Safety And Tolerability Of Twice-Daily Aclidinium Bromide In COPD Patients: ACCORD COPD I

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Introduction

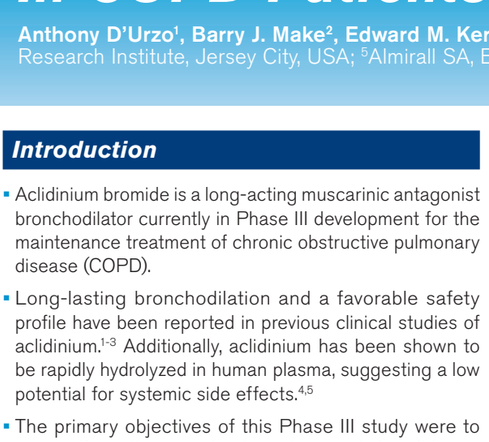
- Aclidinium bromide is a long-acting muscarinic antagonist bronchodilator currently in Phase III development for the maintenance treatment of chronic obstructive pulmonary disease (COPD).
- Long-lasting bronchodilation and a favorable safety profile have been reported in previous clinical studies of aclidinium.[1-3] Additionally, aclidinium has been shown to be rapidly hydrolyzed in human plasma, suggesting a low potential for systemic side effects.[4,5]
- The primary objectives of this Phase III study were to assess the efficacy and safety of twice-daily aclidinium 200 µg and 400 µg administered via the Genuair®† inhaler in patients with moderate-to-severe COPD.
  - Here we present the safety and tolerability of aclidinium 200 µg and 400 µg BID.

Methods

Study Design

- This was a 12-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial evaluating twice-daily aclidinium 200 µg and 400 µg.
- Patients (N=561) were randomized (1:1:1) to aclidinium bromide (200 µg or 400 µg BID) or placebo (Figure 1), administered via the Genuair® inhaler.

Figure 1. Study flow chart



*One patient randomized to aclidinium 200 µg discontinued prior to receiving any study medication and was not included in the safety population.

- Patients were evaluated at screening, at baseline following a 2-week run-in period, at Weeks 1, 4, 8, and 12 during the treatment period, and 2 weeks after treatment end.

Study Population

Inclusion Criteria

- Male and female patients aged ≥40 years
- Diagnosis of moderate-to-severe stable COPD
- Forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio <70%
- FEV_1 ≥30% and <80% of predicted
- Current or ex-smokers with a smoking history of ≥10 pack-years

Exclusion Criteria

- History or current diagnosis of asthma
- Respiratory infection or COPD exacerbation within 6 weeks (3 months if it resulted in hospitalization) prior to screening
- Clinically relevant cardiovascular conditions or respiratory conditions (other than COPD) and abnormalities in laboratory values or electrocardiograms (ECG)

Allowed Concomitant Medications

- Salbutamol as needed
- Inhaled corticosteroids (CS) and oral CS at doses equivalent to 10 mg/day or 20 mg every other day (if stable for 4 weeks before Visit 1)

Study Endpoints

- Safety was assessed via adverse events (AEs), clinical laboratory measures, vital signs, ECGs, and Holter monitoring (subset of patients).

Statistical Analysis

- Safety outcomes were analyzed using the safety population (all randomized patients who took at least 1 dose of double-blind study treatment) and were summarized using descriptive statistics.

Results

Baseline Demographics

- Of the 561 patients randomized, 467 completed the study (87.4% aclidinium 400 µg, 82.2% aclidinium 200 µg, 80.1% placebo). Baseline demographics were similar across all treatment groups (Table 1).

Table 1. Demographic and baseline characteristics (safety population)

Characteristic	Placebo n=186	Aclidinium 200 µg n=184	Aclidinium 400 µg n=190	Total N=560
Age, mean (SD), years	65.1 (9.2)	63.1 (9.5)	64.9 (9.5)	64.3 (9.4)
Male, n (%)	96 (51.6)	101 (54.9)	100 (52.6)	297 (53.0)
Caucasian, n (%)	175 (94.1)	169 (91.8)	181 (95.3)	525 (93.8)
BMI, mean (SD), kg/m²	27.5 (5.2)	27.3 (5.1)	27.6 (5.0)	27.5 (5.1)
Current smoker, n (%)	87 (46.8)	84 (45.7)	80 (42.1)	251 (44.8)
Smoking history, mean (SD), pack-years	52.7 (28.1)	53.0 (23.3)	57.2 (28.5)	54.3 (26.8)
Post-bronchodilator FEV_1 at screening (Visit 1), mean (SD), L	1.56 (0.6)	1.54 (0.5)	1.53 (0.5)	1.54 (0.5)
Post-bronchodilator FEV_1, mean (SD), % of predicted value	54.6 (13.5)	52.8 (13.7)	54.1 (12.9)	53.8 (13.4)
Post-bronchodilator FEV_1/FVC ratio, mean (SD), %	52.7 (10.5)	50.9 (10.6)	51.5 (10.2)	51.7 (10.5)
Bronchodilator reversibility, mean (SD), %	17.1 (15.5	16.7 (15.5)	15.5 (12.0)	16.5 (14.4)
Reversible,[a] n (%)	80 (43.0)	83 (45.1)	77 (40.5)	240 (42.9)

BMI, body-mass index
[a]Reversible was defined as bronchial reversibility ≥12% and change from prebronchodilator FEV_1 ≥0.2 L.

Treatment-emergent AEs (TEAEs)

- The percentage of patients who reported a TEAE was lower in the aclidinium 400 µg group (44.7%) compared with the aclidinium 200 µg and the placebo groups (50.5% and 52.2%, respectively).
- COPD exacerbation was the only TEAE reported by at least 5% of patients; the incidence of COPD exacerbation was lower in the aclidinium groups vs placebo (Table 2). Incidence of COPD exacerbations was lower with the higher dose of aclidinium (7.4%) compared with aclidinium 200 µg (9.2%) or placebo (12.4%).
- The TEAEs reported in at least 2% of the patients in any group and that occurred more frequently in any aclidinium group compared with the placebo group were arthralgia, diarrhea, oropharyngeal pain, headache, nasopharyngitis, back pain, and dizziness.

Table 2. Number (%) of patients with adverse events reported by ≥2% of patients in the aclidinium treatment groups (safety population)

Preferred term	Placebo n=186	Aclidinium 200 µg n=184	Aclidinium 400 µg n=190
COPD exacerbation	23 (12.4)	17 (9.2)	14 (7.4)
Dyspnea	6 (3.2)	4 (2.2)	5 (2.6)
Arthralgia	1 (0.5)	4 (2.2)	5 (2.6)
Cough	5 (2.7)	4 (2.2)	4 (2.1)
Diarrhea	3 (1.6)	3 (1.6)	4 (2.1)
Oropharyngeal pain	3 (1.6)	2 (1.1)	4 (2.1)
Fatigue	4 (2.2)	0 (0)	4 (2.1)
Headache	4 (2.2)	6 (3.3)	3 (1.6)
Nasopharyngitis	2 (1.1)	6 (3.3)	3 (1.6)
Back pain	1 (0.5)	5 (2.7)	3 (1.6)
Dizziness	1 (0.5)	4 (2.2)	2 (1.1)

- The incidence of on-therapy serious AEs (SAEs) was low in all groups (2.2% placebo, 4.3% aclidinium 200 µg, 3.2% aclidinium 400 µg).
  - The most frequently reported SAE was exacerbation of COPD: 1 patient in the placebo group, 1 patient in the aclidinium 200 µg group, and 3 patients in the aclidinium 400 µg group. None of the COPD exacerbations resulted in discontinuation from the study.

Anticholinergic AEs

- Anticholinergic-related effects such as dry mouth and constipation were low and generally comparable between treatment arms (Table 3).

Table 3. Number (%) of patients with potential anticholinergic AEs by system organ class and preferred term (safety population)

System organ class Preferred term	Placebo n=186	Aclidinium 200 µg n=184	Aclidinium 400 µg n=190
Cardiac disorders			
Sinus tachycardia	0 (0)	0 (0)	1 (0.5)
Supraventricular tachycardia	2 (1.1)	2 (1.1)	2 (1.1)
Ventricular tachycardia	1 (0.5)	0 (0)	2 (1.1)
Heart rate increased	1 (0.5)	0 (0)	0 (0)
Gastrointestinal disorders			
Constipation	1 (0.5)	2 (1.1)	0 (0)
Dry mouth	2 (1.1)	3 (1.6)	1 (0.5)
Infections and infestation disorders			
Urinary tract infection	4 (2.2)	2 (1.1)	3 (1.6)
Cystitis	1 (0.5)	1 (0.5)	0 (0)

Cardiac or Cerebrovascular AEs

- The percentages of patients who experienced cardiac AEs in the placebo and aclidinium groups were similar (4.3%, 4.9%, and 4.7% for the placebo, aclidinium 200 µg, and aclidinium 400 µg groups, respectively) and the incidence of any specific cardiac AE was low across groups (<2% for any individual event in any group).
- One patient in the placebo group (0.5%) had a cerebrovascular accident that was considered serious and was discontinued from the study.

Study Discontinuations and Deaths

- The most frequently reported AE resulting in study discontinuation was COPD exacerbation (n=7, placebo; n=4, aclidinium 200 µg; n=1, aclidinium 400 µg) followed by dyspnea (n=2 each, placebo and 400 µg) (Table 4). No other TEAEs resulted in study discontinuation of more than one patient.

Table 4. Adverse events leading to study discontinuation in ≥1 patient in any treatment group (safety population), n (%)

Preferred term	Placebo n=186	Aclidinium 200 µg n=184	Aclidinium 400 µg n=190
COPD exacerbation	7 (3.8)	4 (2.2)	1 (0.5)
Dyspnea	2 (1.1)	0 (0)	2[a] (1.1)
Ventricular tachycardia	1 (0.5)	0 (0)	2 (1.1)

[a]One AE was considered to be related to study treatment.

- One patient in the aclidinium 400 µg group died due to metastatic lung cancer; this was not considered to be related to treatment.

Other Safety Assessments

- The changes from baseline in clinical laboratory tests and vital signs were small and similar across treatment groups, and considered to be of no clinical relevance.
- No patients in the aclidinium groups experienced any potentially clinically significant ECG abnormalities in heart rate or QT interval (Table 5).

Table 5. Number (%) of patients with potentially clinically significant (PCS) 12-lead ECG values (safety population)

Parameter	PCS criteria unit	Placebo n=186	Aclidinium bromide 200 µg n=184	Aclidinium bromide 400 µg n=190
QTcF interval	>500 msec	0 (0)	0 (0)	0 (0)
	Increase ≥60 msec[a]	1 (0.5)	0 (0)	0 (0)
Tachycardia event	≥120 bpm if baseline <120 bpm	0 (0)	0 (0)	0 (0)
Bradycardia event	≤40 bpm if baseline >40 bpm	1 (0.5)	0 (0)	0 (0)

[a]Change from baseline

Conclusions

- In this study, twice-daily treatment with aclidinium 200 µg and 400 µg was safe and well tolerated in patients with moderate to severe COPD.
- The incidence of anticholinergic-related, cardiac, and cerebrovascular adverse events was low and similar between all treatment groups.
- There were no differences in safety profiles between the 200 µg and 400 µg doses of aclidinium bromide administered twice daily.

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†Genuair® is a registered trademark of Almirall SA.

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Safety And Pharmacokinetics Of Multiple Doses Of Aclidinium Bromide Administered Twice Daily In Healthy Volunteers

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Introduction

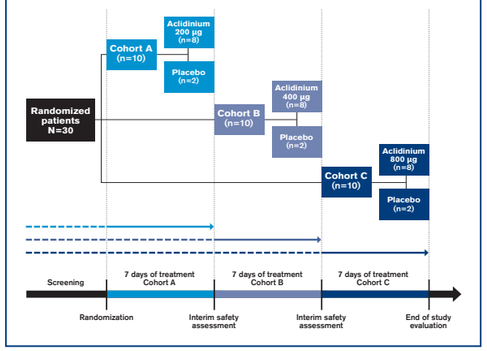
- Aclidinium bromide is a novel, long-acting, muscarinic antagonist currently in Phase III development for the treatment of COPD. Aclidinium is rapidly hydrolyzed in plasma into two main alcohol and acid metabolites, suggesting reduced potential for systemic side effects.[1]
- Single inhaled doses of aclidinium up to 6000 µg and intravenous doses of 400 µg have been well tolerated in healthy subjects.[2,3] Clinical trials in COPD patients with twice-daily administration of aclidinium have demonstrated sustained bronchodilation and a favorable safety and tolerability profile.[4,5]
- This study assessed the safety and pharmacokinetics (PK) of multiple doses of aclidinium administered twice daily in healthy subjects.

Methods

Study Design

- This was a 7-day, single-blind, placebo-controlled, clinical trial evaluating multiple doses of aclidinium bromide 200, 400 and 800 µg administered twice daily.
- Healthy male and female adult subjects (N=30) were randomized to 1 of 3 cohorts of ascending aclidinium doses, with 10 subjects in each cohort randomized (8:2) to twice-daily aclidinium or placebo for 7 consecutive days.
- Cohorts were tested in a stepwise manner; subjects in the preceding cohort were assessed for safety and tolerability prior to starting the higher dose in the next cohort (Figure 1).
- Subjects were admitted into the clinic the day before dosing (Day -1) and remained there until after the last PK sample was collected on Day 9.

Figure 1. Study Design



Study Population

Inclusion Criteria

- Healthy non-smoking male and female patients aged 18-45 years
- BMI ≥18 kg/m^2 and ≤32 kg/m^2

Exclusion Criteria

- Known hypersensitivity to aclidinium bromide or other antimuscarinic agents
- Clinically significant abnormalities with electrocardiogram (ECG) parameters, laboratory tests, or physical examination
- History of alcohol or substance abuse within the previous 5 years
- Any clinical condition that may affect the absorption, distribution, biotransformation, or excretion of aclidinium
- Concomitant medications within 14 days prior to study drug administration
- Previous participation in an investigational study of aclidinium

Safety Assessments

- Standard safety ECGs were recorded and vital signs were assessed at screening, end of study, and interim visits at Day -1 (check-in), Days 1-7, and Days 8-9 (vital signs only).
- Adverse events (AEs) were assessed when vital signs were recorded and at least once per day on all other study days.
- Complete physical examination, vital signs evaluation, clinical laboratory tests, 12-lead ECG, and AE assessments were performed upon study completion, early withdrawal or within 5 days of the final PK blood draw.

Pharmacokinetic Evaluation

- Blood samples were collected at the following scheduled intervals:
  - Day 1 and Day 7: predose, 5, 15, 30 minutes and 1, 1.5, 2, 3, 4, 6, and 8 hours after the morning dose, and 5 minutes before and after the evening dose (12 hours after the morning dose)
  - Days 2 to 6: 5 minutes after the morning and evening doses
  - Day 7: 15, 30 minutes and 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 hours after the evening dose
- Plasma samples were analyzed for aclidinium and its major acid and alcohol metabolites using validated liquid chromatography coupled with tandem mass spectrometry (lower limits of quantification [LLOQ] = 5 pg/mL for aclidinium and the alcohol metabolite, 100 pg/mL for the acid metabolite)
- PK parameters (AUC, C_{max}, t_{max}, and $t_{1/2}$) were determined at Day 1 and at Day 7.

Statistical Analysis

- PK analyses were performed on the PK Analysis Population, which consisted of all subjects who completed the study; descriptive statistics were provided for all PK parameters.
- Wilcoxon signed rank test was used to compare PK parameters on Day 1 and Day 7.
- Safety and demographic analyses were performed on the Safety Population, which consisted of all subjects who received at least one dose of study drug.

Results

Baseline Demographics

- All 30 enrolled subjects completed the study. Baseline demographics were comparable between treatment groups (Table 1).

Safety

- A total of 9 treatment-emergent AEs (TEAEs) were reported (Table 2), all of which were mild in severity.
- No AEs were reported by subjects in the aclidinium 200 µg group. Three patients reported 3 TEAEs in the aclidinium 400 µg group and 3 patients reported 5 TEAEs in the aclidinium 800 µg group. One patient in the aclidinium 800 µg group reported 3 events: somnolence, muscle spasm, and dry throat.
- All AEs were resolved by the end of the study.
- There were no clinically meaningful changes in laboratory values, vital signs, or ECG parameters.

Pharmacokinetic Analyses

Aclidinium Bromide

- On Day 1, mean C_{max} values (following the morning dose) were 102.7, 194.2, and 360.1 pg/mL for the 200 µg, 400 µg, and 800 µg BID treatment groups, respectively (Table 1). In most cases, t_{max} was recorded at the first PK time point (5 minutes). Mean $t_{½}$ estimates on Day 1 ranged from 2.4 to 5.9 hours.
- Examination of consecutive trough concentrations indicated that steady state was achieved on Day 7. This was further confirmed by comparable C_{max} and $AUC_{τ}$ estimates observed after morning and evening administration of aclidinium on Day 7 (Table 3), and the virtually superimposable morning and evening aclidinium plasma concentration vs time profiles on Day 7 (Figure 2). However, considering the short $t_{½}$ of aclidinium, steady state was probably achieved soon after the first dose.

Figure 2. Mean aclidinium plasma concentration-time profiles at Day 7 after morning and evening administration of aclidinium 200 µg, 400 µg, and 800 µg

- The Wilcoxon signed rank test showed no significant differences in C_{max} between Day 1 and Day 7 morning treatment for each dose level, suggesting no accumulation of aclidinium at steady state.
- Comparison of $AUC_{0-∞}$ on Day 1 and $AUC_{τ,ss}$ on Day 7 (Table 3) demonstrates kinetic linearity and time-independent PK since there were no changes in clearance of aclidinium following multiple dosing.
- Following Day 7 evening dosing, sampling was extended until 48 hours postdose. Thus, in order to eliminate methodological sampling differences when estimating and comparing $t_{1/2}$ values between days of treatment, Day 7 evening effective $t_{1/2}$ values for aclidinium and metabolites were calculated (Table 3).
- A high degree of variability in PK parameters was observed for aclidinium 200 µg, which may have been due to the larger number of values below the LLOQ on Day 1 following administration of the lowest dose compared with the higher doses.
- Aclidinium exposure increased with increasing dose, but the increase in exposure was less than dose proportional between the 400 µg and 800 µg doses (Figure 3).

Aclidinium Bromide Metabolites

- PK assessments of the alcohol and acid metabolites on Day 1 and Day 7 demonstrate that steady state was achieved (Table 3).
- C_{max} of the alcohol metabolite generally occurred later than the corresponding t_{max} of aclidinium, but earlier than that of the acid metabolite.
- Comparison of Day 1 and steady-state AUC, shows accumulation of the alcohol and acid metabolites with all dose groups following dosing to steady state.
- Alcohol and acid metabolite exposure increased with increasing dose, but the increase in exposure was less than dose proportional between the 400 µg and 800 µg doses.

Table 1. Demographic characteristics (safety population)

Parameter	Cohort A (n=10)		Cohort B (n=10)		Cohort C (n=10)		All Subjects (N=30)	
	Aclidinium 200 ug BID (n=8) n (%)	Placebo (n=2) n (%)	Aclidinium 400 ug BID (n=8) n (%)	Placebo (n=2) n (%)	Aclidinium 800 ug BID (n=8) n (%)	Placebo (n=2) n (%)	All Aclidinium (n=24) n (%)	Placebo (n=6) n (%)
Age (years), mean (SD)	38.4 (4.4)	35.5 (9.2)	34.8 (8.2)	40.0 (2.8)	39.3 (5.1)	30.5 (14.8)	37.5 (6.2)	35.3 (9.0)
Male, n (%)	5 (62.5%)	1 (50.0%)	5 (62.5%)	1 (50.0%)	4 (50.0%)	2 (100%)	14 (58.3%)	4 (66.7%)
Caucasian, n (%)	7 (87.5%)	1 (50.0%)	7 (87.5%)	1 (50.0%)	8 (100%)	2 (100%)	22 (91.7%)	4 (66.7%)
Weight (kg), mean (SD)	78.0 (13.4)	82.5 (12.0)	74.2 (10.6)	74.8 (8.7)	72.9 (9.3)	67.2 (4.0)	75.0 (10.9)	75.0 (10.5)
Height (cm), mean (SD)	167.7 (9.9)	171.6 (9.1)	165.1 (6.6)	164.0 (1.5)	166.1 (11.8)	165.6 (6.5)	166.3 (9.3)	167.1 (6.2)
BMI (kg/m^2), mean (SD)	27.5 (2.1)	27.9 (1.1)	27.2 (3.3)	27.7 (2.7)	26.5 (3.3)	24.6 (3.4)	27.1 (2.9)	26.8 (2.6)

SD, standard deviation; BMI, body-mass index

Table 2. Incidence of treatment-emergent adverse events, by treatment (safety population)

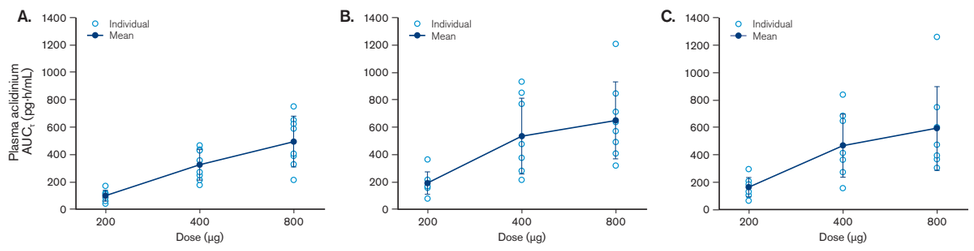
Parameter	Cohort A (n=10)		Cohort B (n=10)		Cohort C (n=10)		All Subjects (N=30)	
	Aclidinium 200 ug BID (n=8) n (%)	Placebo (n=2) n (%)	Aclidinium 400 ug BID (n=8) n (%)	Placebo (n=2) n (%)	Aclidinium 800 ug BID (n=8) n (%)	Placebo (n=2) n (%)	All Aclidinium (n=24) n (%)	Placebo (n=6) n (%)
Subjects ≥1 TEAE	0	0	3 (37.5)	0	3 (37.5)	1 (50)	6 (25.0)	1 (16.7)
Number of TEAEs	0	0	3	0	5	1	8	1
Dry mouth	0	0	0	0	1 (12.5)	1 (50)	1 (4.2)	1 (16.7)
Vessel puncture site pain	0	0	1 (12.5)	0	0	0	1 (4.2)	0
Liver function test abnormal	0	0	1 (12.5)	0	0	0	1 (4.2)	0
Muscle spasm	0	0	0	0	1 (12.5)	0	1 (4.2)	0
Dysgeusia	0	0	0	0	1 (12.5)	0	1 (4.2)	0
Headache	0	0	1 (12.5)	0	0	0	1 (4.2)	0
Somnolence	0	0	0	0	1 (12.5)	0	1 (4.2)	0
Dry throat	0	0	0	0	1 (12.5)	0	1 (4.2)	0

Table 3. Pharmacokinetic parameters of aclidinium and its metabolites

Parameter	Aclidinium 200 µg	Aclidinium 400 µg	Aclidinium 800 µg	Aclidinium 200 µg	Aclidinium 400 µg	Aclidinium 800 µg	Aclidinium 200 µg	Aclidinium 400 µg	Aclidinium 800 µg
	Aclidinium			Alcohol metabolite			Acid metabolite		
Day 1									
$AUC_{τ}$ (pg·h/mL)	97.6 (40.2)	324.9 (34.4)	494.7 (37.6)	229.4 (41.4)	464.7 (19.8)	1106.6 (31.6)	7378.8 (8.5)	16153.3 (7.8)	34997.1 (21.2)
$AUC_{0-∞}$(pg·h/mL)	109.4 (35.8)	386.7 (34.8)	566.3 (46.0)	474.5 (81.7)	809.1 (33.2)	1881.7 (44.0)	8746.2 (7.6)	20022.4 (12.4)	42176.9 (21.7)
C_{max} (pg/mL)	102.7 (51.4)	194.2 (45.2)	360.1 (62.0)	46.7 (43.0)	76.3 (26.0)	174.9 (35.1)	1011.8 (15.7)	2151.9 (13.5)	5272.1 (28.7)
t_{max} (h)	0.08 (0.00)	0.08 (0.00)	0.10 (56.84)	0.26 (192.59)	0.61 (103.02)	0.75 (125.99)	3.38 (15.33)	3.63 (20.52)	3.63 (32.77)
$t_{1/2}$ (h)	2.4 (28.4)	5.9 (39.8)	4.5 (63.0)	12.4 (98.9)	11.3 (67.0)	9.4 (38.7)	3.8 (15.2)	4.2 (22.2)	4.1 (7.6)
Day 7, Morning									
$AUC_{τ, ss}$(pg·h/mL)	190.7 (42.7)	535.8 (51.9)	651.1 (43.5)	636.6 (47.1)	1229.4 (29.8)	2238.1 (27.1)	13045.2 (25.6)	30721.9 (25.4)	39630.0 (12.8)
$C_{max, ss}$ (pg/mL)	119.9 (37.1)	254.7 (57.0)	377.1 (62.7)	90.1 (38.9)	168.6 (26.4)	378.1 (50.4)	1581.5 (26.4)	3726.3 (22.4)	4853.3 (14.9)
t_{max} (h)	0.14 (109.11)	0.20 (164.05)	0.08 (0.00)	0.31 (160.65)	1.35 (149.31)	1.04 (66.81)	3.00 (17.82)	3.31 (26.68)	3.13 (20.51)
$t_{1/2}$ (h)	12.4 (103.9)	6.8 (32.1)	6.4 (24.9)	10.7 (18.4)	9.4 (28.5)	12.5 (73.4)	6.1 (24.4)	6.5 (19.0)	5.5 (15.8)
Day 7, Evening									
$AUC_{τ, ss}$(pg·h/mL)	164.4 (42.7)	468.4 (59.5)	594.4 (51.6)	602.9 (44.1)	1218.8 (28.4)	2203.7 (34.1)	10840.7 (21.4)	25289.5 (28.1)	37975.4 (14.5)
$C_{max, ss}$ (pg/mL)	63.2 (49.9)	240.5 (60.6)	307.8 (86.5)	94.4 (32.8)	185.5 (42.4)	293.1 (37.4)	1125.9 (17.4)	2644.9 (30.1)	3999.9 (15.2)
t_{max} (h)	0.16 (96.65)	0.10 (56.84)	0.10 (56.84)	0.34 (92.10)	0.59 (95.32)	0.36 (127.56)	4.06 (44.83)	3.06 (30.78)	3.88 (25.57)
$t_{1/2}$ (h)	10.9 (61.8)	17.0 (40.8)	16.3 (26.4)	20.9 (21.4)	26.0 (51.7)	19.9 (23.7)	11.8 (28.4)	14.8 (46.6)	12.4 (27.0)
$t_{1/2}$ (h)[a]	9.2 (43.4)	7.0 (25.0)	4.6 (17.6)	17.4 (20.6)	17.3 (31.4)	11.9 (11.9)	7.3 (21.1)	8.2 (30.3)	3.3 (20.9)

All data are presented as mean (%CV).
$AUC_{τ, ss}$, area under the plasma concentration vs time curve during the dosing interval, τ, at steady state; $C_{max,ss}$, maximum plasma drug concentration at steady state; t_{max}, time of maximum plasma drug concentration; $t_{1/2}$, terminal elimination half-life
[a]Data shown here are effective half-life values; all others shown are terminal half-life values.

Figure 3. Plasma aclidinium AUC after the morning dose of aclidinium 200 µg, 400 µg , and 800 µg on Day 1 (A) and after morning (B) and evening administration (C) on Day 7



Conclusions

- PK steady state was achieved for aclidinium and its metabolites within the 7-day treatment period for aclidinium 200 µg, 400 µg, and 800 µg administered twice daily in healthy subjects.
- Aclidinium exhibited time-independent pharmacokinetics following dosing to steady state, indicating that similar concentration-vs-time profiles will occur after repeated administration at the same dose and frequency.
- Exposure for all compounds increased with increasing dose but in a less than dose-proportional manner between the 400 µg and 800 µg doses.
- All doses of twice-daily aclidinium were safe and well tolerated throughout this study.

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Acknowledgements

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Almirall Forest Laboratories, Inc.

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Improvements In Quality Of Life And Dyspnea In COPD Patients With Twice-Daily Aclidinium

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Introduction

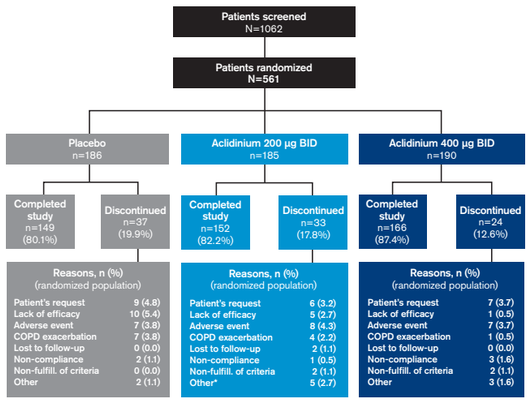
- The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines emphasize that the treatment of stable COPD should include managing symptoms and improving quality of life.[1]
- COPD symptoms often impact patients' capacity to function and the ability to perform normal daily activities. Evaluating and improving symptoms and health-related quality of life (HRQoL) are thus major goals in the treatment of COPD.
  - The St. George's Respiratory Questionnaire is a patient-reported disease-specific instrument used to evaluate quality of life and health status in patients with COPD.[3] The SGRQ focuses on symptoms (frequency and severity), activities (causing or limited by breathlessness), and impact (social functioning, psychological) of the disease.
  - The Transition Dyspnea Index (TDI) is an independent clinician-reported instrument that evaluates breathlessness, a COPD symptom that can have a significant impact on quality of life.
- Aclidinium bromide is a novel, long-acting muscarinic antagonist that is currently in Phase III development for the maintenance treatment of moderate-to-severe COPD using twice-daily administration.
  - Primary efficacy and safety results previously reported from this Phase III study demonstrated that treatment with twice-daily aclidinium 200 µg and 400 µg administered via the Genuair®† inhaler provides clinically relevant sustained bronchodilation compared with placebo and a favorable safety profile in patients with moderate-to-severe COPD.[2]
  - We report here the effects of aclidinium 200 µg and 400 µg BID on health-related quality of life and dyspnea in COPD patients.

Methods

Study Design

- This was a 12-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial evaluating the efficacy and safety of twice-daily aclidinium bromide at 2 dose levels (200 µg and 400 µg).
- Patients (N=561) were randomized (1:1:1) to aclidinium bromide (200 µg or 400 µg BID) or placebo (Figure 1).

Figure 1. Study flow chart



*One patient randomized to aclidinium 200 µg discontinued prior to receiving any study medication and was not included in the safety population..

- Patients were evaluated at screening (for inclusion), at baseline (randomization) following a 2-week run-in period, at Weeks 1, 4, 8, and 12 during the treatment period (6 visits total), and at follow-up 2 weeks after treatment end.

Study Population

Inclusion Criteria

- Male and female patients aged ≥40 years
- Diagnosis of moderate-to-severe stable COPD
- Forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio <70%
- FEV_1 ≥30% and <80% of predicted
- Current or ex-smokers with a smoking history of ≥10 pack-years

Exclusion Criteria

- History or current diagnosis of asthma
- Respiratory infection or COPD exacerbation within 6 weeks (3 months if it resulted in hospitalization) prior to screening
- Clinically relevant cardiovascular conditions or respiratory conditions (other than COPD) and abnormalities in laboratory values or electrocardiogram (ECG) parameters.

Allowed Concomitant Medications

- Albuterol (USA)/Salbutamol (Canada) as needed
- Inhaled corticosteroids (CS) and oral or parenteral CS at doses equivalent to 10 mg/day or 20 mg every other day (if stable at the equivalent dose for 4 weeks before Visit 1)

Study Endpoints

- Change from baseline in SGRQ total score and TDI focal score at Weeks 4, 8, and 12
- Percentage of patients with a clinically meaningful improvement in SGRQ total score (≥4 units decrease) and TDI focal score (≥1 unit increase) at Weeks 4, 8, and 12

Statistical Analysis

- SGRQ and TDI endpoints were analyzed using the ANCOVA model with sex and treatment group as factors and age and baseline SGRQ (total score or dimension score) or baseline dyspnea index (BDI; focal score or dimension score) as covariates.
- The percentages of patients who achieved a change from baseline to Weeks 4, 8, and 12 of ≥4 units in SGRQ total score or ≥1 unit in TDI focal score were analyzed using a logistic regression model with treatment group, sex, age, and baseline SGRQ total score or BDI as explanatory variables, respectively. Statistical significance was based on the Wald test. The effect of aclidinium treatment compared with placebo was estimated by odds ratio and its 95% CI, based on the coefficient and standard error corresponding to the treatment group in the logistic regression model.
- For both SGRQ and TDI, the intent-to-treat (ITT) population was used for analyses.

Results

Baseline Demographics

- Of the 561 patients randomized, 467 completed the study (87.4% in the aclidinium 400 µg group, 82.2% in the aclidinium 200 µg group, and 80.1% in the placebo group). Baseline demographics were similar across all treatment groups (Table 1).

Table 1. Demographic and baseline characteristics (ITT population; N=559)

Characteristic	Placebo n=185	Aclidinium 200 µg n=184	Aclidinium 400 µg n=190	Total N=559
Age, mean (SD), years	65.0 (9.2)	63.1 (9.5)	64.9 (9.5)	64.3 (9.4)
Male, n (%)	96 (51.4)	101 (54.9)	100 (52.6)	296 (53.0)
Caucasian, n (%)	174 (94.1)	169 (91.8)	181 (95.3)	524 (93.7)
BMI, mean (SD), kg/m²	27.5 (5.2)	27.3 (5.1)	27.6 (5.0)	27.5 (5.1)
Current smoker, n (%)	87 (47.0)	84 (45.7)	80 (42.1)	251 (44.9)
Smoking history, mean (SD), pack-years	52.9 (28.1)	53.0 (23.3)	57.2 (28.5)	54.4 (26.8)
SGRQ total score, mean (SD)	45.1 (16.3)	45.9 (17.2)	48.3 (17.8)	46.5 (17.1)
BDI focal score, mean (SD)	6.5 (2.2)	6.4 (2.1)	6.2 (2.1)	6.4 (2.1)
Baseline (Visit 2) FEV_1, mean (SD), L	1.38 (0.6)	1.36 (0.6)	1.33 (0.5)	1.36 (0.5)
Post-bronchodilator FEV_1, mean (SD), % of predicted value	54.7 (13.4)	52.8 (13.7)	54.1 (12.9)	53.9 (13.3)
Post-bronchodilator FEV_1/FVC ratio, mean (SD), %	52.8 (10.5)	50.9 (10.6)	51.5 (10.2)	51.8 (10.4)
COPD severity, n (%)				
Stage II (moderate)	111 (60.0)	98 (53.3)	118 (62.1)	327 (58.5)
Stage III (severe)	72 (38.9)	80 (43.5)	68 (35.8)	220 (39.4)
Stage IV (very severe)	1 (0.5)	3 (1.6)	1 (0.5)	5 (0.9)

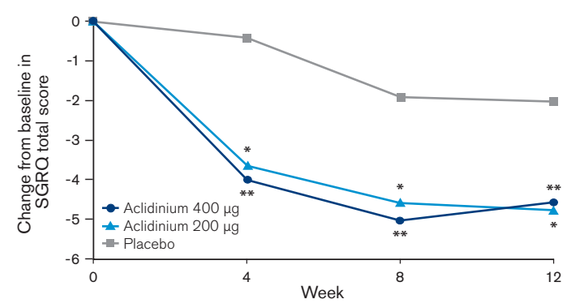
BMI, body mass index

Health-Related Quality Of Life

SGRQ Total Score

- Patients in both the aclidinium 200 µg and 400 µg groups showed a statistically significantly greater improvement in change from baseline SGRQ total score at all time points as compared with placebo (Figure 2).

Figure 2. Change from baseline in SGRQ total score at Weeks 4, 8, and 12



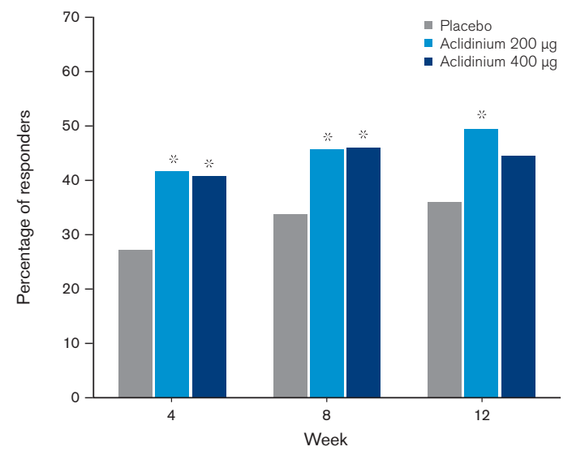
*Aclidinium 200 µg BID vs placebo, p<0.05; **Aclidinium 400 µg BID vs placebo, p<0.05
Minimal clinically important difference is ≥4 point difference in SGRQ total score[4]

- The largest improvement was observed at Week 4 with an adjusted mean difference vs placebo of -3.2 and -3.6 for aclidinium 200 µg and 400 µg, respectively (p<0.001 for both).
- At 12 weeks (study end), the adjusted mean differences vs placebo in the change from baseline in SGRQ total score were -2.7 (aclidinium 200 µg, p=0.013) and -2.5 (aclidinium 400 µg, p=0.019).

Clinically Meaningful Improvements In Quality Of Life

- A statistically significantly higher percentage of patients in each of the aclidinium treatment groups achieved clinically meaningful improvements in SGRQ total score (≥4 point decrease from baseline) compared with placebo at all time points (p<0.05 for all based on odds ratios, except at Week 12 for the aclidinium 400 µg group, p=0.139; Figure 3).

Figure 3. Percentage of patients who achieved a clinically meaningful difference in SGRQ total score at Weeks 4, 8, and 12



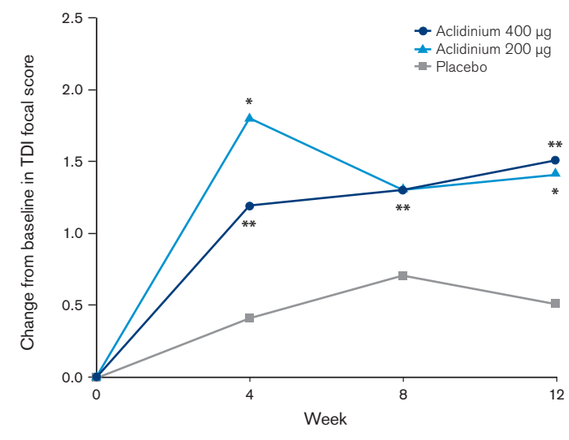
*p<0.05 vs placebo

Dyspnea

TDI

- Both doses of aclidinium resulted in a statistically significantly greater improvement in change from baseline in dyspnea status, as measured by the TDI focal score, compared with placebo across all time points (except at Week 8 for the aclidinium 200 µg group, p=0.060; Figure 4).
- The maximum improvement in TDI focal score was seen at Week 4 for aclidinium 200 µg and at Week 12 for aclidinium 400 µg, with adjusted mean differences vs placebo of 1.4 and 1.0, respectively (p<0.005 for both). Treatment with aclidinium 200 µg resulted in a 0.9 adjusted mean difference in TDI focal score vs placebo at Week 12 (p=0.005).

Figure 4. Change from baseline in TDI focal score at Weeks 4, 8, and 12

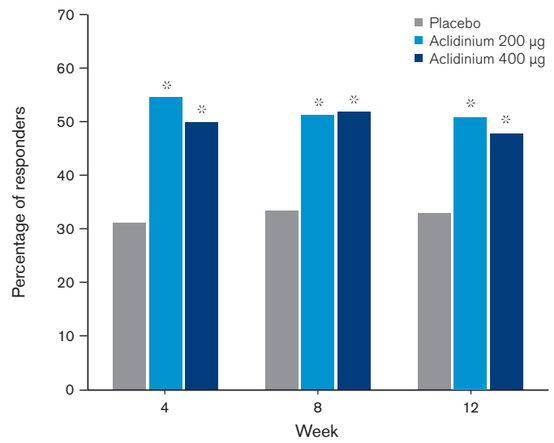


*Aclidinium 200 µg BID vs placebo, p<0.05; **Aclidinium 400 µg BID vs placebo, p<0.05
Minimal clinically important difference is ≥1 point increase in TDI focal score

Clinically Meaningful Improvements In Dyspnea

- Treatment with aclidinium 400 µg resulted in a clinically meaningful improvement in TDI focal score (≥1 unit increase) at Week 12 as compared with placebo (p<0.05).
- A statistically significantly higher percentage of patients in each of the aclidinium treatment groups achieved a clinically meaningful improvement in TDI focal score compared with placebo at all time points (p<0.05 for both, Figure 5).

Figure 5. Percentage of patients who achieved a clinically meaningful difference in TDI focal score at Weeks 4, 8, and 12



*p<0.05 vs placebo

Limitations

- This study was conducted over a short period of time (12 weeks); thus, long-term studies investigating the effects of aclidinium on COPD symptoms are warranted.
- This study was not sufficiently powered to detect differences in improvements in symptoms between the 2 aclidinium doses.

Conclusions

- Treatment with twice-daily aclidinium resulted in improvements in patients' quality of life and dyspnea as measured by SGRQ and TDI.
- In this study, both doses of aclidinium significantly improved patients' SGRQ total scores and TDI focal scores; treatment with aclidinium 400 µg resulted in a clinically meaningful change in TDI focal score at Week 12.
- A significantly greater percentage of patients achieved clinically meaningful differences in both SGRQ total score and TDI focal score during this 12-week study.

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†Genuair® is a registered trademark of Almirall SA.

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Denver, Colorado, May 13-18, 2011

Aclidinium Bromide In Patients With Chronic Obstructive Pulmonary Disease: Efficacy And Safety Results From ATTAIN

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Introduction

- The Global Initiative for Chronic Obstructive Lung Disease (GOLD) recommends the use of long-acting bronchodilators, such as anticholinergics, for the management of patients with chronic obstructive pulmonary disease (COPD).[1]
- Aclidinium bromide is a novel, long-acting muscarinic antagonist, currently in clinical development for the maintenance treatment of patients with COPD.
- A 12-week, Phase III clinical study showed that aclidinium (200 and 400 µg twice daily [BID]) is well tolerated and improves lung function, dyspnea, and quality of life (QoL) in patients with moderate to severe COPD.[2] In a Phase IIa study, aclidinium (400 µg, BID) has also shown comparable morning trough efficacy to tiotropium (18 µg once daily [QD]) and greater bronchodilation in the second half of the day.[3]
- The objectives of this study were to evaluate the long-term efficacy and safety of aclidinium 200 and 400 µg BID versus placebo in patients with moderate to severe COPD.

Methods

Study Design And Treatment

- This was a 24-week, double-blind, Phase III study.
- Patients were randomized (1:1:1) to aclidinium 200 µg, 400 µg, or placebo BID via the Genuair®* inhaler for 24 weeks.
- Patients were evaluated at screening, at baseline following a 2-week run-in period, and at Weeks 1, 4, 8, 12, 18, and 24 during the treatment period. Follow-up contact was made 2 weeks after completion of treatment.

Study Population

Inclusion Criteria

- Male and female patients aged ⩾40 years with moderate to severe stable COPD.
- Post-bronchodilator forced expiratory volume in 1 second (FEV_1)/forced vital capacity ratio <70%.
- Post-bronchodilator FEV_1 ⩾30% and <80% of the predicted value.
- Current or ex-smokers with a smoking history of ⩾10 pack-years.

Exclusion Criteria

- History or current diagnosis of asthma.
- Respiratory infection or COPD exacerbation within 6 weeks (3 months if it resulted in hospitalization) prior to screening.
- Clinically relevant cardiovascular conditions or respiratory conditions.

Allowed Concomitant Medications

- Salbutamol as needed.
- Inhaled corticosteroids (CS) and oral or parenteral CS at doses equivalent to 10 mg/day or 20 mg/day every other day (if stable for 4 weeks before study entry). Oral sustained-release theophyllines were also permitted.

Study Endpoints

Primary Efficacy Endpoint

- Change from baseline in morning pre-dose (trough) FEV_1 at Week 24.

Secondary Efficacy Endpoints

- Change from baseline in peak FEV_1 at Week 24.
- Percentage of patients with a clinically meaningful improvement in dyspnea as measured by a ⩾1-unit increase from baseline on the Transitional Dyspnea Index (TDI) focal score at Week 24.
- Percentage of patients with a clinically meaningful improvement in health-related QoL as measured by a ⩾4-unit decrease from baseline on the St George's Respiratory Questionnaire (SGRQ) total score at Week 24.

Safety Endpoints

- Adverse events (AEs) and serious AEs (SAEs).
- Clinical laboratory measures, vital signs, and 12-lead electrocardiograms (ECGs).

Statistical Analyses

- All efficacy variables were analyzed using the intention-to-treat (ITT) population and safety outcomes were analyzed using the safety population.
- Change from baseline in FEV_1 was analyzed by means of an analysis of covariance (ANCOVA) model.
- TDI focal score and SGRQ total score were analyzed using a logistic regression model.
- Safety outcomes were summarized using descriptive statistics.

Results

Study Population

- A total of 828 patients were randomized to aclidinium 200 µg (n=280), 400 µg (n=272), and placebo (n=276). There were 819 patients in the ITT and safety populations.
- Demographics and baseline characteristics were similar between treatment groups (Table 1).

Table 1. Patient demographics and baseline characteristics (safety population)

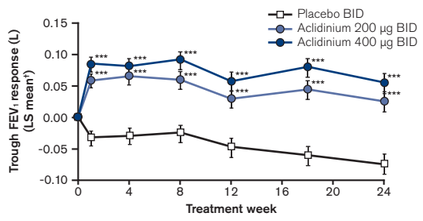
Characteristics	Placebo (n=273)	Aclidinium 200 µg (n=277)	Aclidinium 400 µg (n=269)	Total (n=819)
Age (years) (mean, SD)	62.0 (8.0)	62.3 (7.8)	62.9 (8.4)	62.4 (8.0)
Gender (% male)	69.2	65.3	67.7	67.4
Moderate COPD*	65.9	69.6	68.7	68.1
Severe COPD*	34.1	30.4	31.3	31.9
Current smoker (%)	52.8	50.5	55.0	52.8
Smoking history (pack-years) (mean, SD)	38.9 (18.3)	40.0 (19.8)	41.7 (21.1)	40.2 (19.8)
Baseline FEV_1 (L) (mean, SD)	1.48 (0.5)	1.49 (0.48)	1.48 (0.49)	1.48 (0.49)
Total SGRQ (mean, SD)	45.1 (15.8)	46.3 (16.8)	47.6 (17.7)	46.3 (16.8)
Focal TDI (mean, SD)	6.7 (2.0)	7.0 (2.2)	6.7 (2.1)	6.8 (2.1)

*As classified by the Global Initiative for Chronic Obstructive Lung Disease
COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second; SD, standard deviation; SGRQ, St George's Respiratory Questionnaire; TDI, Transitional Dyspnea Index

Trough FEV_1

- At Week 24, aclidinium 200 µg and 400 µg significantly improved trough FEV_1 from baseline compared with placebo (by 99 mL and 128 mL, respectively; p<0.001 for both; Figure 1).

Figure 1. Change from baseline in trough FEV_1 over time



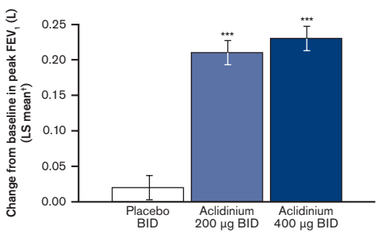
***p<0.001 for both treatments vs placebo; †Adjusted LS mean estimate from ANCOVA model
ANCOVA, analysis of covariance; BID, twice daily; FEV_1, forced expiratory volume in 1 second; LS, least squares

- The improvement in trough FEV_1 provided by aclidinium (200 and 400 µg) was statistically superior to placebo at all timepoints from Week 1 to 24 (p<0.001).
- No statistically significant differences were observed between the two aclidinium doses.

Peak FEV_1

- Peak FEV_1 was significantly improved from baseline with aclidinium 200 µg and 400 µg compared with placebo at Week 24 (by 185 mL and 209 mL, respectively; p<0.001 for both; Figure 2).

Figure 2. Change from baseline in peak FEV_1 at Week 24



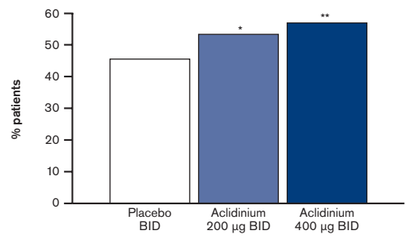
***p<0.001 for both treatments vs placebo; †Adjusted LS mean estimate from ANCOVA model
ANCOVA, analysis of covariance; BID, twice daily; FEV_1, forced expiratory volume in 1 second; LS, least squares

- Peak FEV_1 was also significantly improved with aclidinium 200 and 400 µg at all timepoints from Day 1 through to the end of the study.

Dyspnea And Health-Related Quality Of Life

- More patients treated with aclidinium 200 µg and 400 µg had a clinically meaningful improvement (⩾1-unit increase from baseline) in TDI focal score at Week 24 (53.3% [p<0.05] and 56.9% [p<0.01], respectively, versus placebo, 45.5%; Figure 3).
- At Week 24, the difference in the mean change from baseline in TDI focal score versus placebo was 0.6 units for aclidinium 200 µg (p<0.05) and 1.0 units for aclidinium 400 µg (p<0.001).

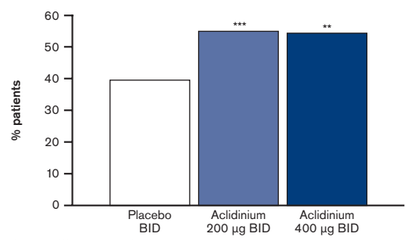
Figure 3. Responders (⩾1-unit improvement) in TDI at Week 24



*p<0.05 vs placebo; **p<0.01 vs placebo
BID, twice daily; TDI, Transitional Dyspnea Index

- A greater percentage of patients treated with aclidinium 200 µg and 400 µg had a clinically meaningful improvement (⩾4-unit decrease from baseline) in SGRQ total score at Week 24 (54.9% [p<0.005] and 54.3% [p<0.0005], respectively, versus placebo, 39.5%; Figure 4).
- At Week 24, the difference in the mean change from baseline in SGRQ total score versus placebo was -3.6 units for aclidinium 200 µg (p<0.001) and -4.3 units for aclidinium 400 µg (p<0.0001).

Figure 4. Responders (⩾4-unit improvement) in SGRQ at Week 24



**p<0.01; ***p<0.001 vs placebo
BID, twice daily; SGRQ, St George's Respiratory Questionnaire

Safety

- The most commonly reported AEs across all treatment groups were COPD exacerbation, headache, nasopharyngitis, diarrhea, and cough (Table 2).
- The incidence of anticholinergic AEs with both aclidinium doses was low (⩽2.5%) and similar to placebo.
- The number of SAEs and number of patients with SAEs were similar across the three treatment groups (4.3%, n=19; 5.6%, n=20; 5.5%, n=18, for aclidinium 200 µg, 400 µg, and placebo, respectively). No SAEs were thought to be related to treatment.
- One patient died from myocardial infarction and one from cardiac failure in the aclidinium 200 and 400 µg treatment groups, respectively. These deaths were not considered to be treatment related.
- No notable differences from baseline in clinical laboratory tests, vital signs, or ECG parameters were observed between treatment groups.

Table 2. Adverse events reported by ⩾2% of patients

	Placebo (n=273)	Aclidinium 200 µg (n=277)	Aclidinium 400 µg (n=269)
COPD exacerbations	56 (20.5)	44 (15.9)	38 (14.1)
Headache	22 (8.1)	30 (10.8)	33 (12.3)
Nasopharyngitis	23 (8.4)	32 (11.6)	30 (11.2)
Rhinitis	7 (2.6)	4 (1.4)	9 (3.3)
Diarrhea	3 (1.1)	5 (1.8)	8 (3.0)
Bronchitis	6 (2.2)	1 (0.4)	7 (2.6)
Hypertension	9 (3.3)	5 (1.8)	7 (2.6)
Cough	5 (1.8)	7 (2.5)	7 (2.6)
Toothache	1 (0.4)	3 (1.1)	6 (2.2)
Back pain	10 (3.7)	12 (4.3)	5 (1.9)
Influenza	6 (2.2)	3 (1.1)	5 (1.9)
Arthralgia	6 (2.2)	5 (1.8)	3 (1.1)
Urinary tract infection	2 (0.7)	6 (2.2)	2 (0.7)
Dyspepsia	6 (2.2)	5 (1.8)	1 (0.4)

Data reported as number (%)
COPD, chronic obstructive pulmonary disease

Conclusions

- **Aclidinium 200 or 400 µg BID in patients with moderate to severe COPD provides statistically significant improvements in airflow limitation (trough FEV_1 and peak FEV_1) compared with placebo.**
- **Statistically significant improvements in symptoms and health status were observed in patients treated with aclidinium 200 or 400 µg BID, compared with placebo.**
- **At both dose levels, aclidinium BID was well tolerated throughout the study, with an incidence of anticholinergic AEs similar to placebo.**

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Acknowledgements

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*Genuair® is a registered trademark of Almirall S.A.

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Twice-Daily Aclidinium Bromide In COPD Patients: Nighttime Symptoms And Rescue Medication Use In ACCORD COPD I

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Introduction

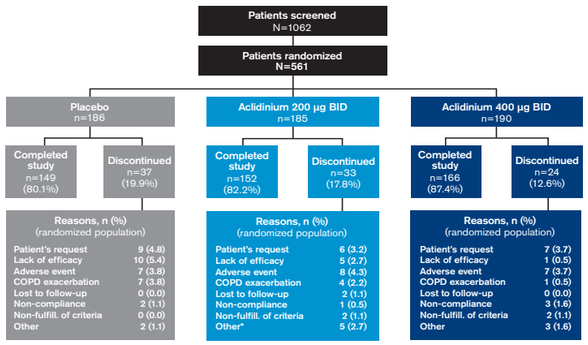
- Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States[1] and is projected to be the third leading cause of death worldwide by 2020.[2]
- COPD symptoms have been reported to be worse at night and in the early morning[3], which may be reflected in disturbed sleep and limitations on morning activities. Little has been published about the effects of currently available COPD medication on nighttime symptoms and sleep.
- Aclidinium bromide is a novel, potent, long-acting muscarinic antagonist being investigated for the maintenance treatment of COPD. Sustained bronchodilation and a favorable safety and tolerability profile were previously reported in clinical studies with twice-daily aclidinium.[4,5]
- The primary objectives of this Phase III study were to assess the efficacy and safety of twice-daily aclidinium 200 µg and 400 µg administered via the Genuair®† inhaler in moderate-to-severe COPD patients.
  - Results for the primary efficacy endpoint of this study showed that change from baseline in morning pre-dose (trough) FEV_1 at Week 12 was statistically and clinically significantly greater for both aclidinium 200 µg and 400 µg BID as compared with placebo (86 mL and 124 mL, respectively; p<0.0001 for both).[5]
  - Here we report the effect of twice-daily aclidinium bromide 200 µg and 400 µg on nighttime symptoms, sleep, and rescue medication use in patients with moderate to severe COPD.

Methods

Study Design

- This was a 12-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group trial evaluating twice-daily aclidinium 200 µg and 400 µg.
- Patients (N=561) were randomized (1:1:1) to aclidinium bromide (200 µg or 400 µg BID) or placebo (Figure 1).

Figure 1. Study flow chart



*One patient randomized to aclidinium 200 µg discontinued prior to receiving any study medication and was not included in the safety population.

Study Population

Inclusion Criteria

- Male and female patients aged ≥40 years
- Diagnosis of moderate to severe stable COPD
- Forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio <70%
- FEV_1 ≥30% and <80% of predicted
- Current or ex-smokers with a smoking history of ≥10 pack-years

Exclusion Criteria

- History or current diagnosis of asthma
- Respiratory infection or COPD exacerbation within 6 weeks (3 months if it resulted in hospitalization) prior to screening
- Clinically significant or relevant cardiovascular conditions, laboratory test, electrocardiogram (ECG) parameters, or respiratory conditions (other than COPD)

Allowed Concomitant Medications

- Albuterol (USA)/Salbutamol (Canada) inhaler as needed
- Inhaled corticosteroids (CS) at any dose and oral or parenteral CS at doses not exceeding 10 mg/day or 20 mg every other day (if stable for 4 weeks before Visit 1)

Health Outcome Measures

- The COPD Nighttime Symptoms Questionnaire and Sleep Diary were self-administered each morning using an electronic diary (eDiary), beginning at screening through study end (Week 12).
- Patients recorded the use of rescue medication (number of puffs) over the last 12 hours and 24 hours every morning in the eDiary, beginning at screening through Week 12.

COPD Nighttime Symptoms Questionnaire

- The questionnaire was designed with a 24-hour or less recall period.
  - The frequency of episodes of the following symptoms the previous night were assessed: breathlessness, cough, sputum production, wheezing when breathing, and rescue medication use.
  - Additional questionnaire items assessed morning activity restriction due to breathlessness, level of breathlessness in the first hour upon getting up, effect of breathlessness and cough on activities in the past 12 hours, amount of sputum production during sleeping hours, amount of sputum production in the last 24 hours, and the effect of COPD symptoms on sleep.

Sleep Diary[6]

- The 10 items within the sleep diary questionnaire assessed the time that the patient went to sleep for the first time the previous night, how long it took to fall asleep, the frequency of waking up during the night, the frequency of waking up and having difficulty falling back to sleep, the time that the patient woke up that morning, whether the patient woke up at the desired time, the total number of hours slept, the overall sleep quality the previous night, how rested the patient felt that morning, and how the patient's sleep the prior night compared to their normal sleep.

Statistical Analysis

- Weekly averages were calculated using the sum of daily averages for each week from baseline until Week 12.
- The change from baseline to Weeks 1, 4, 8, and 12 in the COPD Nighttime Symptoms Questionnaire and Daily Sleep Diary scores, as well as rescue medication use, were analyzed using the intention-to-treat (ITT) population and an ANCOVA model with treatment as factor and the corresponding baseline as covariate.

Results

Baseline Demographics

- A total of 561 patients were randomized and 467 patients completed the study (80.1% of the placebo group, 82.2% of aclidinium 200 µg, and 87.4% of aclidinium 400 µg). Baseline demographics and clinical characteristics were comparable across all treatment groups.
- Baseline (Visit 2) mean (SD) FEV_1 and percent predicted were 1.36 (0.54) L and 47.2 (14.1) %, respectively.
- At baseline, mean (SD) for all nighttime symptom/sleep parameters and rescue medication use were similar between all treatment groups.
- Baseline mean (SD) values for nighttime symptoms are shown in Table 1.
- Baseline mean (SD) values for sleep diary parameters are shown in Table 2.

Table 1. Mean (SD) values of nighttime COPD symptoms at baseline (ITT population)

Parameter	Placebo n=185	Aclidinium 200 µg n=184	Aclidinium 400 µg n=190
Frequency of occurrence in the previous night:			
Breathlessness[a]	1.4 (1.2)	1.5 (1.1)	1.4 (1.3)
Cough[a]	2.1 (1.5)	2.1 (1.6)	1.9 (1.6)
Sputum production[a]	1.3 (1.4)	1.3 (1.5)	1.4 (1.5)
Wheezing[a]	1.3 (1.5)	1.5 (1.5)	1.3 (1.5)
Severity and impact of early morning symptoms			
Usual activities restricted by breathlessness in the morning[b]	1.4 (0.9)	1.4 (0.9)	1.4 (0.9)
Severity of breathlessness for the first hour on getting up in the morning[c]	1.6 (0.9)	1.6 (1.0)	1.5 (0.9)
Severity and impact of nighttime symptoms			
Severity of breathlessness symptoms and impact on activity[b]	1.8 (0.9)	1.8 (0.9)	1.7 (0.9)
Severity of cough and impact on activity[d]	1.5 (0.9)	1.5 (0.9)	1.4 (1.0)
Amount of sputum production			
During sleeping hours[e]	0.7 (0.8)	0.7 (0.8)	0.7 (0.8)
During previous 24 hours across days[e]	1.6 (1.0)	1.5 (1.0)	1.5 (1.1)
Rescue medication:			
Total use, puffs	3.9	3.7	4.4
Daytime use, puffs	3.3	3.1	3.6
Nighttime use, puffs	0.6	0.6	0.8
COPD symptoms affecting sleep			
Breathing symptoms affecting sleep at night[f]	0.8 (0.7)	0.9 (0.8)	0.9 (0.8)

[a]0 = never; 1 = 1-2 times; 2 = 3-4 times; 3 = 5-6 times; 4 = 7 or more times
[b]0 = none; 1 = symptoms present, but caused little or no restriction on morning activities; 2 = mild symptoms that were unpleasant, but caused little restriction on morning activities; 3 = moderate symptoms that caused discomfort and moderately restricted morning activities; 4 = severe symptoms that interfered greatly with morning activities
[c]0 = none; 1 = symptoms present, but caused little or no discomfort; 2 = mild symptoms that were unpleasant, but caused little or no discomfort; 3 = moderate symptoms that caused discomfort, but did not affect normal activities; 4 = severe symptoms that interfered with normal activities
[d]0 = none; 1 = symptoms present, but caused little or no discomfort; 2 = mild symptoms that were unpleasant, but caused little or no discomfort; 3 = moderate symptoms that caused discomfort, but did not affect normal daily activities; 4 = severe symptoms that interfered with normal daily activities
[e]Amount of sputum produced was scored from 0 = none; 1 = amount of 1 teaspoon; 2 = amount of 1 tablespoon; 3 = more than 1 tablespoon
[f]Symptoms causing early awakening or awakening during the night: 0 = none; 1 = once during the night; 2 = 2 or more times during the night; 3 = most times during the night; 4 = symptoms which were so severe that I could not sleep at all

Table 2. Baseline sleep diary parameters (ITT population)

	Placebo n=185	Aclidinium 200 µg n=184	Aclidinium 400 µg n=190
Time it took to fall asleep, minutes, mean (SD)	21. 8 (11.7)	23.2 (11.8)	21.8 (11.2)
Frequency of waking up during the night, mean (SD)	1.8 (1.0)	1.7 (1.0)	1.7 (1.1)
Frequency of waking up and having difficulty falling back to sleep, mean (SD)	0.7 (0.9)	0.8 (0.8)	0.8 (1.0)
Whether the patient woke up at the desired time,[a] n (%)			
Earlier than planned	33 (17.8)	62 (33.7)	52 (27.4)
On time	114 (61.6)	98 (53.3)	95 (50.0)
Later than planned	9 (4.9)	8 (4.3)	13 (6.8)
Earlier than planned/On time	9 (4.9)	6 (3.3)	10 (5.3)
Earlier than planned/Later than planned	1 (0.5)	2 (1.1)	3 (1.6)
On time/Later than planned	3 (1.6)	0 (0.0)	7 (3.7)
Earlier than planned/On time/Later than planned	2 (1.1)	1 (0.5)	0 (0.0)
Total number of hours slept, mean (SD)	7.0 (1.1)	7.0 (1.2)	7.0 (1.2)
Overall sleep quality the previous night,[b] mean (SD)	2.9 (0.7)	2.9 (0.8)	2.9 (0.8)
How rested the patient felt that morning,[c] mean (SD)	2.6 (0.7)	2.5 (0.8)	2.6 (0.8)
How the patient's sleep the prior night compared to their normal sleep,[d] mean (SD)	2.8 (0.6)	2.8 (0.7)	2.8 (0.7)

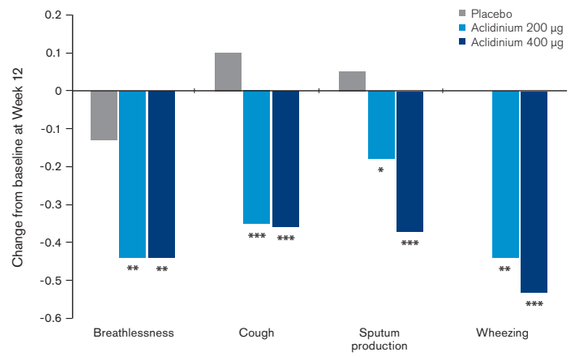
[a]Measured at baseline visit (Visit 2); [b]Rated from 0 (extremely poor) to 5 (extremely good); [c]Rated from 0 (well rested) to 5 (not at all rested); [d]Rated from 0 (much worse than normal) to 5 (much better than normal)

Nighttime COPD Symptoms

Frequency

- Aclidinium 200 µg and 400 µg significantly reduced daily average frequency of nighttime COPD symptom episodes at Week 12 compared with placebo for nighttime breathlessness, cough, sputum production, and wheezing (p<0.05 and p<0.005 for 200 µg and 400 µg, respectively; Figure 2).

Figure 2. Mean change from baseline in daily average frequency of nighttime symptoms† at Week 12 (ITT population)

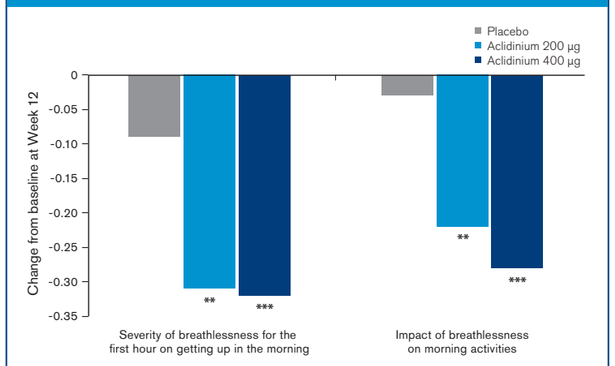


*p<0.05, **p<0.01, ***p<0.001 vs placebo
†Frequency of each of the variables was scored daily as follows: 0 = Never, 1 = 1-2 times, 2 = 3-4 times, 3 = 5-6 times, 4 = 7 or more times; Weekly averages were analyzed.

Severity and Impact of Early Morning Breathlessness

- The severity of early morning (first hour) breathlessness and restriction of activities due to breathlessness were also reduced with aclidinium 200 µg (p<0.01) and 400 µg (p<0.001) vs placebo at Week 12 (Figure 3).

Figure 3. Mean change from baseline in severity† and impact‡ of early morning breathlessness at Week 12 (ITT population)



**p<0.01, ***p<0.001 vs placebo
†Severity of breathlessness was rated from 0 (none) to 4 (severe symptoms that interfered with normal activities).
‡Impact of breathlessness was rated from 0 (none) to 4 (severe symptoms that interfered greatly with morning activities).

- Both aclidinium groups showed significant improvements vs placebo in the severity of 12-hour nighttime breathlessness and cough and their impact on activity at study endpoint (Week 12; Table 3).

Table 3. Mean (SD) change from baseline in the severity and impact of nighttime breathlessness and cough on morning activities[a] at Week 12 (ITT population)

	Placebo n=185	Aclidinium 200 µg n=184	Aclidinium 400 µg n=190
Breathlessness (during previous 12 hours)	-0.19 (0.70)	-0.41 (0.78)**	-0.44 (0.86)***
Cough (during previous 12 hours)	-0.10 (0.78)	-0.28 (0.84)*	-0.24 (0.76)*

[a]Rated from 0 (none) to 4 (severe symptoms that interfered with normal daily activities)
*p<0.05, **p<0.01, ***p<0.001 vs placebo

Sputum Production

- Compared with placebo, the amount of sputum produced over 24 hours was significantly reduced from baseline with aclidinium 200 µg (p<0.05) and 400 µg (p<0.01) at Week 12 (Table 4).
- The amount of sputum produced during sleeping hours at Week 12 was not significantly reduced from baseline with aclidinium compared with placebo, possibly due to a reduction in sputum production in the placebo group at this time point (Table 4).

Table 4. Mean (SD) change from baseline in amount of sputum[a] at Week 12 (ITT population)

	Placebo n=185	Aclidinium 200 µg n=184	Aclidinium 400 µg n=190
24-hour production	0.04 (0.61)	-0.10 (0.68)*	-0.14 (0.67)**
Productiong during sleeping hours	-0.12 (0.52)	-0.17 (0.68)	-0.24 (0.62)

[a]The amount of sputum produced was scored from 0 (none) to 3 (more than 1 tablespoon).
*p<0.05, **p<0.01 vs placebo

Rescue Medication Use

- Aclidinium 200 µg and 400 µg significantly reduced total daily rescue medication use vs placebo over the 12-week treatment period by 0.7 (p=0.0010) and 0.9 (p<0.0001) puffs per day, respectively, driven mostly by reduced daytime use.
- The adjusted mean difference (95% CI) in change from baseline in total rescue medication use at Week 12 was -0.4 (-1.0, 0.1) and -0.6 (-1.1, -0.1) puffs for aclidinium 200 µg and 400 µg, respectively (p<0.001 vs placebo for both).

Sleep Results

- Aclidinium 400 µg significantly improved from baseline the severity and impact of breathing symptoms on sleep vs placebo at 12 weeks (-0.24 vs -0.06, respectively, p<0.01).
- Overall, the results on sleep diary parameters were not statistically significantly different between the aclidinium bromide arms and placebo. However, significant differences in the frequency of nighttime awakenings and ability to fall back asleep were observed with aclidinium 400 µg vs placebo at Week 12 (p<0.05).

Conclusions

- Twice-daily aclidinium bromide 200 µg and 400 µg reduced the frequency of nighttime episodes of breathlessness, cough, sputum production, and wheezing compared with placebo.
- Both doses of aclidinium reduced the severity and impact of nighttime and early morning symptoms compared with placebo.
- Aclidinium 200 µg and 400 µg BID significantly reduced rescue medication use over this 12-week study.
- Treatment with aclidinium 400 µg significantly improved quality of sleep by reducing nighttime awakenings and difficulty in falling back to sleep.
- The relief from nighttime symptoms provided by twice-daily aclidinium may make it a valuable new treatment option for patients with moderate-to-severe COPD.

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†Genuair® is a registered trademark of Almirall SA, Barcelona, Spain.

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Functional Profile Of Aclidinium Bromide In Isolated Human Bronchi And Left Atria

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Introduction

- Anticholinergic treatments for chronic obstructive pulmonary disease (COPD) exert their therapeutic effect by inhibiting pulmonary M_3 receptors, which mediate bronchoconstriction and mucus hypersecretion.[1] If these agents bind to muscarinic receptors outside of the respiratory tract, there is a potential for unwanted side effects; for example, inhibition of cardiac M_2 receptors is known to induce tachycardia.[2]
- Both of the inhaled muscarinic antagonists currently available for the treatment of COPD, the long-acting tiotropium and the short-acting ipratropium, are associated with systemic anticholinergic side effects including tachycardia.[3,4]
- Aclidinium bromide is a novel, long-acting inhaled muscarinic antagonist, currently in clinical development for the maintenance treatment of COPD.
- *In vitro* studies using guinea pig trachea and left atria have shown that, compared with tiotropium, aclidinium has a similar potency and duration of action at M_3 receptors, but a lower potency and a shorter duration of action at M_2 receptors.[5]
- This study investigated the *in vitro* effects of aclidinium at M_3 and M_2 receptors in human bronchial and left-atrial tissue, respectively. Tiotropium and ipratropium were used as comparators.

Methods

Assessment Of M_3-Mediated Smooth Muscle Relaxant Effects In Isolated Human Bronchi

Preparation Of Human Bronchial Strips

- Macroscopically tumor-free bronchial tissue was obtained from patients undergoing surgery for lung carcinoma and used immediately. The protocol was approved by the local ethics committee.
- Bronchial strips were dissected free from parenchyma and mounted in a superfusion chamber containing oxygenated Krebs solution at 37°C. Spontaneous tone, induced by endogenous leukotrienes and histamine, was inhibited by zileuton (10 µM) and fexofenadine (10 µM), respectively.
- Each preparation was connected to a force transducer and isometric changes were recorded using standard software. An initial load of 2 g was used to obtain a stable resting tone prior to the initiation of electrical stimulation.
- Electrical stimulation was delivered by bipolar electrodes in 10-second bursts of square-wave pulses (8 Hz, 40–50 V, and 0.5 ms duration) generated every 120 seconds by a Grass stimulator. The responses to electrical stimulation were allowed to stabilize.

Assessment Of Potency

- Increasing concentrations of aclidinium, tiotropium, or ipratropium (0.3 nM–10 nM) were cumulatively added and the concentration required to obtain a 50% inhibition of tone (IC_{50}) was calculated.
- Antagonist potency was determined as -log IC_{50} (pIC_{50}) values.

Assessment Of Onset And Offset

- Aclidinium, tiotropium, or ipratropium (10 nM) was added to inhibit approximately 75% of baseline contraction. After 30 minutes, the tissue was washed free of antagonist and recovery of tone was recorded for 14–15 hours.
- Onset of action ($t_{½}$) was defined as the time taken from antagonist addition to achieve 50% inhibition of tone.
- Offset of action ($t_{½}$) was defined as the time taken from antagonist washout to achieve 50% recovery of tone.
- Differences between onset and offset values were determined by analysis of variance.

Assessment Of Duration Of Action At M_2 Receptors In Isolated Human Atria

Preparation Of Human Atrial Strips

- Left-atria tissue was obtained from patients undergoing surgery for cardiac bypass and used immediately. The protocol was approved by the local ethics committee.
- Atrial strips were mounted in a superfusion chamber containing oxygenated Krebs solution at 37°C.
- The strips were connected to a force transducer and isometric changes were recorded using standard software. An initial load of 2 g was used to obtain a stable resting tone prior to the initiation of electrical stimulation.
- Electrical stimulation generated by a Grass stimulator was delivered by bipolar electrodes at 1 Hz, 5 ms duration, and 2–5 V (20% higher than the threshold for contraction). The responses to electrical stimulation were allowed to stabilize.

Estimation Of Offset

- The stimulated atrial strips were pre-treated with carbachol (10 µM) to inhibit electrically induced contractions via the M_2 receptor.
- Aclidinium, tiotropium, or ipratropium were added to the carbachol-treated atria at a concentration that inhibited approximately 70% of the maximum carbachol-induced relaxation (70, 50, and 80 nM, respectively).
- After 20–30 minutes, preparations were washed three times to remove free antagonist and the atrial strips were incubated with carbachol (10 µM) for 240 minutes.
- The time to achieve 50% recovery of the maximum carbachol-induced relaxation ($t_{½}$; offset) was calculated using one-phase (aclidinium and tiotropium) or two-phase (ipratropium) exponential decay.

Data Analysis

- Statistically significant differences between onset and offset values were determined by parametric analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test.

Results

M_3-Mediated Smooth Muscle Relaxant Effects In Isolated Human Bronchi

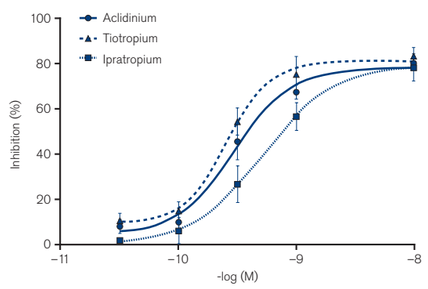
- Aclidinium, tiotropium, and ipratropium inhibited the contractile response induced by electrical stimulation with similar potency (Table 1; Figure 1).

Table 1. Potency of antagonists as inhibitors of the contractile response induced by electrical stimulation of human bronchial strips

	pIC_{50}
Aclidinium	9.3 ± 0.0
Tiotropium	9.6 ± 0.1
Ipratropium	9.5 ± 0.1

Data reported as mean ± standard error for 3–5 experiments using 3 patient samples

Figure 1. Concentration response curves for aclidinium, tiotropium, and ipratropium in electrically stimulated human bronchial strips



Data reported as mean ± standard error for 3–5 experiments using 3 patient samples

- The onset of action of aclidinium was similar to that of ipratropium and significantly faster than tiotropium ($p<0.05$; Table 2).
- The offset time for aclidinium was significantly longer than that of ipratropium ($p<0.05$), whereas no recovery of tone was observed after washout of tiotropium within the duration of the study (Table 2).

Table 2. Onset and offset of aclidinium, ipratropium, and tiotropium against the contraction induced by electrical stimulation of human bronchial strips

	n/p	Maximal inhibition of contraction (%)	Onset time ($t_{½}$; min)	Offset time ($t_{½}$; min)
Aclidinium (10 nM)	8/6	74.9 ± 3.3	4.4 ± 0.7#	334 ± 49*
Tiotropium (10 nM)	5/4	76.6 ± 3.9	7.4 ± 1.3*	NR (⩾10 h)
Ipratropium (10 nM)	5/3	71.1 ± 3.6	3.3 ± 0.6	76 ± 9

*$p<0.05$ vs ipratropium; #$p<0.05$ vs tiotropium
Data reported as mean ± standard error
n, number of individual bronchial strips; NR, no recovery of tension observed after 10 h; p, number of patients

Duration Of Action At M_2 Receptors In Isolated Human Atria

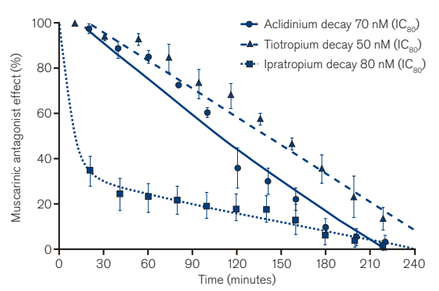
- Aclidinium inhibited the M_2-mediated bradycardiac effect of carbachol with a longer offset time than ipratropium and a shorter offset time than tiotropium (Table 3; Figure 2)

Table 3. Duration of action (offset) for aclidinium, tiotropium, and ipratropium at M_2 receptors in electrically stimulated human left-atrial strips treated with carbachol

	n/p	Inhibition of maximum carbachol-induced relaxation (%)	Offset time ($t_{½}$; min)
Aclidinium	3/3	68.4 ± 5.6	110.2 ± 5.2*#
Tiotropium	3/3	72.1 ± 2.3	159.3 ± 10.5*
Ipratropium	3/3	69.8 ± 1.5	16.6 ± 0.3

*$p<0.01$ vs ipratropium; #$p<0.01$ vs tiotropium
Data reported as mean ± standard error
n, number of individual bronchial strips; p, number of patients

Figure 2. Duration of action (offset) for aclidinium, tiotropium, and ipratropium at M_2 receptors in electrically stimulated human left-atrial strips treated with carbachol



Data reported as mean ± standard error for 3 experiments using different patient samples

Conclusions

- **Aclidinium has similar potency to tiotropium at M_3 receptors in isolated human bronchi, with a faster onset of action. Both aclidinium and tiotropium show a long-lasting pharmacological effect in this model.**
- **Aclidinium demonstrates a shorter duration of action than tiotropium at M_2 receptors in isolated human atria. These data are consistent with previous observations in guinea pig models[5] and suggest that aclidinium may have a lower potential for cardiovascular side effects.**

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Acknowledgements

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Almirall Forest Laboratories, Inc.

Poster presented at the American Thoracic Society International Conference, Denver, Colorado, May 13-18, 2011

Aclidinium Bromide Partially Prevents Human Lung Fibroblast Activation In Vitro

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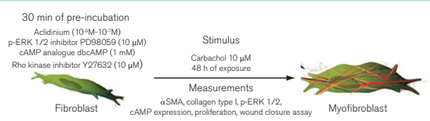
Introduction

- The process of airway remodeling is a contributing factor to the development of chronic obstructive pulmonary disease (COPD) and represents a challenging area of disease management. The activation of lung fibroblasts is known to be involved in this pathologic remodeling process. Upon activation, resident fibroblasts are transformed into a more contractile, proliferative, and secretory-active myofibroblast phenotype characterized by expressing α-smooth muscle actin (αSMA) and collagen type I.
- Muscarinic stimulation has been recently implicated in this process. For example:
  - A non-cholinergic system initiates remodeling propagated by structural cells, for example, fibroblasts and bronchial epithelial cells[1]
  - The muscarinic receptor agonist, carbachol, stimulates collagen synthesis and proliferation of lung fibroblast.
- Aclidinium bromide is a novel, long-acting muscarinic antagonist in Phase III development for COPD treatment. This study explores the effect of aclidinium on human lung fibroblast to myofibroblast transition, following carbachol exposure *in vitro*.

Methods

- αSMA and collagen type-I expression were measured by real-time RT-PCR, western blot, and immunofluorescence (Figure 1).

Figure 1. Experimental procedures



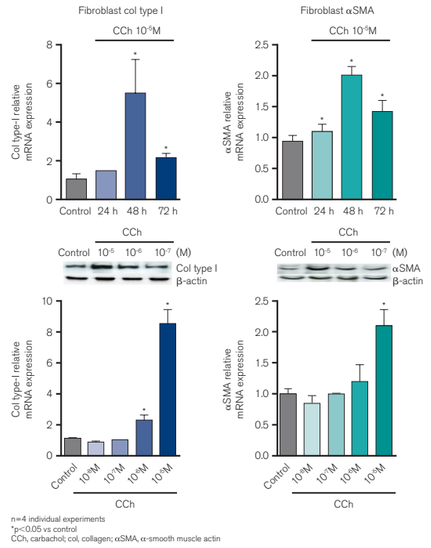
cAMP, cyclic adenoside monophosphate; p-ERK, phosphorylated extracellular signal-regulated kinase; αSMA, α-smooth muscle actin

- p-ERK 1/2 phosphorylation and RhoA-GTP activation were measured by western blot and intracellular cAMP levels by cAMP Biotrak enzyme immunoassay.
- Functional experiments assessed fibroblast proliferation using a BrdU kit, and fibroblast migration by wound closure assay.

Results

- Exposure to carbachol induced a concentration- and time-dependent increase in the mRNA and protein levels of αSMA and collagen type I by 2- and 8-fold, respectively (Figure 2).

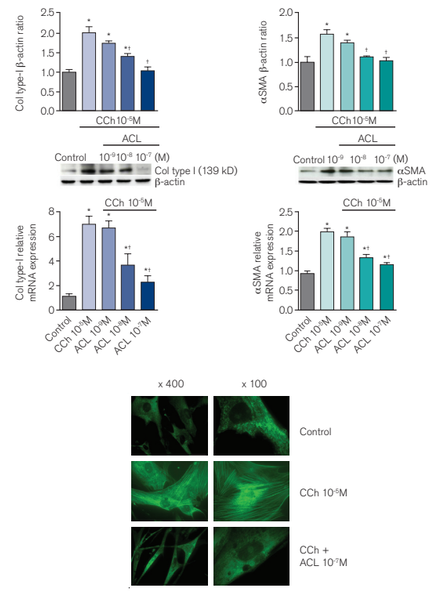
Figure 2. CCh induces αSMA and collagen type-I expression



n=4 individual experiments
*p<0.05 vs control
CCh, carbachol; col, collagen; αSMA, α-smooth muscle actin

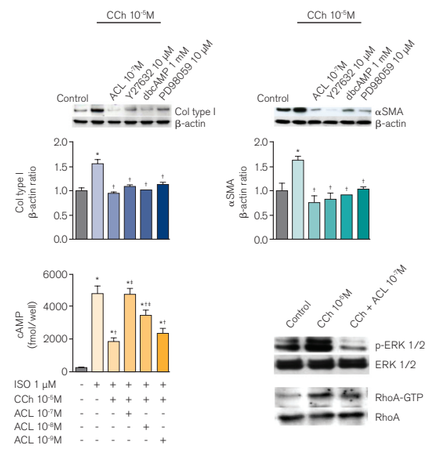
- Aclidinium dose-dependently attenuated the αSMA and collagen type-I expression induced by carbachol, resulting in complete suppression at 10^{-7}M. Furthermore, aclidinium (10^{-7}M) reduced carbachol-induced myofibrillar αSMA formation by 75% (Figure 3).
- Y27632, PD98059, and dbcAMP also prevented the carbachol-induced αSMA and collagen type-I expression (Figure 4).
- Aclidinium prevented phospho-ERK 1/2 and RhoA-GTP increase resulting from stimulation with carbachol.
- Carbachol (10 μM, incubated for 10 min before isoprenaline) effectively prevented the upregulation of cAMP induced by isoprenaline (1 μM) which was completely reversed by aclidinium 10^{-7}M (added 10 min before carbachol).

Figure 3. Aclidinium reduces CCh-induced αSMA and collagen type-I expression



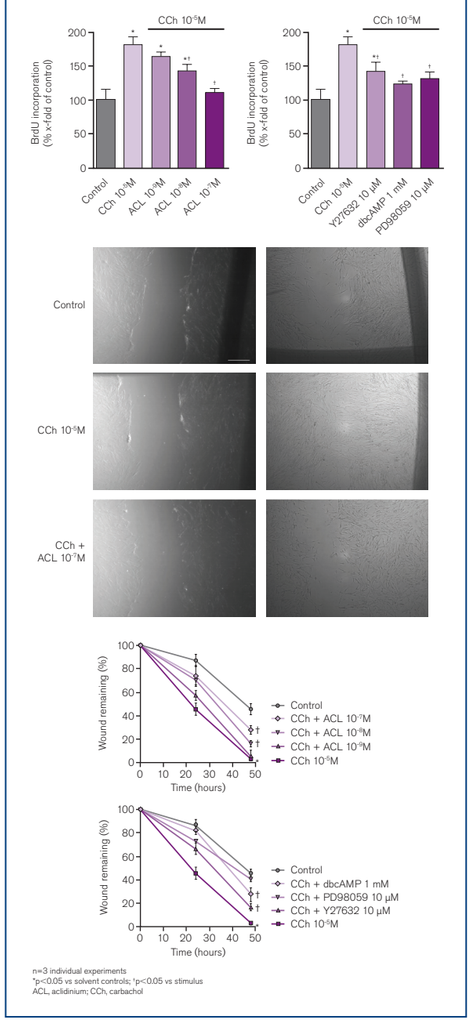
n=3 individual experiments
*p<0.05 vs control; †p<0.05 vs stimulus
Cells were immunostained with monoclonal human anti-αSMA followed by an appropriate secondary-FITC antibody
ACL, aclidinium; CCh, carbachol; col, collagen; αSMA, α-smooth muscle actin

Figure 4. CCh induces αSMA and collagen type I by means of RhoA and ERK1/2 activation and cAMP downregulation



n=3 individual experiments
*p<0.05 vs solvent controls; †p<0.05 vs isoprenaline (ISO); ‡p<0.05 vs CCh plus ISO
ACL, aclidinium; CCh, carbachol; col, collagen; αSMA, α-smooth muscle actin

Figure 5. Aclidinium attenuated CCh-induced fibroblast proliferation and migration



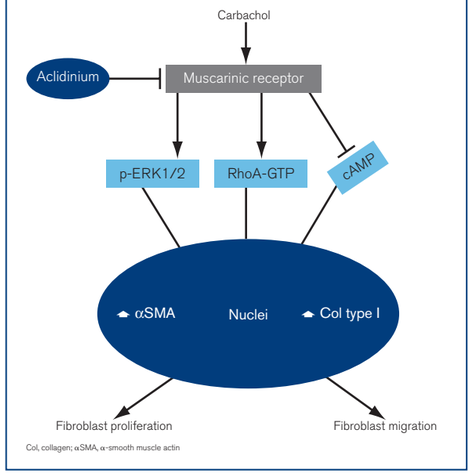
n=3 individual experiments
*p<0.05 vs solvent controls; †p<0.05 vs stimulus
ACL, aclidinium; CCh, carbachol

- Carbachol increased lung fibroblast proliferation by 2-fold which was prevented by aclidinium 10^{-7}M (1.1-fold), Y27632 (1.4-fold), dbcAMP (1.2-fold), and PD98059 (1.3-fold) (Figure 5).
- Fibroblast wound closure was completed after 48 hours of carbachol treatment.
- Fibroblast treated with aclidinium 10^{-7}M, Y27632, PD98059, or dbcAMP reduced wound closure by 30%, 20%, 28%, and 40%, respectively.

Conclusions

- **Carbachol increases myofibroblast markers αSMA and collagen type I.**
- **Aclidinium attenuates carbachol-induced αSMA, collagen type-I protein expression, and αSMA microfilaments, in a dose-dependent manner.**
- **Carbachol-induced αSMA and collagen type-I expression, fibroblast proliferation, and migration are mediated by RhoA-GTP and ERK1/2 activation, and a decrease in cAMP.**
- **Aclidinium attenuates carbachol-induced changes including fibroblast proliferation and migration (Figure 6).**

Figure 6. Aclidinium attenuates CCh-induced lung fibroblast activation



Col, collagen; αSMA, α-smooth muscle actin

Reference

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Almirall Forest Laboratories, Inc.

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Effects of Aclidinium Bromide on Respiratory Function In Guinea Pigs Exposed to Cigarette Smoke For 6 Months

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Introduction

- Cigarette smoke (CS) is a major cause of chronic obstructive pulmonary disease (COPD), a condition characterized by airflow obstruction and clinical symptoms of chronic cough and sputum, dyspnea, wheezing, and fatigue.
- Aclidinium bromide is a long-acting muscarinic antagonist in development for the treatment of COPD.

Objective

- To evaluate the effects of aclidinium on respiratory function and signs of bronchial irritation in guinea pigs chronically exposed to CS for 6 months.

Methods

Animal Groups

- Male Hartley guinea pigs (n=46, ~415 g) were housed under a 12-h light/dark cycle and randomly divided into 6 groups:
  - Vehicle sham: treated with vehicle and exposed to room air (n=8)
  - Vehicle CS: treated with vehicle and exposed to CS (n=10)
  - Ac10 sham: treated with aclidinium 10 µg/mL and exposed to room air (n=7)
  - Ac10 CS: treated with aclidinium 10 µg/mL and exposed to CS (n=6)
  - Ac30 sham: treated with aclidinium 30 µg/mL and exposed to room air (n=7)
  - Ac30 CS: treated with aclidinium 30 µg/mL and exposed to CS (n=8).

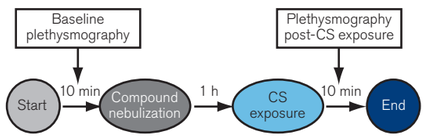
Cigarette Smoke Exposure

- Animals were exposed to the smoke of 6 non-filtered research cigarettes (3R4F Kentucky University) for 5 days/week for 24 weeks using a nose-only system.
- Control animals were sham-exposed to room air for 24 weeks.

Aclidinium Administration

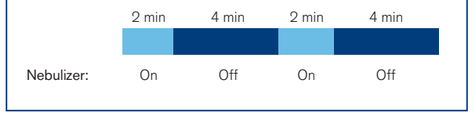
- Animals were nebulized with vehicle (water) or aclidinium in a gas mixture containing 5% CO_2, 21% O_2, and 74% N_2 (ultrasonic Devilbiss Ultraneb 3000 nebulizer, flow of 3 L/min), 1 hour prior to CS exposure (Figures 1 and 2).

Figure 1. Experimental protocol diagram



CS, cigarette smoke

Figure 2. Nebulization protocol



Plethysmography And Respiratory Signs

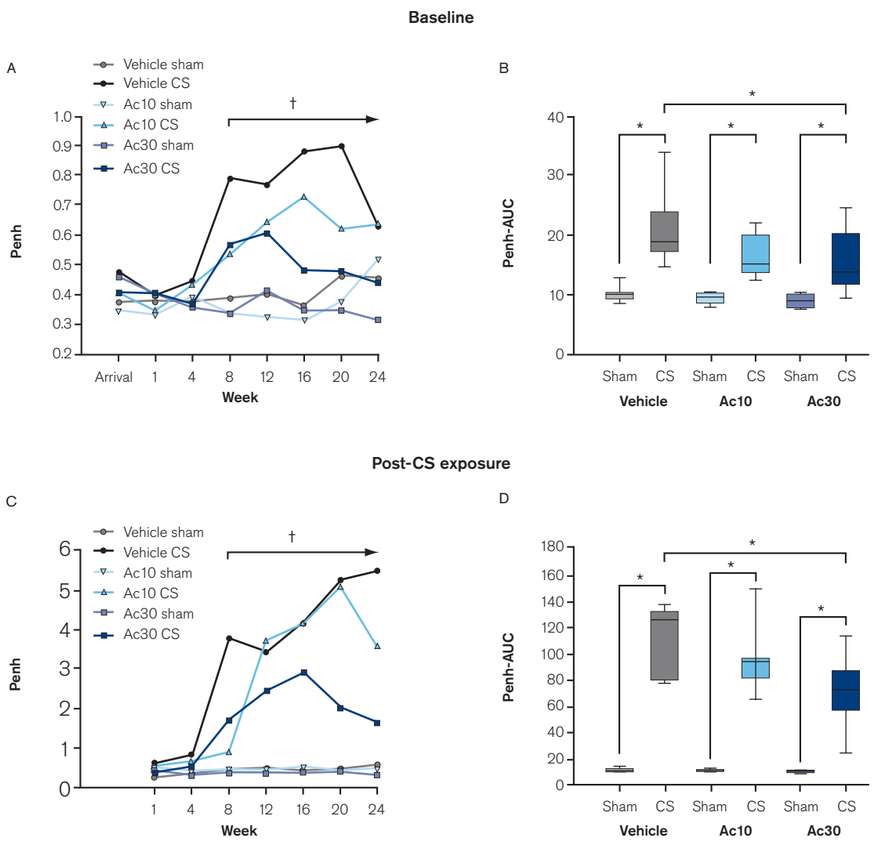
- Pulmonary function was evaluated weekly using an unrestrained plethysmography system (Buxco).
- Plethysmography was performed before (baseline) and 10 minutes after CS exposure (Figure 1).
- Breathing frequency, tidal volume, and enhanced pause (Penh) were recorded for 3 minutes. Penh was used as an indicator parameter of airflow limitation.
- Episodes of cough during the first minute post-CS exposure were counted weekly during Weeks 9–24.
- Episodes of bronchoconstriction during CS exposure were counted through the whole study period.

Results

Respiratory Function

- Guinea pigs exposed to CS showed an increase in Penh, pre- and post-CS exposure (Figure 3).

Figure 3. Penh evolution during the 24 weeks: (A) baseline, (C) post-CS exposure; and box plot of area under curve (AUC): (B) baseline, (D) post-CS exposure



†p<0.05 CS effect, two-way ANOVA; *p<0.05 CS, Mann-Whitney
CS, cigarette smoke; Penh, enhanced pause

Table 1. Respiratory profile at baseline and post-CS exposure

		Vehicle		Ac10 µg/mL		Ac30 µg/mL	
		Sham-exposed (n=8)	CS-exposed (n=8)	Sham-exposed (n=7)	CS-exposed (n=6)	Sham-exposed (n=7)	CS-exposed (n=8)
Breath frequency	Baseline	2409 (2219-2951)	2719 (2625-2818)	2280 (2409-2707)	2812 (2666-3006)*	2071 (2014-2105)	2839 (2447-2957)*
	Post-CS	1845 (1749-1896)	2316 (2010-2492)*	1879 (1764-1949)	2494 (2366-2761)*	1808 (1669-1855)	2463 (2053-2804)*
Tidal volume	Baseline	17192 (15591-18862)	17189 (15034-18190)	17424 (15482-18111)	17419 (16029-20638)	14522 (14255-15344)	16797 (15540-18553)*
	Post-CS	12905 (11862-13534)	22749 (20101-27916)*	13261 (13037-13846)	23744 (22031-26398)*	12433 (11834-13157)	22592 (21018-25651)*

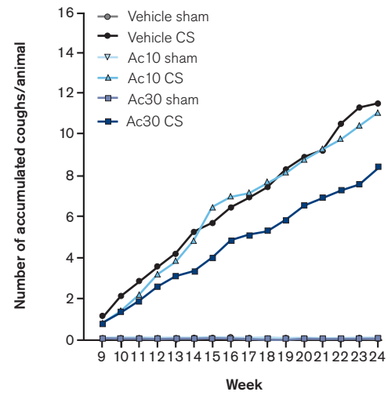
Values are median and inter-quartile range; *p<0.05 vs corresponding sham exposed, Mann-Whitney
CS, cigarette smoke

- Animals exposed to CS and treated with aclidinium 30 µg/mL showed a significant reduction of Penh pre- and post-CS exposure (Figure 3B and 3D).
- No changes in breathing frequency or tidal volume were observed between the vehicle and treatment groups, post-CS exposure (Table 1).

Respiratory Signs: Episodes Of Cough And Bronchoconstriction

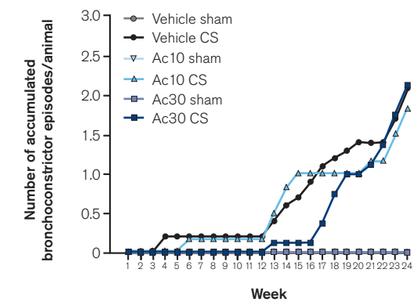
- Animals exposed to CS showed more frequent episodes of cough and bronchoconstriction compared with non-exposed animals.
- Aclidinium 30 µg/mL showed a trend to reduce the occurrence of cough and delay the occurrence of bronchoconstriction episodes (Figures 4 and 5).

Figure 4. Accumulated episodes of cough



CS, cigarette smoke

Figure 5. Accumulated episodes of bronchoconstriction



CS, cigarette smoke

Conclusions

- Treatment with aclidinium 30 µg/mL attenuated airflow limitation induced by CS exposure in the guinea pig.
- Aclidinium (30 µg/mL) showed a trend toward reducing the development of bronchial impairment indicators induced by CS exposure.

Acknowledgements

This study was supported by Almirall S.A., Barcelona, Spain, and Consorcios Estratégicos Nacionales en Investigación Técnica (CENIT), Spain.

Poster presented at the American Thoracic Society International Conference, Denver, Colorado, May 13-18, 2011

Effects Of Aclidinium Bromide On Airway Remodeling In Guinea Pigs Exposed To Cigarette Smoke For 6 Months

David Domínguez-Fandos,[1] Raquel Puig-Pey,[1] Elisabet Ferrer,[1] Cristina Carreño,[2] Mònica Aparici,[2] Jorge Beleta,[2] Neus Prats,[2] Montserrat Miralpeix,[2] Amadeu Gavaldà,[2] Víctor I Peinado,[1,3] Joan Albert Barberà[1,3]

[1]Department of Pulmonary Medicine, Hospital Clínic-IDIBAPS, Barcelona, Spain; [2]Almirall, R&D Centre, Barcelona, Spain; [3]CIBER de Enfermedades Respiratorias, Barcelona, Spain

Introduction

- A significant contributor to the development of airflow obstruction in chronic obstructive pulmonary disease (COPD) is the process of airway remodeling, triggered by inhalation of cigarette smoke (CS) and other noxious substances.
- Aclidinium bromide is a novel, long-acting, inhaled anticholinergic bronchodilator, currently in development for COPD treatment.

Objective

- To investigate the effect of aclidinium on airway remodeling in guinea pigs chronically exposed to CS for 6 months.

Methods

Animal Groups

- Male Hartley guinea pigs (n=46, ~415 g) were housed under a 12-h light/dark cycle and randomly divided into 6 groups:
  - Vehicle sham: treated with vehicle and exposed to room air (n=8)
  - Vehicle CS: treated with vehicle and exposed to CS (n=10)
  - Ac10 sham: treated with aclidinium 10 µg/mL and exposed to room air (n=7)
  - Ac10 CS: treated with aclidinium 10 µg/mL and exposed to CS (n=6)
  - Ac30 sham: treated with aclidinium 30 µg/mL and exposed to room air (n=7)
  - Ac30 CS: treated with aclidinium 30 µg/mL and exposed to CS (n=8).

Cigarette Smoke Exposure

- Animals were exposed to the smoke of 6 non-filtered research cigarettes (3R4F Kentucky University) 5 days/week for 24 weeks using a nose-only system.
- Control animals were sham-exposed to room air for 24 weeks.

Aclidinium Administration

- Animals were nebulized with vehicle (water) or aclidinium in a gas mixture containing 5% CO_2, 21% O_2, and 74% N_2 (ultrasonic Devilbiss Ultraneb 3000 nebulizer, flow of 3 L/min), 1 hour prior to CS exposure (Figures 1 and 2).

Figure 1. Nebulization protocol

Morphological Studies

- Lungs were removed and lobes were inflated and fixed in 10% buffered formalin 4%.

Airway Remodeling

- Thickness of adventitia, muscularis, mucosal layers, and total wall thickness of airways were measured by planimetry in sections.
- Sections were immunostained with a primary monoclonal mouse anti-human smooth muscle actin (SMA).
- To identify airway size and normalize assessments, the median of internal luminal perimeter was used to stratify airways into large (above the median) or small (under the median).

Inflammatory Cells

- The number of neutrophils, eosinophils, and macrophages was counted in alveolar septa and airway adventitia in sections stained with hematoxylin-eosin (H&E), Congo red, and PAS, respectively.

Emphysema And Goblet Cell Metaplasia

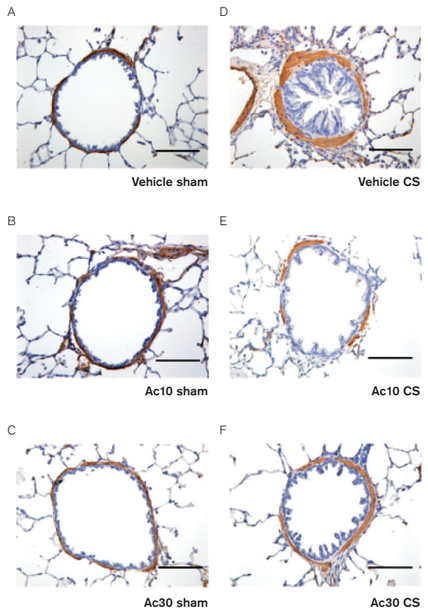
- The presence of emphysema was evaluated in H&E-stained sections by measuring the mean linear intercept of alveolar septa.
- The number of secretory cells in the airway epithelium was counted in sections stained with alcian blue.

Results

Airway Remodeling

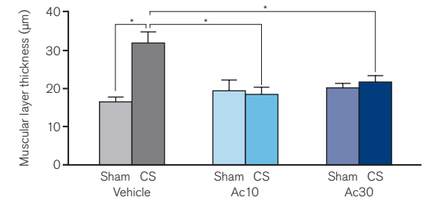
- CS exposure caused enlargement of airway wall layers, particularly in smaller airways (Table 1; Figure 2).
- In animals chronically exposed to CS and treated with aclidinium, thickening of the muscularis in small airways was significantly reduced (Figure 3). The amount of SMA (α-actin) in the small airways was also significantly prevented for both tested doses (10 µg/mL and 30 µg/mL) (Table 1).
- Thickening of adventitial and mucosal layers was not significantly reduced with aclidinium (Table 1).

Figure 2. Airway remodeling



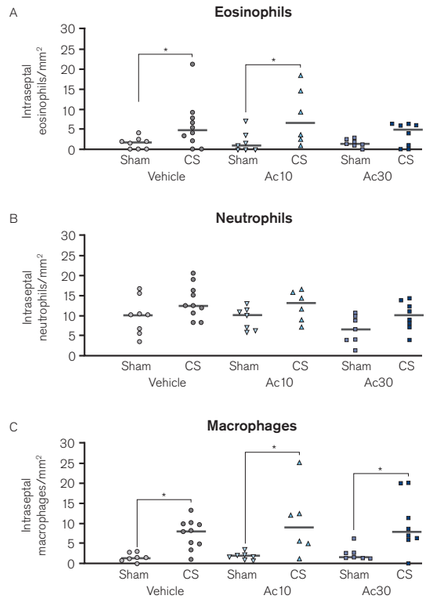
Immunohistochemistry for α-actin. Scale bar, 100 µm
CS, cigarette smoke

Figure 3. Effects of aclidinium on muscular thickness in small airways



Data reported as mean ± standard error; n=6–10
*p<0.02
CS, cigarette smoke

Figure 4. Inflammatory cell counts in alveolar septa



Horizontal bar shows median values
*p<0.05
CS, cigarette smoke

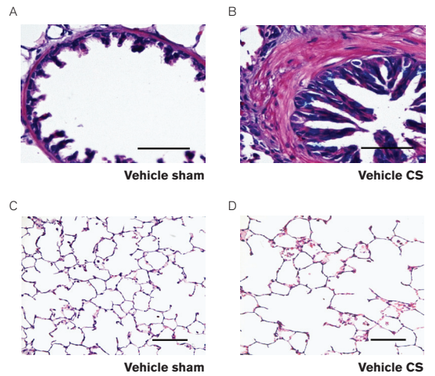
Inflammatory Cells

- CS-exposed animals showed infiltration of inflammatory cells in alveolar septa and airways (data not shown). The number of cells was unaffected by aclidinium treatment (Figure 4).

Emphysema And Goblet Cell Metaplasia

- Emphysematous lesions in parenchyma and goblet cell metaplasia in airways of guinea pigs exposed to CS were not reduced with aclidinium administration (Figure 5; Table 2).

Figure 5. Goblet cell metaplasia and emphysema. Alcian blue staining (A, B) and hematoxilin-eosin (C, D)



Scale bar, 50 µm
CS, cigarette smoke

Table 1. Effects of aclidinium on airway remodeling in guinea pigs exposed to CS

	Airway size (ILP)	Vehicle		Ac10 µg/mL		Ac30 µg/mL	
		Sham (n=8)	CS (n=10)	Sham (n=7)	CS (n=6)	Sham (n=7)	CS (n=8)
Total wall thickness (µm)	Large	66 ± 8	108 ± 9*	73 ± 5	99 ± 8*	79 ± 5	106 ± 6*
	Small	57 ± 9	120 ± 52*	68 ± 15	81 ± 17	66 ± 9	95 ± 24*
Mucosal thickness (µm)	Large	27 ± 2	53 ± 6*	33 ± 3	50 ± 4*	31 ± 2	45 ± 3*
	Small	29 ± 5	59 ± 38*	34 ± 5	41 ± 8	33 ± 4	46 ± 10*
Muscular thickness (µm)	Large	21 ± 2	32 ± 5	23 ± 2	26 ± 3	27 ± 2	31 ± 2
	Small	16 ± 3	32 ± 9*	19 ± 7	18 ± 4#	20 ± 3	21 ± 5#
α-actin+thickness (µm)	Large	19 ± 8	28 ± 14	22 ± 6	24 ± 6	26 ± 7	31 ± 7
	Small	14 ± 4	28 ± 8*	17 ± 7	16 ± 4#	19 ± 3	21 ± 5#
Adventitial thickness (µm)	Large	17 ± 4	23 ± 3	16 ± 3	24 ± 4	21 ± 3	29 ± 4
	Small	12 ± 5	30 ± 17*	15 ± 7	22 ± 6	15 ± 5	29 ± 15*

Data reported as mean ± standard deviation; *p<0.05 compared with sham-exposed under the same treatment; #p<0.05 compared with vehicle+CS-exposed
Results are stratified into large (>median) and small (<median) airways
CS, cigarette smoke; ILP, internal luminal perimeter

Table 2. Goblet cell metaplasia and emphysema

	Airway size (ILP)	Vehicle		Ac10 µg/mL		Ac30 µg/mL	
		Sham (n=8)	CS (n=10)	Sham (n=7)	CS (n=6)	Sham (n=7)	CS (n=8)
Goblet cells (cells/mm)	Large	3.2 ± 4.1	22.8 ± 14.6*	6.2 ± 8.4	17.9 ± 11.4	9.2 ± 10.3	15.9 ± 10.6
	Small	0.2 ± 0.5	6.0 ± 5.5*	1.0 ± 1.9	7.0 ± 6.1*	0.1 ± 0.2	9.9 ± 8.9*
Emphysema (µm)		34.2 ± 2.3	48.5 ± 9.1*	36.8 ± 3.4	41.8 ± 3.9*	38.3 ± 8.0	43.3 ± 5.4

Data reported as mean ± standard error; *p<0.05 vs sham-exposed under the same treatment
Results are stratified into large (>median) and small (<median) airways
CS, cigarette smoke; ILP, internal luminal perimeter

Conclusions

- **Guinea pigs exposed to CS for 6 months showed:**
  - **Thickening of the airway wall**
  - **Inflammatory infiltrate in the airways and alveolar septa containing eosinophils, neutrophils, and macrophages**
  - **Goblet cell metaplasia and emphysema.**
- **Treatment with aclidinium at doses of 10 and 30 µg/mL significantly reduced the muscularization of small airways induced by CS exposure.**
- **The evidence from this chronic model of COPD suggests aclidinium is efficacious in preventing smooth muscle remodeling in small airways.**

Acknowledgements

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Cigarette Smoke-Induced Fibroblast Activation Is Attenuated By Aclidinium In Vitro

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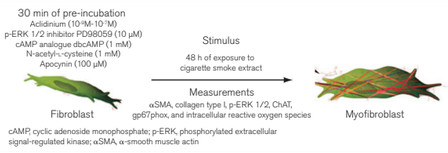
Introduction

- Airway remodeling is a pathologic feature observed in the lungs of patients with chronic obstructive pulmonary disease (COPD).
- The process that contributes to airway remodeling involves the activation of lung fibroblasts. This promotes a more contractile, proliferative, and secretory-active myofibroblast phenotype, characterized by α-smooth muscle actin (αSMA) and collagen type-I expression in the cells.
- The main risk factor for COPD is cigarette smoke (CS), which has recently been shown to promote lung fibroblast proliferation and airway remodeling by means of non-cholinergic system activation.[1]
- Aclidinium bromide is a novel, long-acting muscarinic antagonist in Phase III clinical development for the treatment of COPD. This study explores the effects of aclidinium on human lung fibroblast activation following CS exposure *in vitro*.

Methods

- αSMA and collagen type-I expression were measured by real-time RT-PCR and western blot (Figure 1).

Figure 1. Experimental procedures



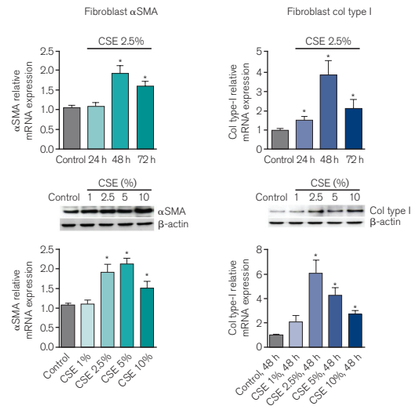
cAMP, cyclic adenoside monophosphate; p-ERK, phosphorylated extracellular signal-regulated kinase; αSMA, α-smooth muscle actin

- ERK 1/2 phosphorylation was measured by western blot.
- Intracellular reactive oxygen species (ROS) were measured by DCFDA fluorescence dye.
- Protein expression from the NADPH complex gp67phox and choline acetyltransferase (ChAT) were measured by western blot.

Results

- Exposure to cigarette smoke extract (CSE) induced a concentration- and time-dependent increase in the mRNA and protein levels of αSMA and collagen type I by 2- and 6-fold, respectively, after 48 hours of CSE 2.5% exposure (Figure 2).

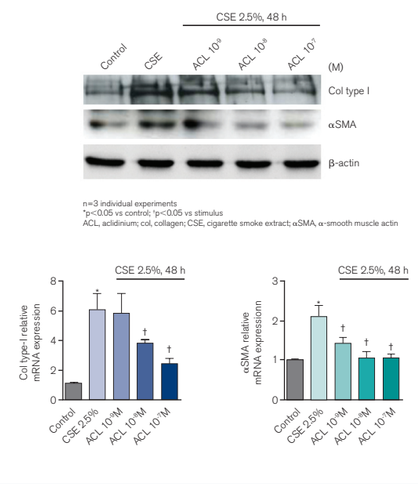
Figure 2. CSE induces αSMA and collagen type-I expression



n=4 individual experiments
*p<0.05 vs control
col, collagen; CSE, cigarette smoke extract; αSMA, α-smooth muscle actin

- Aclidinium dose-dependently attenuated the αSMA and collagen type-I expression induced by CSE 2.5%, resulting in complete suppression at 10^{-7}M (Figure 3).

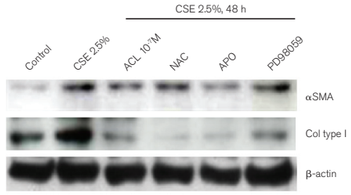
Figure 3. Aclidinium reduces CSE-induced αSMA and collagen type-I expression



n=3 individual experiments
*p<0.05 vs stimulus
ACL, aclidinium; col, collagen; CSE, cigarette smoke extract; αSMA, α-smooth muscle actin

- N-acetyl-L-cysteine (NAC) and apocyanin (both antioxidants), and PD98059 (inhibitor of pERK1/2), also prevented the CSE-induced αSMA and collagen type-I expression (Figure 4).

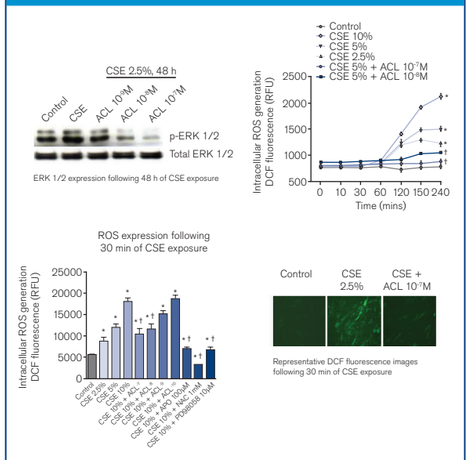
Figure 4. CSE-induced αSMA and collagen type-I expression are prevented by NAC, apocyanin, and PD98059



ACL, aclidinium; col, collagen; APO, apocyanin; CSE, cigarette smoke extract; NAC, N-acetyl-L-cysteine; αSMA, α-smooth muscle actin

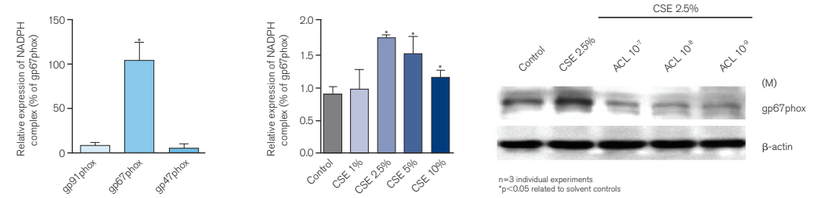
- Aclidinium attenuates CSE-induced phospho-ERK 1/2 and intracellular ROS (Figure 5):
  - Phospho-ERK 1/2 protein synthesis was increased by CSE 2.5%, which was attenuated by aclidinium in a dose-dependent manner
  - CSE promoted an increase of intracellular ROS. This reached its highest concentration after 4 hours
  - ROS generated by CSE was attenuated by aclidinium 10^{-7}M to 50% of control, and by PD98059 to 20% of control
  - Both NAC and apocynin completely suppressed ROS induced by CSE.

Figure 5. CSE-induced phospho-ERK 1/2 and intracellular ROS are attenuated by aclidinium



The fluorescence was generated *in vivo* by the change of non-fluorescence DCFDA to fluorescence DCF induced by intracellular ROS
ACL, aclidinium; CSE, cigarette smoke extract; NAC, N-acetyl-L-cysteine; ROS, reactive oxygen species

Figure 6. CSE-increased NADPH oxidase expression is suppressed by aclidinium

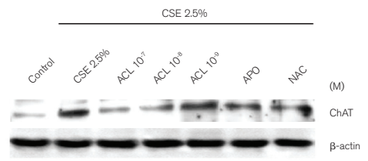


ACL, aclidinium; CSE, cigarette smoke extract

n=3 individual experiments
*p<0.05 related to solvent controls

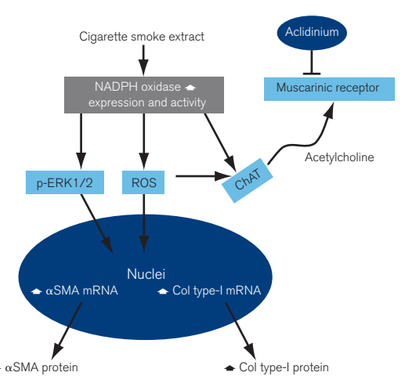
- CSE increased gp67phox expression by 1.75-fold. This was completely suppressed by aclidinium 10^{-7}M (Figure 6).
- CSE 2.5% induced ChAT upregulation, which suggests an autocrine acetylcholine regulation in response to CSE (Figure 7).

Figure 7. CSE-increased ChAT expression is attenuated by aclidinium



n=3 individual experiments
ACL, aclidinium; APO, apocyanin; CSE, cigarette smoke extract; NAC, N-acetyl-L-cysteine

Figure 8. Aclidinium attenuates CSE-induced lung fibroblast activation



ChAT, choline acetyltransferase; col, collagen; p-ERK, phosphorylated extracellular signal-regulated kinase; ROS, reactive oxygen species; αSMA, α-smooth muscle actin

Conclusions

- CSE increases myofibroblast markers αSMA and collagen type I in human lung fibroblast.
- Aclidinium attenuates the CSE-induced αSMA and collagen type-I protein expression in a dose-dependent manner.
- CSE-induced αSMA and collagen type I is mediated by intracellular ROS and ERK1/2 phosphorylation.
- CSE-induced ROS generation is attenuated by aclidinium.
- CSE increases ChAT expression, which suggests an autocrine acetylcholine regulation in response to CSE.
- Aclidinium attenuates CSE-induced lung fibroblast activation (Figure 8).

Reference

1. Profita M, Bonanno A, Siena L, et al. Smoke, choline acetyltransferase, muscarinic receptors, and fibroblast proliferation in chronic obstructive pulmonary disease. *J Pharmacol Exp Ther* 2009;329:753-763.

Acknowledgements

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