Introduction

• Acclidinium 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 acclidinium.1,2 Additionality, acclidinium bromide 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 acclidinium 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 acclidinium 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 acclidinium 200 µg and 400 µg.

• Patients (N=561) were randomized (1:1:1) to acclidinium bromide (200 µg or 400 µg BID) or placebo (Figure 1), administered via the Genuair® inhaler.

Results

Baseline Demographics

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

• Forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) ratio >70%.

• FEV1 >30% and <80% of predicted.

• Current or ex-smokers with a smoking history >20 pack-years.

• History or current diagnosis of asthma.

• Respiratory infection or COPD exacerbation within 6 weeks (3 months if resulted in hospitalization) prior to screening.

• Clinically relevant cardiovascular conditions or respiratory conditions other than COPD and abnormalities in laboratory values or electrocardiograms (ECG).

• Inclusion Criteria

Exclusion Criteria

• FEV1 <50% predicted (at screening visit).

• COPD exacerbation in the 6 weeks prior to screening (in the previous 12 months).

• Any condition considered by the investigator to cause an unacceptable risk to the patient.

• Patients with clinical, laboratory, or endoscopic evidence of active uncontrolled gastro-oesophageal reflux disease (GERD).

• Treatment-emergent AEs (TEAEs)

• The percentage of patients who reported a TEAE was lower in the acclidinium 400 µg group (44.7%) compared with the 200 µg group and the placebo group (50.9% and 50.3%, respectively).

• COPD exacerbation was the only TEAE reported by at least 5% of patients; the incidence of COPD exacerbation was lower in the acclidinium groups vs placebo (Table 2).

• The incidence of COPD exacerbations was lower with the higher dose of acclidinium (7.4%) compared with acclidinium 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 acclidinium group compared with the placebo group were arthralgia, diarrhea, oropharyngeal pain, headache, nasopharyngitis, back pain, and dizziness.

Study Endpoints

• Safety was assessed by 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.

Table 1. Demographic and baseline characteristics (safety population)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo</th>
<th>Acclidinium 200 µg</th>
<th>Acclidinium 400 µg</th>
<th>Total N=561</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), years</td>
<td>65.1 (5.0)</td>
<td>65.7 (5.1)</td>
<td>66.0 (5.3)</td>
<td>65.4 (5.4)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>119 (64)</td>
<td>119 (65)</td>
<td>119 (65)</td>
<td>119 (64)</td>
</tr>
<tr>
<td>Current smoker, n (%)</td>
<td>87 (46)</td>
<td>83 (45)</td>
<td>86 (45)</td>
<td>86 (46)</td>
</tr>
<tr>
<td>BMI, mean (SD), kg/m²</td>
<td>27.5 (5.3)</td>
<td>28.7 (5.7)</td>
<td>28.0 (5.5)</td>
<td>28.0 (5.6)</td>
</tr>
<tr>
<td>FEV1, mean (SD), L</td>
<td>2.2 (0.4)</td>
<td>2.2 (0.4)</td>
<td>2.2 (0.4)</td>
<td>2.2 (0.4)</td>
</tr>
<tr>
<td>Post-bronchodilator</td>
<td>527 (95)</td>
<td>527 (95)</td>
<td>527 (95)</td>
<td>527 (95)</td>
</tr>
<tr>
<td>Asthma, n (%)</td>
<td>28 (18)</td>
<td>29 (18)</td>
<td>27 (17)</td>
<td>28 (18)</td>
</tr>
<tr>
<td>COPD exacerbation</td>
<td>1.9 (0.5)</td>
<td>1.8 (0.5)</td>
<td>1.8 (0.5)</td>
<td>1.9 (0.5)</td>
</tr>
<tr>
<td>Polyps, n (%)</td>
<td>12 (7)</td>
<td>12 (7)</td>
<td>13 (8)</td>
<td>12 (7)</td>
</tr>
<tr>
<td>Treatment-related</td>
<td>0.4 (0.2)</td>
<td>0.3 (0.1)</td>
<td>0.3 (0.1)</td>
<td>0.4 (0.2)</td>
</tr>
</tbody>
</table>

Table 2. Number (%) of patients with adverse events reported by 25% of patients in the acclidinium treatment groups (safety population)

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Placebo</th>
<th>Acclidinium 200 µg</th>
<th>Acclidinium 400 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dyspnea</td>
<td>26 (14)</td>
<td>31 (16)</td>
<td>30 (16)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>11 (6)</td>
<td>10 (6)</td>
<td>8 (4)</td>
</tr>
<tr>
<td>Cough</td>
<td>12 (7)</td>
<td>11 (6)</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>6 (3)</td>
<td>7 (4)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Oropharyngeal pain</td>
<td>5 (3)</td>
<td>6 (3)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>6 (3)</td>
<td>7 (4)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Headache</td>
<td>7 (4)</td>
<td>6 (3)</td>
<td>7 (4)</td>
</tr>
<tr>
<td>Nasopharyngitis</td>
<td>2 (1)</td>
<td>2 (1)</td>
<td>1 (0)</td>
</tr>
<tr>
<td>Back pain</td>
<td>11 (6)</td>
<td>9 (5)</td>
<td>8 (4)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>21 (12)</td>
<td>22 (12)</td>
<td>23 (13)</td>
</tr>
</tbody>
</table>

• The incidence of on-treatment serious AEs (SAEs) was low in all groups (0.2% placebo, 0.4% acclidinium 200 µg, 0.2% acclidinium 400 µg).

• The most frequently reported SAE was exacerbation of COPD: 1 patient in the placebo group, 1 patient in the acclidinium 200 µg group, and 3 patients in the acclidinium 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).

Conclusions

• In this study, twice-daily treatment with acclidinium 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 acclidinium bromide administered twice daily.

References


Acknowledgements

The authors gratefully acknowledge Forest Laboratories, Inc., New York, USA and Almirall SA, Barcelona, Spain. Funding for post hoc development was provided by Forest Laboratories, Inc. Prescott Medical Communications Group, Chicago USA.

*Genuair® is a registered trademark of Almirall SA.

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

Kenneth Lasseter1, Stacy Dilzer2, Josep Maria Jansats, Esther Garcia Gil, Cynthia Caracat, Stephan Ortiz3 • Clinical Pharmacology of Miami, Miami, USA; 2Almirall SA, Barcelona, Spain; 3Forest Research Institute, Jersey City, USA

Introduction

• Aclidinium bromide is a long-acting, muscarinic antagonist currently in Phase II development for the treatment of COPD. Aclidinium bromide is currently under investigation for systemic side effects.
• Single plasma concentration-time profiles at 8000 ng/mL in humans doses of 400 µg have been well tolerated in healthy subjects.1,2 Clinical trials in COPD patients with concomitant medication are underway to evaluate the long-term safety and tolerability of aclidinium bromide.
• 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 5-day, single-blind, placebo-controlled, clinical trial evaluating multiple daily doses of aclidinium bromide in healthy male and female subjects. The study was conducted in four cohorts of 10 subjects each (6 males and 4 females) in a 2:2:2:2 dose-escalation design.
• Subjects were admitted into the clinical day before Day 1 (D0) and remained until the last PK sample was collected on Day 9.

Results

Baseline Demographics

• 30 healthy male and female subjects (13/17) were randomized to 1:1:1:1 cohorts of twice-daily aclidinium, 400 µg and placebo (n=7), 800 µg and placebo (n=7) and placebo (n=6). Cohorts were stratified by gender, smoking status and body mass index (BMI).

Safety

• All 30 enrolled subjects completed the study. Baseline demographics were comparable across treatment groups (Table 1). No serious adverse events (AEs) occurred.
• All 30 enrolled subjects completed the study. Baseline demographics were comparable across treatment groups (Table 1). No serious adverse events (AEs) occurred.

Pharmacokinetic Analyses

• Aclidinium Bromide
• On Day 1, mean Cmax following the morning dose were 102.7, 194.2, and 306.1 pg/mL for the 200, 400, and 800 µg aclidinium bromide doses administered, respectively (Table 2). The PK parameters were estimated using a non-linear mixed-effects model for each subject and treatment.

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-time profiles will occur after repeated administration at the same dose and frequency.
• Exposure for all compounds increased with increasing dose but in 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.

References


Acknowledgements

This study was supported by Forest Laboratories, Inc., New York, USA, and Almirall SA, Barcelona, Spain. Funding for study development was provided by Forest Laboratories, Inc., to Primary Medical Communications, Group USA.


Table 1. Demographic characteristics (safety population)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Aclidinium 200 µg</th>
<th>Aclidinium 400 µg</th>
<th>Aclidinium 800 µg</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.8 (3.3)</td>
<td>38.5 (3.3)</td>
<td>39.1 (2.0)</td>
<td>39.4 (3.3)</td>
</tr>
<tr>
<td>Sex</td>
<td>12 (63)</td>
<td>12 (63)</td>
<td>12 (63)</td>
<td>12 (63)</td>
</tr>
<tr>
<td>Smoker</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Mean BMI (kg/m²)</td>
<td>25.5 (2.3)</td>
<td>25.4 (2.1)</td>
<td>25.6 (2.3)</td>
<td>25.6 (2.3)</td>
</tr>
<tr>
<td>CKD</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>17 (85)</td>
<td>17 (85)</td>
<td>17 (85)</td>
<td>17 (85)</td>
</tr>
<tr>
<td>Race</td>
<td>17 (85)</td>
<td>17 (85)</td>
<td>17 (85)</td>
<td>17 (85)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Event</th>
<th>Aclidinium 200 µg</th>
<th>Aclidinium 400 µg</th>
<th>Aclidinium 800 µg</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Rhinitis</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td>1 (5)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Allergies</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sinusitis</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Dermatologic</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Ophthalmologic</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Neurologic</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Vascular</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Figure 2. Mean aclidinium plasma concentration-time profiles at day 7 and administration and elimination of aclidinium 200 µg, 400 µg, and 800 µg.

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.
**Introduction**

- The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines emphasize that the treatment of stable COPD should be managing symptoms and improving quality of life.
- COPD symptoms often impact patient's quality of life and function, and the disease is associated with increased mortality and healthcare costs.
- The 3rd St. George's Respiratory Questionnaire (SGRQ) is a valid, reliable, and responsive measure of health-related quality of life (HRQoL) that is easy to administer.
- The Transition Dyspnea Index (TDI) is a simple measure of dyspnea.

**Methods**

- **Subjects**: A total of 561 patients with moderate-to-severe COPD were randomized to receive aclidinium 200 µg twice-daily, aclidinium 400 µg twice-daily, or placebo.
- **Study Design**: A 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).
- **primary efficacy and safety endpoints**:
  - **SGRQ Total Score**: A clinically meaningful improvement is ≥4 units from baseline to Week 12.
  - **TDI focal score**: A clinically important improvement is ≥1 unit from baseline.
  - **Number needed to treat (NNT)**: The number of patients who need to be treated to achieve a clinically meaningful improvement in SGRQ total score is 3.2 in the 400 µg group compared with placebo.

**Results**

- **SGRQ Total Score**:
  - Patients in the aclidinium 400 µg group showed a statistically significantly greater improvement in SGRQ total score compared with placebo at Week 12.
  - Both doses of aclidinium resulted in a statistically significantly greater improvement in change from baseline to Week 12 compared with placebo, with adjusted mean differences vs placebo of 6.8 and 6.5 (p<0.001 for both).

- **TDI focal score**:
  - Both doses of aclidinium resulted in a statistically significantly greater improvement in change from baseline to Week 12, with adjusted mean differences vs placebo of 1.4 and 1.0 (p<0.05 for both).

**Conclusions**

- **Treatment with twice-daily aclidinium resulted in improvements in patients’ quality of life and dyspnea as measured by SGRQ and TDI.**
- **Both doses of aclidinium significantly improved patients’ quality of life and dyspnea as measured by SGRQ and TDI.**
- **A clinically significant percentage of patients achieved clinically meaningful differences in both SGRQ total score and TDI focal score during this 12-week study.**

**References**

1. Artur F. Gelb,1 James F. Donohue, 2 Anthony D’Urzo, 3 Ludmyla Rekeda, 4 Diana Jarreta, 5 Jordan Lateiner 4
2. 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.
3. The Transition Dyspnea Index (TDI) is an independent, clinician-reported instrument that evaluates breathlessness that can have a significant impact on quality of life.
4. 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.
5. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) guidelines emphasize that the treatment of stable COPD should be managing symptoms and improving quality of life.

**Acknowledgements**

This study was supported by Forest Laboratories, Inc., New York, USA, and Almirall SA, Barcelona, Spain. Funding for the poster development was provided by Forest Laboratories, Inc., to Prescott Medical Communications Group, Chicago, USA.

**Geiser** is a registered trademark of Almirall SA.
Acldinium Bromide In Patients With Chronic Obstructive Pulmonary Disease: Efficacy And Safety Results From ATTAIN

Paul W Jones,1 Alvar Agustí,2 Eric Bateman,3 David Singh,4 Rosa Lamarca,5 Gonzalo de Miguel,6 Cynthia Caracta,7 Esther Garcia Gil8
1St George’s, University of London, London, UK; 2Thorax Institute, Hospital Clinic, Barcelona, and CIBER Enfermedades Respiratorias and Fundacíó Cautab-Cimerà, Spain; 3University of Cape Town, Cape Town, South Africa; 4Medical Evaluation Unit Ltd, Manchester, UK; 5Amirall S.A., Barcelona, Spain; 6Forest Research Institute, New Jersey, USA

Secondary Endpoint

Change from baseline in trough FEV1, at 24 weeks.

Results

Study Population

A total of 828 patients were randomized to aclidinium 200 µg (n=270), 400 µg (n=270) and placebo (n=288). 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)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Placebo (n=288)</th>
<th>Aclidinium 200 µg (n=270)</th>
<th>Aclidinium 400 µg (n=270)</th>
<th>Total (n=836)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) Median (IQR)</td>
<td>62.0 (59.0-65.0)</td>
<td>62.0 (60.0-64.0)</td>
<td>62.0 (60.0-64.0)</td>
<td>62.0 (60.0-64.0)</td>
</tr>
<tr>
<td>Gender (male) %</td>
<td>60.5</td>
<td>58.5</td>
<td>56.5</td>
<td>59.0</td>
</tr>
<tr>
<td>Moderate COPD %</td>
<td>65.9</td>
<td>66.7</td>
<td>66.7</td>
<td>66.3</td>
</tr>
<tr>
<td>Severe COPD %</td>
<td>34.1</td>
<td>33.5</td>
<td>33.3</td>
<td>33.7</td>
</tr>
<tr>
<td>Current smoker %</td>
<td>50.5</td>
<td>50.0</td>
<td>50.6</td>
<td>50.1</td>
</tr>
<tr>
<td>Smoking history (packs/yr)</td>
<td>30 (15-45)</td>
<td>20 (10-40)</td>
<td>20 (10-40)</td>
<td>20 (10-40)</td>
</tr>
</tbody>
</table>

Trough FEV1

- At Week 24, aclidinium 200 µg and 400 µg significantly improved trough FEV1, from baseline compared with placebo (by 9.9 mL and 12.9 mL, respectively; p<0.001 for both; Figure 1).

- The improvement in trough FEV1, provided by aclidinium (200 and 400 µg) was statistically superior to placebo at all time points from Week 1 to 24 (p<0.001). Accepting a 2-unit difference as clinically meaningful, the improvements in trough FEV1 from placebo were 185 mL and 209 mL, respectively (p<0.001 for both; Figure 1).

- At Week 24, the difference in the mean change from baseline in trough FEV1 decreased from 38 mL at baseline to -3.6 units for aclidinium 200 µg (p<0.001) and -4.3 units for aclidinium 400 µg (p<0.001).

Safety

- The most commonly reported AEs across all treatment groups were COPD exacerbation, headache, nasopharyngitis, diarrhea, and dizziness (Table 2). The incidence of anticholinergic AEs was low. All were mild to moderate in severity and no serious AEs were observed.

- The incidence of anticholinergic AEs with both aclidinium doses was low (<2%). The incidence was lower than placebo (3.1%).

- The number of SAEs and number of patients with SAEs were similar across the three treatment groups (43%, n=119; 5.5%, n=18, for aclidinium 200 µg and 400 µg, 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 µg 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.

Conclusions

- Aclidinium 200 µg and 400 µg BID in patients with moderate to severe COPD provides statistically significant improvements in airflow limitation (trough FEV1 and peak FEV1) compared with placebo.

- Statistically significant improvements in symptoms and health status were observed in patients treated with aclidinium 200 µg and 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.

References


Acknowledgements

The study was supported by Amirall S.A., Barcelona, Spain. “Germán” is a registered trademark of Amirall S.A.

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

Edward Kerwin1, Stephen Rennard1, Arthur Feld2, Ludmila Rekeda3, Esther Garcia Gill1, Cynthia Caracta4,5
1Clinical Research Institute, Medford, USA; 2University of Nebraska Medical Center, Omaha, USA; 3Southern California Clinical Trials, Lakewood, USA; 4Forest Research Institute, Medford, MA, USA; 5Aclidinium SA, Barcelona, Spain

Introduction
Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of death in the United States and is projected to be the third leading cause of death worldwide by 2020.1 Nighttime symptoms are common in patients with COPD, may result in sleep disturbance and affect quality of life.2-4 Patients with COPD are at increased risk of daytime and nighttime symptoms, including increased symptoms and use of rescue medication during the night.5-7 Adenoids are hypothesized to be involved in the development of sleep disturbances in COPD patients.8

Methods
Study Design
This was a 24-week, multinational, randomized, double-blind, placebo-controlled, parallel-group trial evaluating twice-daily aclidinium 200 μg or 400 μg in addition to standard COPD care. Similar previous analyses have been performed.2-4

Study Population
Inclusion criteria were moderate to severe stable COPD with a history of ≥30% and <80% of predicted FEV1. Exclusion criteria were: age ≥25 years; current or ex-smokers with a smoking history of ≥10 pack-years; diagnosis of moderate to severe stable COPD; and rescue medication use over the last 12 hours and 24 hours every night. Inhaled corticosteroids (CS) at any dose and oral or parenteral CS at doses not exceeding 10 mg/day or ≥300 mg/day were allowed.

Statistical Analysis
Baseline characteristics were compared using the Wilcoxon rank-sum test for continuous variables and the χ² test for categorical variables. Response analyses were performed using the ITT population. The mean (SD) difference in change from baseline to Week 12 was assessed using a repeated-measures ANCOVA model with treatment as factor and the corresponding baseline as covariate.

Results
Baseline Demographics
A total of 561 patients were randomized to aclidinium 200 μg or 400 μg and completed the study (ITT population). Baseline demographic and clinical characteristics were comparable across all treatment groups.

Time it took to fall asleep, minutes, mean (SD)

<table>
<thead>
<tr>
<th>Placebo</th>
<th>Aclidinium 200 μg</th>
<th>Aclidinium 400 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.8 (11.7)</td>
<td>23.2 (11.8)</td>
<td>21.8 (11.2)</td>
</tr>
</tbody>
</table>

Cough, sputum production, wheezing when breathing, and rescue medication use.

Baseline Characteristics
Rescue Medication Use
The amount of symptom produced was 0.04 (0.05) for placebo, -0.23 (0.06) for aclidinium 200 μg, and -0.24 (0.06) for aclidinium 400 μg (placebo vs. aclidinium 200 μg, p<0.05). The adjusted mean difference (95% CI) from baseline to total rescue medication use at Week 12 was -0.04 (-0.10, -0.00). Aclidinium 200 μg and 400 μg had similar proportion (p=0.74) of patients for study end (ITT population).

Aclidinium 400 μg significantly improved sleep with respect to time in bed (%) at Week 12 and was greater than placebo at 12 weeks (10.4%, 11.8%, and 12.9% vs. 7.5% in placebo). Figure 1 shows the study flow chart.

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 frequency 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.

References:

Acknowledgments
This study was supported by Forest Laboratories, Inc., New York, USA, and Almirall SA, Barcelona, Spain. Funding for the study was provided by Forest Laboratories, Inc., USA, to Pharmaceutical Communications Group, Chicago, USA. *G另一半是* is a registered trademark of Almirall SA, Barcelona, Spain.

**Introduction**

• Anticholinergic treatments for chronic obstructive pulmonary disease (COPD) exert their therapeutic effect by inhibiting pulmonary M3 receptors, which mediate bronchoconstriction and mucus hypersecretion. 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 M3 receptors is known to induce tachycardia.

• Both of the inhaled muscarinic antagonists currently available for the treatment of COPD, the long-acting ipratropium and the short-acting atropine, are associated with systemic anticholinergic side effects including tachycardia.

• 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 M3 receptors, but a lower potency and a shorter duration of action at M2 receptors.

• This study investigated the in vitro effects of aclidinium at M3 and M2 receptors in human bronchial and left-atrial tissue, respectively. Tiotropium and ipratropium were used as comparators.

**Methods**

**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 (IC50) was calculated.

• Antagonist potency was determined as -log IC50 (pIC50) 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 (t0) was defined as the time taken from antagonist addition to achieve 50% inhibition of tone.

• Offset of action (t½) 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 M3 Receptors In Isolated Human Atrial Strips**

• Pre-treatment of human atrial strips with aclidinium, tiotropium, or ipratropium (10 nM) was added to the Krebs solution at 37ºC. Spontaneous tone, induced by stimulation of the atrial muscarinic receptors in isolated human atrial strips.

• Antagonist potency was determined as -log IC50 (pIC50) values.

• Differences between onset and offset values were determined by analysis of variance.

**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.

• The time to achieve 50% recovery of the maximum carbachol-induced relaxation (%)

• The offset time for aclidinium was significantly longer than that of tiotropium (p<0.05), whereas no recovery of tone was observed after washout of tiotropium within the duration of the study (Table 2).

• 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 M3 receptors is known to induce tachycardia.

**Results**

**M3-Mediated Smooth Muscle Relaxant Effects In Isolated Human Bronchi And Left Atria**

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

• Aclidinium demonstrates a shorter duration of action than tiotropium at M3 receptors in isolated human atria. These data are consistent with previous observations in guinea pig models and suggest that aclidinium may have a lower potential for cardiovascular side effects.

**Duration Of Action At M3 Receptors In Isolated Human Atria**

• Aclidinium inhibited the M3-mediated brady-cardiac effect of carbachol with a longer offset time than ipratropium and a shorter offset time than tiotropium (Table 3; Figure 2).

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

<table>
<thead>
<tr>
<th></th>
<th>pIC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aclidinium</td>
<td>9.3 ± 0.0</td>
</tr>
<tr>
<td>Tiotropium</td>
<td>9.6 ± 0.1</td>
</tr>
<tr>
<td>Ipratropium</td>
<td>9.5 ± 0.1</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th>IC50 (nM)</th>
<th>Maximal Inhibition of contraction (%)</th>
<th>Onset time (t0, min)</th>
<th>Offset time (t½, min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aclidinium</td>
<td>600</td>
<td>63.6 ± 2.5</td>
<td>80.0 ± 6.0</td>
<td>30.0 ± 7.0</td>
</tr>
<tr>
<td>Tiotropium</td>
<td>100</td>
<td>73.0 ± 2.5</td>
<td>100.0 ± 3.0</td>
<td>30.0 ± 5.0</td>
</tr>
<tr>
<td>Ipratropium</td>
<td>500</td>
<td>69.0 ± 1.5</td>
<td>100.0 ± 2.0</td>
<td>20.0 ± 3.0</td>
</tr>
</tbody>
</table>

**Table 3. Duration of action (offset) for aclidinium, tiotropium, and ipratropium at M3 receptors in electrically stimulated human left-atrial strips treated with carbachol**

<table>
<thead>
<tr>
<th></th>
<th>IC50 (nM)</th>
<th>Inhibition of maximum carbachol-induced relaxation (%)</th>
<th>Offset time (t½, min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aclidinium</td>
<td>3/5</td>
<td>68.4 ± 5.6</td>
<td>110.0 ± 5.7</td>
</tr>
<tr>
<td>Tiotropium</td>
<td>3/5</td>
<td>72.1 ± 2.3</td>
<td>150.0 ± 10.0</td>
</tr>
<tr>
<td>Ipratropium</td>
<td>3/5</td>
<td>69.0 ± 1.5</td>
<td>16.6 ± 0.3</td>
</tr>
</tbody>
</table>

**References**


**Conclusions**

• Aclidinium has similar potency to tiotropium at M3 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 M3 receptors in isolated human atria. These data are consistent with previous observations in guinea pig models and suggest that aclidinium may have a lower potential for cardiovascular side effects.

**Acknowledgements**

This study was supported by Almirall S.A., Barcelona, Spain.

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

• 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).

• 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).

• Aclidinium dose-dependently attenuated the αSMA and collagen type-I expression induced by carbachol, resulting in complete suppression at 10⁻⁷M. Furthermore, aclidinium (10⁻⁸M) reduced carbachol-induced myofibillar 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 10⁻⁷M (added 10 min before carbachol).

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

• Carbachol increased lung fibroblast proliferation by 2-fold which was prevented by aclidinium 10⁻⁸M (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 10⁻⁸M, 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

Reference


Acknowledgements

This study was supported by Almirall S.A., Barcelona, Spain.
Effects of Aclidinium Bromide on Respiratory Function In Guinea Pigs Exposed to Cigarette Smoke For 6 Months

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

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
Animals
• Male Hartley guinea pigs (n=46, ~415 g) were housed under a 12-h light/dark cycle and randomly divided into six 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.

Acidimun Administration
• Animals were nebulized with vehicle (water) or aclidinium in a gas mixture containing 5% CO2, 21% O2, and 74% N2 (ultrasonic Devilbiss Ultraneb 3000 nebulizer, flow of 3 L/min), 1 hour prior to CS exposure (Figures 1 and 2).

Results
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 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.

• Aclidinium (30 μg/mL) showed a trend to reduce the occurrence of cough and delay the occurrence of bronchial impairment indicators induced by CS exposure (Figures 4 and 5).

• 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).

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Vehicle sham</th>
<th>CS sham</th>
<th>CS sham</th>
<th>CS sham</th>
<th>Ac10 sham</th>
<th>CS sham</th>
<th>Ac10 sham</th>
<th>Ac30 sham</th>
<th>CS sham</th>
<th>Ac30 sham</th>
<th>CS sham</th>
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<tbody>
<tr>
<td>Week 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(15540-18553)*</td>
<td></td>
<td>(16029-20638)</td>
<td>(2666-3006)*</td>
<td></td>
<td>(22031-26398)*</td>
<td>(1609-2492)*</td>
</tr>
<tr>
<td>Week 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(14255-15464)</td>
<td></td>
<td>(15482-18111)</td>
<td>(2053-2804)*</td>
<td></td>
<td>(21018-25651)*</td>
<td>(1548-18558)*</td>
</tr>
<tr>
<td>Week 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(15540-18553)*</td>
<td></td>
<td>(16029-20638)</td>
<td>(2666-3006)*</td>
<td></td>
<td>(22031-26398)*</td>
<td>(1609-2492)*</td>
</tr>
<tr>
<td>Week 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(14255-15464)</td>
<td></td>
<td>(15482-18111)</td>
<td>(2053-2804)*</td>
<td></td>
<td>(21018-25651)*</td>
<td>(1548-18558)*</td>
</tr>
<tr>
<td>Week 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(15540-18553)*</td>
<td></td>
<td>(16029-20638)</td>
<td>(2666-3006)*</td>
<td></td>
<td>(22031-26398)*</td>
<td>(1609-2492)*</td>
</tr>
<tr>
<td>Week 6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(14255-15464)</td>
<td></td>
<td>(15482-18111)</td>
<td>(2053-2804)*</td>
<td></td>
<td>(21018-25651)*</td>
<td>(1548-18558)*</td>
</tr>
</tbody>
</table>

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 Ciberes: capacité investigadora en enfermedades respiratorias (CIBERES), Instituto de Salud Carlos III, Spain.

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

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1Department of Pulmonary Medicine, Hospital Clinic-IDIBAPS, Barcelona, Spain; 2Almirall, R&D Centre, Barcelona, Spain; 3CIBER 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.
- CS, cigarette smoke; ILP, internal luminal perimeter

**Objective**

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

**Methods**

**Animal Groups**

- Male Han; 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=6)

**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.

**Results**

**Airway Remodeling**

- CS exposure caused enlargement of airway wall layers, particularly in smaller airways (Table 1; Figure 3).
- *p < 0.05 compared with sham-exposed under the same treatment; #p < 0.05 compared with sham-exposed airway wall thickness (ILP)

**Morphological Studies**

- Losses were removed and lobes were inflated and fixed in 10% buffered formaldehyde.
- Airway Remodeling:
  - Thickness of adventitial and mucosal layers was not significantly reduced with aclidinium (Table 1).
- Inflammatory Cells:
  - Treatment with aclidinium at dosages of 10 and 30 μg/mL significantly reduced the muscularization of small airways induced by CS exposure.

**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 administration (Figure 4).

**Empysema And Goblet Cell Metaplasia**

- Goblet cell metaplasia and emphysema. Airway blue staining (A, B) and hematoxylin-eosin (C, D).

**Conclusions**

- Guinepigps 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**

- This study was supported by Almirall S.A., Barcelona, Spain, and Consorcios Epidemiológicos Facilitadores en Investigación Tecnica (CEFIT), Spain.

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

<table>
<thead>
<tr>
<th>Airway size</th>
<th>Vehicle</th>
<th>Ac10 μg/mL</th>
<th>Ac30 μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total wall thickness</td>
<td>22.6 ± 1.4</td>
<td>29.7 ± 6.4*</td>
<td>30.3 ± 3.6*</td>
</tr>
<tr>
<td>Mucosal thickness</td>
<td>15.0 ± 1.0</td>
<td>18.5 ± 1.5*</td>
<td>18.8 ± 1.0*</td>
</tr>
<tr>
<td>Adipose thickness</td>
<td>11.2 ± 0.5</td>
<td>11.0 ± 0.5</td>
<td>11.4 ± 0.5</td>
</tr>
</tbody>
</table>

* Results are stratified into large (ILP) and small (ILP) airways.

**Table 2. Goblet cell metaplasia and emphysema**

<table>
<thead>
<tr>
<th>Airway size</th>
<th>Vehicle</th>
<th>Ac10 μg/mL</th>
<th>Ac30 μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Goblet cells (μm)</td>
<td>6.4 ± 0.6</td>
<td>5.6 ± 0.4</td>
<td>6.0 ± 0.5*</td>
</tr>
<tr>
<td>Emphysema (μm)</td>
<td>36.8 ± 1.0</td>
<td>41.8 ± 3.5*</td>
<td>36.9 ± 8.0</td>
</tr>
</tbody>
</table>

* Results are stratified into large (ILP) and small (ILP) airways.

---

**Figure 1. Nebulization protocol**

**Figure 2. Airway remodeling**

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

**Figure 4. Inflammatory cell counts in alveolar septa**

**Figure 5. Goblet cell metaplasia and emphysema.**

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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 a non-cholinergic system activation. 1
- Acridinium bromide is a novel, long-acting muscarinic antagonist in Phase III clinical development for the treatment of COPD. This study explores the effects of acridinium 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).
- 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 oxidase 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 5-fold, respectively, after 48 hours of CSE 2.5% exposure (Figure 2).
- N-acetyl-L-cysteine (NAC) and apocynin (both antioxidants), and PD98059 (inhibitor of pERK1/2), also prevented the CSE-induced αSMA and collagen type-I expression (Figure 4).

**Conclusions**

- CSE increases myofibroblast markers αSMA and collagen type-I in human lung fibroblasts.
- Acridinium 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 increases ROS generation is attenuated by acridinium. CSE increases ChAT expression, which suggests an autocrine acetylcholine regulation in response to CSE.
- Acridinium attenuates CSE-induced lung fibroblast activation (Figure 8).

**Reference**


**Acknowledgements**

This study was supported by Almirall S.A., Barcelona, Spain.