, Small I, Pearce L, Pinnock H, Stephenson P. Summary of the 2008 BTS/SIGN British Guideline on the management of asthma. *Prim Care Respir J* 2009;18: S1–16.

3. National Asthma Education and Prevention Program. Expert panel report 3 (EPR3): guidelines for the diagnosis and management of asthma-summary report 2007. *J Allergy Clin Immunol* 2007;**120**:S94–138.
4. Bateman ED, Boushey HA, Bousquet J, et al. Can guideline-defined asthma control be achieved? The gaining optimal asthma control study. *Am J Respir Crit Care Med* 2004;**170**: 836–44.
5. Rabe KF, Adachi M, Lai CK, et al. Worldwide severity and control of asthma in children and adults: the global asthma insights and reality surveys. *J Allergy Clin Immunol* 2004;**114**: 40–7.
6. McFadden Jr ER. Methylxanthines in the treatment of asthma: the rise, the fall, and the possible rise again. *Ann Intern Med* 1991;**115**:323–4.
7. Persson CG, Draco AB. Xanthines as airway anti-inflammatory drugs. *J Allergy Clin Immunol* 1988;**81**:615–7.
8. Sullivan P, Bekir S, Jaffar Z, Page C, Jeffery P, Costello J. Anti-inflammatory effects of low-dose oral theophylline in atopic asthma. *Lancet* 1994;**343**:1006–8.
9. Kidney J, Dominguez M, Taylor PM, Rose M, Chung KF, Barnes PJ. Immunomodulation by theophylline in asthma: demonstration by withdrawal of therapy. *Am J Respir Crit Care Med* 1995;**151**:1907–14.
10. Evans DJ, Taylor DA, Zetterstrom O, Chung KF, O'Connor BJ, Barnes PJ. A comparison of low-dose inhaled budesonide plus theophylline and high-dose inhaled budesonide for moderate asthma. *N Engl J Med* 1997;**337**:1412–8.
11. Ukena D, Harnest U, Sakalauskas R, et al. Comparison of addition of theophylline to inhaled steroid with doubling of the dose of inhaled steroid in asthma. *Eur Respir J* 1997;**10**: 2754–60.
12. Nathan RA, Sorkness CA, Kosinski M, et al. Development of the Asthma Control Test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004;**113**:59–65.
13. Ringdal N, Eliraz A, Pruzinec R, et al. The salmeterol/-fluticasone combination is more effective than fluticasone plus oral montelukast in asthma. *Respir Med* 2003;**97**:234–41.
14. Hanxiang N, Jiong Y, Yanwei C, et al. Persistent airway inflammation and bronchial hyperresponsiveness in patients with totally controlled asthma. *Int J Clin Pract* 2008;**62**: 599–605.
15. Gendo K, Lodewick MJ. Asthma economics: focusing on therapies that improve costly outcomes. *Curr Opin Pulm Med* 2005; **11**:434–50.
16. Tulic MK, Hamid Q. New insights into the pathophysiology of the small airways in asthma. *Clin Chest Med* 2006;**27**:41–52.
17. Hamid Q, Song Y, Kotsimbos TC, et al. Inflammation of small airways in asthma. *J Allergy Clin Immunol* 1997;**100**:44–51.
18. van Veen IH, Sterk PJ, Schot R, Gauw SA, Rabe KF, Bel EH. Alveolar nitric oxide versus measures of peripheral airway dysfunction in severe asthma. *Eur Respir J* 2006;**27**:951–6.
19. Bergeron C, Hauber HP, Gotfried M, et al. Evidence of remodeling in peripheral airways of patients with mild to moderate asthma: effect of hydrofluoroalkane-flunisolide. *J Allergy Clin Immunol* 2005;**116**:983–9.
20. Kraft M. The distal airways: are they important in asthma? *Eur Respir J* 1999;**14**:1403–17.
21. Tulic MK, Christodoulopoulos P, Hamid Q. Small airway inflammation in asthma. *Respir Res* 2001;**2**:333–9.
22. Burgel PR. The role of small airways in obstructive airway diseases. *Eur Respir Rev* 2011;**20**:23–33.
23. Grootendorst DC, Sont JK, Willems LN, et al. Comparison of inflammatory cell counts in asthma: induced sputum vs bronchoalveolar lavage and bronchial biopsies. *Clin Exp Allergy* 1997;**27**:769–79.
24. Pavord ID, Pizzichini MM, Pizzichini E, Hargreave FE. The use of induced sputum to investigate airway inflammation. *Thorax* 1997;**52**:498–501.
25. Leach C, Colice GL, Luskin A. Particle size of inhaled corticosteroids: does it matter? *J Allergy Clin Immunol* 2009;**124**: S88–93.
26. Howarth P. Small airway inflammation and asthma. *Int J Clin Pract Suppl* 1998;**96**:15–22.
27. Makino S, Fueki M, Fueki N. Efficacy and safety of methylxanthines in the treatment of asthma. *Allergol Int* 2004;**53**:13–22.