



Anti-inflammatory effects of salmeterol/fluticasone, tiotropium/fluticasone or tiotropium in COPD

D-W. Perng^{*,#,+}, C-W. Tao^{†,+}, K-C. Su^{*}, C-C. Tsai^{*}, L-Y. Liu^{*} and Y-C. Lee^{*,#}

ABSTRACT: The anti-inflammatory effects of salmeterol/fluticasone (SFP), tiotropium/fluticasone (Tio+FP) and tiotropium (Tio) alone were investigated on the inflammatory cells and mediators in sputum induced from chronic obstructive pulmonary disease patients.

Subjects were either newly diagnosed or had not taken any medication for 3 months prior to the study. Subjects (n=99) were randomised (not double blinded) and received either SFP (100/1,000 µg daily), Tio+FP (18/1,000 µg daily) or Tio (18 µg daily) for 12 weeks. Induced sputum and serum C-reactive protein (CRP) were analysed prior to and at the end of treatment.

The results showed that treatment with SFP caused a significant reduction in interleukin (IL)-8 and matrix metalloprotease (MMP)-9 in induced sputum, compared with treatment with Tio alone. There were no treatment differences between the SFP and Tio+FP groups in decreasing IL-8 and MMP-9 levels. The reduction in IL-8 showed significant association with the reduction in MMP-9. All treatment groups failed to significantly reduce the numbers of total cells, neutrophils, macrophages and eosinophils in induced sputum; in addition, there were no treatment differences in terms of improvement of forced expiratory volume in one second, forced vital capacity, CRP or quality of life between the three groups.

The anti-inflammatory effects of salmeterol/fluticasone probably contribute to the clinical benefits seen in chronic obstructive pulmonary disease patients.

KEYWORDS: Chronic obstructive pulmonary disease, inflammation, interleukin-8, matrix metalloprotease, salmeterol/fluticasone, tiotropium

Chronic obstructive pulmonary disease (COPD) is characterised by the airflow limitation associated with an abnormal inflammatory response [1]. The inflammatory cells present in COPD are heterogeneous; neutrophils, macrophages, eosinophils, mast cells and CD8+ lymphocytes have been shown to play important roles in inflammatory processes in COPD [2–5]. Neutrophils are one of the major inflammatory cell types in the airways of patients with COPD [6], and increased numbers of neutrophils in sputum were found to be correlated with a rapid decline in forced expiratory volume in one second (FEV₁) in a 15-yr follow-up study [7]. In stable COPD patients, FEV₁ showed a significant inverse correlation with the number of neutrophils in induced sputum [8] and in the subepithelium from bronchial biopsies [9], suggesting that neutrophilic inflammation of the airways may contribute to the pathogenesis of COPD.

The combinations of an inhaled corticosteroid and a long-acting β_2 -agonist or of an inhaled long-acting anticholinergic are currently used in COPD treatment to improve lung function and

quality of life, prevent exacerbation and reduce hospitalisation [10–12]. Inhaled corticosteroids alone have limited anti-inflammatory effect in COPD patients [3, 13, 14]. Treatment with a combination of salmeterol and fluticasone has been shown to reduce the numbers of macrophages, neutrophils and CD8+ T-lymphocytes in bronchial biopsy tissue and in induced sputum from patients with COPD [15, 16]. M₃-selective muscarinic antagonists have been shown to inhibit the release of chemoattractant for neutrophils from alveolar macrophages that were stimulated by acetylcholine [17]. However, the anti-inflammatory effect of tiotropium on the airways of COPD patients remains to be elucidated.

The aim of the present study was to investigate the anti-inflammatory effects of salmeterol/fluticasone (SFP), tiotropium/fluticasone (Tio+FP) and tiotropium (Tio) alone on inflammatory cells and mediators in induced sputum from patients with COPD. The changes in levels of neutrophils, macrophages, eosinophils, interleukin (IL)-8 and matrix metalloprotease (MMP)-9 in sputum were

AFFILIATIONS

*Dept of Chest Medicine, Taipei Veterans General Hospital,
#School of Medicine, National Yang-Ming University, and
†Cheng Hsin Rehabilitation Medical Center
Taipei, Taiwan
+These authors contributed equally to the study.

CORRESPONDENCE

D-W. Perng
School of Medicine, National Yang-Ming University, Department of Chest Medicine
Taipei Veterans General Hospital
201
Section 2
Shih-Pai Road
Taipei 11217
Taiwan
Fax: 886 228761009
E-mail: dwperng@vghtpe.gov.tw

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STATEMENT OF INTEREST

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assessed after 12 weeks of therapy; furthermore, lung function was also measured and health-related quality of life was assessed using the St George's Respiratory Questionnaire (SGRQ).

MATERIALS AND METHODS

Patient population

A total of 113 subjects with a clinical diagnosis of COPD [1] were enrolled between July 2006 and July 2007 from outpatient clinics in the Taipei Veterans General Hospital, Taipei, Taiwan. Subjects were eligible for the study if they were: aged 40–85 yrs; were a current or former smoker (history ≥ 20 pack-yrs); had a post-bronchodilator FEV₁ <80% of the predicted value and FEV₁/forced vital capacity (FVC) <70%; and had no history of asthma, atopy (as defined by a positive reaction to one or more allergen in a fluoroenzyme immunoassay) or any other active lung disease. Subjects were either newly diagnosed or had not taken corticosteroids (either oral or inhaled), or any other bronchodilators or theophylline, for a minimum of 3 months prior to the commencement of the study. All subjects had been free from respiratory tract infections or COPD exacerbation for ≥ 12 weeks prior to the pulmonary function tests and sputum induction. The hospital ethics committee approved the study and written informed consent was obtained from all subjects before the study commenced.

Study design

The study was a randomised (not double blinded) clinical trial, with subjects allocated to receive treatment with one of the following: SFP 25/250 $\mu\text{g}\cdot\text{puff}^{-1}$, two puffs twice daily by

Evohaler (GlaxoSmithKline, Ware, UK); Tio 18 μg once daily by Handihaler (Boehringer Ingelheim Pharma, Ingelheim, Germany) plus FP 250 $\mu\text{g}\cdot\text{puff}^{-1}$, two puffs twice daily by Acuhaler (GlaxoSmithKline); or Tio 18 μg once daily only. After screening, subjects were randomised to one of the treatment groups: randomisation was performed using a computer-generated list of random numbers. The treatment duration was 12 weeks, with clinical visits in weeks 4, 8 and 12. Pulmonary function, serum C-reactive protein (CRP), sputum induction and assessment of health-related quality of life were carried out in the morning at screening and in week 12. Health status was assessed using the SGRQ, and the total score and scores from the three categories (symptoms, activity, and impact) were calculated [18]. Induced sputum was processed as described previously [8] and the supernatant was aspirated and frozen at -80°C prior to measurement of the inflammatory mediators IL-8 and MMP-9. The levels of IL-8 and MMP-9 in supernatants were assayed by ELISA (R&D Systems, Abingdon, UK), according to the manufacturer's instructions.

Statistics

In light of the results of previous studies [8, 19, 20], IL-8 was selected as the reference for calculation of the estimated sample size. It was assumed that the mean difference in IL-8 was 300 $\text{pg}\cdot\text{mL}^{-1}$ between groups, with an SD of 420 $\text{pg}\cdot\text{mL}^{-1}$; the level of significance was 0.05, the power of the test was 0.9, and the calculated sample size was 21 per treatment group. The differences between the results (pre-treatment

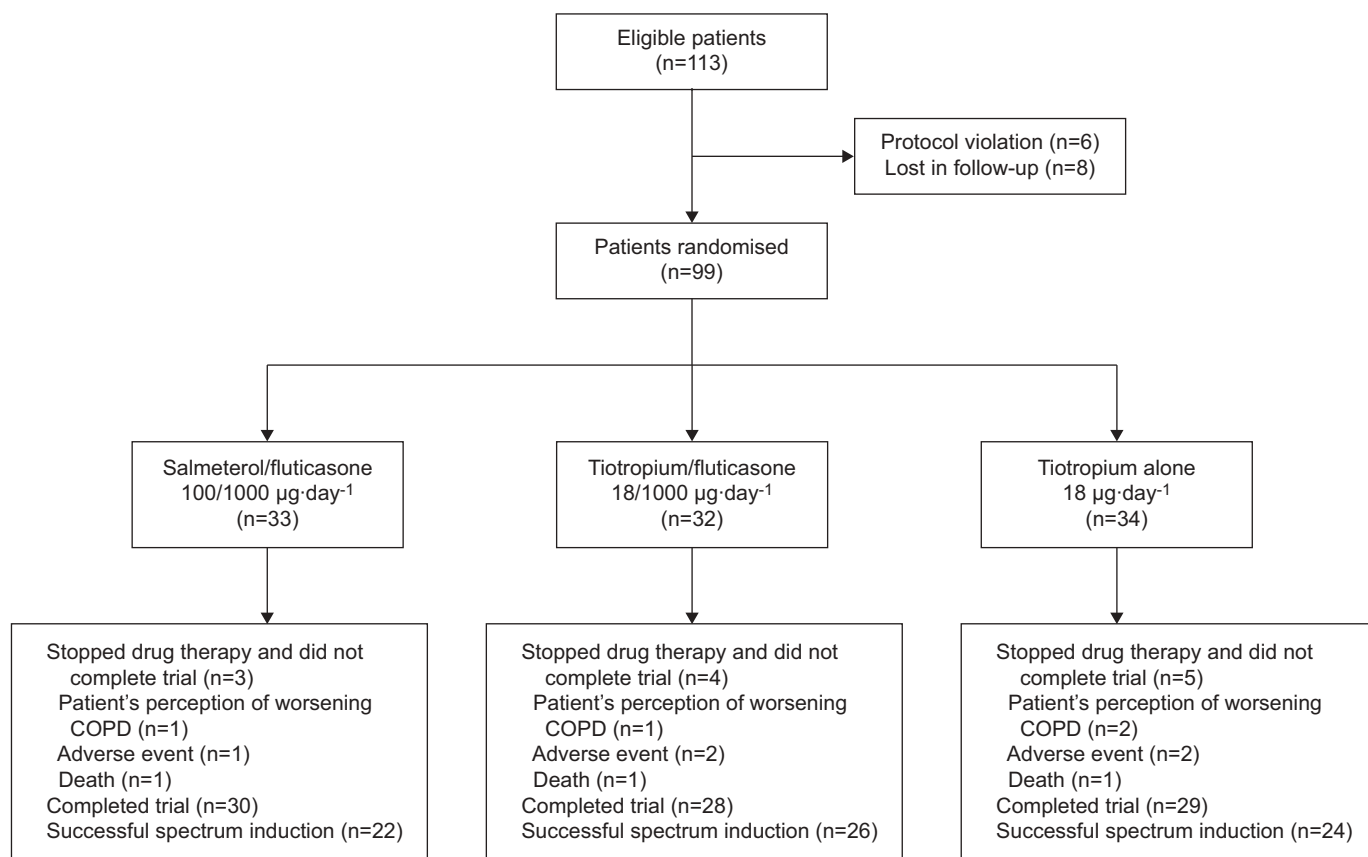


FIGURE 1. Flow diagram of patients in the study. COPD: chronic obstructive pulmonary disease.

TABLE 1 Patient characteristics

	All completed cases			Completed cases with successful sputum induction		
	SFP	Tio+FP	Tio	SFP	Tio+FP	Tio
Subjects n	33	32	34	22	26	24
Mean age yrs	72.0±8.0	74.1±8.1	74.3±7.2	74.3±8.0	76.5±7.9	76.1±4.3
Males/females n	31/2	32/0	32/2	21/1	26/0	22/2
Current smoker %	57	60	65	55	58	60
Smoking history pack-yrs	49.5	50.4	47.4	48.5	48.4	49.5
Pre-bronchodilator FEV ₁ L	1.24±0.34	1.28±0.34	1.18±0.28	1.21±0.28	1.30±0.24	1.25±0.27
Pre-bronchodilator FEV ₁ % pred	53.4±11.1	57.8±13.6	53.1±11.0	52.8±10.5	58.7±9.1	54.1±10.3
Pre-bronchodilator FVC L	2.35±0.47	2.43±0.56	2.28±0.54	2.32±0.56	2.31±0.41	2.36±0.59
Pre-bronchodilator FVC % pred	72.2±13.9	76.5±16.3	73.5±14.5	70.5±16.0	72.2±10.1	74.1±16.2
Reversibility FEV ₁ % pred	6.8±4.5	5.5±4.6	6.8±5.6	7.2±4.2	6.5±4.3	6.8±4.6
Serum CRP mg·L ⁻¹	0.8±0.3	1.0±0.5	0.8±0.2	0.6±0.3	0.9±0.2	0.7±0.3
SGRQ score	44.3±13.2	47.9±10.5	47.8±15.7	45.3±11.3	46.7±11.2	45.7±10.5

Data are presented as mean ±SD, unless otherwise stated. SFP: salmeterol/fluticasone; Tio+FP: tiotropium and fluticasone combination; Tio: tiotropium only; FEV₁: forced expiratory volume in one second; % pred: % predicted; FVC: forced vital capacity; CRP: C-reactive protein; SGRQ: St George's Respiratory Questionnaire.

minus post-treatment), including FEV₁, FVC, serum levels of CRP and cortisol, and all cells and mediators measured in the induced sputum, passed the test for normality. Data are expressed as mean ±SD. Statistical analysis for multiple comparisons was performed using ANOVA, and the *post hoc* test applied for pairwise comparison following ANOVA was the least significance difference test. Association between IL-8 and MMP-9 in treatment differences (pre-treatment minus post-treatment) was measured using Spearman's rank correlation test. A p-value <0.05 was considered significant for all tests.

RESULTS

The details of the 113 eligible patients screened, randomised and withdrawn during the study are shown in figure 1. In total, 33 patients were treated with SFP, 32 with Tio+FP and 34 with Tio alone. Demographic details, smoking history, baseline lung function and health-related quality life were matched among treatment groups (table 1). Table 2 reveals the characteristics of the inflammatory cells and mediators in the induced sputum: no differences in baseline inflammatory cells and mediators were found among the groups. The overall yield rate of sputum induction was 72.7% (72 out of 99) at the end of study.

Inflammatory cells and markers

The mean treatment differences in total cell numbers, neutrophils, macrophages and eosinophils between the SFP, Tio+FP and Tio groups failed to reach statistical significance (fig. 2). The levels of serum CRP showed significant association with total cell numbers ($r=0.273$, $p=0.02$) and with the numbers of neutrophils ($r=0.247$, $p=0.037$) in induced sputum. IL-8 concentrations showed strong association with those of MMP-9 before ($r=0.639$, $p<0.001$) and following ($r=0.704$, $p<0.001$) treatment. The decrease in CRP was 0.3 ± 0.2 , 0.6 ± 0.5 and 0.3 ± 0.2 mg·L⁻¹ in the SFP, Tio+FP and Tio groups, respectively; there were no statistically significant differences between the three groups. In contrast, there was a significant

reduction in IL-8 and MMP-9 in the SFP group compared with patients treated with Tio alone ($p=0.03$ and $p=0.004$, respectively; fig. 3). Interestingly, IL-8 ($p=0.041$) and MMP-9 ($p=0.02$) levels increased in the Tio group, but no difference was found between the SFP and Tio+FP groups. In addition, the reduction in IL-8 was found to be strongly associated with that in MMP-9 (fig. 4).

Lung function and health-related quality of life

The improvement in FEV₁, FVC and health-related quality of life as assessed by the SGRQ in each treatment group is shown in table 3. No treatment differences were found between the groups in terms of FEV₁, FVC and SGRQ score.

DISCUSSION

The present study is the first randomised (not double blinded) clinical trial to compare the anti-inflammatory effects of SFP, Tio+FP and Tio alone on the airways of patients with COPD. The study showed that 12 weeks of treatment with SFP caused a significant reduction in the levels of inflammatory mediators IL-8 and MMP-9 compared with treatment with tiotropium alone. There were no differences between the SFP and Tio+FP groups in terms of decreasing IL-8 and MMP-9 levels. The reduction in IL-8 was significantly associated with the reduction in MMP-9. Although treatment with SFP resulted in a mean reduction of 1.53×10^6 neutrophils per g of sputum in the present study, no treatment group showed a reduction in the numbers of total cells, which included neutrophils, macrophages and eosinophils, of statistical significance. In addition, there were no differences in terms of improvement in FEV₁, FVC and health-related quality of life between the three groups.

BOURBEAU *et al.* [15] reported that 3 months of treatment for COPD with SFP (50/500 µg, twice daily) significantly reduced CD8+ T-cells and CD68+ macrophages in bronchial biopsy tissue compared with a placebo, but that this treatment did not

TABLE 2 Baseline of cells and mediators in induced sputum

	SFP	Tio+FP	Tio	p-value
Subjects n	22	26	24	
Total cells [#] × 10 ⁶	7.0 ± 1.6	7.9 ± 3.6	6.3 ± 1.6	NS
Macrophages %	9.7 ± 2.3	14.8 ± 3.2	10.2 ± 2.1	NS
Neutrophils %	84.2 ± 3.7	81.8 ± 3.6	86 ± 2.3	NS
Eosinophils %	3.3 ± 2.5	1.9 ± 1.6	2.3 ± 0.9	NS
Lymphocytes %	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	NS
Epithelial cells %	0.6 ± 0.1	1.3 ± 0.6	1.2 ± 0.3	NS
MMP-9 [#] ng·mL ⁻¹ (CV %)	352.2 ± 38.7 (10.9)	366.3 ± 51.1 (13.9)	373.2 ± 49.1 (13.1)	NS
IL-8 [#] ng·mL ⁻¹ (CV %)	7.9 ± 1.9 (24.6)	7.4 ± 1.6 (21.9)	7.6 ± 1.7 (22.6)	NS

Data are presented as mean ± sd, unless otherwise stated. SFP: salmeterol/fluticasone; Tio+FP: tiotropium and fluticasone combination; Tio: tiotropium only; MMP: metalloprotease; CV: coefficient of variation; IL: interleukin; NS: nonsignificant. #: per g of sputum.

significantly affect the numbers of neutrophils and eosinophils. In contrast, the same dose with a similar treatment duration was reported as significantly reducing neutrophils, eosinophils and CD8+ T-cells in biopsies by BARNES *et al.* [16]. CD68+ macrophages were not affected in the present study. In conjunction with the current results, these aforementioned studies show that heterogeneity exists in the COPD patient population and that treatment response varies. Therefore, the

factors that influence anti-inflammatory response deserve urgent investigation.

Long-term use of Tio has been shown to increase sputum IL-8, although it decreases the frequency of exacerbation in COPD patients [21]. In the present study, both IL-8 and MMP-9 levels significantly increased in the group treated with Tio alone. It is difficult to link the increases in inflammatory mediators with

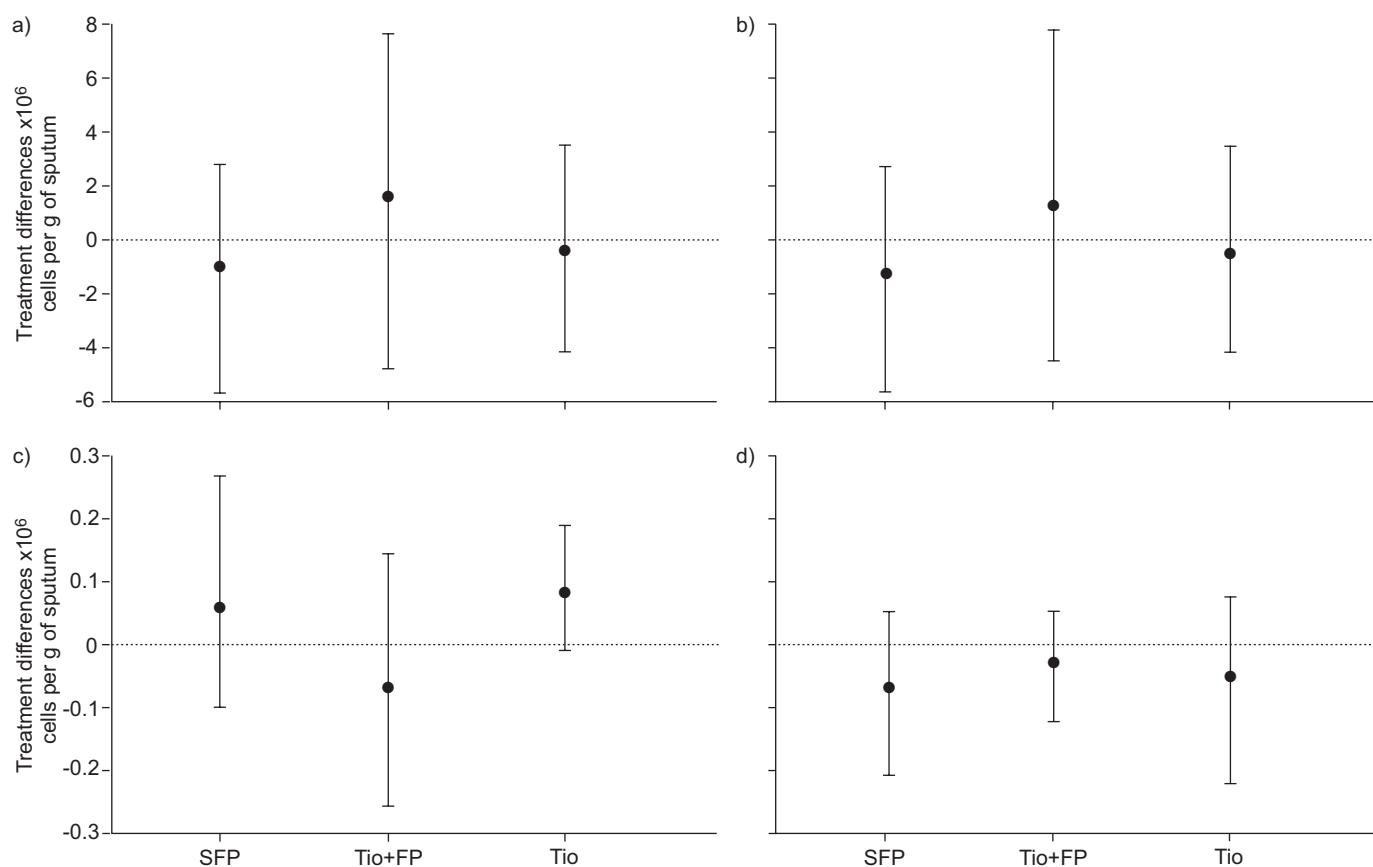


FIGURE 2. Treatment differences in terms of a) number of total cells, b) neutrophils, c) macrophages and d) eosinophils. Data are expressed as mean difference in the number of cells with 95% confidence intervals. SFP: salmeterol/fluticasone; Tio+FP: tiotropium/fluticasone; Tio: tiotropium only.

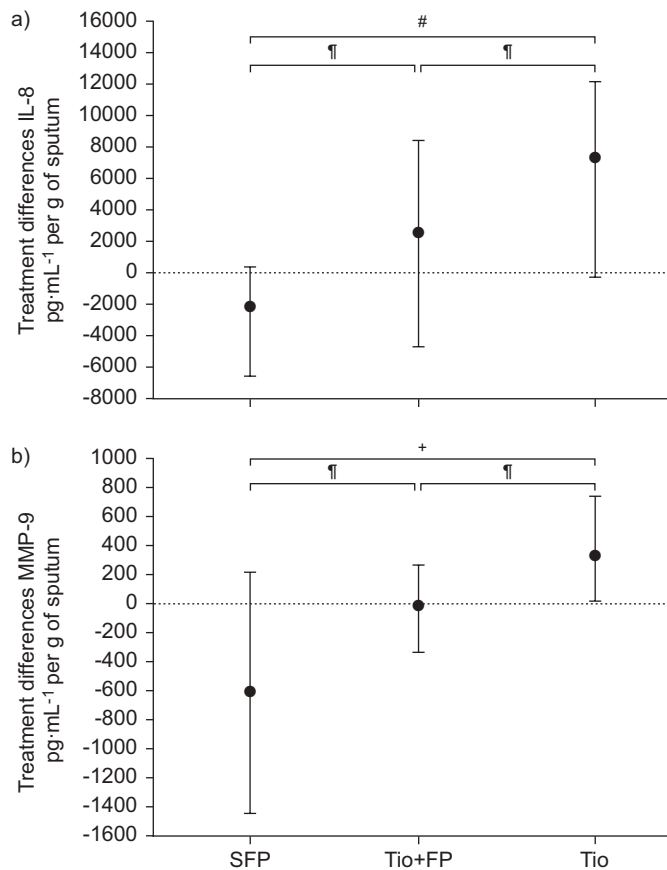


FIGURE 3. Effects of treatments on a) interleukin (IL-8) and b) matrix metalloproteinase (MMP)-9. Data are expressed as mean difference in the concentration of induced sputum with 95% confidence intervals. SFP: salmeterol/fluticasone; Tio+FP: tiotropium/fluticasone; Tio: tiotropium only. #: $p=0.022$; †: $p>0.05$; †: $p=0.012$.

decreased exacerbation rates in COPD. Presumably, Tio may influence the depth of airway surface liquid through inhibition of cholinergic stimulation [22]. Serous and mucous-secreting cells in the airways may reduce secretion under such motor

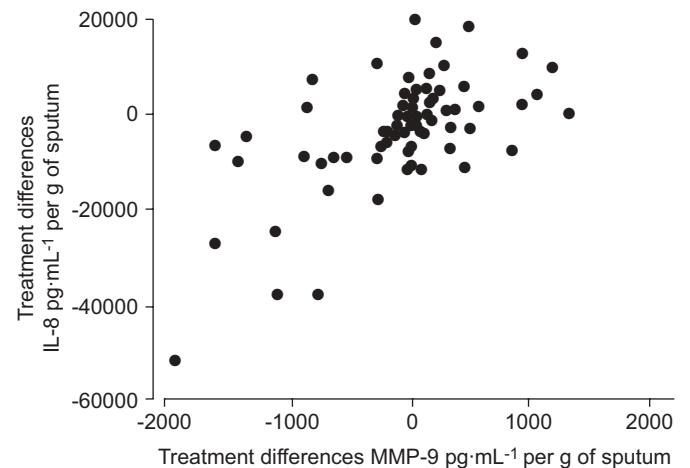


FIGURE 4. Associations between the changes in levels (pre-treatment minus post-treatment) of interleukin (IL)-8 and metalloproteinase (MMP)-9 in induced sputum from the chronic obstructive pulmonary disease patients ($n=72$) who completed the treatment course. $r=0.628$, $p<0.001$.

control, leading to increased concentrations of mediators in the airways. Neither IL-8 nor MMP-9 increased in the Tio+FP arm of the study. SFP treatment significantly reduced sputum IL-8 and MMP-9 levels; such anti-inflammatory effects may be attributed partly to the action of salmeterol. Introduction of salmeterol into the airways of asthmatics has been found to reduce IL-8 and myeloperoxidase levels in bronchoalveolar lavage fluid; in the same study, high-dose inhaled corticosteroid treatment increased rather than decreased airway neutrophils [23]. However, *in vitro* studies have suggested that there are dose-dependent effects of fluticasone on the inhibition of IL-8 and other mediators released from various types of cells, including airway epithelial cells [24], smooth muscle cells [25] and fibroblasts [26]. A synergistic effect of SFP on the inhibition of mediator release has also been demonstrated [27–30].

The reductions in IL-8 and MMP-9 levels may explain the clinical benefit of SFP in decreasing airway inflammation. IL-8 is a potent chemoattractant for neutrophils, and MMP-9 is

TABLE 3 Effect of treatment on lung function and quality of life

	All completed cases			Completed cases with successful sputum induction		
	SFP	Tio+FP	Tio	SFP	Tio+FP	Tio
Subjects n	33	32	34	22	26	24
Baseline						
FEV ₁ L	1.24	1.28	1.18	1.21	1.30	1.25
FVC L	2.35	2.43	2.28	2.32	2.31	2.36
SGRQ score	44.3	47.9	47.8	45.3	46.7	45.7
Treatment difference						
FEV ₁ mL	129 (19–238)	115 (–6–216)	128 (67–229)	121 (29–243)	115 (–16–223)	124 (58–248)
FVC mL	194 (–30–237)	222 (–42–303)	179 (–32–324)	204 (–15–221)	212 (–34–293)	189 (–42–302)
SGRQ score	12 (6–19)	11 (4–18)	9 (5–14)	11 (5–19)	10 (3–16)	12 (4–17)

Data are presented as mean (95% confidence interval), unless otherwise stated. SFP: salmeterol/fluticasone; Tio+FP: tiotropium and fluticasone combination; Tio: tiotropium only; FEV₁: forced expiratory volume in one second; FVC: forced vital capacity; SGRQ: St George's Respiratory Questionnaire.

expressed and released from neutrophils and alveolar macrophages and is present in the airways of COPD patients at an increased level [31]. The level of MMP-9 has been found to be associated with emphysema formation [32] and lung remodeling [33]. The relationship between decreasing MMP-9 and the slowing of lung function decline by SFP [10, 34] deserves further investigation.

Elevated CRP levels have been associated with a higher probability of cardiac infarction [35]. Inhaled and oral corticosteroids may reduce serum CRP levels in patients with COPD, suggesting a potential application for corticosteroids in improving cardiovascular outcomes in COPD [36]. However, a recent study demonstrated that neither FP nor SFP had a significant effect on serum CRP levels [37]. In the present study, SFP, Tio+FP and Tio treatments did not result in reduced serum CRP levels at the end of treatment. The levels of CRP may be too low to reflect the differences between groups.

Limitations of the current study were that the number of study subjects was small and there was no placebo group for comparison. A placebo group was not included because of ethical considerations. All three treatments are believed to be beneficial to COPD patients. The improvement in terms of FEV₁, FVC and SGRQ score showed no differences between groups, and the potential benefit of adding an inhaled corticosteroid to Tio is not clear. The present study may provide some information that can be used as a basis for further investigation of the anti-inflammatory effects of Tio with or without inhaled corticosteroid.

In conclusion, the anti-inflammatory effects of salmeterol/fluticasone likely contribute to the clinical benefits seen in chronic obstructive pulmonary disease patients. A long-term, large-scale study is needed in order to determine whether adding inhaled corticosteroid to either a long-acting β_2 -agonist or a long-acting anticholinergic or both can modify the progressive nature of chronic obstructive pulmonary disease.

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