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Combining tiotropium and salmeterol in COPD: Effects on airflow obstruction and symptoms☆

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Chronic obstructive pulmonary disease;
Combination therapy;
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Inhaled long-acting β_2-agonist;
Lung function;
Dyspnea

Summary
Background: Clinical information on 24-h spirometric efficacy of combining tiotropium and salmeterol compared to single-agent therapy is lacking in patients with COPD.
Methods: A randomized, double-blind, four-way crossover study of 6-week treatment periods comparing combination therapy of tiotropium 18 μg *plus* qd or bid salmeterol 50 μg versus single-agent therapy. Serial 24-h spirometry (FEV_1, FVC), effects on dyspnea (TDI focal score) and rescue salbutamol use were evaluated in 95 patients.
Results: Tiotropium *plus* qd salmeterol was superior to tiotropium or salmeterol alone in average FEV_1 (0–24 h) by 72 mL and 97 mL ($p < 0.0001$), respectively. Compared to this qd regimen, combination therapy including bid salmeterol provided comparable daytime (0–12 h: 12 mL, $p = 0.38$) bronchodilator effects, but significantly more bronchodilation during the night-time (12–24 h: 73 mL, $p < 0.0001$). Clinically relevant improvements in TDI focal score were achieved with bronchodilator combinations including salmeterol qd or bid (2.56 and 2.71; $p < 0.005$ versus components). Symptom benefit of combination therapies was also reflected in less need for reliever medication. All treatments were well tolerated.
Conclusion: Compared to single-agent therapy, combination therapy of tiotropium *plus* salmeterol in COPD provided clinically meaningful improvements in airflow obstruction and dyspnea as well as a reduction in reliever medication.

☆ Study not registered in the Clinical Trials Registry; all patients were entered before implementation of the registry.

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Introduction

Inhaled bronchodilators are the mainstay in the pharmacological management of chronic obstructive pulmonary disease (COPD) and are recommended for treatment of symptoms at all stages of the disease. When maintenance therapy is required to adequately control symptoms of COPD, the guidelines highlight long-acting bronchodilators as being more effective and convenient, and recommend mono- or combination therapy of these agents in moderate-to-severe COPD.[1,2]

To date treatment options with long-acting bronchodilators include the once-daily anticholinergic tiotropium, providing sustained 24-h bronchodilation, and the two β_2-adrenoceptor agonists formoterol and salmeterol, requiring twice daily dosing over a 24-h period. Numerous controlled trials have addressed the efficacy and safety of these agents as mono-therapies in COPD.[3–8] Clinical information on the combination of these two types of long-acting bronchodilator is emerging. The potential benefit of co-administration of tiotropium and twice daily β_2-adrenoceptor agonists has been demonstrated in terms of lung function improvement and clinical outcomes.[9–19] Most of these studies evaluated combination therapy of tiotropium with formoterol and to date limited data exist on combination therapy with salmeterol. Previously, we showed that co-administration of tiotropium and formoterol is superior to single-agent therapy throughout a 24-h dosing interval.[10,11] Spirometric improvements of tiotropium *plus* salmeterol with serial FEV_1 and FVC measurements over a full 24-h observation period are not available. The present study was designed to characterize the 24-h bronchodilating profile of tiotropium in combination with salmeterol, to evaluate symptom relief assessed by a reduction in dyspnea and need for reliever medication.

Methods

Patients

Patients, aged $\geq$40 years and with documented diagnosis of COPD,[20] had to have a pre-bronchodilator forced expiratory volume in one second (FEV_1) $\leq$ 60% predicted[21] and an $FEV_1/FVC \leq 70\%$. All were current or ex-smokers with $\geq$10 pack-yr smoking history. Specific exclusion criteria included diagnosis of asthma, atopy, allergic rhinitis or an elevated blood eosinophil count ($\geq$600 mm^3). Also, patients with a recent history of myocardial infarction, heart failure or cardiac arrhythmia requiring drug therapy, known symptomatic prostatic hypertrophy and narrow-angle glaucoma were excluded. Randomization of patients who suffered from a COPD exacerbation in the 6 weeks prior to screening or during the run-in period was postponed till 6 weeks following recovery from the event.

Design

The hospital medical ethics committees approved the study protocol (study code 1184.7) and written informed consent by the patients was obtained prior to any study-related procedure. The study had a randomized, double-blind (double-dummy), crossover design with four 6-week treatment periods (Fig. 1): tiotropium 18 μg *qd* via HandiHaler®, salmeterol 50 μg *bid* via metered-dose inhaler (MDI), tiotropium *qd plus* salmeterol *qd* or *bid*. The time interval between inhalation of the morning and evening medication was ~12 h. Patients completed a 2-week run-in period following screening to ensure clinical stability; this period was also used for training of appropriate recording of daily use of salbutamol and twice-daily peak expiratory flow rate (PEFR). All inhaled short-acting bronchodilators (8 h) and long-acting β_2-agonists (48 h) were withdrawn at randomization (identical washout periods before screening visit); tiotropium and theophylline preparations were not allowed for $\geq$4 weeks prior to screening. Patients continued to use inhaled steroids and oral steroids up to the equivalent of daily 10 mg prednisone. Eligible patients were provided open-label salbutamol for use of acute symptom relief.

Assessments

Following the qualifying pre-bronchodilator spirometric test (FEV_1 and FVC), severity of COPD was assessed 45 min following inhalation of 4 puffs of salbutamol 100 μg.[1] After completion of the run-in period study baseline FEV_1 and

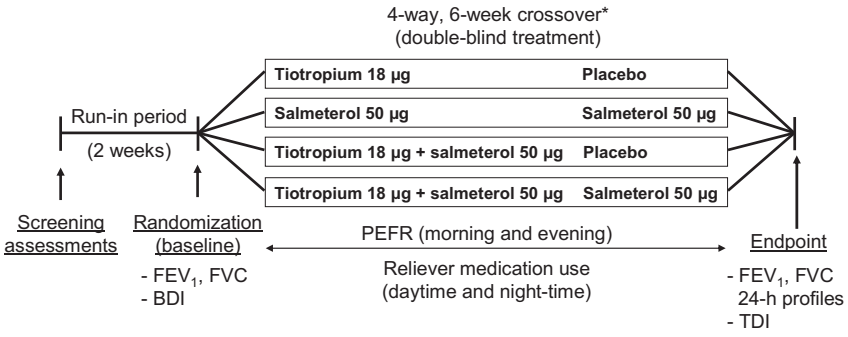

Figure 1 Study design of the three-centre, randomized, double-blind, crossover study with four 6-week treatment periods. Tiotropium (Spiriva®) and tiotropium-matched placebo powder capsule via HandiHaler® (Boehringer Ingelheim); Salmeterol (Serevent®, GlaxoSmithKline) and salmeterol-matched placebo via metered-dose inhaler. FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEFR, peak expiratory flow rate; BDI, Baseline Dyspnea Index; TDI, Transitional Dyspnea Index. *No washout period between each 6-week period of randomized treatment.

FVC were determined before inhalation of the first dose of study medication. Serial spirometry was conducted at the end of each treatment period including readings 10 min prior to and ½, 1, 2, 3, 4, 6, 8, 10 and 12 h after inhalation of the morning dose, and continued at ½, 1, 2, 7, 10, 11 and 12 h after inhalation of the evening dose of study medication. Testing started between 08:00 and 10:00 h with 30 min maximum difference between the start at the randomization visit and the tests on each 24-h pulmonary function test-day. Measurements were performed according to American Thoracic Society (ATS) criteria[22]; the highest values of FEV_1 and FVC from three technically adequate measurements were retained.

Dyspnea was evaluated using the Baseline Dyspnea Index (BDI) and the Transition Dyspnea Index (TDI).[23] The BDI (at randomization visit) and TDI (at the end of each 6-week period) were administered before any other study-related assessments were performed. Patients completed a daily diary card recording morning and evening peak expiratory flow rate (PEFR) always before inhalation of study medication. Use of rescue salbutamol was recorded separately for daytime and night-time.

Safety assessments included a medical examination, laboratory testing and a 12-lead ECG recording on entry and upon completion of the study. At each visit clinical status and adverse events were recorded; at the 24-h pulmonary function test-days vital signs were recorded for the first six hours after the morning dose.

Statistical analysis

Primary efficacy endpoint was the average FEV_1 over the 24-h observation period (0—24 h). This was calculated as the area under the curve from zero time (i.e. the pre-dose FEV_1) to 24 h using the trapezoidal rule divided by the corresponding duration (i.e. 24 h). Secondary spirometry-based endpoints were trough, peak, average FEV_1 over the first 12 h (0—12 h) and the second 12 h (12—24 h), and FEV_1 values at individual time points. The average FEV_1 (0—12 h) and FEV_1 (12—24 h) were calculated similar to FEV_1 (0—24 h). Trough was defined as the pre-dose value measured at the beginning of the observation period. Peak FEV_1 was the highest reading observed within 3 h after inhalation of the morning dose of study medication. Trough, peak and average responses were defined as the change from the study baseline FEV_1, i.e. the FEV_1 determined at the randomization visit before inhalation of the first dose of study medication. Analogous definitions were used for FVC-based parameters. The effect on dyspnea was evaluated using the TDI focal score; a difference of $\geq$1 unit was considered clinically meaningful. Morning and evening PEFR, and 'as-needed' salbutamol (daytime and night-time) were the diary-based endpoints. Data collected in the first 3 weeks of each period were discarded in order to eliminate possible carry-over effects[24,25] and the means over remaining days of each period calculated.

The planned sample size was 80 completed patients. Assuming a standard deviation of 135 mL for paired differences,[10] this sample size provides a power of 95% to detect a difference of 55 mL in average FEV_1 (0—24 h) (type I error rate: 0.05), resulting in an overall power for both comparisons (combination once-daily *versus* single-agent therapy) of 90%.

For all endpoints, adjusted means for the treatments were calculated using a fixed-effects analysis of variance model with terms for centre, patients within centre, treatment and period. Patients with on-treatment data were included in the analysis (safety: 97, diary and spirometric endpoints: 93 and 95, respectively). Sensitivity analysis did neither reveal a period effect nor a treatment by centre interaction. For the primary endpoint, treatment means were compared in a prespecified order to control type I error rate (fixed sequence testing). For the other endpoints, no adjustments for multiple comparisons were utilized. Statistical significance was considered at $p < 0.05$. Missing spirometry values were imputed using other values recorded for the patient on that test-day. Data were also analyzed based on patients who completed all 24-h pulmonary function tests. This sensitivity analysis showed consistent results.

Results

Ninety-seven out of 103 screened patients were randomized; 8 of them prematurely discontinued the trial (Fig. 2). All treated patients were included in the safety analysis. For two patients no efficacy data were available as they discontinued the trial in the first three weeks of the first treatment period. Therefore, 95 patients were included in the efficacy analysis; Table 1 provides demographics and baseline characteristics.

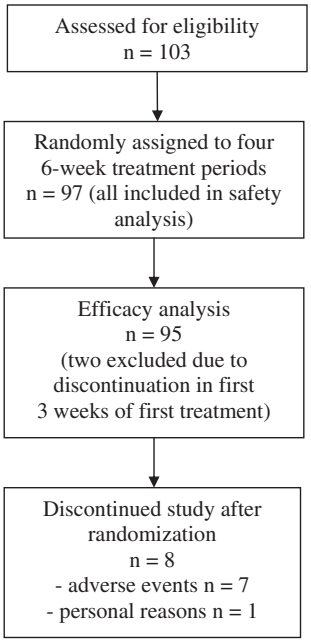

Figure 2 Flow diagram of the three-centre, randomized, double-blind (double-dummy), crossover study with four 6-week treatment periods. The diagram presents information on the number of patients screened for eligibility, randomized to treatment, included in the efficacy analysis and the number discontinued in the study.

Table 1 Demographics and baseline disease characteristics of patients included in the efficacy population.[a]

Variable	Data[b]
No. of patients	95
Age, yr	64 ± 9
Males/females	76/19
Smoking status	
Current smokers, *n* (%)	25 (26)
Ex-smokers, *n* (%)	70 (74)
Smoking history (pack-years)	36 ± 17
Prebronchodilator FEV_1 (L)	1.09 ± 0.34
Prebronchodilator FEV_1% predicted	39 ± 10
Postbronchodilator FEV_1 (L)	1.28 ± 0.39
Postbronchodilator FEV_1% predicted	45 ± 11
FEV_1 reversibility[c]	
L	0.19 ± 0.14
% baseline	18 ± 13
% predicted	7 ± 5
COPD severity according to GOLD	
Moderate, *n* (%)	31 (33)
Severe, *n* (%)	57 (60)
Very severe, *n* (%)	7 (7)
Prebronchodilator FVC (L)	2.86 ± 0.76
Prebronchodilator FEV_1/FVC %	39 ± 9
Postbronchodilator FVC (L)	3.22 ± 0.82
Postbronchodilator FEV_1/FVC %	40 ± 10
Prestudy respiratory medication use[d]	
Inhaled anticholinergics	75 (79)
Inhaled β_2-adrenergics	93 (98)
Inhaled steroid	81 (85)
Oral steroid	5 (5)

FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; COPD, chronic obstructive pulmonary disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease.[1]

[a] For 2 of the 97 randomized patients no efficacy data available as they discontinued in the first 3 weeks of the first 6-week period of randomized treatment.

[b] Values are presented as mean SD or No. (%) unless otherwise stated.

[c] Response 45 min following four puffs of 100 μg of salbutamol (Ventolin® metered-dose inhaler, GlaxoSmithKline).

[d] Patients could have received more than one of these medications.

Spirometry

The 24-h FEV_1 time—response curves are shown in Fig. 3; the endpoints derived from these curves are presented in Table 2.

Combination treatment with tiotropium *plus* once-daily salmeterol provided significantly greater bronchodilation compared with the individual components as measured by the average FEV_1 (0—24 h). During the daytime (0—12 h) the additional improvement in FEV_1 *versus* salmeterol ranged from 0.128 L to 0.148 L ($p < 0.0001$), whereas improvements compared with tiotropium ranged from 0.069 L to 0.113 L ($p < 0.0001$). Also during the night-time (12—24 h) this combination regimen performed significantly ($p < 0.001$) better compared with each of the components; its sustained bronchodilator effect was also reflected in a significantly higher trough value. In terms of average FEV_1 (0—24 h), co-administration of tiotropium *plus* twice daily salmeterol was superior ($p < 0.0011$) to the once-daily combination as a result of an additional increase in FEV_1 after the evening salmeterol dose. During the daytime (0—12 h) no difference was found, whereas during the night-time (12—24 h) all mean FEV_1 values observed at all time points for combination therapy including salmeterol *bid* were significantly higher compared with once-daily combination therapy, resulting in a superior average FEV_1 ($p < 0.0001$).

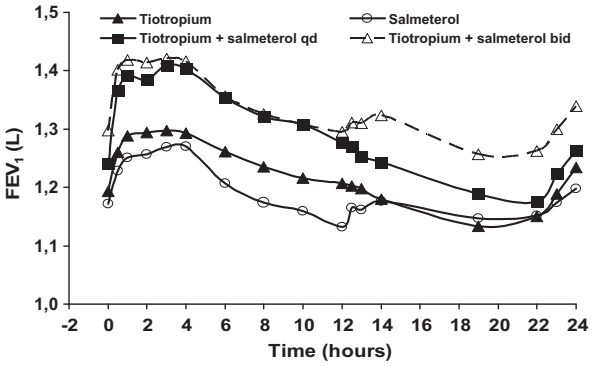

Figure 3 Mean* FEV_1 before (trough) and during 24 h after the inhalation of tiotropium *q.d.* (morning) (▲), salmeterol *b.i.d.* (morning and evening) (○), tiotropium *q.d. plus* salmeterol *q.d.* (both in the morning) (■), and tiotropium *q.d.* (morning) *plus* salmeterol *b.i.d.* (morning and evening) (△) at the end of 6-week treatment periods. The baseline mean FEV_1 at the randomization visit is 1.116 L. *Adjusted for centre, patient within centre and period.

Treatment with tiotropium resulted in significantly ($p < 0.05$) greater bronchodilation compared with salmeterol in terms of average FEV_1 (0—24 h), mainly due to the superior daytime (0—12 h) spirometric efficacy of tiotropium. In addition to a higher peak FEV_1 ($p < 0.02$), tiotropium provided a significantly greater improvement from 6 to 12 h in the range of 0.054 L to 0.075 L ($p < 0.002$). During the night-time no difference in average FEV_1 (12—24 h) was observed between tiotropium and salmeterol.

The 24-h FVC profiles are depicted in Fig. 4 and the derived endpoints are shown in Table 2. The results for FVC paralleled the results found for FEV_1. Both combination therapies provided significantly higher FVC values at all time points during the 24-h observation period compared to each of the single agents, resulting in a significantly better performance of combination therapy in all FVC-derived endpoints. Also for single-agent therapy, the pattern of improvement in FVC was similar to that in FEV_1. Tiotropium was superior to salmeterol in average FVC (0—12 h, 0—24 h) and peak FVC, whereas no difference was found for average FVC (12—24 h).

PEFR

PEFR data for the treatments were in line with FEV_1 and FVC results (Table 3). Both combination regimens provided significantly additional improvements in morning and

Table 2 Average, peak and trough response[a] in FEV_1 and FVC, and the comparison between the four treatment regimens.

Variable	Average[b]			Peak	Trough
	0–12 h	0–24 h	12–24 h		
FEV1 response (L)					
[T + S]	0.208 ± 0.010	0.142 ± 0.010	0.076 ± 0.010	0.314 ± 0.012	0.101 ± 0.012
[T + S] + S	0.221 ± 0.010	0.185 ± 0.009	0.148 ± 0.010	0.331 ± 0.012	0.158 ± 0.012
T	0.115 ± 0.011	0.070 ± 0.010	0.026 ± 0.010	0.214 ± 0.012	0.055 ± 0.012
S + S	0.068 ± 0.011	0.045 ± 0.010	0.021 ± 0.010	0.173 ± 0.012	0.033 ± 0.013
[T + S] versus T, *p*-value	0.093 (0.065–0.122), <0.0001	0.072 (0.046–0.097), <0.0001	0.050 (0.023–0.077), 0.0003	0.100 (0.067–0.133), <0.0001	0.047 (0.013–0.080), 0.0061
[T + S] versus S + S, *p*-value	0.140 (0.112–0.169), <0.0001	0.097 (0.072–0.123), <0.0001	0.054 (0.027, 0.081), <0.0001	0.141 (0.108–0.174), <0.0001	0.069 (0.036–0.102), <0.0001
[T + S] + S versus T, *p*-value	0.106 (0.078–0.134), <0.0001	0.114 (0.089–0.140), <0.0001	0.122 (0.096–0.149), <0.0001	0.117 (0.085–0.150), <0.0001	0.103 (0.070–0.137), <0.0001
[T + S] + S versus S + S, *p*-value	0.153 (0.125–0.181), <0.0001	0.140 (0.114–0.165), <0.0001	0.127 (0.100–0.154), <0.0001	0.158 (0.125–0.191), <0.0001	0.125 (0.092–0.158), <0.0001
[T + S] + S versus [T + S], *p*-value	0.012 (−0.016 to 0.040), 0.38	0.043 (0.017–0.068), 0.0011	0.073 (0.046–0.100), <0.0001	0.017 (−0.015 to 0.050), 0.29	0.057 (0.024–0.090), 0.0009
T versus S + S, *p*-value	0.047 (0.019–0.075), 0.0011	0.026 (0.000–0.051), 0.0495	0.004 (−0.023 to 0.031), 0.75	0.040 (0.007–0.074), 0.0166	0.022 (−0.011 to 0.055), 0.20
FVC response (L)					
[T + S]	0.357 ± 0.017	0.251 ± 0.016	0.144 ± 0.019	0.535 ± 0.021	0.198 ± 0.025
[T + S] + S	0.353 ± 0.017	0.292 ± 0.016	0.230 ± 0.019	0.553 ± 0.020	0.252 ± 0.024
T	0.200 ± 0.017	0.114 ± 0.016	0.028 ± 0.020	0.401 ± 0.021	0.081 ± 0.025
S + S	0.096 ± 0.017	0.046 ± 0.016	−0.005 ± 0.020	0.305 ± 0.021	0.026 ± 0.025
[T + S] versus T, *p*-value	0.158 (0.112–0.203), <0.0001	0.137 (0.093–0.181), <0.0001	0.116 (0.064–0.169), <0.0001	0.134 (0.079–0.190), <0.0001	0.117 (0.051–0.184), 0.0006
[T + S] versus S + S, *p*-value	0.261 (0.216–0.306), <0.0001	0.205 (0.162–0.249), <0.0001	0.149 (0.097–0.201), <0.0001	0.230 (0.174–0.285), <0.0001	0.172 (0.106–0.239), <0.0001
[T + S] + S versus T, *p*-value	0.153 (0.108–0.198), <0.0001	0.178 (0.134–0.221), <0.0001	0.202 (0.150–0.255), <0.0001	0.153 (0.098–0.208), <0.0001	0.171 (0.105–0.238), <0.0001
[T + S] + S versus S + S, *p*-value	0.257 (0.211–0.302), <0.0001	0.246 (0.202–0.289), <0.0001	0.235 (0.183–0.287), <0.0001	0.248 (0.193–0.304), <0.0001	0.226 (0.160–0.293), <0.0001

(*continued on next page*)

Table 2 (*continued*)

Variable	Average[b]			Peak	Trough
	0–12 h	0–24 h	12–24 h		
[T + S] + S versus [T + S], *p*-value	−0.005 (−0.050 to 0.040), 0.84	0.041 (−0.003 to 0.084), 0.0652	0.086 (−0.034 to 0.138), 0.0013	0.019 (−0.036 to 0.074), 0.50	0.054 (−0.012 to 0.120), 0.11
T versus S + S, *p*-value	0.103 (0.058–0.149), <0.0001	0.068 (0.024–0.112), 0.0024	0.033 (−0.020 to 0.085), 0.22	0.095 (0.040–0.151), 0.0008	0.055 (−0.012 to 0.122), 0.11

T, tiotropium (morning); S + S, salmeterol *bid* (morning and evening); [T + S], tiotropium *plus* salmeterol *qd* (both in the morning); [T + S] + S, tiotropium (morning) *plus* salmeterol *bid* (morning and evening).
FEV_1, forced expiratory flow in 1 s; FVC, forced vital capacity.
[a] Data are presented as mean ± SE and mean (95% confidence interval) unless otherwise stated. Study baseline values are 1.12 L (FEV_1) and 2.93 L (FVC). Treatment responses are adjusted for centre, patient within centre and period.
[b] Average was calculated as the area under the curve from zero time to 12 or 24 h (or 12–24 h), respectively, using the trapezoidal rule divided by the corresponding duration (i.e. 12 or 24 h) to give results in litres.

evening PEFRs compared with single-agent therapy. No difference was found between the single agents in morning PEFR, whereas tiotropium was superior to salmeterol in terms of evening PEFR.

Dyspnea and use of rescue salbutamol

The mean (±SE) BDI focal score was 7.0 (±2.5). Both combination regimens provided a clinically and statistically significant ($p < 0.005$) improvement in mean TDI focal score compared to each of the single agents (Table 3). The proportion of patients who achieved a clinically meaningful improvement in TDI focal score was greater during treatment with tiotropium *plus* salmeterol *qd* (67%) or *bid* (72%) than for the salmeterol (48%) or tiotropium (57%) periods.

The improvement in TDI focal score is associated with significantly less need of salbutamol (Fig. 5). When on combination therapy patients used significantly ($p < 0.001$) less salbutamol over a 24-h period compared with single-agent therapy, which reflected mainly the reduced use during the daytime (Table 3). No difference was observed between the combination regimens as well as between the two single long-acting agents.

Safety

Seven patients discontinued the study due to an adverse event: two patients when on combination therapy (tiotropium *plus* salmeterol *qd*: hospitalization due to lung cancer; tiotropium *plus* salmeterol *bid*: increase of dyspnea), one patient in the tiotropium period (hepatic cysts) and four patients when on salmeterol. Of these four patients, one was withdrawn due to drug sensitivity (considered related to study medication), another patient due to atrial fibrillation requiring hospitalization, and two patients hospitalized due to a COPD exacerbation; in addition to the COPD exacerbation in one patient a deterioration of dementia was noted, the patient died during hospitalization (death unexplained).

Although the incidence of adverse events was generally balanced between the treatment periods (Table 4), during combination therapy a lower incidence was noted for COPD exacerbations and complaints of dyspnea. Measurements of blood pressure and pulse rate did not reveal any difference between combination and single-agent therapies. The post-study ECG recordings and laboratory safety screen did not indicate any study drug-related changes.

Discussion

This is the first study evaluating benefit of combination therapy of tiotropium and salmeterol in patients with COPD compared with the individual components in pharmacodynamic steady state. Both combination regimens were superior to single-agent therapy in terms of 24-h lung function improvement, TDI focal score and use of reliever medication. The superior bronchodilator effects were not only restricted to the relevant period of daily activities (0–12 h), but were also observed during the night-time (12–24 h) and did not increase the incidence of side effects.

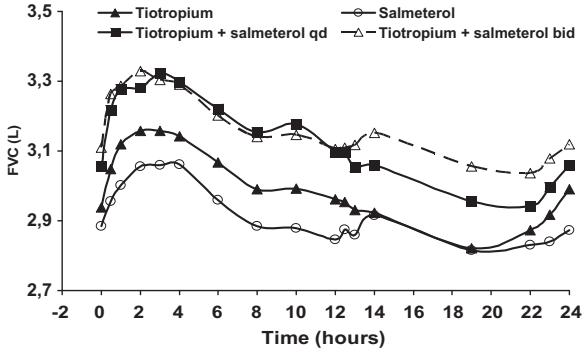

Figure 4 Mean* FVC before (trough) and during 24 h after the inhalation of tiotropium *q.d.* (morning) (▲), salmeterol *b.i.d.* (morning and evening) (○), tiotropium *q.d. plus* salmeterol *q.d.* (both in the morning) (■), and tiotropium *q.d.* (morning) *plus* salmeterol *b.i.d.* (morning and evening) (△) at the end of 6-week treatment periods. The baseline mean FVC at the randomization visit is 2.934 L. *Adjusted for centre, patient within centre and period.

During daytime substantially higher peak and average FEV_1 and FVC responses were observed for the once-daily combination regimen compared to either component. This combination including only one dose of salmeterol provided significantly higher trough FEV_1 and FVC values, indicating sustained 24-h bronchodilator effects, which is supported by significantly higher average FEV_1 and FVC values during the nigh-time (12—24 h). The spirometric endpoints trough and average night-time response demonstrate that the individual components, tiotropium (full-daily dose) and salmeterol (half-daily dose), both contributed to the overall 24-h bronchodilator effect of the once-daily combination. As expected, due to the evening salmeterol dose, the combination regimen including twice daily salmeterol was the most effective of all treatments in particular during the night-time period (12—24 h); no additional spirometric benefit was found during daytime (0—12 h).

One of the management goals of maintenance bronchodilator therapy is to achieve improvement in dyspnea on activities of daily living. In this respect, both combination regimens provided significantly ($p < 0.001$) and clinically ($\geq$1 unit change in focal score[26]) greater relief of dyspnea compared with either agent alone. The magnitude of the improvement in dyspnea with tiotropium combined with salmeterol amounted to approximately a mean TDI focal score of 2.64, substantially higher than with tiotropium or salmeterol alone where improvements in mean TDI focal score of 1.18 and 0.97, respectively, were observed. The improvements of >2 units observed with tiotropium *plus* salmeterol are in line with recent combination studies including tiotropium.[14,15,18] Generally, this standardized instrument to measure breathlessness related to activities of daily living is used in trials employing a parallel design, however, also in the present crossover trial the TDI instrument appeared to be sensitive enough to discriminate patients' responsiveness in perceived breathlessness between single-agent and combination therapy. The marked bronchodilator-mediated symptomatic benefit of combination therapy is associated with a decreased need of salbutamol for acute symptom relief. Rescue medication use was approximately 5—6 times higher during the period when patients tend to be active (i.e. daytime) compared to the period when they are in rest (i.e. night-time) as observed previously as well.[10] Remarkably, in the tiotropium *plus* salmeterol *bid* period, the additional need for salbutamol was comparable to the period when patients inhaled salmeterol only in the morning on top of tiotropium. This indicates that the omission of the evening salmeterol dose, which can be seen as equivalent to four puffs of salbutamol over a 12 h period,[27] did not result in more symptoms requiring rescue salbutamol use.

As indicated above, published data on pulmonary effects of combination therapy with tiotropium and salmeterol is limited. Interestingly, the present results of once-daily combination in pharmacodynamic steady state can be compared with a single dose study conducted by Cazzola et al.,[9] in which the functional impact of adding salmeterol to tiotropium was also examined over 24 h. Following acute dosing, combination therapy elicited a significantly faster onset of action and showed a trend for a greater maximum bronchodilation than the single components alone, while in

Table 3 Three-weekly peak flow (morning and evening) and number of puffs per day of rescue salbutamol, and Transition Dyspnea Index (TDI) focal score results at the end of 6-week treatment periods.

	Tiotropium	Salmeterol *bid*	Tiotropium *plus* salmeterol *qd*	Tiotropium *plus* salmeterol *bid*
Peak flow (L/min)				
Morning	263 ± 1.9	260 ± 1.9	272 ± 1.9*	278 ± 1.9*,****
Evening	278 ± 1.8***	268 ± 1.8	289 ± 1.8*	288 ± 1.8*
Rescue salbutamol use				
Daytime (0—12 h)	1.88 ± 0.11	1.81 ± 0.12	1.25 ± 0.11*	1.28 ± 0.11*
Night-time (12—24 h)	0.39 ± 0.04	0.37 ± 0.04	0.27 ± 0.04**	0.19 ± 0.04*
TDI focal score	1.18 ± 0.28	0.97 ± 0.28	2.56 ± 0.27*	2.71 ± 0.27*

*$p < 0.005$ versus tiotropium and versus salmeterol; **$p < 0.05$ versus tiotropium; ***$p < 0.0001$ versus salmeterol; ****$p < 0.05$ versus tiotropium plus salmeterol qd.
Data are presented as mean ± SE.
Treatment responses are adjusted for centre, patient within centre and period.

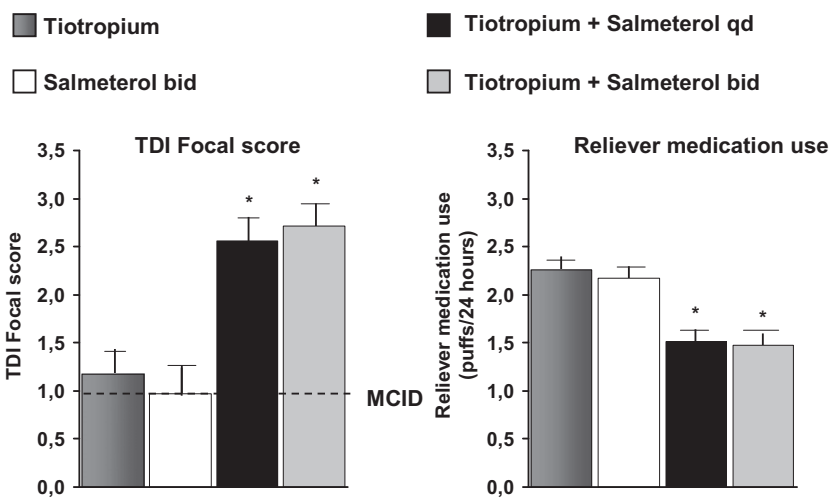

Figure 5 Improvement of Transition Dyspnea Index focal score (left panel) is associated with less need for rescue salbutamol (right panel). Baseline Dyspnea Index (BDI) is 7.00. *$p < 0.001$ compared with either single agent alone. MCID: minimal clinically important difference (i.e. improvement of $\geq$1 unit).

terms of duration of effect (i.e. 12 and 24 h post-dosing) combination therapy was only statistically significant when compared to salmeterol. The present study shows that definitive conclusions on the pulmonary effects of single long-acting bronchodilators in relation to their combination can only be drawn following maintenance therapy, i.e. when pharmacodynamic steady state is achieved. In line with Cazzola et al., during the first hours post-dosing the once-daily combination regimen provided superior bronchodilation compared to either component and the onset of action, as judged from the FEV_1 improvements at 30 min after inhalation, was significantly faster ($p < 0.0001$, both) as well. However, in pharmacodynamic steady state also during the night-time hours [average FEV_1 (12—24 h)] the improvements in spirometric parameters with the once-daily combination were sustained, with significantly higher trough FEV_1 and FVC values. The additive effects in pre-bronchodilator (trough) FEV_1 observed with tiotropium *plus* twice daily salmeterol in the present study differ from the findings in the 1-year intervention study by Aaron et al.,[12] who found that tiotropium *plus* twice daily salmeterol did not statistically improve the pre-bronchodilator FEV_1 compared to treatment with tiotropium alone. However, in particular when trough values are assessed after treatment with long-acting drugs like tiotropium[28,29] or combined tiotropium *plus* LABA,[10,11] it is preferred to standardize the timing (and time window) of the morning pre-bronchodilator lung function measurement, in view of circadian variations, with a substantial rise in the early morning FEV_1. Since the timing of the pulmonary assessments was not given, and more than 40% of patients discontinued therapy prematurely, it is difficult to compare the results from both studies. In addition, the reversible component of the airflow obstruction in the present study population was higher (0.19 L) compared to the population in the 1-year intervention study (0.07 L). Improvement of airflow obstruction (FEV_1) and hyperventilation-evoked hyperinflation (IC) has been documented by Eguchi et al.[13] Compared to tiotropium alone, combination therapy with salmeterol provided greater improvements in FEV_1, while no additional benefit was found for IC. Since this trial was designed as an open-label, non-randomized study (treatment with tiotropium alone followed by combination therapy), this study is most probably suffering from methodological issues, as indicated by the authors. Employing a double-blind, cross-over design we have evaluated dynamic hyperinflation (IC) induced by an increase in

Table 4 Adverse events.[a]

Event	Tiotropium	Salmeterol *bid*	Tiotropium *plus* salmeterol *qd*	Tiotropium *plus* salmeterol *bid*
Total treated n	93	93	92	92
Total with any adverse event	47 (50.5)	47 (50.5)	40 (43.5)	42 (45.7)
Influenza	0 (0.0)	1 (1.1)	2 (2.2)	4 (4.3)
Nasopharyngitis	14 (15.1)	12 (12.9)	13 (14.1)	15 (16.3)
Headache	1 (1.1)	1 (1.1)	0 (0.0)	3 (3.3)
COPD exacerbated	10 (10.8)	15 (16.1)	5 (5.4)	7 (7.6)
Dyspnea exacerbated	12 (12.9)	13 (14.0)	1 (1.1)	2 (2.2)

Data are presented as *n* (%), unless otherwise stated.
COPD, chronic obstructive pulmonary disease.
[a] Occurring in $\geq$3% of the patients.

breathing frequency during combination treatment with tiotropium plus salmeterol as well as during tiotropium and salmeterol alone.[30] Combination therapy resulted in the greatest reduction in dynamic hyperinflation compared to single-agent therapy.

To explore the benefit of combination therapy of tiotropium *plus* salmeterol it was considered mandatory to include 24-h spirometric assessments. Therefore, the present study provides the opportunity to compare the improvements in FEV_1 and FVC of the single-agent therapies in their approved posology over a 24-h period. The superior daytime bronchodilator efficacy of tiotropium over salmeterol is consistent with previous findings[31] and is explained by the longer duration of action of tiotropium. During the night-time period no difference was found in average FEV_1 and FVC response between the two long-acting bronchodilators.

Previously, we reported superior improvement in airflow limitation with combination treatment of tiotropium plus formoterol.[10] Interestingly, the results also suggested a more than additive effect, i.e. the combined effect was greater or longer lasting than predicted from addition of components. Also in the present study it appears that the morning dose of salmeterol, in addition to tiotropium, still provides added efficacy after 12 h, whereas salmeterol alone had returned to the test-day (morning) baseline 8 h after the morning dose. This finding could be explained by the fact that acetylcholine-stimulated muscarinic M_3 receptors, generating inositol-1,4,5-trisphosphate and diacylglycerol (DAG) as second messengers, may have a major influence on β_2-adrenoceptor function. This is due to DAG-induced activation of protein kinase C which may phosphorylate the β_2-adrenoceptor and the G_s-protein, causing β_2-receptor uncoupling and desensitisation,[32,33] and phosphorylate and activate β-adrenoceptor kinases [βARKs; members of the G-protein receptor kinase (GRK) family], amplifying β-agonist-induced desensitisation.[34] Hence, M_3-receptor blockade by tiotropium may not only antagonise acetylcholine-mediated airway constriction, but may also amplify and prolong salmeterol-induced β_2-receptor activation after receptor occupancies have reached their steady state, and the in time diminishing β_2-adrenoceptor activation by salmeterol is being increasingly potentiated by (slower dissociating) tiotropium. Confirmation of this hypothesis based on the current findings is hampered by the lack of a placebo control period. FEV_1 values have been shown to drop below the pre-treatment morning baseline due to the circadian variation in airflow limitation.[11,28] This means that without a correction for the placebo response, the calculated sum of the average FEV_1 responses for the individual components will be underestimated when compared to the response observed for the free combination regimen. Therefore, an additional study including a placebo control period is required to establish the magnitude of the additive effect during a 24-h period.

In summary, the present study confirms and supports the recommendations of the guidelines on pharmacotherapy of COPD[1,2] to combine two long-acting bronchodilators with different pharmacological mechanisms in patients who require both classes of drugs for optimal control of their disease. Optimal bronchodilation, relief of breathlessness and reduced use of reliever medication were achieved with combination therapy of tiotropium and salmeterol.

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