Efficacy And Safety Of Aclidinium Bromide 400 μg BID Compared With Placebo And Tiotropium In Patients With Moderate To Severe COPD

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Introduction
• Chronic obstructive pulmonary disease (COPD) is a highly prevalent lung disease characterized by gradual loss of lung function and airflow limitation that is not fully reversible.
• Only one long-acting anticholinergic is currently available for COPD treatment; however, high rates of morbidity and mortality associated with COPD necessitate the investigation of additional therapeutic options.
• Aclidinium bromide is a new, potent, long-acting, muscarinic antagonist being investigated for the maintenance treatment of COPD.
• Clinical trials in patients with COPD have demonstrated sustained bronchodilation and a favorable tolerability profile.

Methods

Study Design
• This was a multicenter, randomized, double-blind, double-dummy, placebo- and active comparator-controlled, 8-period crossover trial.
• Patients (N=30) were randomized (1:1:1) to receive one of three treatments (aclidinium bromide 400 μg BID, tiotropium 18 μg OD, or placebo); each treatment arm consisted of three 16-day treatment periods separated by a 5- to 9-day washout period.
• Patients were evaluated at screening (for inclusion), at baseline following a 5- to 9-day run-in period, on Days 1 and 15 during each treatment period (8 visits total), and at follow-up.

Study Population

Inclusion Criteria
• Male and non-pregnant, non-lactating females aged ≥40 years.
• Post-bronchodilator forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) ratio <70%.
• Post-bronchodilator FEV1 ≥200 and <800 predicted.
• Current or ex-smokers with a smoking history of ≥10 pack-years.

Exclusion Criteria
• History or current diagnosis of asthma.
• Other clinically relevant respiratory or cardiovascular conditions.
• Respiratory infection or COPD exacerbation within 6 weeks (or 3 months if hospitalization was required) prior to screening.
• Clinically relevant abnormalities in laboratory values, ECG, or physical examination.

Study Endpoints

Primary Endpoint
• Change from baseline in normalized FEV1, area under the curve for the 12 hours immediately following morning dose administration (AUC0,12) on Day 15.

Secondary Endpoints
• Change from baseline in normalized FEV1, AUC12-24, and FEV1, AUC0,12 on Days 1 and 15.
• Change from baseline in normalized morning pre-dose and peak FEV1, on Days 1 and 15.
• Change from baseline in FEV1 at each specific time point on Days 1 and 15.

Safety
• Safety assessments included adverse events (AEs), 12-lead ECGs, and blood pressure and laboratory parameters.

Statistical Analysis
• All efficacy analyses were performed using the Intention-to-treat (ITT) population (defined as all randomized patients who took at least one dose of trial medication).
• Safety outcomes were analyzed using the safety population, defined as all randomized patients who took at least one dose of study medication.
• All efficacy analyses were performed using the ANCOVA model for crossover designs. Safety outcomes were summarized with descriptive statistics.

Results

Baseline Demographics
• A total of 30 patients were randomized, and 27 patients completed the study. Baseline demographics and clinical characteristics are presented in Table 1.

Table 1. Demographic and baseline characteristics (safety population)
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mean (SD)</th>
<th>Min–Max (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>64.9 (7.8)</td>
<td>45–85 (27)</td>
</tr>
<tr>
<td>BMI, kg/m2</td>
<td>24.6 (4.1)</td>
<td>19–39 (27)</td>
</tr>
<tr>
<td>BMI, SD (kg/m2)</td>
<td>24.6 (4.1)</td>
<td>19–39 (27)</td>
</tr>
<tr>
<td>Smoking history, pack-years</td>
<td>3.5 (5.0)</td>
<td>0–30 (27)</td>
</tr>
<tr>
<td>FEV1, mean (SD), L</td>
<td>1.37 (0.17)</td>
<td>0.50–2.30 (27)</td>
</tr>
<tr>
<td>FEV1/FVC ratio, %</td>
<td>74.7 (16.1)</td>
<td>30–105 (27)</td>
</tr>
<tr>
<td>FEV1, mean (SD), %</td>
<td>74.7 (16.1)</td>
<td>30–105 (27)</td>
</tr>
<tr>
<td>Bronchodilator reversibility, mean (SD), %</td>
<td>18.2 (18.6)</td>
<td>0–60 (27)</td>
</tr>
</tbody>
</table>

Efficacy
• Normalized FEV1, AUC0,12 at Day 15 was significantly increased in patients with stable moderate to severe COPD treated with aclidinium (0.24 L) and tiotropium (0.26 L) compared with placebo (0.02 L) (p<0.0001 for both, Figure 1A).
• At Day 15, normalized FEV1, AUC0,12 was significantly greater for both aclidinium and tiotropium versus placebo (p<0.001 for both and for aclidinium versus tiotropium (p<0.05), Figure 1A).

Figure 1. Change from baseline in normalized FEV1, AUC12-24, and AUC0,12 on Day 1 and Day 15

Figure 2. Change from baseline in FEV1, on Day 15 (L/S mean ±SE; ITT population)

Conclusions
• Aclidinium 400 μg twice-daily provides bronchodilation over 24 hours that is statistically superior and clinically meaningful compared with placebo.
• In this study, improvements in Day 1 normalized FEV1, AUC0,12, FEV1, AUC12-24 and FEV1, AUC0,12 with aclidinium indicate that optimal bronchodilation is achieved as early as the first day of treatment and is sustained over time.
• Aclidinium treatment resulted in significantly greater improvements than tiotropium in normalized FEV1, AUC0,12 and FEV1, AUC12-24 on Day 1 and in normalized FEV1, AUC0,12 on Day 15.
• Treatment with twice-daily aclidinium was safe and well tolerated.

References

Acknowledgements
This study was supported by Amirall SA, Barcellona, Spain, and Forest Laboratories, Inc., New York, USA.

Figure 1A. Mean (S.E.M.) change in FEV1 vs baseline.

Figure 2. Mean (S.E.M.) change in FEV1 vs placebo.

Figure 2A. Change from baseline in normalized FEV1, AUC12-24, and AUC0,12 on Day 1 and Day 15.

Table 2. Change from baseline in morning FEV1, measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Day 1</th>
<th>Day 15</th>
<th>Day 1</th>
<th>Day 15</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dose FEV1, L</td>
<td>1.37 (0.17)</td>
<td>1.51 (0.19)</td>
<td>0.96 (0.17)</td>
<td>0.99 (0.18)</td>
<td>1.46 (0.21)</td>
</tr>
<tr>
<td>Peak FEV1, L</td>
<td>2.62 (0.35)</td>
<td>3.06 (0.40)</td>
<td>2.09 (0.35)</td>
<td>2.22 (0.38)</td>
<td>2.82 (0.44)</td>
</tr>
</tbody>
</table>

Time after first dose (h)

Aclidinium vs Placebo
Acclidinium Bromide Improves Exercise Endurance, Dyspnea and Inspiratory Capacity in Patients With Moderate to Severe COPD

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Introduction

• Activities of daily living may be compromised in patients with COPD due to diminished exercise capacity. Improved exercise tolerance and dyspnea, and alleviating lung hyperinflation, are important goals in managing COPD, treatment with bronchodilators, including anticholinergics is associated with improvements in these measures.1,2

• Acclidinium bromide is a novel, long-acting muscarinic antagonist currently in Phase III development for the maintenance treatment of COPD.

Objective

• The purpose of this study was to examine the effect of once-daily acclidinium 200 µg on exercise endurance and lung hyperinflation in patients with COPD.

Methods

Study Design

• This was a Phase II, randomized, double-blind, placebo-controlled, multicenter trial.

• Following a 2-week run-in phase, patients were randomized (1:1) to receive once-daily acclidinium 200 µg or placebo for 6 weeks via the Genuair® inhaler, a novel multi-dose dry powder inhaler.

Study Population

Inclusion Criteria

• Male and non-pregnant, non-lactating female patients aged ≥40 years

• Post-bronchodilator forced expiratory volume in 1 second (FEV1)/forced vital capacity (FVC) ratio ≥70%

• FEV1 <80% and 20% predicted

• Functional residual capacity (FRC) ≥100% of predicted

• Current or ex-smokers with a smoking history of ≥10 pack-years

• Baseline dyspnea index (BDI) score ≤7

Exclusion Criteria

• History or current diagnosis of asthma

• Other clinically relevant respiratory or cardiovascular conditions, including laboratory and ECG abnormalities and contradictions to clinical exercise testing

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

• Cycled ≥20 minutes during the constant work-rate exercise tests conducted during the run-in period

Assessments

• Taken at baseline, after the first dose of study medication, and at Week 3 and Week 6:
  - Spirometry and body plethysmography at pre-dose and 2 hours post-dose
  - Constant work-rate cycling exercise (75% peak exercise capacity) for 3 hours post-dose
  - Baseline BDI, and the Transitional Dyspnea Index (TDI) at Week 3 and Week 6

Study Endpoints

Primary Endpoint

• Change in endurance time (ET) the time from increased work rate at 75% Wmax to the point of symptom limitation, from baseline to Week 6

Secondary Endpoint

• Changes in ET from baseline to Day 1 and Week 3

• Changes from baseline in FEV1, IC, FRC, IC/TLC at trough and 2 hours post-dose at all visits

Other Outcomes

• Dyspnea, leg fatigue, and ventilatory responses to exercise; dynamic hyperinflation at rest, best (defined as the minimum ET among the constant work-rate tests at 75% Wmax, at each study visit), and end of exercise (peak)

Safety

• Safety and tolerability were assessed using adverse event (AE) reporting, physical examinations, vital signs, electrocardiograms (ECGs), and clinical laboratory tests.

Efficacy

• Significant improvements in ET were observed in patients treated with acclidinium as early as the first dose (Day 1) and continued to Week 6 (p<0.0005 vs placebo at all visits) (Figure 1).

• Treatment differences in trough FEV1, IC, and IC/TLC were significantly higher for acclidinium over placebo at Week 6 (Figure 2), similar results were observed at 1 Week and Week 3 (Table 1).

• Trough FRC showed a trend towards improvement between acclidinium versus placebo at Week 6 but did not reach statistical significance; similar changes were observed at Week 3.

Conclusions

• Treatment with acclidinium 200 µg once-daily significantly improved exercise endurance time in patients with COPD as early as Day 1; this effect was sustained through study endpoint (Week 6).

• Treatment with acclidinium results in rapid and significant bronchodilation, and reductions in static lung hyperinflation and dyspnea.

• Acclidinium was safe and well tolerated and may be a valuable new option for the treatment of COPD.

References


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

Acknowledgements

This study was supported by Forest Laboratories, Inc., New York, NY, USA and Almirall, SA, Barcelona, Spain.


Table 1. Baseline demographics and clinical characteristics at screening (safety population)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Acclidinium 200 µg (n=98)</th>
<th>Placebo (n=98)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>64 (10)</td>
<td>66 (9)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>52 (53)</td>
<td>52 (53)</td>
</tr>
<tr>
<td>Maca, n (%)</td>
<td>60 (97)</td>
<td>60 (97)</td>
</tr>
<tr>
<td>Cervical, n (%)</td>
<td>63 (97)</td>
<td>63 (97)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.1 (4.3)</td>
<td>26.6 (4.7)</td>
</tr>
<tr>
<td>Current smoker</td>
<td>36 (37)</td>
<td>36 (37)</td>
</tr>
<tr>
<td>Coughing history, pack years</td>
<td>1.96 (0.67)</td>
<td>1.97 (0.54)</td>
</tr>
<tr>
<td>Pulmonary function (spirometry and body plethysmography)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-bronchodilator FEV1, L</td>
<td>1.18 (0.44)</td>
<td>1.23 (0.43)</td>
</tr>
<tr>
<td>Pre-bronchodilator FVC, % predicted</td>
<td>48 (12)</td>
<td>51 (14)</td>
</tr>
<tr>
<td>Post-bronchodilator FEV1/ FVC, %</td>
<td>44 (6)</td>
<td>47 (9)</td>
</tr>
<tr>
<td>FEV1, L</td>
<td>3.13 (12)</td>
<td>3.40 (12)</td>
</tr>
<tr>
<td>FRC, % predicted</td>
<td>105 (20)</td>
<td>109 (20)</td>
</tr>
<tr>
<td>TLC, L</td>
<td>0.65 (0.05)</td>
<td>0.68 (0.07)</td>
</tr>
<tr>
<td>IC/L</td>
<td>1.06 (0.07)</td>
<td>1.07 (0.07)</td>
</tr>
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</table>

Table 2. Resting (post-dose) and exercise measures in lung function and dyspnea (ITT population)

<table>
<thead>
<tr>
<th>Measure</th>
<th>Week 1</th>
<th>Week 3</th>
<th>Week 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1, L</td>
<td>2.26</td>
<td>2.26</td>
<td>2.26</td>
</tr>
<tr>
<td>IC, L</td>
<td>1.18</td>
<td>1.18</td>
<td>1.18</td>
</tr>
<tr>
<td>IC/TLC</td>
<td>1.32</td>
<td>1.32</td>
<td>1.32</td>
</tr>
<tr>
<td>Inspiratory Capacity</td>
<td>4.8</td>
<td>4.8</td>
<td>4.8</td>
</tr>
<tr>
<td>Dyspnea, Borg</td>
<td>1.85</td>
<td>1.85</td>
<td>1.85</td>
</tr>
<tr>
<td>Resting Measure of Dyspnea</td>
<td>1.85</td>
<td>1.85</td>
<td>1.85</td>
</tr>
<tr>
<td>Changes from baseline (L)</td>
<td>0.02</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Changes from baseline (%)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Figure 1. Exercise endurance

Figure 2. Resting lung function and dyspnea at Week 6 (trend)

Figure 3. Resting lung function and dyspnea at 2 weeks post-dose

**p≤0.005, ***p≤0.001 vs placebo

Safety and Tolerability Of Aclidinium Administered Intravenously And Its Bioavailability Of Inhalated Aclidinium In Healthy Subjects

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Introduction
• Aclidinium bromide is a novel, long-acting muscarinic antagonist currently in Phase II development as a monotherapy treatment for COPD.
• Aclidinium bromide exhibits long residence time at M3 receptors and is rapidly hydrolysed in human plasma to two inactive metabolites.

Study Design
• This was a Phase I, two-part study, in which 24 healthy male subjects were randomized to 1 of 2 treatment groups (1:1).
• Part (1:1=2) was a 3-period, single-blind, placebo-controlled study of alternating, single-ascending IV doses of aclidinium bromide.
• Subjects were randomized to 1 of 2 treatment groups (Group A or B) and treated with a single aclidinium bromide dose or placebo via intravenous (IV) infusion over a 5-minute period (Table 1A).
• Part II (1:1=2) was an open-label, single-dose, two-period crossover study of IV and inhaled administration of a single dose of aclidinium bromide 200 µg (Table 1B).
• Aclidinium bromide was administered IV in 5 minutes (Period 0) and as a slow nebulizer inhalation (Period 1).

Objective
• To evaluate the safety and tolerability of single ascending doses of IV aclidinium bromide in healthy male subjects.

Methods
• Pharmacokinetic Assessments
• Safety and tolerability were assessed via AEIs, vital signs, ECGs, clinical laboratory tests, and physical examinations.

Results
• No treatment-related SAEs were reported. A total of 1164 AEs were reported in Part I (866, 298) and Part II (298, 866).
• There were no clinically relevant changes in laboratory values, vital signs, or ECG parameters.
• No anticholinergic AEs were reported in Part I, whereas 66.6% vs 49.9% of the adverse events reported in Part II were anticholinergic in nature. The most common TEAEs were infusion site pain (n=9), headache (n=4), puncture site pain (n=3), application site rash (n=2), and erythema (n=2).

Part I Plasma Pharmacokinetics Of Aclidinium Following Intravenous Administration
• Baseline demographics and clinical characteristics were similar between treatment groups.
• Aclidinium appeared rapidly in plasma following aclidinium 200 µg inhalation, with maximum plasma concentrations occurring at a median tmax of 5 minutes (0.09 hours) post-dose (Table 4); the IV aclidinium pharmacokinetic profile was similar in Part II compared with Part I (Tables 2 and 4).
• The amount of unchanged aclidinium excreted in urine was low when measured up to 48 hours post-dose for both inhalation (0.16%) and IV administration (8% in 25% subjects).

Conclusions
• Absolute bioavailability of aclidinium was low (≤5%) following a single 200 µg inhalated dose.
• Aclidinium appeared rapidly in plasma following inhalation and was rapidly cleared from the body after both IV and IN administration.
• Urinary excretion of unchanged aclidinium was very low (0.16% for inhalation and 2.73% for IV).
• Single ascending doses of aclidinium bromide 25 µg to 400 µg administered by IV and single inhaled doses of aclidinium 200 µg were safe and well tolerated in this study.
• Based on results from this study aclidinium demonstrates a favorable pharmacological profile and a low potential for adverse events associated with systemic exposure.

References
2. Altor and Genuair (registered trademark of Almirall, Barcelona, Spain.
3. Genuair ® is a registered trademark of Almirall, Barcelona, Spain.
4. The MITD was not determined in this study.
5. There were no clinically relevant changes in laboratory values, vital signs, or ECG parameters.
8. Altor and Genuair (registered trademark of Almirall, Barcelona, Spain.
9. Pulm Pharmacol Ther

Pharmacokinetic Assessments
• Blood samples for pharmacokinetic assessments were taken pre-dose, at 5, 15, 30, 45 min, and 1, 2, 3, 4, 5, 6, 8, 12, 18, 24, 36, and 38-48 hours post-dose for measurement of the urinary concentration of aclidinium bromide.
• The lower limit of quantitation (LLOQ) for the plasma measurements was 5 pg/mL, for the acid metabolite 30 pg/mL, for the alcohol metabolite and 105 pg/mL for the acid metabolite.
• The LLOQ for the urine measurements was 20 pg/mL for aclidinium bromide, 250 pg/mL for the alcohol metabolite, and 2000 pg/mL for the acid metabolite.

Safety Assessments
• Safety and tolerability were assessed via AEIs, vital signs, ECGs, clinical laboratory tests, and physical examinations.

Statistical Analysis
• All pharmacokinetic analyses were performed on the PK Analysis population, defined as all subjects who received aclidinium bromide and completed the study.
• Pharmacokinetic calculations included AUC0-∞, Cmax, AUC0-12, Cmax, Cmax, and t1/2, for aclidinium bromide and its two inactive metabolites (acid metabolite and alcohol metabolite); and absolute bioavailability (F) for aclidinium bromide.

Results
• Table 2 shows the mean plasma pharmacokinetic parameters of aclidinium bromide following single IV administration (Part I) analysis population.

Part II Plasma Pharmacokinetics Of Aclidinium Bromide Following Inhalation And IV Administration
• The total plasma bioavailability of inhaled aclidinium 200 µg was low at a mean of <1% (Table 4), with values for individual subjects ranging from 0 to 9.1%.
• Aclidinium appeared rapidly in plasma following aclidinium 200 µg inhalation, with maximum plasma concentrations occurring at a median tmax of 5 minutes (0.09 hours) post-dose (Table 4); the IV aclidinium pharmacokinetic profile was similar in Part II compared with Part I (Tables 2 and 4).

Statistical Analysis
• Table 3 shows the mean plasma pharmacokinetic parameters of acid and alcohol metabolites following IV administration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>25 µg</th>
<th>50 µg</th>
<th>100 µg</th>
<th>200 µg</th>
<th>400 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0-∞ (pg.h/mL)</td>
<td>42.0 (14.7)</td>
<td>45.8 (16.0)</td>
<td>47.5 (17.2)</td>
<td>49.5 (18.3)</td>
<td>50.0 (18.5)</td>
</tr>
<tr>
<td>Cmax (pg/mL)</td>
<td>1.00 (0.24)</td>
<td>1.00 (0.24)</td>
<td>1.00 (0.24)</td>
<td>1.00 (0.24)</td>
<td>1.00 (0.24)</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>0.09 (0.01)</td>
<td>0.09 (0.01)</td>
<td>0.09 (0.01)</td>
<td>0.09 (0.01)</td>
<td>0.09 (0.01)</td>
</tr>
<tr>
<td>F (%)</td>
<td>0.08 (0.06)</td>
<td>0.08 (0.06)</td>
<td>0.08 (0.06)</td>
<td>0.08 (0.06)</td>
<td>0.08 (0.06)</td>
</tr>
</tbody>
</table>

Part II Plasma Pharmacokinetics Of Aclidinium Bromide After Inhalation and IV Administration
• Baseline demographics and clinical characteristics were similar between treatment groups.
• Table 3 shows the mean plasma pharmacokinetic parameters of acid and alcohol metabolites following IV administration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>25 µg</th>
<th>50 µg</th>
<th>100 µg</th>
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<td>45.8 (16.0)</td>
<td>47.5 (17.2)</td>
<td>49.5 (18.3)</td>
<td>50.0 (18.5)</td>
</tr>
<tr>
<td>Cmax (pg/mL)</td>
<td>1.00 (0.24)</td>
<td>1.00 (0.24)</td>
<td>1.00 (0.24)</td>
<td>1.00 (0.24)</td>
<td>1.00 (0.24)</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>0.09 (0.01)</td>
<td>0.09 (0.01)</td>
<td>0.09 (0.01)</td>
<td>0.09 (0.01)</td>
<td>0.09 (0.01)</td>
</tr>
<tr>
<td>F (%)</td>
<td>0.08 (0.06)</td>
<td>0.08 (0.06)</td>
<td>0.08 (0.06)</td>
<td>0.08 (0.06)</td>
<td>0.08 (0.06)</td>
</tr>
</tbody>
</table>

Part II Plasma Pharmacokinetics Of Aclidinium Bromide After Inhalation and IV Administration
• Baseline demographics and clinical characteristics were similar between treatment groups.
• Table 4 shows the mean plasma and urinary pharmacokinetic parameters of aclidinium bromide, Part II PK analysis population.
Pharmacokinetics Of Aclidinium Bromide 200 μg And 400 μg In Young And Elderly Patients With Chronic Obstructive Pulmonary Disease

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1Harrison Clinical Research GmbH, Munich, Germany; 2Insal Respiratory Research Institute, Wesbaden, Germany; 3APEX GmbH, Munich, Germany; 4Almirall, R&D Centre, Barcelona, Spain.

Introduction

Post-bronchodilator pulmonary function decreases (FEV1) has been increased prevalence in the elderly population. Elderly patients are also given less of other conditions including low body fat and more frequent infections compared to younger adults which should be considered.

Aclidinium bromide is a rapid-acting long-acting inhaled antimuscarinic currently is clinically developed for the maintenance therapy of COPD.

In clinical studies, aclidinium demonstrated long-lasting bronchodilatory activity, and a favorable safety profile.1,2 Postural changes and clinical studies have shown aclidinium rapidly hydrolyzed in human plasma to two major inactive alcohol and acid metabolites, suggesting a low risk potential for systemic side effects.2

The aim of the study was to assess the pharmacokinetics profile of aclidinium (200 μg and 400 μg) in elderly patients and determine mean steady state plasma concentration at a steady state day in young and elderly patients with COPD.

Methods

Study Design And Treatment

• The study was a Phase I, open-label, multiple-dose study in young (aged ≥ 18-40 years) and elderly (≥ 65 years) patients with COPD.

• Patients received one dose of aclidinium (200 μg) (single oral intake) for three days. Following a washout, second patients received two consecutive aclidinium 400 μg (two calls 200 μg) for three days.

• Aclidinium was administered via the FeNO™ inhalator via a sonic nebulizer by patient intubate.

Assessments

• GI Day 1 and 3 treatment with aclidinium 200 μg and 400 μg. Urine samples were collected on Day 1, pre-dose and at 0, 0.5, 1, 2, 3, 4, and 6 hours post-dose.

• Urine samples were collected pre-dose on Day 1, at 2, 4, 6, 8, 12 and 24 hours post-dose and on Day 3 post-dose with aclidinium 200 μg and 400 μg.

• Determination of aclidinium and its metabolites in urine and plasma were performed by liquid chromatography tandem mass spectrometry. These analyses were fully validated, allowing for linear quantification in plasma and urine samples.

• These procedures were developed using validated analytical laboratory practices, ensuring the accuracy and precision of the data obtained.

Statistical Analysis

• Pharmacokinetic analysis was performed using non-compartmental methods calculated from a single-dose analysis (Day 1 study at single dose (Day 2). Measurements included areas under the plasma concentration-time curve (AUC), maximum observed plasma concentration (Cmax), apparent plasma terminal elimination half-life (t1/2,app), renal clearance (CLr), and percentage of dose excreted in urine (FE).

• All data were analyzed using descriptive statistics.

Results

Patients

• A total of 24 young (n=12) and elderly (n=12) patients were enrolled in the study. All patients completed the study.

• Baseline demographic and clinical characteristics are shown in Table 1.

Pharmacokinetics

Aclidinium

• The mean plasma concentration time profiles of aclidinium were similar for young and elderly patients at each dose and day (Figures 1 and 2).

• For both age groups, aclidinium approached steady state with a median t1/2 between 10.1 and 10.6 hours following Cmax plasma levels of aclidinium achieved with 30 minutes between 1.9 and 2.0 hours (Table 3). The plasma exposure on the 400 μg dose was approximately two-fold higher than the 200 μg dose in all patients at both steady state days 1 and 2 (Table 4).

• The plasma exposure on 24 hours was 10% to 15% of the dose for young and elderly patients each day (Table 2). The mean CLr of aclidinium was greater in young (0.75 mL/min) versus elderly (0.49 mL/min) patients at each dose and day (Table 3).

Safety

• Maximum concentrations of the two metabolites occurred later than aclidinium with a median t1/2 between 10.1 and 10.6 hours following Cmax plasma levels of aclidinium achieved with 30 minutes between 1.9 and 2.0 hours (Table 3). The plasma exposure on the 400 μg dose was approximately two-fold higher than the 200 μg dose in all patients at both steady state days 1 and 2 (Table 4).

• Urinary excretion over 24 hours was greater for the two metabolites than aclidinium, ranging from 2.7% to 3.6% of the dose for the alcohol metabolite, and 1.0% to 1.2% of the dose for the acid metabolite, for young and elderly patients across each dose and day (Table 3).

Conclusion

• Once-daily dosages of aclidinium 200 μg and 400 μg (administered at two consecutive 200 μg doses) are well tolerated with a favorable safety profile in young and elderly patients with COPD.

• Aclidinium showed a similar pharmacokinetic profile in young and elderly patients at each dose level, allowing administration of aclidinium as a single dose and at steady state, with an approximately two-fold greater plasma exposure at the 400 μg versus 200 μg dose. Therefore, the pharmacokinetics of aclidinium are linear and time-independent irrespective of patient age.

• Exposure to the alcohol and acid metabolites was reported to be somewhat higher in elderly patients; however, this is not considered to be clinically relevant since these metabolites have been shown to be devoid of activity at a wide array of receptor sites and enzymes, including no neurotoxic or related effects.

• These findings suggest that no dose adjustment of aclidinium is required when treating elderly patients with COPD.

Acknowledgements

Aclidinium bromide was developed and sponsored by Almirall, S.A., Barcelona, Spain. This study was supported by Almirall S.A., Barcelona, Spain.

Table 1. Baseline demographic and clinical characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young patients (n=12)</th>
<th>Elderly patients (n=12)</th>
<th>All patients (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>40 ± 10</td>
<td>70 ± 5</td>
<td>55 ± 5</td>
</tr>
<tr>
<td>FEV1% predicted (baseline)</td>
<td>70 ± 10</td>
<td>70 ± 10</td>
<td>70 ± 10</td>
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<tr>
<td>FEV1% predicted (steady state)</td>
<td>65 ± 10</td>
<td>65 ± 10</td>
<td>65 ± 10</td>
</tr>
<tr>
<td>Exercise capacity (mL/min. / kg)</td>
<td>22 ± 4</td>
<td>12 ± 4</td>
<td>17 ± 4</td>
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<tr>
<td>Current smoking status</td>
<td>7 (58.3)</td>
<td>6 (50.0)</td>
<td>13 (54.2)</td>
</tr>
<tr>
<td>Current smoking status baseline</td>
<td>7 (58.3)</td>
<td>7 (58.3)</td>
<td>14 (58.3)</td>
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</table>

Table 2. Pharmacokinetic parameters for aclidinium after a single dose (Day 1) and at steady state (Day 3) in young and elderly patients with COPD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young patients (n=12)</th>
<th>Elderly patients (n=12)</th>
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<tr>
<td>Aclidinium dose</td>
<td>200 µg</td>
<td>200 µg</td>
<td>200 µg</td>
</tr>
<tr>
<td>Day 1</td>
<td>45.0 (8.0)</td>
<td>45.0 (8.0)</td>
<td>45.0 (8.0)</td>
</tr>
<tr>
<td>Day 3</td>
<td>45.0 (8.0)</td>
<td>45.0 (8.0)</td>
<td>45.0 (8.0)</td>
</tr>
</tbody>
</table>

Table 3. Pharmacokinetic parameters for aclidinium after a single dose (Day 1) and at steady state (Day 3) in young and elderly patients with COPD

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Young patients (n=12)</th>
<th>Elderly patients (n=12)</th>
<th>All patients (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aclidinium dose</td>
<td>400 µg</td>
<td>400 µg</td>
<td>400 µg</td>
</tr>
<tr>
<td>Day 1</td>
<td>90.0 (16.0)</td>
<td>90.0 (16.0)</td>
<td>90.0 (16.0)</td>
</tr>
<tr>
<td>Day 3</td>
<td>90.0 (16.0)</td>
<td>90.0 (16.0)</td>
<td>90.0 (16.0)</td>
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</tbody>
</table>

Table 4. Number of adverse events reported by n/2 patients by patient age and dose

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>Young patients (n=12)</th>
<th>Elderly patients (n=12)</th>
<th>Young patients (n=10)</th>
<th>Elderly patients (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aclidinium 200 µg</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Aclidinium 400 µg</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

References


Metabolism And Excretion Of Aclidinium Bromide Following Intravenous Administration Of [14C] Aclidinium Bromide In Healthy Subjects

Stephen Flach, Josep M. Jansà, John Ho, Esther García Gil, Cynthia Caracta, Stephán Ortiz • Covance Clinical Research Unit Inc, USA; Amalrill SA, Spain; Forest Research Institute, USA

Introduction
Aclidinium bromide is an inhaled, novel, long-acting, muscarinic antagonist currently in Phase III development for the treatment of COPD.

Previous studies have shown aclidinium bromide has longer residence time at M2, receptors and lower plasma concentrations than the marketed long-acting anticholinergic, tiotropium.

Aclidinium bromide is rapidly hydrolyzed in human plasma to two inactive metabolites, limiting the potential for systemic exposure.

Clinical trials in patients with COPD have demonstrated sustained bronchodilation and a favorable tolerability profile.

Objective
To determine the time and amount of aclidinium elimination and to identify characteristics in metabolites when administered as a single 400 µg intravenous dose to healthy male subjects.

Pharmacokinetic Parameters

Plasma
Following IV administration of 400 µg (phenyl-U-14C)– and [glycolyl-U-14C]–aclidinium bromide, the plasma levels of the acid metabolite were quantifiable (≥100 pg/mL) for 8–16 hours post-dose (Figure 1).

Excretion
The predominant route of excretion for both treatments was renal (Figure 2).

Safety
A total of 27 of 12 subjects reported at least one adverse event (AE), which resulted in early discontinuation on treatment in 5 subjects. AEs were considered related to [glycolyl-U-14C]–treatment and were mild to moderate in intensity.

Conclusions
Intravenous aclidinium bromide is metabolized rapidly and extensively to its inactive metabolites via hydrolysis directly (hydrolysis alone) or indirectly (metabolic transformation plus hydrolysis).

Only 1.2% of the aclidinium dose was excreted as unchanged drug. The predominant route of excretion was renal for both the acid and alcohol metabolites.

Based on results from this study, aclidinium demonstrates a favorable pharmacokinetic profile and a low potential for systemic exposure.

References

Acknowledgements
This study was supported by Almirall SA, Barcelona, Spain, and Forest Research Institute, Inc, New York, USA.

Figure 1. Plasma concentration-time profile of aclidinium metabolites

Figure 2. Mean cumulative excretion of total radioactivity

Figure 3. The proposed metabolic pathway of aclidinium

Conclusions
Intravenous aclidinium bromide is metabolized rapidly and extensively to its inactive metabolites via hydrolysis directly (hydrolysis alone) or indirectly (metabolic transformation plus hydrolysis).

Only 1.2% of the aclidinium dose was excreted as unchanged drug. The predominant route of excretion was renal for both the acid and alcohol metabolites.

Based on results from this study, aclidinium demonstrates a favorable pharmacokinetic profile and a low potential for systemic exposure.

References

Acknowledgements
This study was supported by Almirall SA, Barcelona, Spain, and Forest Research Institute, Inc, New York, USA.

Table 1. Mean pharmacokinetic parameters for the acid and alcohol metabolites following single IV doses of 400 µg [phenyl-U-14C]- and [glycolyl-U-14C]-aclidinium bromide: PK analysis population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acid Metabolite</th>
<th>Alcohol Metabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC0-t, µg eq/mL</td>
<td>1144±404</td>
<td>354±112</td>
</tr>
<tr>
<td>Cmax, µg eq/mL</td>
<td>5.09 (0.99-0.20)</td>
<td>9.08 (0.83-0.15)</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>3.41 (0.53)</td>
<td>2.70 (0.26)</td>
</tr>
</tbody>
</table>

Table 2. Pharmacokinetic parameters for total radioactivity

<table>
<thead>
<tr>
<th>Parameter</th>
<th>[Phenyl-U-14C]-aclidinium bromide 400 µg</th>
<th>[Glycolyl-U-14C]-aclidinium bromide 400 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax, µg eq/mL</td>
<td>12.6 (0.69)</td>
<td>24.5 (14.6)</td>
</tr>
<tr>
<td>Tmax, h</td>
<td>0.08 (0.08-0.20)</td>
<td>0.08 (0.08-0.20)</td>
</tr>
</tbody>
</table>

Table 3. The proposed metabolic pathway of aclidinium

The proposed metabolic pathway of aclidinium in healthy male subjects is shown in Figure 3.
In Vitro M₂/M₃ Kinetic Selectivity Of Acclidinium Bromide And Glycopyrrolate

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Introduction

• Anticholinergic agents are frequently used to treat chronic obstructive pulmonary disease (COPD) due to their bronchodilatory effect, which is mediated primarily via the blockade of pulmonary M₂ receptors. However, this effect is often accompanied by the blockade of other muscarinic receptor subtypes, particularly M₂ receptors.

• Kinetic selectivity for M₃ over M₂ receptors is desirable, since the M₂ subtype can down-regulate parasympathetic nerve activity. Moreover, as blockade of M₂ receptors in cardiac muscle is associated with unwanted cardiovascular effects, differences in the pharmacological selectivity of anticholinergic agents may impact on their tolerability profiles in vivo.

• Aclidinium bromide is a novel, long-acting muscarinic antagonist, currently in clinical development for the maintenance treatment of patients with COPD.

• Preclinical studies have shown that aclidinium is a potent muscarinic receptor antagonist, with a long time to reach M₂ receptors and a shorter residence time at M₃ receptors. Furthermore, aclidinium has demonstrated a reduced potential for cardiovascular effects compared with glycopyrrolate in animal models, which may be due to its relatively faster dissociation rate from M₂ receptors and rapid hydrolysis in plasma.

• The aim of this study was to investigate the effects of glycopyrrolate on M₂ and M₃ in vitro systems and compare the results with previously presented data for aclidinium, using tiotropium and ipratropium as comparators, under similar study conditions.

Methods

Affinity For Human M₂-M₃ Muscarinic Receptors

• The affinity (Kᵢ) of each antagonist for muscarinic M₂-M₃ receptors was evaluated by displacement of 1-(N-methyl-H)oxcopolonium (±H-NMS) binding to membrane preparations from cells expressing human muscarinic receptors:

  - M₂, M₃, M₂-M₃, and M₃ receptor membrane preparations (100 μg/well each) were incubated at room temperature with ±H-NMS (0.3 nM for M₂ and M₃, and 0.1 nM for M₂-M₃) in the presence of a range of antagonist concentrations (10⁻⁴ to 10⁻¹ M) or 1 μM atropine (to measure non-specific binding).

  - After a 1- or 6-hour incubation period (M₂-M₃ and M₃, respectively) to ensure that equilibrium was reached, bound ±H-NMS was separated from free ±H-NMS by rapid vacuum filtration and radioactivity in the bound fraction was quantified using a scintillation counter.

• Kᵢ values were calculated as described by Cheng and Prusoff.

Dissociation From Human M₂ And M₃ Muscarinic Receptors

• Membranes expressing M₂ and M₃ receptors (final protein concentration 15 μg/mL) were incubated at room temperature with ±H-acilidinium (2.5 μM), ±H-glycopyrrolate (15 μM and 1 μM for M₂ and M₃, respectively), ±H-tiotropium (2.5 μM), or ±H-ipratropium (10 μM) for 135 minutes. These conditions allowed for the radioligands to reach equilibrium with approximately 90% occupancy of the binding sites.

• Aclidinium (final concentration 10 μM) was then added to initiate the dissociation and to occupy the binding sites as they became available, thereby preventing reassociation.

• The amount of ±H-NMS that remained bound at different time points was determined, using the free ±H-NMS by rapid vacuum filtration and quantifying radioactivity using a scintillation counter.

• Dissociation half-lives (t₁/₂) were calculated using one-phase exponential decay.

Results*

Affinity For Human M₂-M₃ Muscarinic Receptors

• At equilibrium, acilidinium and tiotropium displayed higher affinity for all muscarinic receptor subtypes compared with glycopyrrolate and ipratropium (Table 1).

• Glycopyrrolate appeared to show some preferred affinity for M₃ receptors; however, the magnitude of the effect was limited (approximately 3-fold; Table 1).

Table 1. Binding affinity of human, M₂, M₃, and M₂-M₃ receptors

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>M₁</th>
<th>M₂</th>
<th>M₃</th>
<th>M₂-M₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aclidinium</td>
<td>3.0 ± 0.7</td>
<td>0.13 ± 0.04</td>
<td>0.13 ± 0.04</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>Tiotropium</td>
<td>8.10 ± 0.45</td>
<td>4.12 ± 0.34</td>
<td>3.22 ± 0.15</td>
<td>4.82 ± 0.45</td>
</tr>
<tr>
<td>Glycopyrrolate</td>
<td>15.11 ± 1.57</td>
<td>4.12 ± 0.34</td>
<td>3.22 ± 0.15</td>
<td>4.82 ± 0.45</td>
</tr>
<tr>
<td>Ipratropium</td>
<td>20.11 ± 3.04</td>
<td>4.12 ± 0.34</td>
<td>3.22 ± 0.15</td>
<td>4.82 ± 0.45</td>
</tr>
</tbody>
</table>

Figure 1. Dissociation of ±H-acilidinium, ±H-glycopyrrolate, ±H-tiotropium, and ±H-ipratropium from human M₂ and M₃ receptors.

Analysis

• The concentrations required for 50% inhibition (EC₅₀) of the electrically stimulated contraction (M₃ receptors) and maximum carbamol-induced relaxation (M₂ receptors) were calculated using non-linear regression analysis.

• The time taken to achieve 50% recovery (tₑ) of the electrically stimulated contraction (M₃ receptors) was calculated using non-linear regression analysis. The tₑ for recovery of the maximum carbamol-induced relaxation (M₂ receptors) was calculated using one-phase (acilidinium, glycopyrrolate, and tiotropium) or two-phase (ipratropium) exponential decay.

Table 2. Assay for potency and duration of action of aclidinium bromide, glycopyrrolate, tiotropium, and aclidinium in M₂ receptors (pameline pgp/trachexes) (Table 1).

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>M₁</th>
<th>M₂</th>
<th>M₃</th>
<th>M₂-M₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aclidinium</td>
<td>1.76 ± 0.20</td>
<td>1.76 ± 0.20</td>
<td>1.76 ± 0.20</td>
<td>1.76 ± 0.20</td>
</tr>
<tr>
<td>Tiotropium</td>
<td>18.11 ± 3.04</td>
<td>18.11 ± 3.04</td>
<td>18.11 ± 3.04</td>
<td>18.11 ± 3.04</td>
</tr>
<tr>
<td>Glycopyrrolate</td>
<td>20.11 ± 3.04</td>
<td>20.11 ± 3.04</td>
<td>20.11 ± 3.04</td>
<td>20.11 ± 3.04</td>
</tr>
<tr>
<td>Ipratropium</td>
<td>30.20 ± 3.04</td>
<td>30.20 ± 3.04</td>
<td>30.20 ± 3.04</td>
<td>30.20 ± 3.04</td>
</tr>
</tbody>
</table>

• Data are recorded as mean ± standard error of the mean; n=3.

Conclusion

• All four muscarinic antagonists showed similar potency at M₂ receptors in the isolated trachea strip assay (Table 3).

• Aclidinium, glycopyrrolate, and tiotropium had a similar offset time for ipratropium, which was of greater magnitude than that observed for ipratropium (Table 3).

Table 3. Dissociation half-lives of ±H-acilidinium, ±H-glycopyrrolate, ±H-tiotropium, and ±H-ipratropium from human M₂ and M₃ receptors.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>M₂</th>
<th>M₃</th>
<th>M₂-M₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aclidinium</td>
<td>1.76 ± 0.20</td>
<td>1.76 ± 0.20</td>
<td>1.76 ± 0.20</td>
</tr>
<tr>
<td>Tiotropium</td>
<td>18.11 ± 3.04</td>
<td>18.11 ± 3.04</td>
<td>18.11 ± 3.04</td>
</tr>
<tr>
<td>Glycopyrrolate</td>
<td>20.11 ± 3.04</td>
<td>20.11 ± 3.04</td>
<td>20.11 ± 3.04</td>
</tr>
<tr>
<td>Ipratropium</td>
<td>30.20 ± 3.04</td>
<td>30.20 ± 3.04</td>
<td>30.20 ± 3.04</td>
</tr>
</tbody>
</table>

• Data are recorded as mean ± standard error of the mean; n=3.

Acknowledgements

This study was supported by Almirall S.A., Barcelona, Spain. "Methods and results for acilidinium, glycopyrrolate, and ipratropium are as previously reported.

Dr. Jorge Beleta

References

7. Cheng Y, Prusoff WH. Relationship between the inhibition constant (Kᵢ) and the concentration of inhibitor which causes 50% increase of EC₅₀ of an enzymatically catalyzed reaction. Biochem Pharmacol 1973;22:3099-3108.
Introduction

• Inhaled anticholinergic agents are commonly used for the treatment of chronic obstructive pulmonary disease (COPD). Ideally, these agents should be potent, long-acting bronchodilators with low systemic availability to limit the potential for unwanted anticholinergic effects. Currently available inhaled anticholinergic agents, including ipratropium and tiotropium, have been associated with systemic side effects including dry mouth.1,2

• Aclidinium bromide is a novel, inhaled, long-acting muscarinic antagonist, currently in development for the treatment of patients with COPD. Preclinical studies have shown aclidinium to have high affinity for muscarinic receptors with a long residence time at M₁ receptors and a shorter residence time at M₂ receptors.3 In addition, aclidinium is rapidly hydrated in human plasma to two inactive metabolites. These features suggest aclidinium may provide sustained bronchodilation with a reduced potential for systemic anticholinergic effects.4,5

• Herein we report the results of two in vivo studies:
  • The first study was designed to assess the onset, potency, and duration of action of glycopyrrolate in guinea pigs, compared with data previously obtained for aclidinium, tiotropium, and ipratropium using identical assays and conditions.1
  • The second study investigated the effects of either glycopyrrolate or aclidinium on salivation in rats, using tiotropium as a comparator.6

Methods*

Study 1: Assessment Of Onset, Potency, And Duration Of Action In Anesthetized Guinea Pigs

• Aclidinium, glycopyrrolate, tiotropium, and ipratropium (1–1000 µg/ml) or vehicle were administered to male Dunkin–Hartley guinea pigs (400–600 g) by nebulization for two 1-minute periods separated by an interval of 5 minutes.

• At various time points after exposure to antagonist (1, 2, 4, 6, 18, and 24 hours), guinea pigs were anesthetized, artificially ventilated, and their tracheas were cannulated and connected to a pneumotachograph to record airway resistance.

• Bronchodilatation was induced by an intravenous administration of a single bolus dose of acetylcholine (30 µg/kg), and the inhibitory effect of the antagonists was tested in comparison to vehicle.

• Potency was defined as the concentration required to induce 50% inhibition (EC₅₀) of bronchodilatation, determined from a dose-response curve constructed using the inhibition values obtained at each of the time points studied. Onset of action (t₁/₂) for each compound was defined as the time required to achieve the maximal potency. The duration of action (t₅₀) defined as time to achieve 50% recovery of a submaximal inhibitory concentration of antagonist, was derived from time-course bronchodilatation inhibition curves using one-phase exponential decay.

Study 2: Salivation Studies In Conscious Rats

• Aclidinium (0.1–1000 µg/kg, s.c.), glycopyrrolate (0.1–10 µg/kg, s.c.), tiotropium (0.1–100 µg/kg, s.c), or vehicle were administered to male Wistar rats (180–260 g; fasted for 18 hours).

• After 30 minutes, pilocarpine (0.5 mg/kg) was administered via the caudal vein.

• During the following 15 minutes, the presence of any saliorhea (excess saliva) was recorded by gently pressing filter paper on the animal’s mouth.

• Proportions of animals showing salivation were compared with vehicle-treated animals by Fischer’s exact test. ED₅₀ values (the dose of test compound inducing pilocarpine-induced salivation in 50% of rats) were calculated by non-linear regression.

Results*

Study 1: Onset, Potency, And Duration Of Action In Guineas Pigs

• Aclidinium, glycopyrrolate, and ipratropium achieved maximal inhibition of bronchodilatation at 2 hours post-administration, compared with 4 hours for tiotropium (Table 1).2

• All four antagonists showed similar potency at the time of maximal effect (Table 1).

• The duration of action was longer for aclidinium (t₁/₂ = 64 h) and aclidinium (t₁/₂ = 29 h) compared with glycopyrrolate and ipratropium (t₁/₂ = 13 h and t₁/₂ = 8 h, respectively; Figure 1).

• At the concentrations selected, all four compounds achieved an inhibitory effect on bronchodilatation of 97–98% at 1 hour post-administration (Figure 1).

Study 2: Salivation Studies In Conscious Rats

• Aclidinium inhibited pilocarpine-induced salivation to a lesser extent than tiotropium (ED₅₀ [µg/kg] = 38 and 0.88, respectively; Figure 2a).

• Inhibition of pilocarpine-induced salivation by glycopyrrolate was very similar to that of tiotropium (ED₅₀ [µg/kg] = 0.74 and 0.72, respectively; Figure 2b).

Table 1. Onset of action and potency of aclidinium, glycopyrrolate, tiotropium, and ipratropium in reversing acetylcholine-induced bronchodilatation in guinea pigs*

<table>
<thead>
<tr>
<th>Dose µg/kg, s.c.</th>
<th>Aclidinium</th>
<th>Glycopyrrolate</th>
<th>Tiotropium</th>
<th>Ipratropium</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3</td>
<td>75%</td>
<td>80%</td>
<td>100%</td>
<td>95%</td>
</tr>
<tr>
<td>1.0</td>
<td>95%</td>
<td>90%</td>
<td>100%</td>
<td>85%</td>
</tr>
<tr>
<td>5.0</td>
<td>100%</td>
<td>95%</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>10.0</td>
<td>100%</td>
<td>95%</td>
<td>100%</td>
<td>90%</td>
</tr>
</tbody>
</table>

*Data are mean ± standard error of the mean; n=4–9

Conclusions

• The onset of bronchodilatory effect for aclidinium in guinea pigs is faster than that of tiotropium and similar to that of glycopyrrolate and ipratropium.

• At the time of maximal effect, aclidinium, glycopyrrolate, tiotropium, and ipratropium are equipotent inhibitors of bronchodilatation in guinea pigs.

• Aclidinium has a longer duration of effect in anesthetized guinea pigs compared with glycopyrrolate and ipratropium but a shorter duration of effect than tiotropium.

• The lower potency of aclidinium compared with glycopyrrolate on the inhibition of salivation in conscious rats suggests a lower propensity for xerostomia (dry mouth) in the clinical setting.

Acknowledgements

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

References

Efficacy And Safety Of Single Inhaled Doses Of LAS100977, A Novel Long-Acting β2-Agonist, In Patients With Persistent Asthma

Jutta Beier,1 Rainard Fuhr,2 Eric Massana,3 Eulàlia Jiménez,3 Beatriz Seoane,1 Gonzalo de Miquel,1 Sandrine Ruiz2
1Institut für Atemwegsforschung (INSAF), Wiesbaden, Germany; 2Parexel International GmbH, Berlin, Germany; 3Almirall, R&D Centre, Barcelona, Spain

Introduction
LAS100977 is a novel, potent, and selective long-acting β2-agonist in clinical development for once-daily treatment of asthma in combination with inhaled corticosteroid (ICS) therapy. In vitro studies have shown that LAS100977 displays high potency and selectivity at β2-receptors, with a rapid onset and long duration of action.1 Furthermore, in vivo studies in dogs suggest that LAS100977 may provide more potent bronchodilatation, a longer duration of action, and a reduced potential for cardiac side effects compared with salmeterol.2

A Phase I clinical trial, the first in humans, demonstrated that once-daily LAS100977 significantly decreased airway resistance, increased airway conductance, and was well tolerated in healthy subjects.3

Objective
The purpose of this study was to assess the efficacy, safety, and tolerability of single inhaled doses of LAS100977 in patients with mild-to-moderate persistent asthma.

Methods

Patients
Inclusion Criteria
- Male patients aged 18–70 years, inclusive.
- Clinical diagnosis of persistent asthma for at least six months prior to screening.
- Maintenance therapy of stable doses of ICSs during the six weeks prior to the run-in, either alone or in combination with a short- or long-acting β2-agonist.
- A forced expiratory volume in one second (FEV1) between 60% and 85% of the predicted normal post-bronchodilator value at screening.
- FEV1 reversibility >12% and an absolute increase of at least 200 mL over baseline value following salbutamol inhalation.
- Pre-dose FEV1 of each treatment period within 80%–120% of pre-dose FEV1, at screening.

Exclusion Criteria
- History of smoking during preceding 12 months or >10 pack-years.
- Presence of clinically significant diseases, other than asthma.
- Hospitalization or emergency-room treatment for acute asthma in the six weeks prior to screening.
- History of severe allergy or drug hypersensitivity.
- Treatment with β2-agonists.

Study Design
- This was a Phase IIa, randomized, double-blind, double-dummy, placebo- and active-comparator-controlled, five-way crossover study.
- After an initial screening and run-in period of up to 14 days, randomized 1:1:1:1:1 LAS100977 since day 5 (5, 10, or 25 µg), salmeterol 50 µg twice daily (bid), or placebo. Ongoing asthma medications were withdrawn during the run-in period, with the exception of rescue β2-agonists, if required.

- At 5 minutes post-dose, LAS100977 (5 µg, 10 µg, and 25 µg) significantly increased FEV1 compared with placebo and salmeterol (50 µg bid).
- At all doses tested, LAS100977 provided sustained bronchodilatation for the entire duration of the study period. Increases in FEV1 were observed at all time points and were significantly greater than placebo (p < 0.001) and salmeterol 50 µg bid (p < 0.05; Figure 3).
- All LAS100977 doses decreased mean values of Raw from baseline as early as 1 hour post-dose and at all remaining time points post-dose (Figure 4).
- Compared with baseline, mean values of iQase increased with all LAS100977 doses as early as 1 hour post-dose and at all remaining time points post-dose (Figure 5).
- There were no apparent dose-response relationships for LAS100977 on either airway resistance or airway conductance.

Conclusions
- LAS100977 showed potent, rapid, and long-acting bronchodilatory effect at all doses and had a favorable safety profile in patients with persistent mild-to-moderate asthma.
- Single inhalations of LAS100977® administered at 5 µg, 10 µg, and 25 µg doses, provided significant improvements in lung function over 24 hours, compared with salmeterol 50 µg twice daily; these results demonstrate that LAS100977 is suitable for once-daily dosing.
- LAS100977 doses of 5 µg and 10 µg were well tolerated and had a comparable bronchodilatory profile to the higher 25 µg dose, suggesting that the lower doses are still at or near the top of the LAS100977 dose-response curve.

References

Acknowledgements
The study was supported by Almirall S.A., Barcelona, Spain.
Single Doses Of LAS100977, A Novel Long-Acting β₂-Agonist, Show High Activity And Long Duration In Healthy Subjects

Wolfgang Timmer,1 Éric Massana,1 Eulàlia Jiménez,2 Beatriz Seoane,2 Gonzalo de Miguel,2 Sandrine Ruiz2

1Clinical Research Services Mannheim GmbH, Germany; 2Almirall S.A., Barcelona, Spain

Introduction

- LAS100977 is a novel, potent, and selective long-acting β₂-agonist (LABA) in clinical development for once-daily treatment of asthma in combination with inhaled corticosteroid therapy.
- In vitro studies have shown that LAS100977 displays high potency and selectivity at β₂ receptors, with a rapid onset and long duration of action.1 Furthermore, in vivo studies in dogs suggest that LAS100977 may provide more potent bronchodilation, a longer duration of action, and a reduced potential for cardiac side effects compared with salmeterol.2

Objective

- The purpose of this study, the first human trial, was to examine the safety, tolerability, and activity of different doses of LAS100977 in healthy subjects.

Methods

Subjects

- All subjects were healthy Caucasian males, aged between 18 and 45 years, and with a body mass index of 18.5 to 30 kg/m².
- Concomitant medications were not permitted.

Study Design

- This was a Phase I, randomized, parallel, single-blind, placebo-controlled, single-center, dose-escalation study.
- Within 14 days of a screening visit, subjects were randomized to receive a single dose of LAS100977 (5 µg, 10 µg, 25 µg, or 50 µg) or placebo. The randomization ratio was 2:1 for LAS100977 versus placebo at each dose level.
- LAS100977 and matching placebo were provided as dry powder contained in hard capsules and were administered in the morning by inhalation via a rechargeable device (Cyclohaler®).

Results

Assessments

- All the pharmacodynamic assessments were of an exploratory nature (descriptive statistics only).
- Airway resistance (Raw) was measured using whole-body plethysmography immediately before drug administration (baseline) and at 1, 2, 4, 6, 12, 24, and 36 hours post-dose with the subject in the seated position. Five technically satisfactory measurements were recorded at each time point.
- Raw was converted to airway conductance (Gaw) and divided by the functional residual capacity to obtain specific airway conductance (sGaw).
- Normalized area under the curve between 0 and 24 hours after dosing (AUC24) for sGaw and Raw was calculated using the trapezoidal method.
- Safety assessments included adverse events (AEs), physical examination, vital signs including pulse rate, 12-lead electrocardiograms (ECGs), and laboratory tests.

Effect On Airway Caliber

Table 1. Baseline demographic and other characteristics (n=48)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (n=12)</th>
<th>LAS100977 5 µg (n=12)</th>
<th>LAS100977 25 µg (n=12)</th>
<th>LAS100977 50 µg (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.8 (7.4)</td>
<td>40.6 (7.6)</td>
<td>39.9 (7.2)</td>
<td>39.2 (7.0)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.5 (2.3)</td>
<td>26.0 (4.6)</td>
<td>27.3 (2.7)</td>
<td>26.5 (2.9)</td>
</tr>
<tr>
<td>Smoking history, n (%)</td>
<td>Non-smokers</td>
<td>9/12 (75.0)</td>
<td>8/12 (66.7)</td>
<td>8/12 (66.7)</td>
</tr>
<tr>
<td></td>
<td>Current smokers</td>
<td>3/12 (25.0)</td>
<td>4/12 (33.3)</td>
<td>4/12 (33.3)</td>
</tr>
<tr>
<td></td>
<td>% cigarette/day</td>
<td>19 (39.6)</td>
<td>20 (33.3)</td>
<td>23 (33.3)</td>
</tr>
<tr>
<td>Systolic blood pressure, mean (SD mmHg)</td>
<td>129.7 (18.8)</td>
<td>129.8 (16.1)</td>
<td>132.9 (16.0)</td>
<td>132.7 (14.9)</td>
</tr>
<tr>
<td>Diastolic blood pressure, mean (SD mmHg)</td>
<td>75.4 (13.5)</td>
<td>75.0 (12.3)</td>
<td>75.6 (12.9)</td>
<td>75.5 (13.1)</td>
</tr>
<tr>
<td>Pulse rate, mean (SD) beats/min</td>
<td>64.1 (11.0)</td>
<td>63.7 (10.3)</td>
<td>64.3 (10.1)</td>
<td>63.8 (10.0)</td>
</tr>
<tr>
<td>Heart rate, mean (SD) beats/min</td>
<td>63 (10.1)</td>
<td>61 (10.1)</td>
<td>62 (10.1)</td>
<td>61 (10.1)</td>
</tr>
<tr>
<td>sGaw (L/sec kPa⁻¹)</td>
<td>0.190 (0.078)</td>
<td>0.184 (0.082)</td>
<td>0.189 (0.086)</td>
<td>0.191 (0.080)</td>
</tr>
<tr>
<td>Raw (kPa . sec/L)</td>
<td>0.291 (0.033)</td>
<td>0.290 (0.033)</td>
<td>0.290 (0.031)</td>
<td>0.295 (0.032)</td>
</tr>
<tr>
<td>sGaw at baseline (AUC0-24)</td>
<td>1.000 (0.069)</td>
<td>0.999 (0.070)</td>
<td>0.998 (0.068)</td>
<td>0.998 (0.067)</td>
</tr>
</tbody>
</table>

Table 2. Mean (SD) normalized AUC24 for sGaw and Raw

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Placebo</th>
<th>LAS100977 5 µg</th>
<th>LAS100977 25 µg</th>
<th>LAS100977 50 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-24</td>
<td>0.190</td>
<td>0.184</td>
<td>0.189</td>
<td>0.191</td>
</tr>
<tr>
<td>0-24</td>
<td>0.291</td>
<td>0.290</td>
<td>0.290</td>
<td>0.295</td>
</tr>
<tr>
<td>0-24</td>
<td>1.000</td>
<td>0.999</td>
<td>0.998</td>
<td>0.998</td>
</tr>
</tbody>
</table>

Results

- All the pharmacodynamic assessments were of an exploratory nature (descriptive statistics only).
- Baseline demographic and other characteristics are shown in Table 1.

Effect On Airway Caliber

- At 24 hours post-dose, all LAS100977 doses decreased sGaw mean values by -0.018 to -0.067 kPa·sec/L versus baseline. However, in subjects receiving placebo, there was no improvement in Raw at 24 hours post-dose compared with baseline.
- Raw mean values were lower with all LAS100977 doses compared with placebo at all time points over 36 hours post-dose.
- All doses of LAS100977 decreased Raw AUC0-24 versus placebo (Table 2).
- There were no deaths, serious AEs, or withdrawals due to AEs observed during the study.

Conclusions

- LAS100977 increased airway conductance and decreased airway resistance over the dose range studied (5 to 50 µg). This effect was sustained for at least 24 hours post-dose.
- At all doses tested, LAS100977 was safe and well tolerated.

References


Acknowledgements

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

- LAS100977 is a novel β2-receptor agonist currently in Phase II clinical development for the treatment of asthma in combination with inhaled corticosteroid therapy.
- This study evaluated the human β-2-adrenergic receptor-binding profile of LAS100977. Additionally, the potency, onset, and duration of action of LAS100977 in human bronchi were compared with three other long-acting β2-agonists (LABAs): salmeterol, formoterol, and indacaterol.

**Methods**

**Human β1/β2/β3-Adrenergic Receptor Affinity**

- Radioligand displacement binding studies for human β1, β2, and β3-adrenergic receptors were performed in Sf9 cell membrane preparations expressing the recombinant human β1, β2, and β3-adrenergic receptors. Membranes were incubated in 1 nM I-CYP ((-)-[125I]-adrenergic antagonist, and different concentrations of the compounds. Non-specific binding was measured in the presence of 1 µM propranolol.

- Binding reactions were terminated by filtration binding reactions were terminated by filtration and incubated with the β2-adrenergic blocker H-CGP12177 (0.14 nM) and different concentrations of the compounds. Non-specific binding was measured in the presence of 1 µM propranolol.

- Potency was assessed by cumulative relaxation-response curves of agonist obtained in preparations at ST and following subsequent changes in tone for up to 14–15 hours.

**Functional β2-Adrenergic Activity In Rat Left Atria**

- Left atria were dissected from euthanized male Dunkin Hartley guinea pigs, dissected into rings, suspended in an organ bath containing Krebs Henseleit solution and connected to a force transducer. Cumulative relaxation-response curves were performed for each compound in preparations at spontaneous tone (ST). 

- β2-adrenergic activity was expressed as the concentration of agonist required to induce 50% of the maximum relaxation induced by isoprenaline 0.1 µM (EC50).

**Results**

**Affinity And Selectivity For Human β2-Adrenergic Receptor Subtypes**

- In cell lines expressing human β2-adrenergic receptors, LAS100977 had the highest affinity for the β2 receptor as measured by I-CYP, formoterol, and indacaterol (Table 1).

- LAS100977 demonstrated higher selectivity for human β2 receptor (β2/β1, binding affinity ratio) than formoterol and indacaterol and lower than salmeterol (Table 1).

**Potency, Onset, And Duration Of Action In Isolated Human Bronchi**

- Human bronchi were obtained from patients who were undergoing surgery for lung carcinoma without history of asthma. The protocol was approved by the Ethics Committee of University Clinic Hospital (València, Spain) and informed consent was obtained from all patients. Bronchial rings were dissected from lung tissue as described by Cortijo et al and suspended in standard organ baths.

- Potency was assessed by cumulative relaxation-response curves of agonist obtained in preparations at ST, and expressed as the concentration of compound required to produce 50% of the maximum relaxation induced by theophylline 3 mM (EC50).

- Onset and duration of action were determined by applying an EC50 of LABA in preparations at ST and following subsequent changes in tone for up to 14-15 hours.

**Conclusions**

- The results of this study show that LAS100977 is a potent β2-adrenergic agonist.
- LAS100977 is a selective β2-adrenergic agonist with a β2/β3 selectivity ratio comparable with salmeterol but superior to indacaterol and formoterol.
- LAS100977 has a rapid onset and a sustained duration of action in isolated human bronchial tissue that is comparable with indacaterol.

**References**

Introduction

Las100977 is a novel β2 receptor agonist currently in phase II clinical development for the treatment of asthma in combination with inhaled corticosteroid therapy.

This study evaluated the human β-adrenergic receptor-binding profile of Las100977. Additionally, the potency, onset, and duration of action of Las100977 in human bronchi were compared with three other long-acting β-agonists (LABAs): salmeterol, formoterol, and indacaterol.

Methods

Human β1/β2/β3-Adrenergic Receptor Affinity

Radioligand displacement binding studies for human β1, β2, and β3-adenrenergic receptors were performed in Sf9 cell membrane preparations expressing the recombinant human β1, β2, and β3-adenrenergic receptors. Membranes were incubated in assay buffer and incubated with the β-adrenergic blockers [125]I-CYP ([(-)]-3-[125]I-CYP, 0.6 nM) and [3H]CGP12177 ([3H]H-CGP12177, 3 nM) against specific and non-specific binding, respectively. Cumulative relaxation-response curves were performed for each compound at concentrations ranging from 0.1 nM to 1 µM, and curve analysis was performed with non-linear regression analysis using SASS.

Potency, Onset, and Duration of Action in Human Bronchi

Human bronchi were obtained from patients who were undergoing surgery for lung carcinoma without history of asthma. The protocol was approved by the Ethics Committee of the University Hospital Clínico San Carlos (Valencia, Spain) and informed consent was obtained from all patients. Bronchial rings were dissected from lung tissue as described by Cortijo et al and stored in standard organ baths.

Potency was assayed by cumulative relaxation-response curves of agonist obtained in preparations at ST, and expressed as the concentration of compound required to produce 50% of the maximum relaxation induced by isoprenaline 0.1 µM (EC50).

Results

Affinity and Selectivity for Human β2-Adrenergic Receptor Subtypes

In cell lines expressing human β2-adrenergic receptors, Las100977 had the highest affinity for the β2 receptor compared with salmeterol, formoterol, and indacaterol (Table 1). Las100977 demonstrated higher selectivity for human β2 receptor (EC50, binding affinity ratio) than formoterol and indacaterol and lower than salmeterol (Table 1).

Conclusions

The results of this study show that Las100977 is a potent β2-adrenergic agonist.

Las100977 is a selective β2-adrenergic agonist with a β2/β3 selectivity ratio comparable with salmeterol but superior to indacaterol and formoterol.

Las100977 has a rapid onset and a sustained duration of action in isolated human bronchial tissue that is comparable with indacaterol.

References


Acknowledgements

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

Affinity and selectivity for human β2-adrenergic receptors

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC50 (nM)</th>
<th>β2/β3 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmeterol</td>
<td>10000</td>
<td>65</td>
</tr>
<tr>
<td>Formoterol</td>
<td>10000</td>
<td>65</td>
</tr>
<tr>
<td>Indacaterol</td>
<td>10000</td>
<td>65</td>
</tr>
</tbody>
</table>

Table 1. Functional β2-Adrenergic Receptor Activity in Guinea Pig Trachea

Las100977 relaxed the tracheal rings from euthanized male Dunkin Hartley guinea pigs, disected into rings, in a dose-dependent manner (Figure 1). The relaxant effect of Las100977 was greater than that of formoterol and indacaterol (Figure 2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (nM)</th>
<th>Potency (EC50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmeterol</td>
<td>30000</td>
<td>50</td>
</tr>
<tr>
<td>Formoterol</td>
<td>30000</td>
<td>50</td>
</tr>
<tr>
<td>Indacaterol</td>
<td>30000</td>
<td>50</td>
</tr>
</tbody>
</table>

Table 2. Functional β2-Adrenergic Receptor Selectivity

In isolated guinea pig tracheal rings, Las100977 exhibited the most potent relaxant activity of the compounds tested, demonstrating 60%, 40%, and 3-fold more relaxant potency than salmeterol, formoterol, and indacaterol, respectively (Table 2).

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC50 (nM)</th>
<th>relaxant potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmeterol</td>
<td>10</td>
<td>50</td>
</tr>
<tr>
<td>Formoterol</td>
<td>10000</td>
<td>3</td>
</tr>
<tr>
<td>Indacaterol</td>
<td>10000</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 3. Onset and duration of action of Las100977 in isolated human bronchi

<table>
<thead>
<tr>
<th>Compound</th>
<th>Onset (minutes)</th>
<th>Duration (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmeterol</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>Formoterol</td>
<td>10</td>
<td>60</td>
</tr>
<tr>
<td>Indacaterol</td>
<td>10</td>
<td>60</td>
</tr>
</tbody>
</table>

Figure 1. Relaxation-response curves of Las100977 and reference compounds in isolated human bronchial tissues

Figure 2. Onset of Las100977 and reference compounds in isolated human bronchial tissues

Figure 3. Relaxation (%)

Figure 4. Time (minutes)


The In Vitro Pharmacological profile of Las100977 – A potent Beta-2 Adrenergic Receptor Agonist

Mónica Aparicí1, Mireia Gómez-Angelats1, Dolores Vilella1, Julio Cortijo2, Esteban Morcillo2, Carla Carcasona3, Amadeu Galvàda3, Jordi Beleta3, Carlos Puig3, Hamish Ryder3,Montserrat Miralpeix1

1Almirall, R&D Centre, Barcelona, Spain; 2Department of Pharmacochemistry, University of Barcelona, Barcelona, Spain; 3Almirall, R&D Centre, Barcelona, Spain; 4Department of Pharmacochemistry, University of Barcelona, Barcelona, Spain
Stability Of Aclidinium Bromide Inhalation Powder (200 μg Per Dose) Delivered From The Genuair® Inhaler

Kathrin Block, Sonja Folger, Beatriz Fymys • Almirall Sofotec GmbH, Bad Homburg, Germany

Introduction

• Aclidinium bromide is a novel, long-acting muscarinic antagonist, currently in clinical development for the maintenance treatment of patients with chronic obstructive pulmonary disease (COPD).

• The Genuair® inhaler (Figure 1) is a multidose dry powder inhaler (MDPI) that functions as a breath-actuated delivery device for inhalation drugs used in COPD and asthma.

• The Genuair® inhaler incorporates a number of technological advances to ensure effective deagglomeration of inhalation powder and consistent delivery of the fine particle drug dose on every actuation.1

• A study conducted in healthy volunteers showed that administration of aclidinium using the Genuair® inhaler resulted in efficient drug deposition in the lungs, indicating that this novel device is an effective MDPI for aclidinium.2

• All investigations were performed according to the European Pharmacopoeia, United States Pharmacopoeia, and the Food and Drug Administration requirements.

Methods

• Three pilot-scale (PS) and three laboratory-scale (LS) batches of aclidinium were tested at a pressure drop of 4 kPa (~65 L/min flow rate; 2 L and 4 L inhalation volumes) through a sample collection tube connected to an Andersen Cascade Impactor, respectively.

• Resulting solutions were analyzed using an isocratic high performance liquid chromatography (HPLC) system with ultraviolet (UV) detection and a C18 column.

• Impeller speed of all samples was assessed using a gradient HPLC method with UV detection and a C18 column.

• All investigations were performed according to the European Pharmacopoeia, United States Pharmacopoeia, and the Food and Drug Administration requirements.

Results

Stability Of Aclidinium Dose Delivered From The Genuair® Inhaler

• There was no change in mean delivered dose of aclidinium (LS 25°C/60% RH & PS 45–300 batches) following storage of up to two and three years, respectively, in various environmental conditions (Table 1, Figure 2).

• The mean delivered dose remained within ±15% of the LC dose of 181 μg for all batches and storage conditions.

• The standard deviation of the mean-delivered dose was <2.95% for all batches and storage conditions.

• Stability samples were taken periodically from all batches and analyzed for critical inhalation performance attributes (delivered dose and particle size) and purity.

• The total delivered dose and fine particle dose of aclidinium using the Genuair® inhaler were tested at a pressure drop of 4 kPa (~65 L/min flow rate; 2 L and 4 L inhalation volumes) through a sample collection tube connected to an Andersen Cascade Impactor, respectively.

• Resulting solutions were analyzed using an isocratic high performance liquid chromatography (HPLC) system with ultraviolet (UV) detection and a C18 column.

• The following specification limits were applied:
  - ±15% of the label claim (LC) dose of 181 μg aclidinium
  - A fine particle dose of 40-85 μg aclidinium

• The purity profile of all samples was assessed using a gradient HPLC method with UV detection and a C18 column.

• There was also no change in the mean fine particle dose (LS 25°C/60% RH & PS 25–300 batches), indicating that particle size of aclidinium was not affected by storage in various environmental conditions (Table 1, Figure 3).

• The mean fine particle dose delivered remained well within specification limits (40–85 μg) for all batches and storage conditions.

Purity Of Aclidinium Delivered From The Genuair® Inhaler

• The initial impurity level of all batches of aclidinium was:
  - <0.3% for LS batches
  - Below the limit of quantification (0.09%) and 0.09% for aclidinium and unidentified impurities, respectively, and 0.10% for the two main degradation products for PS batches.

• The impurity level of aclidinium remained largely unchanged throughout storage in various environmental conditions:
  - 0.24–1.82% for LS batches (Table 2)
  - <0.2% for PS batches (data not shown).

• Stability samples were taken periodically from all batches and analyzed for critical inhalation performance attributes (delivered dose and particle size) and purity.

• The mean delivered dose remained within ±15% of the LC dose of 181 μg for all batches and storage conditions.

Conclusion

• This study demonstrates that the Genuair® inhaler delivers a reproducible fine particle dose of aclidinium dry powder for inhalation, which remains unaffected under various storage conditions for a stability period of up to three years.

• There was no significant increase of impurities observed in aclidinium when stored under various conditions for a stability period of up to three years.

• This novel MDPI fulfills US and European regulatory requirements and can therefore be used as a delivery device for various monotherapy and combination therapy inhalation drugs for COPD and asthma, including aclidinium, which is in clinical development as a maintenance treatment for patients with COPD.

References


Acknowledgements

This study was supported by Almirall S.A., Barcelona, Spain. "Genuair®" is a registered trademark of Almirall S.A. 

Figure 1. General design and features of the Genuair® inhaler.

Figure 2. Mean (±SD) aclidinium dose delivered with the Genuair® inhaler following storage in various environmental conditions.

Table 1. Mean (±SD) aclidinium dose delivered with the Genuair® inhaler following storage in various environmental conditions.

Table 2. Impurity profile of laboratory-scale batches of aclidinium following storage in various environmental conditions.

Table 3. Mean (±SD) aclidinium dose delivered with the Genuair® inhaler following storage in various environmental conditions.

Table 4. Impurity profile of laboratory-scale batches of aclidinium following storage in various environmental conditions.
The Genuair® Inhaler: A Reliable Device Technology In Inhalation Therapy
Roland Greguletz, Beatrix Fyrns, Joachim Goede, Martin Herder • Almirall Sofotec GmbH, Bad Homburg, Germany

Introduction

• The Genuair® inhaler (Figure 1) is a novel, breath-actuated, multidose, dry powder inhaler designed for the effective delivery of various types of inhaled drugs, including long-acting muscarinic antagonists such as aclidinium bromide.

This inhaler incorporates a new design of the dispersion set (mouthpiece and cyclone unit) which enhances fluid and particle dynamics to ensure effective deagglomeration of the inhalation powder into a suitable aerosol, even at a low inhalation flow rate and volume.

• Features of this inhaler include:
  - A dose indicator
  - A control window that provides visual feedback by changing color when the inhaler is ready to use and again when the dose is inhaled correctly (Figure 2)
  - An audible click upon successful actuation of each dose

The aim of this study was to test the device under a variety of thermal and mechanical stress conditions to assess the reliability of the inhaler and its functions. In addition, inhalers from large-scale production batches were tested to assess the reliability of the lock-out and trigger threshold mechanisms.

Methods

Reliability Under Thermal And Mechanical Stress

• A total of 48 inhalers, randomly selected from one production batch, were subjected to one of the following stress treatments (6 inhalers per treatment):
  - Cold storage (2–8°C) for 3 or 8 days
  - Storage at 40°C and 75% relative humidity for 3 or 8 days
  - Hot storage (60°C) for 1 or 6 hours
  - Vibrational loading for 1 hour at an amplitude of approximately 1.5 mm/s through the Genuair® inhaler for the delivery of various types of inhalation powder.

• After stress treatment, the following features and functions of the inhalers were tested: fastening of the slide cover and mouthpiece, colored control window, double-dosing prevention (correct behavior of the control window when the dosage button is pressed multiple times), dose indicator (dose counter) mechanism, lock-out mechanism (activation between 30 and 38 doses), and trigger threshold mechanism (flow rate >0.5 L/min required to activate trigger mechanism).

Results

Reliability Of The Lock-Out And Trigger Threshold Mechanisms

• A total of 310 inhalers were randomly selected from five large-scale production batches (batch size >50,000 inhalers).

• The lock-out and trigger threshold mechanisms were tested for each inhaler as described above. Four trigger threshold measurements were performed for each inhaler and the mean trigger flow rate was calculated.

Conclusions

• The features and functions of the Genuair® inhaler were found to be reliable under conditions of thermal and mechanical stress.

• The inhaler lock-out and trigger mechanisms worked correctly across large-scale production batches.

• The defined trigger threshold flow rate is considerably lower than the peak inspiratory flow rates achieved through the inhaler by patients with moderate to severe chronic obstructive pulmonary disorder.

• The reliability and quality of the inhaler, in conjunction with feedback features that inform patients that they have inhaled correctly, suggest that the Genuair® inhaler may be a useful device in the clinical setting.

References


Acknowledgements

This study was supported by Almirall S.A., Barcelona, Spain. *Genuair® is a registered trademark of Almirall S.A.

Figure 2. Visual and audible feedback by the Genuair® inhaler following inhalation

Figure 3. Lock-out mechanism of the Genuair® inhaler blocks the device when the last dose is loaded and ready for inhalation

Figure 4. Reliability of the Genuair® inhaler’s lock-out mechanism: results for individual inhalers across different batches (n=310)

Figure 5. Trigger flow rates for individual inhalers across different batches (n=310)

Table 1. Test results after thermal and mechanical stress treatment of Genuair® inhalers (n=48)

<table>
<thead>
<tr>
<th>Cold storage</th>
<th>Cold storage</th>
<th>Storage at 40°C and 75% relative humidity</th>
<th>Storage at 40°C and 75% relative humidity</th>
<th>Hot storage (60°C) for 1 hour</th>
<th>Hot storage (60°C) for 6 hours</th>
<th>Vibration of empty inhalers for 1 hour</th>
<th>Vibration of inhalers loaded with empty cartridges for 1 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>(2–8°C) for 3 days (n=6)</td>
<td>(2–8°C) for 8 days (n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
<td>(n=6)</td>
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</tr>
</tbody>
</table>

*Conformance (✓) or non-conformation (✗) to the defined technical specification is indicated above.
Impact Of Different Inhalation Volumes On The Aerodynamics Of Acidinium Bromide Delivered Through The Genuair® Inhaler

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Introduction

• Accurate and consistent delivery of inhaled medication is an important consideration for the effective treatment of chronic obstructive pulmonary disease (COPD).
• Regulatory authorities of different countries require testing of inhaled drugs at different inhalation volumes prior to granting marketing approval; the in vitro testing requirements for the United States and Europe are 2 liters and 4 liters, respectively.
• The Genuair® inhaler (Figure 1) is a breath-actuated, multidose dry powder inhaler (MDPI) that features advanced fluid and particle dynamics to ensure effective deagglomeration and drug delivery, even at low inhalation flow rates and volumes.\(^1\)

Methods

• Aerodynamic assessments were conducted to evaluate the effects of inhalation volume (2 L versus 4 L) on the total delivered dose, fine particle dose, and particle size distribution of acidinium delivered using the Genuair\(^2\) inhaler. The stability of the aerodynamic performance and acidinium particle size distribution, including fine particle dose, was also assessed.

Effect Of Inhalation Volume On Acidinium Dose Content Uniformity

The mean total dose and fine particle dose of acidinium (50 μg and 400 μg) were consistent for both inhalation volumes (Figure 2).

Effect Of Flow Rate And Inhalation Volume On Acidinium Dose Content Uniformity

The mean total dose and fine particle dose of PS batches of acidinium (200 μg) at various flow rates were not significantly affected by the different inhalation volumes (Figure 4).

Conclusions

• Administration of acidinium inhalation powder with the Genuair\(^2\) inhaler produces a consistent dose delivery and aerodynamic particle size distribution, which is independent of inhalation volume.
• Storage of the inhalers for up to three years in a range of environmental conditions has no impact on the aerodynamic behavior of acidinium at different inhalation volumes.
• This MDPI therefore fulfills the regulatory requirements of both the US and EU and does not require adjustments of acidinium inhalation powder used based on inhalation volume.

Acknowledgements

This study was supported by Almirall S.A., Barcelona, Spain. "Genuair" is a registered trademark of Almirall S.A.

References
