



Effect of tiotropium on sputum and serum inflammatory markers and exacerbations in COPD

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ABSTRACT: Chronic obstructive pulmonary disease (COPD) patients experiencing frequent exacerbations demonstrate increased stable-state airway inflammation. Tiotropium has been shown to reduce exacerbation frequency, but its effect on airway inflammation is unknown. The aim of the present study was to investigate the effect of tiotropium on sputum inflammatory markers and exacerbation frequency.

Patients (n=142) were randomised to receive tiotropium or placebo in addition to their usual medication for 1 yr. Sputum and serum cytokines were assayed by ELISA and exacerbation frequency calculated using a symptom-based diary.

There was no difference in the area under the curve for sputum interleukin (IL)-6 or myeloperoxidase between the groups, but sputum IL-8 level was increased in the tiotropium arm. There was no difference between start and end of study in serum IL-6 or C-reactive protein level. Tiotropium was associated with a 52% reduction in exacerbation frequency (1.17 versus 2.46 exacerbations·yr⁻¹). Of patients on tiotropium, 43% experienced at least one exacerbation, compared with 64% on placebo. The total number of exacerbation days was reduced compared with placebo (17.3 versus 34.5 days).

Tiotropium reduces exacerbation frequency in chronic obstructive pulmonary disease, but this effect does not appear to be due to a reduction in airway or systemic inflammation.

KEYWORDS: Anticholinergic, cytokines, sputum

Chronic obstructive pulmonary disease (COPD) is an inflammatory disease of the airways, resulting in a progressive irreversible decline in respiratory function. Patients with COPD experience acute exacerbations characterised by worsening symptoms, deterioration in lung function and increased airway inflammation [1, 2]. Exacerbations are a major cause of morbidity and mortality, and have major resource implications for the treatment of COPD [3, 4].

Patients suffering more frequent exacerbations report worse quality of life, are more likely to become housebound and exhibit increased mortality [3, 5, 6]. Frequent exacerbations are associated with a faster decline in lung function, and it has been shown that patients experiencing more frequent exacerbations demonstrate increased airway inflammation in the stable state

[7, 8]. Therefore, reducing exacerbation frequency is clearly an important objective in the management of COPD.

Tiotropium is a once-daily inhaled anticholinergic bronchodilator. Long-term studies have demonstrated improvements in forced expiratory volume in one second (FEV₁), forced vital capacity (FVC), lung volumes, health-related quality of life and exercise capacity [9–13]. In addition, previous studies have reported reductions in exacerbation frequency [9–11]. Recent prospective multicentre studies have shown an increase in time to first exacerbation with tiotropium compared with placebo, as well as a reduction in exacerbation frequency, hospitalisations and healthcare utilisation [14, 15]; however, the mechanism of this reduction in exacerbations is unknown.

It has been shown that acetylcholine increases neutrophil chemotactic activity in COPD, and

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STATEMENT OF INTEREST

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that this effect is attenuated *in vitro* by tiotropium, thus providing a possible anti-inflammatory mechanism of action [16, 17]. There is a close relationship between airway inflammation and exacerbation frequency, and it might be postulated that a reduction in stable-state airway inflammation would result in a reduction in exacerbation frequency.

There have been several short-term studies on the effect of COPD therapies on airway inflammation [18–21]. The present authors hypothesised that tiotropium might reduce airway inflammation and that this would lead to a reduction in exacerbation frequency. Thus the aim of the present trial was to study, for the first time, the effect of inhaled tiotropium on airway and systemic inflammatory markers in COPD in a 1-yr randomised placebo-controlled study. The effect of tiotropium on exacerbation frequency was also studied, using symptom-based diary cards. Results from the present study have previously been presented in the form of abstracts [22, 23].

METHODS

Study design

The present study was a 1-yr, single-centre, double-blind, randomised, placebo-controlled trial to assess the effect of tiotropium on sputum inflammatory markers and exacerbation frequency. Ethics approval was obtained from the East London and the City Research Ethics Committee (London, UK), the study was registered on ClinicalTrials.gov (NCT00405236) [24] and all patients gave written informed consent. The primary end-point was the concentration of interleukin (IL)-6 in sputum. Secondary end-points included sputum IL-8 and myeloperoxidase (MPO) levels, serum IL-6 and C-reactive protein (CRP) levels, sputum bacterial colonisation, FEV₁ and exacerbation frequency.

Patient selection

Patients aged ≥ 40 yrs with a diagnosis of COPD (FEV₁ $< 80\%$ of the predicted value and FEV₁/FVC $< 70\%$) and a minimum 10-pack-yr smoking history were recruited from primary care or the outpatients department of the London Chest Hospital (London, UK). Patients with a history of asthma or atopy were excluded, as were those on long-term oxygen therapy or with another clinically significant disease. Anticholinergics other than the study drug were not permitted during the course of the study.

Study protocol

Subjects were randomised to 18 μ g tiotropium *q.d.* or placebo *via* the HandiHaler device (Boehringer-Ingelheim, Bracknell, UK). They were provided with diary cards for recording daily symptoms, morning peak flow and drug compliance, and baseline sputum and blood samples were collected.

Patients were seen at weeks 4, 16, 32 and 52 after randomisation. Spirometry was performed, a sputum sample obtained and diaries were examined. Additionally, at weeks 32 and 52, serum samples were collected and patients asked about any change in sputum production.

Laboratory analyses

Patients provided spontaneous or induced sputum samples as previously described [25]. Following processing, sputum IL-6, IL-8 and MPO and serum IL-6 and CRP were quantified by

ELISA. A portion of the sputum sample underwent quantitative and qualitative bacteriological analysis [26, 27]. Further details of these analyses are provided in the online data supplement.

Exacerbations

Patients were asked to record any increase in their respiratory symptoms above their normal stable condition using a letter-annotated system in their diary. The diagnosis of an exacerbation was based upon symptomatic criteria previously validated by the East London COPD Study Group (London, UK) [1, 5, 7]. An exacerbation was defined as the presence, for ≥ 2 days consecutively, of an increase in any two major symptoms (dyspnoea, sputum purulence and sputum volume) or in one major and one minor symptom (wheeze, sore throat, cough and symptoms of a common cold).

Statistics

Analyses were carried out using the full analysis dataset (all randomised treated patients with efficacy data), using ANCOVA that adjusted for smoking status and exacerbation history over the previous year (< 3 or ≥ 3 exacerbations), recorded on recruitment. For sputum markers, the area under the curve (AUC) was calculated for each patient, with missing data replaced by interpolation or the last observation carried forward. The model also included baseline inflammatory marker levels as a covariate. AUCs for IL-6 and MPO were skewed and therefore log₁₀-transformed. For lung function, comparisons were made between changes from the start to the end of the study. The pre-specified data analysis with AUCs was chosen for its ability to estimate the total effect of tiotropium on sputum and serum cytokines over 1 yr rather than the effect at a single time-point [28]. Additional analysis was also carried out using cross-sectional regression models. Systemic inflammatory markers were not sampled at weeks 4 and 16; thus comparisons were made by Wilcoxon signed-rank test between changes from baseline to the final sample.

The effect of tiotropium on individual annual rates (number of exacerbations multiplied by 365/days on drug) was tested using a Wilcoxon test. Differences in time to the next exacerbation were examined using a log-rank test.

Exacerbation recovery times were calculated as the time for a 3-day moving average of peak flow to return, post-exacerbation, to a baseline taken as the average on days 14–8 prior to exacerbation onset.

RESULTS

Figure 1 shows the trial profile of the 237 COPD patients who were enrolled into the study, 142 of whom were randomised (69 to tiotropium and 73 to placebo). The two treatment groups were well matched (table 1); 99 patients had a history of < 3 exacerbations·yr⁻¹ (41 nonsmokers and 58 current smokers) and 43 patients > 3 exacerbations·yr⁻¹ (18 nonsmokers and 25 current smokers; $p = 0.960$ using Chi-squared test).

Sputum and serum inflammatory markers

As a proportion of the patients remaining active in the study, 91, 57, 70, 78 and 67% of patients on tiotropium, and 90, 60, 66, 62 and 63% on placebo, produced a sputum sample at baseline, 1, 3, 6 and 12 months, respectively. Figure 2 shows the results

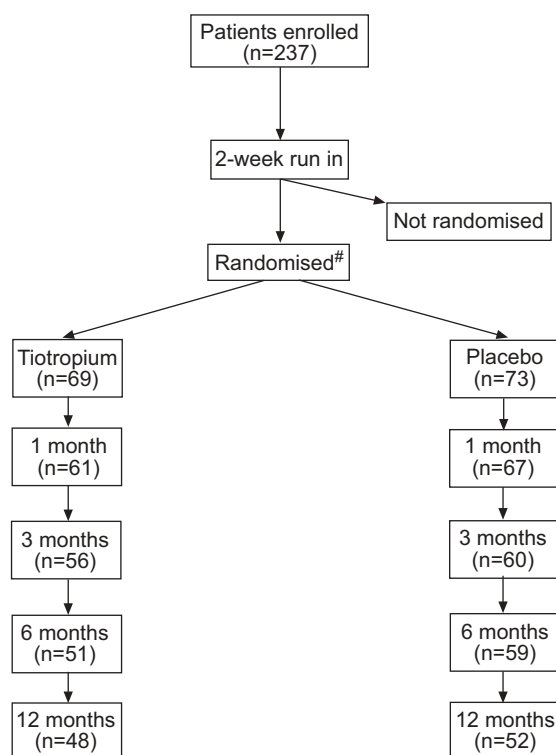


FIGURE 1. Profile of patients remaining in the study. #: all randomised patients received at least one dose of the study treatment.

of the AUC analysis of sputum inflammatory markers. Table 2 shows that there was no difference in the log₁₀-transformed AUC sputum IL-6 level in patients on tiotropium from that in those on placebo after adjustment for baseline values, exacerbation frequency and smoking status ($p=0.324$). The AUC for sputum IL-8 level was 15.4% higher ($p=0.043$) in patients on tiotropium than in those on placebo. In the analysis of sputum IL-8 level, smokers showed significantly higher AUCs ($p=0.029$). There were no significant differences in sputum MPO levels in patients on tiotropium compared with placebo ($p=0.079$). Inclusion in the analysis of whether the patients were taking inhaled steroids did not alter the findings. There was still no effect of tiotropium on sputum IL-6 level ($p=0.327$) or sputum MPO level ($p=0.082$). The AUC for sputum IL-8 level remained higher in patients on tiotropium ($p=0.047$).

There was no significant difference between the groups in change in levels of serum IL-6 ($p=0.691$ using Wilcoxon test) or CRP ($p=0.700$) from baseline to week 52 (table 2). Further analysis using cross-sectional regression models, with similar allowance for exacerbation frequency, smoking habits and baseline levels, gave similar results. Tiotropium did not increase log₁₀ sputum IL-6 concentration ($p=0.540$). The effect of tiotropium on sputum IL-8 level was not significant ($p=0.055$), whereas it caused log₁₀ MPO level to rise by $0.156 \log_{10} \text{IU}\cdot\text{L}^{-1}$ ($p=0.006$). No significant differences were seen in log₁₀ CRP ($p=0.456$) or log₁₀ serum IL-6 concentration ($p=0.058$).

Lung function

During the trial, the mean \pm SD FEV₁ of patients taking tiotropium rose from 1.35 ± 0.47 at baseline to 1.39 ± 0.55 L at

TABLE 1 Patient physical, smoking and exacerbation characteristics and pre-study medication

	Tiotropium	Placebo	p-value
Subjects n	69	73	
Males	69.6	56.2	0.099
≥ 3 exacerbations last year	30.4	30.1	0.969
Current smoker	59.4	57.5	0.820
Age yrs	66.3 ± 8.1	66.4 ± 9.8	0.947
Body mass index $\text{kg}\cdot\text{m}^{-2}$	27.1 ± 6.71	27.1 ± 6.15	0.993
Smoking history pack-yrs	54.6 ± 25.5	55.7 ± 28.0	0.807
FEV₁ L	1.35 ± 0.47	1.23 ± 0.51	0.148
FEV₁ % pred	50.9 ± 14.8	49.2 ± 15.6	0.507
FVC L	2.29 ± 0.75	2.16 ± 0.79	0.317
PEFR weekly mean $\text{L}\cdot\text{min}^{-1}$	261.6 ± 82.5	236.4 ± 84.5	0.075
Pulmonary medication at baseline			
Daily inhaled steroids	73.9	76.7	0.699
Daily oral steroids	4.3	1.4	0.284
Short-acting anticholinergics	44.9	45.2	0.973
Short-acting β -adrenergics	94.2	93.2	0.797
Long-acting β -adrenergics	42.0	43.8	0.828

Data are presented as per cent or mean \pm SD, unless otherwise stated. FEV₁: forced expiratory volume in one second; % pred: % predicted; FVC: forced vital capacity; PEFR: peak expiratory flow rate.

1 yr, whereas patients taking placebo experienced a fall from 1.26 ± 0.49 at baseline to 1.20 ± 0.49 L at 1 yr ($p=0.027$).

The FVC of patients on tiotropium was unchanged between baseline and the end of treatment, at 2.26 ± 0.79 L, whereas patients in the placebo group experienced a decrease in FVC of 0.20 L, from 2.21 ± 0.81 at baseline to 2.01 ± 0.81 L at the end of treatment ($p=0.003$).

Exacerbations

The patients in the tiotropium-treated group experienced 60 exacerbations compared with 134 in the placebo arm (table 3). Patients on tiotropium showed fewer exacerbations per year (mean \pm SD 1.17 ± 2.25 ; median (interquartile range) 0 (0–1.0)) compared with those on placebo (2.46 ± 3.82 ; 1.6 (0–3.0); $p=0.001$ using Wilcoxon test), representing a 52% reduction in exacerbation frequency on tiotropium. The number of exacerbations in those treated with antibiotics or steroids was also reduced (0.98 ± 2.11 in the tiotropium arm compared with 1.73 ± 2.46 on placebo; $p=0.007$). Of the patients on tiotropium, 43% experienced at least one exacerbation during the study period, compared with 64% of those on placebo ($p=0.01$; fig. 3). Survival-time analysis indicated the mean time to first exacerbation was 236 ± 143 days (SEM 17.2) with tiotropium, compared with 157 ± 124 days (SEM 14.6) with placebo ($p=0.0092$ using log-rank test). There were significantly fewer exacerbation days in patients on tiotropium than in those receiving placebo (17.3 ± 33.6 (median 0) versus 34.5 ± 47.5 (median 21.7) days $\cdot\text{yr}^{-1}$; $p=0.002$ using Wilcoxon test). This was due to a reduction in the number of exacerbations, as there was no difference in the length of the exacerbations, as

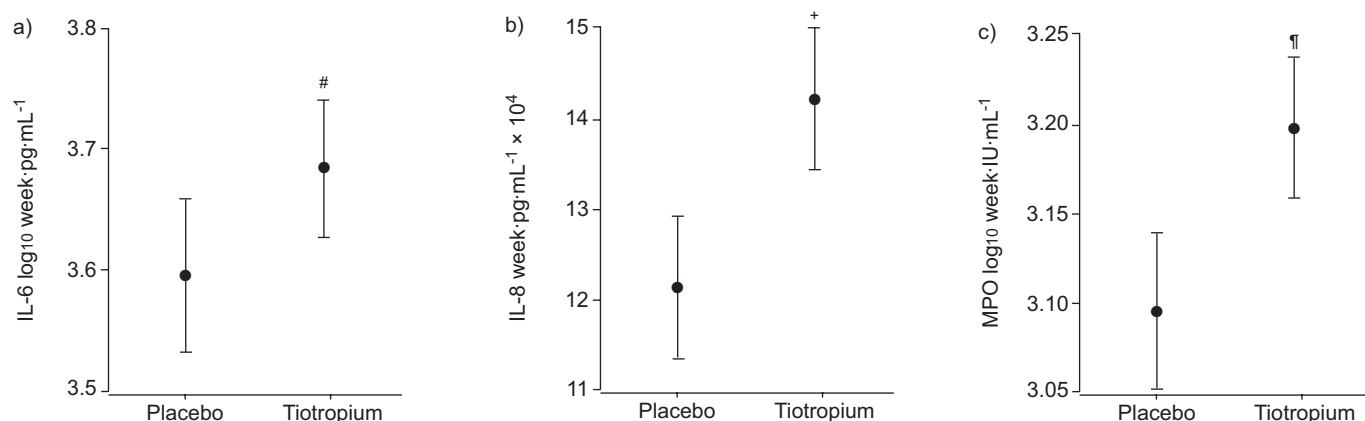


FIGURE 2. Areas under the curve (AUCs) of sputum inflammatory markers: a) interleukin (IL)-6; b) IL-8; and c) myeloperoxidase (MPO). AUCs for IL-6 and MPO were log₁₀-transformed. Data are presented as mean ± SEM. #: $p=0.324$; †: $p=0.079$; +: $p=0.043$ versus placebo (ANCOVA with adjustment for exacerbation frequency and smoking).

indicated by symptom count recovery times: 9.89 ± 6.94 days on tiotropium versus 9.54 ± 6.96 days on placebo ($p=0.748$).

There were no differences in the symptoms recorded at exacerbation onset in the 60 exacerbations of the tiotropium group and 134 exacerbations of the placebo group. Tiotropium was associated with a subjective reduction in sputum production in 33% of patients compared with 7.9% in the placebo group ($p=0.001$ using Chi-squared test).

There was no difference in the proportion of treated exacerbations in the tiotropium group, 50 (83.3%) out of 60, compared with 108 (80.6%) out of 134 in the placebo group ($p=0.650$ using Chi-squared test). Two patients who experienced exacerbations on tiotropium were hospitalised, compared with three on placebo ($p=0.657$).

Bacteria

There were no differences in the proportion of any bacterial species identified at any time-point between tiotropium and placebo. The commonest species isolated was *Haemophilus influenzae*, present in 10–20% of patients at each time-point. Mean bacterial counts were similar at all time-points (at baseline: $1 \times 10^{6.6}$ colony-forming units (cfu)·mL⁻¹ and $1 \times 10^{6.9}$ cfu·mL⁻¹

for patients on tiotropium and placebo, respectively; week 52: $1 \times 10^{7.2}$ and $1 \times 10^{6.9}$ cfu·mL⁻¹, respectively).

Study compliance and withdrawals

Of patients receiving tiotropium, 82.6% took >90% of their medication, compared with 90.4% of those on placebo ($p=0.172$). Compliance with diary cards was also high. Patients on tiotropium recorded diary data for 94.5% of the time, whereas those on placebo recorded diary card data for 94.1% of the time.

Figure 1 shows that during the study, 21 (30.4%) out of 69 patients on tiotropium withdrew before 1 yr, compared with 21 (28.8%) out of 73 on placebo ($p=0.828$). There were no significant differences in withdrawals at any other time-points. Seven (10.1%) patients on tiotropium and 14 (19.2%) on placebo withdrew due to an adverse event ($p=0.130$).

Adverse events

Table 4 shows adverse events during treatment, excluding exacerbations. Most adverse events were mild or moderate in intensity. None of the adverse events were considered to be therapy related, with the exception of one episode of pruritus in the tiotropium group and one of palpitations in the placebo group.

TABLE 2 Areas under the curve of sputum inflammatory markers for tiotropium and placebo treatment arms

	Tiotropium		Placebo		p-value
	Subjects n	Mean	Subjects n	Mean	
IL-6 [#] week·pg·mL ⁻¹	55	4898.5 (3894.0–6162.3)	58	4198.1 (3326.7–5297.7)	0.324 [§]
IL-8 [†] week·pg·mL ⁻¹	55	143325 (129623–157027)	58	124223 (110331–138116)	0.043 [§]
MPO [#] week·IU·L ⁻¹	51	1512.5 (1255.5–1822.0)	58	1210.0 (1006.0–1455.3)	0.079 [§]
Serum marker⁺					
IL-6 pg·mL ⁻¹	50	-20.4 ± 6.6	58	-3.3 ± 6.3	0.691 ^f
CRP mg·L ⁻¹	34	-0.80 ± 1.2	37	-0.05 ± 1.8	0.700 ^f

Data are presented as mean ± SEM, unless otherwise stated. IL: interleukin; MPO: myeloperoxidase; CRP: C-reactive protein. #: means presented as geometric mean (95% confidence interval), adjusted for baseline, exacerbation frequency and smoking status; †: means presented as mean (95% confidence interval), adjusted for baseline, exacerbation frequency and smoking status; +: change from baseline to week 52; §: ANCOVA; f: Wilcoxon signed-rank test.

TABLE 3	Exacerbation parameters in tiotropium and placebo group		
	Tiotropium	Placebo	p-value
Subjects n	69	73	
Rate exacerbations·yr ⁻¹	1.17±2.25	2.46±3.82	0.001
Rate treated exacerbations·yr ⁻¹	0.98±2.11	1.73±2.46	0.007
Patients with no exacerbations during study %	56.5	35.6	0.012
Time to first exacerbation days	236±143	157±124	0.009
Days of exacerbation days·yr ⁻¹	17.3±33.6	34.5±47.5	0.002
Symptom recovery time days	9.89±6.94 [#]	9.54±6.96 [†]	0.748

Data are presented as mean±SD, unless otherwise stated. [#]: 54 exacerbations; [†]: 125 exacerbations.

DISCUSSION

The present study is the first to assess, using a symptom-based exacerbation definition, the effect of an inhaled anticholinergic therapy on sputum and systemic inflammatory markers and exacerbations over a 12-month period in patients with COPD. There were no significant differences in levels of sputum IL-6 and MPO or systemic inflammatory markers between the groups, but sputum IL-8 concentration was significantly higher in the tiotropium group at the end of the study. A significant reduction of 52% in exacerbation frequency, together with a reduction in total exacerbation days and the proportion of patients suffering at least one exacerbation, was demonstrated in the tiotropium group compared with placebo.

COPD is associated with progressive airway and systemic inflammation, and exacerbations are associated with increases in levels of corresponding inflammatory markers [8, 29, 30]. These exacerbations are important events in the natural history of the disease, but the mechanisms by which therapies reduce exacerbation frequency are not clearly understood. It has previously been demonstrated that there are increased levels of sputum IL-6 and IL-8 in COPD patients experiencing frequent exacerbations, suggesting that frequent exacerbators show increased airway inflammation in the stable state [8]. Therefore, it might be postulated that a reduction in airway inflammation might lead to a reduction in exacerbation frequency.

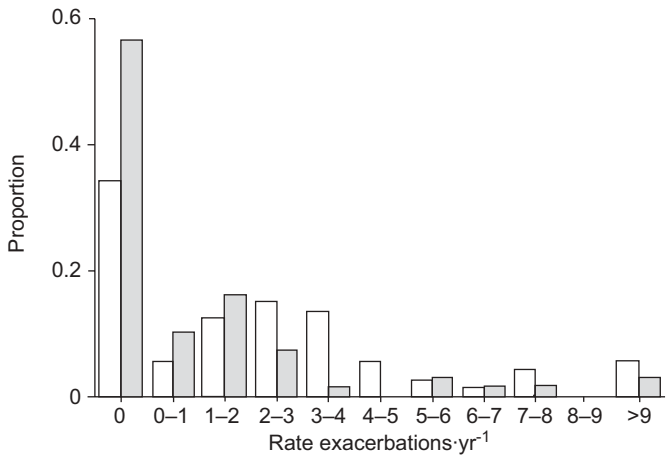


FIGURE 3. Proportion of patients on tiotropium (■) or placebo (□) during the study by annual exacerbation rate.

Short-term biopsy studies have demonstrated a reduction in numbers of epithelial mast cells and CD8 cells after therapy with fluticasone and salmeterol/fluticasone [19, 21]. However, no studies have demonstrated an effect of inhaled therapy on sputum cytokines. It has recently been shown that acetylcholine increases neutrophil chemotactic activity and production of leukotriene B₄ in COPD, and this action is attenuated *in vitro* by tiotropium, thus indicating a potential anti-inflammatory action of tiotropium [16, 17]. However, in the present study, no effect of tiotropium on sputum IL-6 or MPO level was demonstrated, and thus the reduction in exacerbation frequency cannot be related to changes in airway inflammation.

Surprisingly, tiotropium therapy was associated with a significant increase in the concentration of sputum IL-8. Tiotropium could potentially inhibit cholinergic stimulation of mucus-secreting airway goblet cells, thus causing a reduction in airway mucus and increasing the concentration of cytokines. This is supported by the subjective reporting of reduced sputum production by patients in the present study. In common with previous studies of COPD therapies, no reduction in levels of sputum inflammatory markers was demonstrated, and it is possible that measurement of sputum cytokines is not the optimal means of assessing airway inflammation.

TABLE 4	Adverse events occurring in >3% of patients during the treatment period [#]	
	Tiotropium	Placebo
Subjects	69	73
Hypertension	5 (7.2)	1 (1.4)
Myocardial infarction	3 (4.3)	1 (1.4)
Urinary tract infection	3 (4.3)	1 (1.4)
Pharyngitis	3 (4.3)	1 (1.4)
Pneumonia	2 (2.9)	3 (4.1)
Ear infection	1 (1.4)	3 (4.1)
Tooth extraction	0 (0.0)	3 (4.1)

Data are presented as n or n (%). The treatment period was defined as the period between the start of treatment and end of treatment plus 30 days following the end of treatment. [#]: excluding exacerbation of chronic obstructive pulmonary disease.

It is well known that systemic inflammation is a factor in COPD. Inhaled fluticasone has been shown to reduce levels of serum CRP, thus indicating a reduction in systemic inflammation [31]. Serum CRP and IL-6 levels were studied in order to assess systemic inflammation, but no differences were found between tiotropium and placebo.

The present trial demonstrated a significant reduction in exacerbation frequency in the tiotropium group, in keeping with previous studies, although a larger (52%) reduction in exacerbation frequency was found than previously reported. This is the first intervention trial to employ the previously validated method of a daily diary card over 1 yr for the detection of exacerbations [1, 5]. There has been much debate as to what constitutes a COPD exacerbation, and several definitions have been proposed [5, 32, 33]. Some have included an increase in respiratory symptoms from baseline requiring a change in usual therapy. This relies on the patient seeking healthcare assistance, and, as such, presents some associated difficulties. Factors other than the severity of symptoms may prevent the patient from seeking input from a healthcare practitioner. It has previously been shown that 50% of exacerbations go unreported and that these unreported events also impact on quality of life and are associated with disease progression [1, 5, 7]. The advantage of the diary cards is that all exacerbations, whether reported or unreported, can be detected. Compliance in using the diary card was extremely good in the present trial, indicating their utility as a tool for detecting exacerbations.

Patients in the tiotropium arm demonstrated an improvement in FEV₁ and FVC over the year of the study, whereas patients on placebo demonstrated a decline in lung function. Tiotropium has been shown to improve inspiratory capacity, thus reducing dyspnoea, which is an important symptom of a COPD exacerbation [12, 13]. It is this reduction in dyspnoea that is probably a major factor in the reduction in exacerbation frequency demonstrated in the tiotropium group.

Mucus secretion is frequently increased in patients with COPD. This mucus hypersecretion might be associated with airway obstruction and disruption of the mucociliary escalator, and provide an attractive milieu for airway bacterial colonisation. These changes might be expected to be associated with increased inflammation and exacerbation frequency. Secretion from mucous glands is mediated by cholinergic pathways and potentially inhibited by anticholinergics. TAMAOKI *et al.* [34] showed a reduction in sputum volume using the anticholinergic agent oxitropium. Therefore, a reduction in mucus hypersecretion is a further potential mechanism by which tiotropium might reduce exacerbation frequency. Although quantitative measurements of sputum were not performed in the present study, patients on tiotropium reported a subjective reduction in sputum volume, suggesting that tiotropium might indeed have an effect on mucus secretion.

Patients with COPD may demonstrate lower airway bacterial colonisation, which is associated with increased airway inflammation, disease progression and increased exacerbation frequency [27, 35]. Therefore, rates of lower airway bacterial colonisation were studied in the present trial, but no differences were found between the tiotropium and placebo arms of the study.

The principal limitation of the present study was difficulty in the use of sputum inflammatory markers, since sputum samples were difficult to obtain, although there was no significant difference between the groups. In addition, there was marked variability in levels of sputum cytokines in the trial, thus reducing the power of the study to detect a significant difference. This may also explain the failure of previous studies to demonstrate an effect of COPD therapies on airway inflammatory markers. Many patients in the present study were taking inhaled steroids, and it might be argued that this was a potentially confounding factor; however, no significant differences in inflammatory markers were demonstrated between those taking and those not taking inhaled steroids.

This was a cohort of patients with moderate-to-severe disease and, as it has been shown that steroid withdrawal results in a deterioration in lung function and increase in exacerbation frequency [36], it was decided that the patients should remain on inhaled corticosteroids.

In summary, the present study was the first year-long trial using the present symptom-based exacerbation definition and daily diary card monitoring. The results of the study showed a significant reduction in exacerbation frequency in chronic obstructive pulmonary disease patients treated with tiotropium, compared with those receiving placebo. However, this effect does not seem to be related to a reduction in airway or systemic inflammation and is likely to be related to reductions in dynamic hyperinflation or airway mucus production. Further studies are now required in order to evaluate the mechanisms of this effect of inhaled tiotropium on exacerbation frequency in chronic obstructive pulmonary disease.

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