Comparison between the Effects of Generic and Original Salmeterol/Fluticasone Combination (SFC) Treatment on Airway Inflammation in Stable Asthmatic Patients

Kittipong Maneechotesuwan MD, PhD*, Jirawatana Assawabhumi BSc*,
Kanokwan Rattanasaengloet BSc*, Tasneeya Suthamsmai MSc*,
Sutat Pipopsuthipaiboon BSc*, Suthipol Udompunturak MSc**

** Division of Respiratory Diseases and Tuberculosis, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*
*** Department of Health Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand*

Background: *Little is known about the effect of inhaled corticosteroids (ICS)/long-acting β_2 agonists (LABA) in combination on inflammatory markers in asthma. In addition, therapeutic equivalence of generic salmeterol/fluticasone combination (SFC) and original SFC is as yet unknown.*
Objective: *To determine the effects of SFC and the effects of generic and original SFC on airway inflammation in patients with mild-to moderate stable asthma.*
Material and Method: *A randomized double-blinded, crossover non-inferiority study was conducted to compare the anti-inflammatory effects of generic SFC and original SFC on sputum eosinophils as a primary outcome and fractional exhaled nitric oxide (FENO) as a secondary outcome.*
Patients: *The authors studied 51 mild-to-moderate asthmatic patients who ranged from 18 to 80 years of age and were treated with ICS or ICS/LABA of any dose, and whose asthma was stable without an exacerbation episode for at least 3 months prior to study entry.*
Results: *Both sputum eosinophils percentage and absolute eosinophil counts well correlated with FENO levels at baseline prior to the initiation of study medications. Significant reduction in sputum eosinophil percentage was observed following generic SFC and original SFC treatment. The degree of sputum eosinophil suppression by generic SFC was not inferior to original SFC, and this was not affected by treatments with the sequence of generic SFC first vs. original SFC second or original SFC first vs. generic SFC. In addition, there was no significant difference between treatments in terms of normalized gain in asthma control scores, including the number of patients found to have improved asthma control, irrespective of sequence, as change from baseline. However, this was not the case for the magnitude of FENO reduction that occurred after generic SFC treatment to a significantly larger extent than original SFC treatment.*
Conclusion: *This short-term study demonstrated that there was no significant difference between generic SFC and original SFC in terms of anti-inflammatory activity and the control of asthma symptoms. However, it is completely unknown whether generic SFC could effectively prevent the development of asthma exacerbations on a long-term basis. Therefore, longer-term studies are indicated to evaluate generic SFC's relative efficacy on asthma exacerbations.*

Keywords: *Asthma, Salmeterol/fluticasone combination, Sputum eosinophils*

J Med Assoc Thai 2014; 97 (Suppl. 3): S91-S100
Full text. e-Journal: *http://www.jmatonline.com*

Asthma is characterized by chronic airway inflammation that primarily involves eosinophils[1-4]. Eosinophilic airway inflammation in mild to moderate asthmatics is driven by Th2 cytokines[5-8] while neutrophilic airway inflammation in severe asthmatics is at least in part driven by Th17 cytokines[9,10]. The important effector cell eosinophils increase and are activated in asthma[11,12]. They play several critical roles in asthma, which are to induce asthma exacerbation[13,14] and undermine asthma control[15-17]. There are several studies addressing the fact that eosinophils can be

Correspondence to:
Maneechotesuwan K, Division of Respiratory Disease and Tuberculosis, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, 2 Prannok Road, Bangkok 10700, Thailand.
Phone: 0-2419-7757, Fax: 0-2419-7760
E-mail: sikmn@mahidol.ac.th

used as an inflammatory marker for adjusting asthma therapy for the individuals to enhance asthma control and reduce asthma exacerbations[18-21].

Exhaled nitric oxide is a non-invasive marker for monitoring the inflammatory activity in asthma. Nitric oxide (NO) in orally exhaled air is mainly derived from the respiratory epithelium. NO is synthesized by inducible NO synthase (iNOS), which is regulated by signal transducer and activator of transcription (STAT)-1 under the influence of homeostatic interferon-γ[22-24]. In patients with asthma, iNOS expression is upregulated by interleukin (IL)-4 and IL-13 via the activation of STAT-6 in the bronchial epithelium[25-28]. Therefore, exhaled NO primarily signals local T-helper cell type 2-driven inflammation in the bronchial mucosa. With these characteristics, exhaled NO will be a suitable marker for predicting the response to inhaled corticosteroids, and to monitor the anti-inflammatory effect. Fractional nitric oxide (FENO) measurements have been shown to correlate with sputum eosinophil counts in adults with mild asthma[29]. Berry et al examined the relationship between FENO levels and sputum eosinophils in adults with asthma and found a significant, but moderate, correlation[30]. Another study demonstrated that there was a significant but weak correlation between FENO and eosinophil differential counts in subjects with steroid-naive asthma[31]. FENO levels were significantly lower in the subjects with asthma taking steroids compared with those not doing so[31].

Inhaled corticosteroids (ICS) are by far the most effective therapy for controlling airway inflammation in asthma. The addition of long-acting β_2 agonists (LABAs) is necessary for asthmatic patients whose symptoms are unable to be controlled with ICS only. The underlying molecular mechanisms of the synergistic anti-inflammatory effects of combined ICS/LABA are partly mediated by enhancing glucocorticoid receptor nuclear translocation and restoring anti-inflammatory enzyme indoleamine 2, 3 dioxygenase (IDO)[32,33]. However, in clinical perspective, there are only few data that demonstrate the effects of ICS/LABA in combination on eosinophilic airway inflammation[34-37] although the combination therapy is widely used in clinical practice. In addition, none of the above studies demonstrated consistent results. Some studies demonstrated that there was no significant difference between salmeterol plus fluticasone propionate in combination (SFC) and fluticasone (FP) with montelukast treatment in the control of eosinophilic airway inflammation in asthma[37]. In contrast, other studies suggested that SFC treatment could initially reduce sputum eosinophils to a greater extent than FP treatment alone[35,36]; however, this effect was not sustainable[35]. Therefore, overall, during the 1-yr treatment period there were no significant differences between FP and SFC in the reduction of the numbers and percentages of sputum eosinophils[35], which is consistent with the data of combined budesonide/formoterol studies[38]. In addition to these data, some studies addressed the point that double dose of FP alone in comparison with SFC provides superior anti-inflammatory effects on surrogate inflammatory markers[39]. All the above conflicting data led us to test direct anti-inflammatory effects of combined FP/salmeterol (generic SFC and original SFC) and determine whether there was a difference in the efficacy of generic and original SFC used as a maintenance therapy on airway inflammation of stable asthmatic patients.

Material and Method

Subjects

Eligible non-smoker patients were stable and had experiences of mild to moderate persistent asthma for at least 3 months prior to study entry (15 men and 36 women; age range, 26 to 79 years; mean [$\pm$ SEM] age, 55.98$\pm$1.72 yrs); none had received a course of therapy with oral corticosteroids prior to study entry. Asthma was diagnosed using the American Thoracic Society criteria. All subjects had been treated with constant daily dose of inhaled corticosteroids (a minimum of 250 µg/day of fluticasone propionate, 400 µg/day of budesonide, 500 µg/day of beclomethasone dipropionate) in the presence or the absence of long-acting β_2 agonists (LABAs). Subjects had a baseline FEV_1 of $\geq$70% predicted and demonstrated a reversibility of FEV_1 after therapy with inhaled salbutamol (400 µg) of $\geq$12% and a provocative concentration of a substance (methacholine) causing a 20% fall in FEV_1 (PC_{20}) of $\leq$8 mg/mL. Exclusion criteria were asthma exacerbation, a respiratory tract infection within 4 weeks before study initiation or during the study period. Four patients were withdrawn from study during the randomized treatment period because of their asthma exacerbations, accident or study medication-related rash. Written informed consent was obtained from each patient, and the study was approved by the Ethics Review Committees of Siriraj Hospital.

Study design

In this double-blinded, non-inferiority study, patients received each of 2 treatments in a random

sequence for 4 weeks in a crossover design (2 puffs of 25/125 μg of generic SFC (Seroflo™; Cipla, India) and equivalent dose of comparator original SFC (Seretide™; GlaxoSmithKline, Brentford, UK) via metered dose inhaler twice a day), with a 2-week washout phase between rounds of therapy. Patients entered an initial 2-week run-in period in which all previous asthma controllers were discontinued and inhaled short-acting β_2-agonist, which was administered via metered-dose inhaler, used as rescue medication during the study period. The primary end point was the change in sputum eosinophil number after generic SFC treatment compared with the comparator original SFC, with changes in fractional exhaled nitric oxide (FENO) being the secondary end point. Measurements for sputum eosinophil numbers, FENO levels, and asthma control test (ACT)[47] and Siriraj asthma control questionnaire (ACQ)[48] scores were made before and after each randomized treatment. The randomized code was withheld from the investigators until completion of the present study. The present study medication was packed by the central pharmacy according to the randomization code.

Fractional exhaled nitric oxide (FENO)

FENO was measured simultaneously by a chemiluminescence analyzer (model LR2000; Logan Research; Rochester, UK). The analyzer is sensitive to nitric oxide (NO) concentrations from 1 to 500 parts per billion (ppb) by volume, with a resolution of 0.3 ppb. The analyzer was calibrated using certified NO mixtures (i.e, 90 and 436 ppb) in nitrogen (BOC Special Gases; Guildford, UK). Measurement was made by slow exhalation (5 to 6 L/min) from total lung capacity for 15 to 20 s against a low resistance (5 cm H_2O) to exclude nasal contamination.

Sputum induction and processing

Sputum was induced by the inhalation for 15 min of a 3.5% saline solution via an ultrasonic nebulizer (model 2000; DeVilbiss Co; Heston, UK), as previously described. Briefly, the whole sputum sample was processed with dithiothreitol (Sigma Chemicals; Poole, UK). The homogenized sputum was centrifuged at 300 g for 10 min. The supernatant was separated and frozen at -70°C until further analysis. Total cell counts were made on a hemocytometer slide, using Kimura stain, and slides were prepared with a cytospin device (Shandon; Runcorn, UK) and were stained with May-Grunwald-Giemsa stain. Differential cell counts were made by a blinded observer. A minimum of three hundred non-squamous cells were counted on two slides for each sample. Differential cell counts are expressed as the percentages of non-squamous cells.

Statistical analysis

The analysis was performed for testing non-inferiority of 50/250 μg twice daily of generic SFC versus 50/250 μg twice daily of original SFC for the primary end point (15% non-inferiority margin, 3% sputum eosinophils). The secondary end point were the changes in exhaled nitric oxide levels. The within-patient treatment differences were calculated and then analyzed by Mann-Whitney U test. An ANOVA mixed model was fitted for all end points, with the factors sequence, period, and treatment fitted as fixed effects and subject within the sequence fitted as a random effect. If the assumptions of the model were satisfied, adjusted means, differences versus comparator (original SFC), standard error of the mean (SEM), 95% CIs, and p-values based on a 1-sided test were presented. The test The Z-test for non-inferiority margin was the pre-specified analysis, and the power was based on testing whether generic SFC was non-inferior to comparator. Statistical analysis was performed with PASW statistics 18 (SPSS, IBM, Somers, NY).

Assuming a success rate of 90% in reducing at least 3% sputum eosinophil at the end of inhaled therapy for both generic SFC and original SFC, the number of patients required to establish 15% non-inferiority margin with 80% power was 50 in each study group.

Because of the ceiling and floor effects, normalized gain in ACT and ACQ scores calculated according to Hake et al[40] were used to evaluate the efficacy of generic SFC and original SFC in controlling asthma symptoms using Wilcoxon signed ranks test.

Results

Patient characteristics

The majority of asthmatic patients was female. All patients had severe airway hyperresponsiveness. About half of the participant patient had other comorbid illnesses. Most patients were treated with ICS and LABA in combination at the medium dose of ICS (Table 1). The authors also analyzed patients' compliance to the study medications using the differences of canister weight before use and after return. We found that there was no significant difference in their compliance to generic SFC and original SFC (mean $\pm$ SEM, 8.26$\pm$0.35 g vs. 8.54$\pm$0.46 g, respectively, p = 0.7).

There was no significant difference in anti-

Table 1. Characteristics of study asthmatic patients

	n = 51
Mean age (year) (SD)	55.9±12.3
Male sex, n (%)	29.4
Median equivalent beclomethasone daily dose (μg) (IQR)	640.0 (400.0-1,000)
LABA use (%)	70.5
Other asthma controllers (%)	29.4
Geometric mean PC_{20} (mg/ml) (CV)	1.7 (103.9)
Comorbid illness (%)	52.9

LABA = long-acting β_2 agonist

inflammatory effects of generic SFC and original SFC treatment.

The authors determined anti-inflammatory effects of generic SFC and original SFC treatment on sputum inflammatory cells in asthmatic patients. Both generic SFC and original SFC exerted their anti-inflammatory action on eosinophils by significantly reducing the percentage of eosinophils in the sputum after 4 weeks of treatment (means ± SEM, 7.6%±1.5% vs. 3.6%±0.8%, p = 0.036; 6.6%±1.6% vs. 3.6%±0.9%, p = 0.047, respectively) (Fig. 1A and 1B). This was consistent with FENO data in that after treatment, both generic SFC and original SFC could significantly suppress FENO levels (means ± SEM, 55.8 ppb±6.2 ppb vs. 31.2 ppb±2.9 ppb, p = 0.002; 50.4 ppb±5.2 ppb vs. 33.7 ppb±4.6 ppb, p = 0.01). On the other hand, there was no significant difference in the magnitude of reduction in the percentage and absolute number of sputum eosinophils (means ± SEM [95% CI], -4.7%±1.3% [-7.3 to -2.1] vs. -3.0%±1.5% [-6.0 to -0.04], p = 0.37 and -0.05±0.01 [-0.07 to -0.02] vs. -0.03±0.02 [-0.1 to 0.01], p = 0.26), neutrophils (3.9%±2.7% [-1.5 to 9.1] vs. 1.5%±3.0% [-4.5 to 7.5], p = 0.54 and -0.01±0.04 [-0.1 to 0.1] vs. -0.01±0.07 [-0.1 to 0.1], p = 0.98) and macrophages (0.1%±2.7% [-5.3 to 5.5] vs. 2.2%±3.1% [-4.0 to 8.5], p = 0.47 and -0.04± 0.03 [-0.1 to 0.02] vs. -0.05±0.03 [-0.1 to 0.01], p = 0.87, respectively) including total cell count (-0.1±0.1 [-0.2 to 0.01] vs. -0.1±0.1 [-0.2 to 0.1], p = 0.66) between generic and original SFC treatment (Fig. 1C and 1D) (Table 2). Moreover, there was no difference between treatments for the sequence of generic SFC first vs. original SFC second or original SFC first vs. generic SFC second (Table 2). However, the magnitude of the reduction in FENO levels was significantly greater in generic SFC group than that in original SFC group (p = 0.036) irrespective of the treatment sequence (Fig. 1E). There was non-inferiority in the percentages of asthmatic patients who were treated with generic and original SFC and whose sputum eosinophils was reduced by at least 3% (39.2% vs. 29.4%, p = 0.004 for 15% non-inferiority margin, respectively).

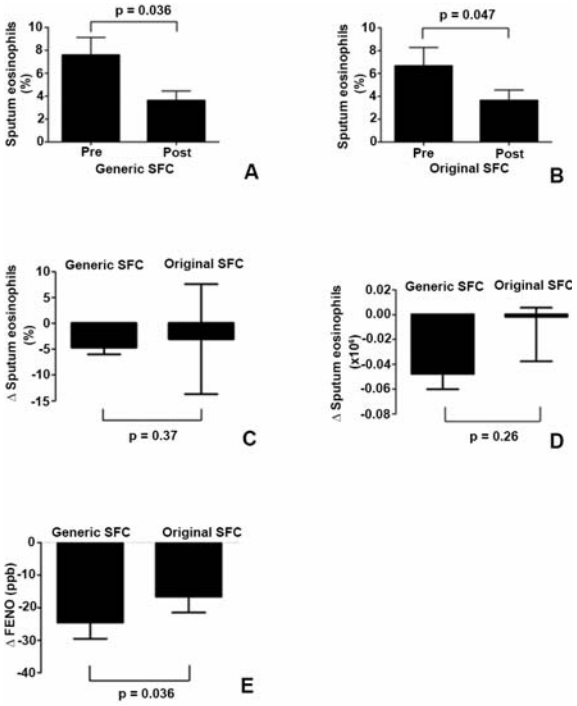

Fig. 1 The effect of generic and original salmeterol/fluticasone combination (SFC) on sputum eosinophil %, absolute eosinophil count and fractional exhaled nitric oxide (FENO). Reduction in sputum eosinophil % after treatment with generic SFC (A) and original SFC (B). Comparison in Δ sputum eosinophil % (C) and Δ absolute sputum eosinophils (D) between generic and original SFC treatment. (E) Comparison in Δ FENO between generic and original SFC treatment.

Table 2. Anti-inflammatory effect of generic SFC and original SFC treatment

Sputum cells	Generic SFC treatment	Original SFC treatment	p-value	95% CI for mean difference	Effects by order p-value
Δ Total cells ($x10^6$)	-0.1±0.1 [-0.2 to 0.01]	-0.1±0.1 [-0.2 to 0.1]	0.66	-0.1 to 0.2	0.39
Δ Neutrophil number ($x10^6$)	-0.01±0.04 [-0.1 to 0.1]	-0.01±0.07 [-0.1 to 0.1]	0.98	-0.16 to 0.2	0.59
Δ Neutrophils (%)	3.9±2.7 [-1.5 to 9.1]	1.5±3.0 [-4.5 to 7.5]	0.54	-9.9 to 5.3	0.8
Δ Eosinophil number ($x10^6$)	-0.05±0.01 [-0.07 to -0.02]	-0.03±0.02 [-0.1 to 0.01]	0.26	-0.02 to 0.1	0.32
Δ Eosinophils (%)	-4.7±1.3 [-7.3 to -2.1]	-3.0±1.5 [-6.0 to -0.04]	0.37	-2.1 to 5.4	0.27
Δ Macrophage number ($x10^6$)	-0.04±0.03 [-0.1 to 0.02]	-0.05±0.03 [-0.1 to 0.01]	0.87	-0.01 to 0.1	0.84
Δ Macrophages (%)	0.1±2.7 [-5.3 to 5.5]	2.2±3.1 [-4.0 to 8.5]	0.47	-5.6 to 9.7	0.59
Δ Lymphocyte number ($x10^6$)	0.0004±0.0003 [-0.0002 to 0.001]	0.01±0.01 [-0.006 to 0.02]	0.33	-0.01 to 0.02	0.37
Δ Lymphocytes (%)	0.09±0.06 [-0.02 to 0.2]	0.06±0.03 [-0.01 to 0.1]	0.5	-0.16 to 0.1	0.24
Δ Airway epithelial cell number ($x10^6$)	0.01±0.005 [-0.003 to 0.02]	0.003±0.002 [-0.003 to 0.01]	0.36	-0.02 to 0.01	0.62
Δ Airway epithelial cells (%)	0.6±0.05 [-0.3 to 1.6]	-0.2±0.4 [-0.9 to 0.6]	0.19	-2.0 to 0.4	0.36
Δ FENO (ppb)	-24.6±4.9 [-34.5 to -14.6]	-16.6±4.8 [-26.3 to -7.0]	0.036	0.5 to 15.3	0.87

All data are expressed as mean ± SEM [95% CI]
FENO = fractional exhaled nitric oxide; PC_{20} = concentration of methacholine that reduces the FEV_1 by 20%

There was a significantly positive correlation between FENO levels and sputum eosinophil numbers. The authors determined whether FENO levels correlated with sputum eosinophil percentage and the absolute number of sputum eosinophils prior to the treatment with generic and original SFC. The authors demonstrated that there was a significant association between FENO levels and sputum eosinophil % ($r = 0.57$, $p<0.001$ and $r = 0.42$, $p = 0.002$, respectively) (Fig. 2A and 2B) as well as absolute eosinophil counts ($r = 0.53$, $p<0.001$ and $r = 0.45$, $p = 0.0007$, respectively) in generic SFC and original SFC groups (Fig. 2C and 2D), suggesting that FENO can be used as an inflammatory marker in the present study. After treatment, the reduction in FENO levels significantly and positively, albeit weakly, correlated with the reduction of sputum eosinophil percentage in original SFC but not with generic SFC treatment groups ($r = 0.35$, $p = 0.01$ and $r = 0.24$, $p = 0.08$, respectively) (Fig. 2E and 2F).

There was no significant difference in asthma symptoms using ACT and ACQ scores following treatment with generic SFC and original SFC

The authors evaluated the effects of generic SFC and original SFC on asthma symptom control. Because of ceiling and floor effects, the authors used normalized gain in ACT and ACQ scores to demonstrate the therapeutic efficacy of generic SFC and original SFC. The authors found no significant differences of the gains in ACT and ACQ scores between generic SFC and original SFC treatment groups (median [IQR], 50 [0, 87.5] and 50 [0, 75], $p = 0.62$ for ACT; 100 [66.6, 100] and 100 [25, 100], $p = 0.11$ for ACQ, respectively). The authors also found no significant differences in the number of patients who were at either well controlled or uncontrolled status according to both ACT and ACQ scores before and after treatment with generic SFC and original SFC ($p = 0.38$ and $p = 0.75$ for pre- and post-treatment ACT scores; $p = 0.57$ and $p = 1.0$ for pre- and post-treatment ACQ scores). In addition,

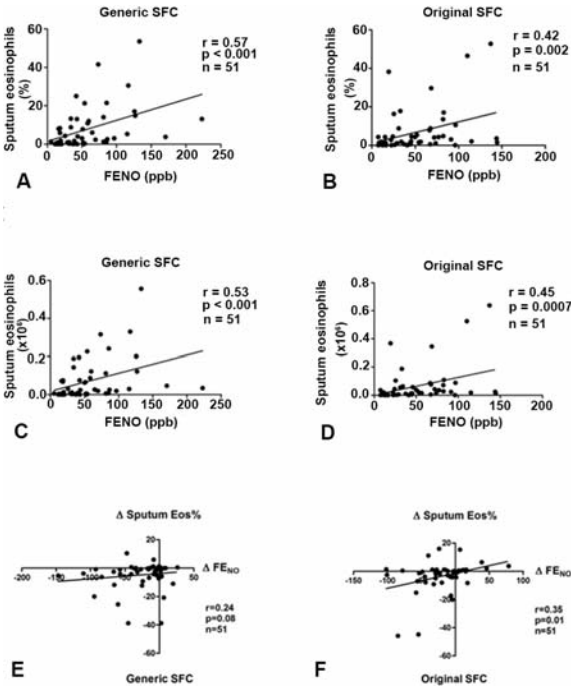

Fig. 2 Correlation between sputum eosinophil % and FENO prior to generic (A) and original SFC treatment (B). Relationship between absolute sputum eosinophils and FENO prior to generic (C) and original SFC treatment (D). Correlation between Δ sputum eosinophil % and Δ FENO after treatment with generic (E) and original SFC treatment (F).

there was no significant difference of the number of patients with transition of asthma control from uncontrolled to well controlled state according to ACT scores between generic SFC and original SFC treatment groups (61.1% vs. 63.6%, respectively).

Discussion

The present randomized double-blinded crossover study demonstrates that the combined ICS/LABA inhaler, either generic SFC or original SFC, could effectively reduce sputum eosinophils and FENO in a comparable manner irrespective of the sequence of treatment. Our initial assessment showed that there was a significantly positive correlation between the percentage and absolute number of sputum eosinophils and the levels of FENO prior to treatment with generic SFC and original SFC, indicating the appropriateness of the use of these inflammatory markers for our efficacy evaluation of the present study medications. With regard to the primary endpoint, the authors found that there was no significant difference in the degree of the reduction in sputum eosinophils in either percentage or absolute number of asthmatic patients who had been treated with generic SFC and original SFC for 4 weeks on separate occasions. This was in agreement with the FENO data. In clinical asthma control, there was also no significant difference in normalized gain in ACT and ACQ scores, including the number of patients who achieved full asthma control status after treatment with generic SFC and original SFC.

Eosinophils and their mediators are consistently identified in asthmatic but not healthy lungs, and suppression of eosinophilic airway inflammation in asthma by glucocorticoids is usually associated with an amelioration of symptoms and disordered airway function[41]. The authors chose 3% of sputum eosinophil count for the non-inferiority margin because this count identifies individuals with corticosteroid-responsive asthma[42]. Consistent with previous studies[34,43,44] their results indicated that 4 weeks' duration was appropriate for the detection of the anti-inflammatory effects of low dose fluticasone or low dose fluticasone/salmeterol combination on sputum eosinophilia. In addition, such a duration and double-blinded cross-over randomized trial as used for the present study can be appropriate for comparing similar treatments to determine non-inferiority. In this context, it is of interest to note that although the primary analysis of sputum eosinophil reduction difference at endpoint showed non-inferiority, the secondary analysis of the magnitude of exhaled nitric oxide reduction difference at the end of each treatment period favored generic SFC.

Although this trial was only of 4 weeks' duration and, as such, does not provide insight into the long-term clinical efficacy of generic SFC, such duration was adequate for assessing airway inflammation and asthma symptom control[45]. Our results showed the short-term clinical relevance of anti-inflammatory effects of generic SFC and original SFC in comparably improving asthma control scores (ACT and ACQ) because there were no significant differences in normalized gain in asthma symptom improvement and the numbers of patients with achievable well control status between the two treatment groups. This correlation is consistent with a recent study addressing the point that fractional exhaled nitric oxide has an inverse correlation with ACT scores[46]; this might suggest that FENO is more sensitive than sputum eosinophils for short-term monitoring anti-inflammatory activity of inhaled corticosteroids. However, the authors could not explain exactly why there was no significant correlation of the magnitude of the changes

in sputum eosinophils % with FENO levels after treatment with generic SFC although the weak but significant correlation was present in original SFC. The authors postulated that it may be the fact that not so very intense was eosinophilic airway inflammation in stable asthmatics, therefore there was the little room for both SFC's suppression, and the fact that FENO was more sensitive marker than sputum eosinophils for the short-term evaluation of anti-inflammatory therapy in asthma. It should be noted that the correlation between the magnitude of changes in ACT and FENO in response to generic SFC and original SFC was rather weak, possibly due to the fact that most patients in the present study were stable asthmatics.

Despite non-inferiority of the generic and original SFC in terms of anti-inflammatory activity, the present study has certain limitations. First, this short-term study could not evaluate their relative efficacy on asthma exacerbations, which is similar to any other short-term studies[45], and therefore long-term studies are further required for this evaluation. Second, all patients in the present study were stable asthma, therefore it is unknown whether generic SFC would provide the comparable efficacy to original SFC, when it is administered to uncontrolled asthmatics.

The present study addressed a 4-week treatment with SFC, which provided anti-inflammatory effects in mild-to-moderate stable asthmatics. In addition, the efficacy of generic or original SFC was comparable in terms of the reduction in eosinophilic airway inflammation at least in short-term duration.

Acknowledgement

The authors wish to thank Professor Arth Nana, Head of Division of Respiratory Disease and TB for his encouraging and supporting the authors and Professor Visanu Thammalikitkul, Former Deputy Dean of Research for giving the study assignment to the authors to conduct the present study. Finally, the authors wish to thank our patients who participated actively in the present study. The present study was supported from a grant from Healol Pharmaceutical

What is already known on this topic?

Inhaled corticosteroids (ICS) are by far the most effective therapy for asthma. The addition of long-acting β_2 agonist (LABA) to a low dose of ICS provides better asthma control including improvement in lung function than a higher dose of ICS. This combination has yet been demonstrated their effects on airway inflammation.

What this study adds?

The authors demonstrated ICS in combination with LABA could effectively suppress airway inflammation in this short-term study.

Potential conflicts of interest

KM, JA, KR, TS, SP and SU have declared that no competing interests exist. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Kay AB. Eosinophils and asthma. N Engl J Med 1991; 324: 1514-5.
2. Kay AB, Corrigan CJ. Asthma. Eosinophils and neutrophils. Br Med Bull 1992; 48: 51-64.
3. Kay AB. "Helper" (CD4+) T cells and eosinophils in allergy and asthma. Am Rev Respir Dis 1992; 145 (2 Pt 2): S22-6.
4. Bradley BL, Azzawi M, Jacobson M, Assoufi B, Collins JV, Irani AM, et al. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma: comparison with biopsy specimens from atopic subjects without asthma and normal control subjects and relationship to bronchial hyperresponsiveness. J Allergy Clin Immunol 1991; 88: 661-74.
5. Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, Djukanovic R. The relationship between airways inflammation and asthma severity. Am J Respir Crit Care Med 2000; 161: 9-16.
6. Tomita K, Caramori G, Ito K, Sano H, Lim S, Oates T, et al. STAT6 expression in T cells, alveolar macrophages and bronchial biopsies of normal and asthmatic subjects. J Inflamm (Lond) 2012; 9: 5.
7. Hamzaoui A, Maalmi H, Berraies A, Abid H, Ammar J, Hamzaoui K. Transcriptional characteristics of CD4 T cells in young asthmatic children: RORC and FOXP3 axis. J Inflamm Res 2011; 4: 139-46.
8. Choy DF, Modrek B, Abbas AR, Kummerfeld S, Clark HF, Wu LC, et al. Gene expression patterns of Th2 inflammation and intercellular communication in asthmatic airways. J Immunol 2011; 186: 1861-9.
9. Aujla SJ, Alcorn JF. T(H)17 cells in asthma and inflammation. Biochim Biophys Acta 2011; 1810: 1066-79.
10. Alcorn JF, Crowe CR, Kolls JK. TH17 cells in asthma and COPD. Annu Rev Physiol 2010; 72: 495-516.
11. Bentley AM, Menz G, Storz C, Robinson DS,

Bradley B, Jeffery PK, et al. Identification of T lymphocytes, macrophages, and activated eosinophils in the bronchial mucosa in intrinsic asthma. Relationship to symptoms and bronchial responsiveness. Am Rev Respir Dis 1992; 146: 500-6.
12. Azzawi M, Johnston PW, Majumdar S, Kay AB, Jeffery PK. T lymphocytes and activated eosinophils in airway mucosa in fatal asthma and cystic fibrosis. Am Rev Respir Dis 1992; 145: 1477-82.
13. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med 2009; 360: 973-84.
14. Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. Lancet 2002; 360: 1715-21.
15. Nair P, Pizzichini MM, Kjarsgaard M, Inman MD, Efthimiadis A, Pizzichini E, et al. Mepolizumab for prednisone-dependent asthma with sputum eosinophilia. N Engl J Med 2009; 360: 985-93.
16. Green RH, Brightling CE, McKenna S, Hargadon B, Neale N, Parker D, et al. Comparison of asthma treatment given in addition to inhaled corticosteroids on airway inflammation and responsiveness. Eur Respir J 2006; 27: 1144-51.
17. Bacci E, Cianchetti S, Bartoli M, Dente FL, Di Franco A, Vagaggini B, et al. Low sputum eosinophils predict the lack of response to beclomethasone in symptomatic asthmatic patients. Chest 2006; 129: 565-72.
18. Petsky HL, Cates CJ, Lasserson TJ, Li AM, Turner C, Kynaston JA, et al. A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils). Thorax 2012; 67: 199-208.
19. Patil SU, Long AA. The usefulness of biomarkers of airway inflammation in managing asthma. Allergy Asthma Proc 2010; 31: 259-68.
20. Malerba M, Ragnoli B, Radaeli A, Tantucci C. Usefulness of exhaled nitric oxide and sputum eosinophils in the long-term control of eosinophilic asthma. Chest 2008; 134: 733-9.
21. van Rensen EL, Straathof KC, Veselic-Charvat MA, Zwinderman AH, Bel EH, Sterk PJ. Effect of inhaled steroids on airway hyperresponsiveness, sputum eosinophils, and exhaled nitric oxide levels in patients with asthma. Thorax 1999; 54: 403-8.
22. Kleinert H, Pautz A, Linker K, Schwarz PM. Regulation of the expression of inducible nitric oxide synthase. Eur J Pharmacol 2004; 500: 255-66.
23. Xu W, Comhair SA, Zheng S, Chu SC, Marks-Konczalik J, Moss J, et al. STAT-1 and c-Fos interaction in nitric oxide synthase-2 gene activation. Am J Physiol Lung Cell Mol Physiol 2003; 285: L137-48.
24. Uetani K, Thomassen MJ, Erzurum SC. Nitric oxide synthase 2 through an autocrine loop via respiratory epithelial cell-derived mediator. Am J Physiol Lung Cell Mol Physiol 2001; 280: L1179-88.
25. Guo FH, Uetani K, Haque SJ, Williams BR, Dweik RA, Thunnissen FB, et al. Interferon gamma and interleukin 4 stimulate prolonged expression of inducible nitric oxide synthase in human airway epithelium through synthesis of soluble mediators. J Clin Invest 1997; 100: 829-38.
26. Guo FH, Comhair SA, Zheng S, Dweik RA, Eissa NT, Thomassen MJ, et al. Molecular mechanisms of increased nitric oxide (NO) in asthma: evidence for transcriptional and post-translational regulation of NO synthesis. J Immunol 2000; 164: 5970-80.
27. Suresh V, Mih JD, George SC. Measurement of IL-13-induced iNOS-derived gas phase nitric oxide in human bronchial epithelial cells. Am J Respir Cell Mol Biol 2007; 37: 97-104.
28. Chibana K, Trudeau JB, Mustovich AT, Hu H, Zhao J, Balzar S, et al. IL-13 induced increases in nitrite levels are primarily driven by increases in inducible nitric oxide synthase as compared with effects on arginases in human primary bronchial epithelial cells. Clin Exp Allergy 2008; 38: 936-46.
29. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. Thorax 1998; 53: 91-5.
30. Berry MA, Shaw DE, Green RH, Brightling CE, Wardlaw AJ, Pavord ID. The use of exhaled nitric oxide concentration to identify eosinophilic airway inflammation: an observational study in adults with asthma. Clin Exp Allergy 2005; 35: 1175-9.
31. Berlyne G, Barnes N. No role for NO in asthma? Lancet 2000; 355: 1029-30.
32. Usmani OS, Ito K, Maneechotesuwan K, Ito M, Johnson M, Barnes PJ, et al. Glucocorticoid receptor nuclear translocation in airway cells after inhaled combination therapy. Am J Respir Crit Care Med 2005; 172: 704-12.

33. Maneechotesuwan K, Supawita S, Kasetsinsombat K, Wongkajornsilp A, Barnes PJ. Sputum indoleamine-2, 3-dioxygenase activity is increased in asthmatic airways by using inhaled corticosteroids. J Allergy Clin Immunol 2008; 121: 43-50.
34. Currie GP, Syme-Grant NJ, McFarlane LC, Carey FA, Lipworth BJ. Effects of low dose fluticasone/salmeterol combination on surrogate inflammatory markers in moderate persistent asthma. Allergy 2003; 58: 602-7.
35. Koopmans JG, Lutter R, Jansen HM, van der Zee JS. Adding salmeterol to an inhaled corticosteroid: long term effects on bronchial inflammation in asthma. Thorax 2006; 61: 306-12.
36. Hoshino M, Handa H, Miyazawa T. Effects of salmeterol and fluticasone propionate combination versus fluticasone propionate on airway function and eosinophilic inflammation in mild asthma. Allergol Int 2009; 58: 357-63.
37. Pavord I, Woodcock A, Parker D, Rice L. Salmeterol plus fluticasone propionate versus fluticasone propionate plus montelukast: a randomised controlled trial investigating the effects on airway inflammation in asthma. Respir Res 2007; 8: 67.
38. Kips JC, O'Connor BJ, Inman MD, Svensson K, Pauwels RA, O'Byrne PM. A long-term study of the antiinflammatory effect of low-dose budesonide plus formoterol versus high-dose budesonide in asthma. Am J Respir Crit Care Med 2000; 161: 996-1001.
39. Currie GP, Bates CE, Lee DK, Jackson CM, Lipworth BJ. Effects of fluticasone plus salmeterol versus twice the dose of fluticasone in asthmatic patients. Eur J Clin Pharmacol 2003; 59: 11-5.
40. Hake RR. Interactive-engagement versus traditional methods: a six-thousand-student survey of mechanics test data for introductory physics courses. Am J Phys 1998; 66: 64-74.
41. Wardlaw AJ, Brightling C, Green R, Woltmann G, Pavord I. Eosinophils in asthma and other allergic diseases. Br Med Bull 2000; 56: 985-1003.
42. Pavord ID, Brightling CE, Woltmann G, Wardlaw AJ. Non-eosinophilic corticosteroid unresponsive asthma. Lancet 1999; 353: 2213-4.
43. Gershman NH, Wong HH, Liu JT, Fahy JV. Low- and high-dose fluticasone propionate in asthma; effects during and after treatment. Eur Respir J 2000; 15: 11-8.
44. Vignola AM, Riccobono L, Profita M, Foresi A, Di Giorgi R, Guerrera D, et al. Effects of low doses of inhaled fluticasone propionate on inflammation and remodelling in persistent-mild asthma. Allergy 2005; 60: 1511-7.
45. Lee DK, Haggart K, Currie GP, Bates CE, Lipworth BJ. Effects of hydrofluoroalkane formulations of ciclesonide 400 microg once daily vs fluticasone 250 microg twice daily on methacholine hyperresponsiveness in mild-to-moderate persistent asthma. Br J Clin Pharmacol 2004; 58: 26-33.
46. Soto-Ramos M, Castro-Rodriguez JA, Hinojos-Gallardo LC, Hernandez-Saldana R, Cisneros-Castolo M, Carrillo-Rodriguez V. Fractional exhaled nitric oxide has a good correlation with asthma control and lung function in latino children with asthma. J Asthma 2013; 50: 590-4.
47. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol 2004; 113: 59-65.
48. Saejong R, Dejsomritrutai W. Validity and reliability assessment of the Siriraj Asthma Control Questionnaire. J Med Assoc Thai 2011; 94 (Suppl 1): S72-6.

การศึกษาเปรียบเทียบฤทธิ์ต้านการอักเสบของยาผสมระหว่าง salmeterol และ fluticasone (SFC) ในหลอดยาเดียวกันชนิด generic และ original ในการรักษาหลอดลมอักเสบของผู้ป่วยโรคหืด

กิตติพงศ์ มณีโชติสุวรรณ, จิรวัฒน์ อัศวภูมิ, กนกวรรณ รัตนแสงเลิศ, ทัศนียา สุธรรมสมัย, สุทัศน์ พิภพสุทธิไพบูลย์, สุทธิพล อุดมพันธุรัก

ภูมิหลัง: *ฤทธิ์ต้านการอักเสบของยาสูดพ่นแบบผสมระหว่างคอร์ติโคสเตอรอยด์และยาขยายหลอดลมชนิด β_2 agonists ชนิดออกฤทธิ์นาน (long-acting β_2 agonist หรือชื่อย่อว่า LABA) มีการศึกษากันน้อยในผู้ป่วยโรคหืดนอกจากนั้นยังไม่เป็นที่ทราบกันว่าประสิทธิภาพการรักษาของยาผสมระหว่าง salmeterol และ fluticasone ในหลอดยาเดียวกัน (SFC) ชนิด generic และ original เทียบเท่ากันหรือไม่*

วัตถุประสงค์: *ศึกษาผลของ SFC และผลของ generic และ original SFC ต่อการอักเสบของหลอดลมของผู้ป่วยโรคหืดที่อาการคงที่ ในขั้นความรุนแรงของโรคน้อยถึงรุนแรงปานกลาง*

วัสดุและวิธีการ: *การศึกษานี้ดำเนินแบบสุ่มปกปิดสองทางในรูปแบบไขว้ยารักษาและไม่ด้อยกว่ากัน เพื่อเปรียบเทียบฤทธิ์ต้านการอักเสบของยาสูดพ่น SFC ชนิด generic และ original โดยติดตามการลดจำนวนของเซลล์ eosinophil เป็นวัตถุประสงค์หลักและการลดลงของระดับของก๊าซไนตริคออกไซด์ในลมหายใจออก (FENO) เป็นวัตถุประสงค์รอง*

ผู้ป่วยที่เข้าการศึกษา: *การศึกษานี้รวบรวมผู้ป่วยโรคหืดชนิดรุนแรงน้อยถึงปานกลางจำนวน 51 ราย อายุระหว่าง 18-80 ปี ที่ได้รับการรักษาด้วย inhaled corticosteroids (ICS) หรือ ICS/LABA ในขนาดใดก็ได้และไม่มีอาการหืดกำเริบอย่างน้อย 3 เดือน ก่อนเข้าการศึกษา*

ผลการศึกษา: *พบว่าทั้งจำนวนร้อยละและจำนวนเต็มของเซลล์ eosinophil ในเสมหะสัมพันธ์อย่างดีกับ FENO ก่อนการรักษาแสดงว่า inflammatory markers ทั้งสองมีความสอดคล้องกัน จำนวนร้อยละของเซลล์ eosinophil ลดลงชัดเจนตามหลังการรักษาด้วยยา generic SFC และ original SFC อย่างมีนัยสำคัญ สัดส่วนการลดลงของ sputum eosinophil ระหว่างช่วงที่รักษาด้วยยา generic SFC ไม่ด้อยกว่าช่วงที่รักษาด้วย original SFC โดยที่ไม่เกี่ยวกับลำดับการรักษาด้วย generic SFC เป็นลำดับแรก แล้วตามด้วย original SFC เป็นลำดับสอง หรือในทางกลับกัน original SFC เป็นลำดับแรก และ generic SFC เป็นลำดับสอง นอกจากนั้นไม่มีความแตกต่าง ระหว่างการรักษาด้วยยาทั้งสองชนิดในการทำให้คะแนนควบคุมอาการหืดดีขึ้น รวมทั้งจำนวนผู้ป่วยที่มีอาการหืดดีขึ้น โดยผลที่เกิดขึ้นไม่เกี่ยวข้องกับลำดับการรักษาที่ระบุไว้ข้างต้น อย่างไรก็ตามไม่ใช่ในกรณีของระดับของ FENO ที่มีสัดส่วนการลดลง มากกว่าอย่างมีนัยสำคัญทางสถิติตามหลังรักษาด้วย generic SFC เมื่อเปรียบเทียบกับหลังรักษาด้วย original SFC*

สรุป: *การศึกษานี้เป็นการศึกษาระยะสั้นที่แสดงให้เห็นว่าฤทธิ์ต้านการอักเสบของหลอดลมและการควบคุมอาการหืดของยา generic SFC ไม่แตกต่างจากยา original SFC อย่างไรก็ตามการศึกษาไม่ได้ตอบคำถามในเรื่องประสิทธิภาพของยา generic SFC ในระยะยาวโดยเฉพาะการป้องกันการเกิดอาการโรคหืดกำเริบเฉียบพลัน ดังนั้นควรมีการดำเนินการศึกษาระยะยาวในเชิงเปรียบเทียบเพื่อประเมินประสิทธิภาพของยาชนิดนี้ว่าดีเท่ากับยา original SFC ในการช่วยลดอาการหืดกำเริบ*