Respiratory Medicine (2008) **102**, 1701–1707

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

Rational timing of combination therapy with tiotropium and formoterol in moderate and severe COPD

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Received 22 January 2008; accepted 14 July 2008
Available online 28 August 2008

KEYWORDS
COPD Therapy;
Tiotropium;
Formoterol;
Spirometry

Summary
Aim: To determine which timing of therapy with formoterol (FOR) and/or tiotropium (TIO) shows the greater and more continuous functional improvement during 24 h in patients with moderate to severe COPD.
Methods: In this randomised, blind, crossover study 80 patients with stable COPD (40 moderate and 40 severe) received 5 different bronchodilator 30-day treatments in a random order. Treatments (Tr) were: Tr1: TIO 18 μg once-daily (8 am); Tr2: TIO 18 μg (8 am) + FOR 12 μg (8 pm); Tr3: FOR 12 μg twice-daily (8 am and 8 pm); Tr4: TIO 18 μg (8 am) + FOR 12 μg twice-daily (8 am and 8 pm); Tr5: FOR 12 μg twice-daily (8 am and 8 pm) + TIO 18 μg (8 pm). Spirometries were performed during 24 h (13 steps) on Day1 and Day30. End-points were: gain of FEV_1 (ΔFEV_1) from baseline of the Day1 and Day30, AUC (Area Under Curve), Dyspnoea Index, and as-needed use of salbutamol.
Results: Sixty-eight patients completed all treatments. The greater and continuous daily functional improvement was showed during Tr4 and Tr5 (Day1 +135.8 mL and +119.1 mL; Day30 +160.2 mL, and +160.5 mL, respectively). Daily means of ΔFEV_1 were significantly different between single-drug treatments and combination therapy. Dyspnoea was greater in single-drug treatments. Less use of rescue salbutamol was reported in Tr4 (0.80 puffs/die) and Tr5 (0.71 puffs/die).
Conclusions: In patients with moderate to severe COPD, combination therapy with tiotropium administered in the morning (Tr4) was the most effective; in patients with prevailing night-symptoms, treatment with tiotropium in the evening (Tr5) reduced symptoms and use of salbutamol. Tr5 showed less variability of FEV_1 during the 24 h (CV = 0.256). These results are relevant for opening new ways in clinical practice.

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doi:10.1016/j.rmed.2008.07.012

Introduction

Chronic obstructive pulmonary disease (COPD) is a progressive disease characterized by irreversible or not fully reversible airflow obstruction and increasing symptoms of dyspnoea and limitation in physical activity.

Long-acting bronchodilators, β_2-agonists and tiotropium bromide, are the most effective drugs for controlling symptoms of COPD.[1] The primary aim of treatment is to relieve symptoms, and to reduce the progression of the disease and the number of exacerbations.[2]

Functionally, forced expiratory volume in 1 s (FEV_1) is the widely used parameter for classifying the disease, and for monitoring the therapeutic effects of these drugs.[3]

Generally, irreversible airway obstruction shows an unpredictable and limited response to the bronchodilators in COPD population. A small number of patients show a coexistence of COPD and asthma functional features.

Combination therapy with bronchodilators improves efficacy without additional side effects, compared with increasing the dose of a single bronchodilator.[4]

Currently, two types of inhaled long-acting bronchodilators are commonly utilized in COPD: long-acting β_2-agonists (LABAs) with duration of action of 12 h, and a long-acting anticholinergic (tiotropium) with duration of action $>$24 h.[1,4]

In contrast to LABAs, which have a twice-daily dose regimen, once-daily tiotropium maintains bronchodilation over 24 h. Large studies over a period of 1 year showed the effectiveness of tiotropium in sustaining functional improvements with no evidence of tolerance.[5,6] Combination therapy of these long-acting agents can provide important benefits, since these drugs have complementary actions on the airways.[7]

Clinical data on combination therapy with tiotropium and inhaled LABAs have been published in the last years with an evidence of additive effects in COPD.[8] In addition, long-term studies demonstrated a greater efficacy of tiotropium compared to placebo, ipratropium,[5,6] as well as to salmeterol.[9]

Several studies also showed that tiotropium and LABAs could lead to hyperinflation reduction, which is accompanied by improvements in exertion dyspnoea and exercise endurance.[10]

In their study van Noord et al. reported that a maintenance therapy of combined tiotropium and formoterol, both once-daily, provides additive effects on FEV_1 throughout the 24 h in patients with COPD.[11]

Add-on therapy of formoterol in the morning to maintenance therapy of tiotropium significantly improved FEV_1, forced vital capacity (FVC) and inspiratory capacity (IC) in COPD. A second formoterol dose in the evening provided a further improvement in average FEV_1, FVC and IC during the night-time hours.[12]

COPD patients can differently report symptoms of dyspnoea and cough during day-time and/or night-time, as well as the use of rescue salbutamol. Therefore, an accurate timing of bronchodilation therapy is essential to achieve the best control of airway obstruction and symptoms.

In order to determine the efficacy of bronchodilation and the best timing of administration, we designed a blind, randomised, crossover study, in which all the patients received 5 different 30 day-treatments with tiotropium (TIO) and/or formoterol (FOR).

Patients and methods

Eighty consecutive patients with stable COPD (40 moderate and 40 severe, ERS/ATS classification) were enrolled from April 2005 to December 2006. A minimum period of 1 month without exacerbations was required for inclusion. All the patients were ex-smokers, and all received an adequate training in the use of inhalers. Demographic and baseline characteristics of the patients are reported in Table 1.

Patients with history of asthma, severe cardiovascular disease, neurological diseases, prostate hyperplasia, glaucoma, inability to perform lung function test correctly were excluded. The study was approved by the Hospital Ethics Committee, and informed written consent was obtained from each patient.

All the patients received, in random order, 5 different 30 day-treatments with a washout of 96 h between each treatment, during which patients used salbutamol metered-dose inhaler (MDI) as-needed.

Tiotropium 18 μg was administrated via HandiHaler dry powder inhaler (Spiriva®), and formoterol 12 μg via metered-dose inhaler (MDI) (Foradil®). Placebo Handihaler and Placebo MDI were utilized, if drugs were not scheduled. Inhalation sequence in contemporaneous administration was FOR as first and subsequently TIO, in all the patients. Treatments are reported in Fig. 1.

Pre-inhalation spirometry at Day1 and Day30 of each treatment was performed at 8 am, 10 min before the morning dose of medication. Spirometry was repeated 5′, 30’, 1 h, 2 h, and 10 h after inhalations. In the same way, spirometries were performed at 8 pm, 10 min before the second inhalation, and repeated after 5′, 30’, 1 h, 2 h, and 10 h. Finally, a 24-h spirometry was performed at 8 am of the following day (Fig. 1).

A PFT Quark 4 spirometer (Cosmed srl, Pavona, Italy) was used to measure the functional parameters, and 3 valid manoeuvres were performed in accordance with guidelines.[13]

Table 1 Baseline characteristics of the patients

COPD	40 Moderate, 40 severe
Sex	58 M, 22 F
Age	69.7 ± 7.3
Smoke	All ex-smokers
FEV_1 pre in L (% predicted)	1.27 ± 0.4 (50% ± 11.6)
FEV_1 post in L (% predicted)	1.37 ± 0.5 (54.3% ± 11.8)
FVC pre in L (% predicted)	2.34 ± 0.67 (72.3% ± 10.2)
	Mean ± SD
Patients completing the study	
Patients	68
COPD	38 Moderate, 30 severe
Sex	49 M, 19 F
Withdrawn	
Exacerbation	2 Moderate, 6 severe
No follow-up	4 Severe

Dyspnoea was assessed with the Baseline and Transitional Dyspnoea Index (BDI/TDI).[14] In comparison to Baseline Dyspnoea Index (Day1), Transitional Dyspnoea Index measured the changes in functional impairment (from +3 to −3), magnitude of task (from +3 to −3), and magnitude effort (from +3 to −3).

Patients were trained in the appropriate daily recording on a diary of as-needed use of salbutamol MDI during the treatments. The use of salbutamol was recorded separately for day-time (8 am−8 pm) and night-time (8 pm−8 am).

Details of clinical status, BDI/TDI, adverse events, and withdrawals were recorded at the beginning and at the end of each treatment.

Data analysis

The primary efficacy end-point was the ΔFEV_1 (in mL), calculated as the difference between FEV_1 value of each step during 24 h and the baseline FEV_1 (FEV_1 at time 0 of Day1 of each treatment). Secondary end-points were Dyspnoea Index (BDI/TDI) and the as-needed use of salbutamol.

All values are expressed as means ± SD (Standard Deviation). Daily mean ΔFEV_1 is the mean bronchodilation over 24 h for each treatment. Area Under Curve (AUC) was calculated for each treatment on Day1 and Day30 to assess the 24 h bronchodilation. At steady state, Coefficient of Variation (CV = Standard Deviation (SD)/mean) of the daily mean ΔFEV_1 was considered to assess the variability of bronchodilation over 24 h.

Statistical analysis was performed with Prism 4 GraphPad Software. A two-tailed, paired *T* test, with confidence intervals of 95%, was carried out to analyse the difference between Day1 and Day30 and among each treatment. One-way ANOVA with repeated measure test and Bonferroni post-test was carried out to compare all the treatments at Day1 and Day30. *P* values < 0.05 were considered significant.

BDI/TDI index of dyspnoea was calculated as the addition of improvement in functional impairment, magnitude of task, and magnitude of effort (ANOVA).

Results

Sixty-eight patients (49 M, 19 F; 38 with moderate and 30 with severe COPD) completed all the treatments. Twelve patients prematurely discontinued the study: 8 patients because of exacerbations (2 with moderate and 6 with severe COPD), and 4 patients with severe COPD because of unavailability at follow-up (Table 1). There were no notable side effects.

Twenty-four hours improvements of FEV_1 (ΔFEV_1) in mL and AUC, on Day1 and Day30, for all the treatments are reported in Table 2 and 3.

On Day1, Tr4 showed the best daily mean functional improvement of FEV_1 (ΔFEV_1 +135.8 ± 65.9 mL, AUC 197,708). Other combination treatments, Tr2 (ΔFEV_1 +109.8 mL, AUC 171,343) and Tr5 (ΔFEV_1 +119.1 mL, AUC 175,675), showed a greater effect than single-drug treatments, Tr1 (ΔFEV_1 +60.2 mL, AUC 98,060) and Tr3 (ΔFEV_1 +86.2 mL, AUC 114,485). During 24 h of Day1, less bronchodilation variability was observed in Tr4 and Tr5 (CV = 0.485 and 0.524, respectively) (Table 3).

On Day30, combination treatments confirmed the significant greater daily bronchodilation, especially in full dosage treatments of Tr4 (ΔFEV_1 +160.2 mL, AUC 222,518) and Tr5 (ΔFEV_1 +160.5 mL, AUC 226,235).

At steady state, Tr1 (TIO once-daily) showed a significant improvement of daily mean ΔFEV_1 (+75.6 mL) compared to Day1 value (+60.2 mL) ($p = 0.0032$). On the contrary in Tr3 (FOR twice-daily), a not significant decrement of daily mean ΔFEV_1 was observed at Day30 (+79 mL) compared with the Day1 value (+86.2 mL) ($p = 0.22$).

Comparing treatments with single-drug (Tr1 and Tr3) not significant differences were observed at Day1 ($p = 0.077$) and Day30 ($p = 0.788$).

Daily mean ΔFEV_1 and AUC were significantly different between treatments with single drug (Tr1 and Tr3) and treatments with combination of tiotropium and formoterol (Tr2, Tr4, and Tr5) (Table 4).

Significant differences in mean ΔFEV_1 and AUC were observed between the Day1 and Day30 in Tr1, Tr2, Tr4, and Tr5. Statistical analysis of ΔFEV_1 comparing each treatment was reported in Table 4.

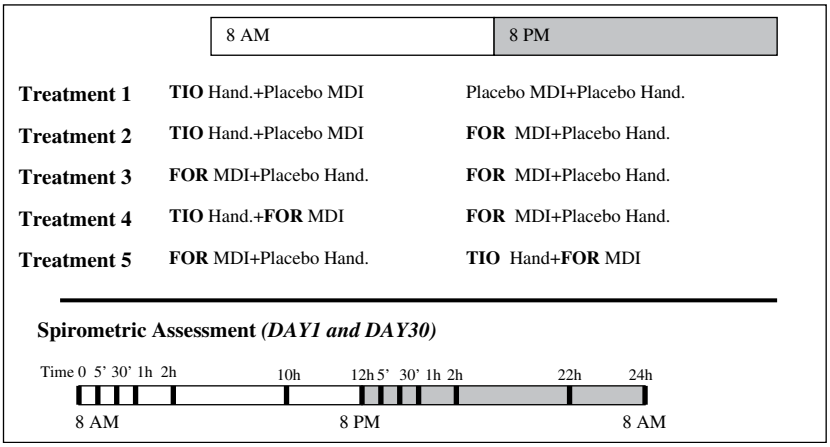

Figure 1 Thirty-day treatments and functional assessment at Day1 and Day30 of each treatment. Patients received in random order all the treatments.

Table 2 ΔFEV_1 (mL) measured for each step of Day1 and Day30 of treatment (Tr)

	Time0	5′	30′	1 h	2 h	10 h	12 h	12 h 5′	12 h 30′	13 h	14 h	22 h	24 h	Daily mean ΔFEV_1 ± SD in mL
Day1														
TIO 8 am	0	42	100	148	165	75	64	51	40	41	38	8	10	60.2 ± 50.8
TIO 8 am, and FOR 8 pm	0	60	125	151	176	82	65	114	175	185	186	52	56	109.8 ± 61.6
FOR 8 am, and FOR 8 pm	0	95	136	157	126	61	48	102	140	134	135	−8	−5	86.2 ± 60.3
TIO + FOR 8 am, and FOR 8 pm	0	110	165	185	180	122	94	184	208	196	198	58	66	135.8 ± 65.9
FOR 8 am, and TIO + FOR 8 pm	0	91	138	132	129	60	51	107	215	208	194	108	115	119.1 ± 62.4
Day30														
TIO 8 am	33	55	94	132	168	112	91	62	60	55	52	35	34	75.6 ± 41.4
TIO 8 am, and FOR 8 pm	60	96	158	184	183	107	72	143	187	175	171	63	68	128.2 ± 51.5
FOR 8 am, and FOR 8 pm	15	90	112	110	115	58	41	103	115	120	114	16	18	79 ± 42.7
TIO + FOR 8 am, and FOR 8 pm	84	172	215	208	216	140	128	189	192	196	190	78	75	160.2 ± 53
FOR 8 am, and TIO + FOR 8 pm	118	205	196	186	188	113	85	138	197	195	201	130	135	160.5 ± 41.9

<Inhalation 8 am

<Inhalation 8 pm

TIO = tiotropium, FOR = formoterol Placebo HandiHaler and MDI were utilized if not scheduled drugs

In addition, at Day30 (steady state), Tr5 showed the greater functional 24 h-improvement (daily mean ΔFEV_1) in comparison to Day1: (daily mean ΔFEV_1 of Day30 − daily mean ΔFEV_1 of Day1 =) Tr1: +15.4 mL (AUC +27,655), Tr2: +18.4 mL (AUC +13,195), Tr3: −7.2 mL (AUC −7287), Tr4: +24.4 mL (AUC +24,810), and Tr5: +41.4 mL (AUC +50,560).

During the Day30, an interesting pharmacodynamic behaviour of formoterol was observed in Tr4 and Tr5: after the contemporaneous administration of FOR and TIO (Tr4 in the morning, and Tr5 in the evening) the post 5′ spirometry showed less functional improvement (Tr4 +172 mL, Tr5 +138 mL) than administration of formoterol alone (Tr4 +189 mL and Tr5 +205 mL). This behaviour resulted inverted in post 30′, 1 h, and 2 h spirometric measurements (Table 2).

BDI/TDI showed a greater improvement of symptoms in treatments with combination therapy than single drug ($p = 0.0004$) (Table 5).

The evaluation of as-needed use of salbutamol showed a greater number of administrations during Tr1 (a mean of 2.14 puffs/die) than other treatments. Less use was reported during Tr4 and Tr5 (a mean of 0.80 puffs/die and 0.71 puffs/die, respectively). Use of salbutamol was generally less during the night-time in all the treatments. Mean of puffs/die was reported in Table 5.

Discussion

This study investigated the possible variable effects of a different timing in administration of bronchodilator drugs, alone and in combination, in stable moderate and severe COPD.

As reported in recent relevant studies, combination of tiotropium and formoterol shows a superior bronchodilation efficacy compared to either of them administered separately.[11,12,15–17]

The aim of our study was to find out the best timing of administration for these drugs.

Generally in other studies authors' questions about therapy were: *what* and *how* to administer. In this study our main question was: *when* during the 24 h.

A rational timing, keeping a constant daily bronchodilation, may be a further chance to improve the effects of therapy in COPD patients, improving functional parameters, quality of life, and potentially decreasing the frequency of exacerbations.

In this crossover study, the subjects received all the treatments in random order, avoiding interindividual variability. A washout period of 96 h between each treatment was applied. The random order of administration reduced the possible interference of the residual effect of prior tiotropium.

Conducted on stable moderate and severe COPD patients, our study was designed in order to collect data on reversibility, symptoms, and rescue salbutamol. Therefore periods of treatments were limited at 30 days. In this way, it was not possible to determine the evaluation on exacerbations or long-term loss of functional capacity.

Authors did not consider studying a 6th regimen with TIO 8 am and FOR 8 am, which was one of the regimens reported in van Noord's paper,[11] because our study aimed to achieve a constant bronchodilation during the 24 h.

Table 3 AUC (Area Under Curve) and CV (coefficient of variation) at Day1 and Day30 of each treatment (Tr)

	AUC	CV
Day1		
Tr 1 − TIO 8 am	98,060	0.846
Tr 2 − TIO 8 am, and FOR 8 pm	171,343	0.561
Tr 3 − FOR 8 am, and FOR 8 pm	114,485	0.699
Tr 4 − TIO + FOR 8 am, and FOR 8 pm	197,708	0.485
Tr 5 − FOR 8 am, and TIO + FOR 8 pm	175,675	0.524
Day30		
Tr 1 − TIO 8 am	125,715	0.548
Tr 2 − TIO 8 am, and FOR 8 pm	184,538	0.402
Tr 3 − FOR 8 am, and FOR 8 pm	107,198	0.541
Tr 4 − TIO + FOR 8 am, and FOR 8 pm	222,518	0.331
Tr 5 − FOR 8 am, and TIO + FOR 8 pm	226,235	0.256

This study was performed applying 2 single-drug treatments (Tr1 and Tr3) with standard dosage, and 2 combination treatments of formoterol and tiotropium (Tr4 and Tr5) with different timing of administration (Tiotropium in the morning or in the evening). Tr2 (Tiotropium once-daily and formoterol once-daily) was considered to compare the results of recent studies on this type of administration.[8,12]

Spirometric measurements were similarly distributed during the day-time (6) and the night-time (6), in order to have an equal weight on data analysis. However, in this study lung volumes were not measured, mainly because this study is rather complex to allow these measurements to be performed as well. It would have been important to show the correlation between improvement in the dyspnea index and IC.

At Day1 and Day30, combination treatments confirmed the significant greater daily bronchodilation, especially in full dosage treatments (Tr4 and Tr5), than single-drug treatments.

Interestingly, at steady state, Tr1 showed an improvement of daily mean ΔFEV_1 compared with Day1 value ($p = 0.0032$); whereas in Tr3 a not significant decrement of daily mean ΔFEV_1 was observed at Day30 compared with Day1 ($p = 0.22$). This decrement may be hardly linked to a rapid beta-receptor tolerance. In fact, several long-term studies have shown no tolerance to the bronchodilation effect of formoterol in COPD.[18,19]

In our study at Day1 and Day30, formoterol twice-daily provided a not significant difference in bronchodilation than tiotropium once-daily.

In Tr4 and Tr5, the pharmacodynamic behaviour of the contemporaneous administration of FOR and TIO, showing a less functional improvement in the post 5′ spirometry than administration of formoterol alone, was an intriguing result. This behaviour resulted inverted in the post 30′, 1 h, and 2 h spirometric measurements.

A hypothetical mechanism of that feature might be the effect of TIO powder. It might delay the bronchodilation effect of FOR MDI for a few minutes, when contemporaneously administered. This is supposedly associated to a mild provoking effect of the powder on airway smooth muscle or a delayed effect when the two different bronchodilating receptors are activated at the same time. It would be interesting to confirm this hypothesis in next studies comparing the early effect of FOR MDI and FOR DPI during 24 h in moderate and severe COPD and asthma.

As supposed in some studies,[11,20] nocturnal administration of combination therapy may be linked to a modulation of anticholinergic effect on bronchial receptors (vagal tone), and to a synergism with adrenergic system. An endogenous circadian rhythm may play an important and intricate role in the circadian modulation of the inflammatory processes, and susceptibility of airway smooth muscle and vasculature. In fact, an increase in vagal tone may induce nocturnal bronchoconstriction which is further enhanced by falling catecholamine levels. This is consistent with the findings by Postma et al.,[20] who demonstrated that activity of adrenergic system is most prominent during the day; whereas an increased activity of parasympathetic system was found during the night. Compared with normal controls, this vagal activity appeared significantly increased in COPD patients.[20]

Moreover, in the nocturnal administration, anticholinergic effect on mucus glands can be exploited for inhibition of mucus secretion, and consequent reduction of nocturnal bronchial build-up.[7]

At steady state, further important result was the minor bronchodilation variability of Tr5 during the 24 h (CV 0.256).

As previously shown by van Noord et al.,[15] in our study an optimal bronchodilation response by tiotropium was

Table 4 Statistical analysis of daily ΔFEV_1 and AUC (ANOVA and *T* test)

			Day1	Day30
Day1 vs Day30		Tr 1 vs Tr 2	0.007*	0.001*
Tr 1 − TIO 8 am	0.003*	Tr 1 vs Tr 3	0.077	0.788
Tr 2 − TIO 8 am, and FOR 8 pm	0.006*	Tr 1 vs Tr 4	0.0005*	0.0001*
Tr 3 − FOR 8 am, and FOR 8 pm	0.221	Tr 1 vs Tr 5	0.015*	0.0001*
Tr 4 − TIO + FOR 8 am, and FOR 8 pm	0.009*	Tr 2 vs Tr 3	0.017*	0.0001*
Tr 5 − FOR 8 am, and TIO + FOR 8 pm	0.003*	Tr 2 vs Tr 4	0.0007*	0.0001*
		Tr 2 vs Tr 5	0.311	0.004*
	ANOVA	Tr 3 vs Tr 4	0.0001*	0.0001*
All treats at Day1	0.0001*	Tr 3 vs Tr 5	0.033*	0.0001*
All treats at Day30	0.0001*	Tr 4 vs Tr 5	0.160	0.946
*$p < 0.05$		*T test* *$p < 0.05$		

Table 5 BDI/TDI index of dyspnoea (mean ± SD) and rescue salbutamol

Dyspnoea		Mean of puffs of salbutamol as needed/die		
Baseline DI (68 pts) Grade 2.17 ± 0.72	Transitional DI (68 pts)	8 am–8 pm day-time	8 pm–8 am night-time	TOT. puffs/die
Tr 1 –TIO 8 am	+1.0 ± 1.59	1.51	0.63	2.14
Tr 2 –TIO 8 am, and FOR 8 pm	+1.5 ± 1.44	1.38	0.23	1.61
Tr 3 –FOR 8 am, and FOR 8 pm	+1.38 ± 1.61	1.15	0.38	1.53
Tr 4 –TIO + FOR 8 am, and FOR 8 pm	+2.61 ± 2.0*	0.59	0.21	0.80*
Tr 5 –FOR 8 am, and TIO + FOR 8 pm	+2.32 ± 1.77*	0.65	0.05	0.71*
	T test $*p < 0.05$			*T test* $*p < 0.05$

achieved in pharmacodynamic steady state. It means that conclusions on pulmonary effects of the single drugs in relation to their combination can only be assessed following maintenance therapy.

On the other hand, in our study, Tr4 showed some advantages compared with other treatments: a rapid achievement of high bronchodilation values already in the first day of treatment (AUC 197,708), and the attainment of the highest bronchodilation peaks during the 24 h.

In this study, Dyspnoea Index showed a significant improvement of symptoms in combination treatments, confirming their superiority on other treatments in reducing symptoms.

In addition, daily as-needed use of salbutamol was significantly less during treatments with combination regimens. Previous studies in COPD have also shown less salbutamol consumption during the night-time.[21] In our study use of reliever medications was significantly less during the night-time and patients required lower daily use of salbutamol during Tr5 (Table 5).

In the study conducted by van Noord et al.,[11] comparing the effects of TIO and FOR, the most pronounced bronchodilation effect was achieved with the combination treatment. Confirming these data, our study completes the knowledge on bronchodilation effects of these drugs. In fact, we can assert that combinations Tr4 and Tr5, with TIO administered in the morning or in the evening, allow to achieve a greater and constant level of bronchodilation in the 24 h than treatment with TIO and FOR once-daily (Tr2 in our study).

Certainly, the different administration of TIO (in the morning or in the evening), can be preferred in relation to the patient's characteristics (daily activity, nocturnal dyspnoea, etc.). Van Noord[15] observed once-daily TIO provides more bronchodilation during day-time compared with twice-daily FOR, and no different results between the two long-acting bronchodilator treatment regimens during the night-time. In our study FOR twice-daily and TIO once-daily showed a similar 24 h bronchodilation at steady state, although TIO once-daily provides more bronchodilation during the first 12 h, whereas FOR twice-daily shows a greater bronchodilation in the second 12 h.

Conclusion

As recommended by the Global Initiative for Chronic Obstructive Lung Disease guidelines,[2] a combination of two long-acting bronchodilators with different pharmacological mechanisms of action should be considered in all patients with moderate to severe COPD.

In terms of improvement in lung function, combination of formoterol and tiotropium is more effective in patients with moderate to severe COPD than single drugs administered alone. In our crossover study, the most effective timing to achieve a constant and greater bronchodilation was resulted in Tr4 and Tr5. These results are important to assure the best level of bronchodilation in COPD patients, and to use such combination for such patients in clinical practice.

A rational timing for administration of the combination therapy, in the morning or in the evening, can be selected in relation to COPD patient's characteristics.

Our study can open the way to trials with combination therapy in different timing of administration, monitoring nocturnal hypoxic episodes, sleep disorders, as-needed use of salbutamol, and the correlation with the activity of adrenergic system during the day and the activity of parasympathetic system during the night. Although present study supplies new data, it can be considered a preliminary one supporting the design of new projects on long-term impact of a given timing of administration on exacerbations, quality of life, and lung function loss. This study may open the way to a therapy modelled on the COPD patients' characteristics.

Conflict of interest statement

The authors have no conflict of interest.

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