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.[1]
- 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.[2,3]

Objective

- This Phase IIa study assessed the efficacy, safety, and tolerability of aclidinium bromide 400 µg BID in patients with moderate to severe COPD.

Methods

Study Design

- This was a multicenter, randomized, double-blind, double-dummy, placebo- and active comparator-controlled, 3-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 QD, or placebo); each treatment arm consisted of three 15-day treatment periods separated by a 9- to 15-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 (6 visits total), and at follow-up.

Study Population

Inclusion Criteria

- Male and non-pregnant, non-lactating female patients aged ≥40 years
- Post-bronchodilator forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio <70%
- Post-bronchodilator FEV_1 ≥30% and <80% of predicted
- Current or ex-smokers with a smoking history of ≥10 pack-years

Exclusion Criteria

- History or current diagnosis of asthma
- 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 FEV_1 area under the curve for the 12 hours immediately following morning dose administration (AUC_{0-12}) on Day 15

Secondary Endpoints

- Change from baseline in normalized FEV_1 AUC_{0-12} on Day 1
- Change from baseline in normalized FEV_1 AUC_{0-24} and FEV_1 AUC_{12-24} on Days 1 and 15
- Change from baseline in normalized morning pre-dose and peak FEV_1 on Days 1 and 15
- Change from baseline in FEV_1 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 variables were analyzed using the Intention-to-treat (ITT) population (defined as all randomized patients who took at least one dose of trial medication, had a baseline FEV_1 measurement, and at least one post-dose FEV_1 measurement).
- Safety outcomes were analyzed using the safety population, defined as all randomized patients who took at least one dose of study medication.
- All efficacy outcomes were analyzed 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)

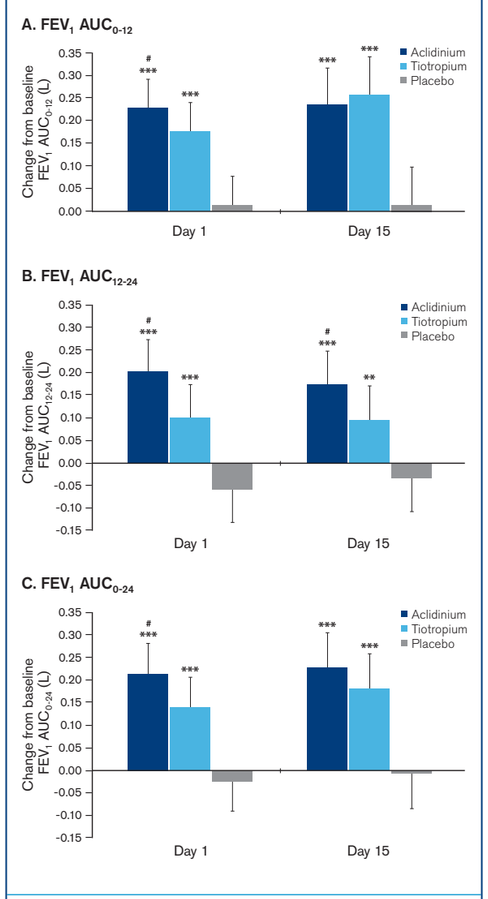
Characteristic	
Age, mean (SD), years	58.4 (7.9)
Male, n (%)	19 (63.3)
White, n (%)	30 (100.0)
BMI, mean (SD), kg/m²	26.1 (4.4)
Current smoker, n (%)	19 (63.3)
Smoking history, mean (SD), pack-years	41.1 (15.9)
Baseline FEV_1 at screening, mean (SD), L	1.47 (0.47)
Post-bronchodilator FEV_1, mean (SD), L	1.7 (0.5)
Post-bronchodilator FEV_1, mean (SD), % of predicted value	55.8 (13.7)
Post-bronchodilator FEV_1/FVC ratio, mean (SD), %	46.2 (10.3)
Bronchodilator reversibility, mean (SD), %	18.2 (11.9)

BMI, body mass index; SD, standard deviation.

Efficacy

- Normalized FEV_1 AUC_{0-12} 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 1, normalized FEV_1 AUC_{0-12} 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).
- Normalized FEV_1 AUC_{12-24} and AUC_{0-24} (Days 1 and 15) were significantly greater for both aclidinium and tiotropium versus placebo ($p<0.001$ for all).
  - Compared with tiotropium, aclidinium produced a significantly greater increase in normalized FEV_1 AUC_{12-24} on Days 1 and 15 (Figure 1B) and on Day 1 for FEV_1 AUC_{0-24} (Figure 1C).

Figure 1. Change from baseline in normalized FEV_1 AUC_{0-12}, AUC_{12-24}, and AUC_{0-24} on Day 1 and Day 15



***p<0.0001, **p<0.001 vs placebo; #p<0.05 vs tiotropium bromide.

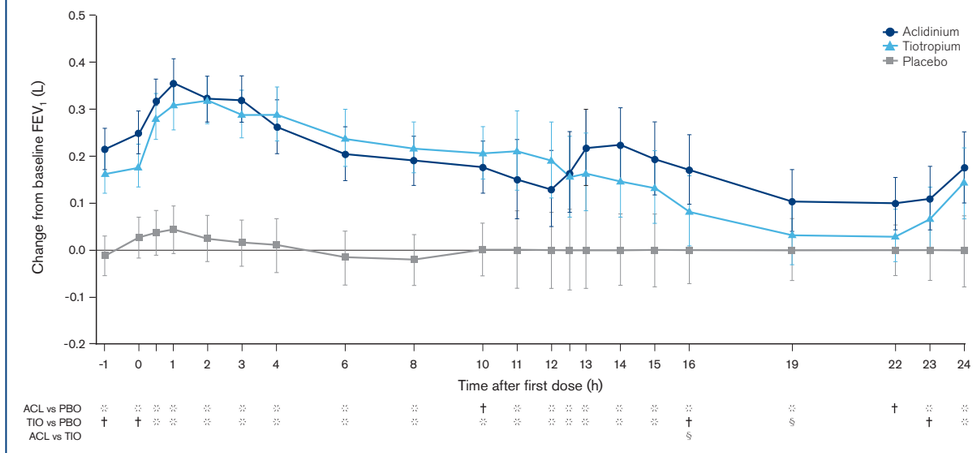
- FEV_1 morning pre-dose and peak values on Days 1 and 15 were significantly higher for both aclidinium and tiotropium treatment versus placebo (Table 2). Aclidinium treatment did not significantly differ from tiotropium treatment at any measurement.

Table 2. Change from baseline in morning FEV_1 measurements

	Aclidinium bromide		Tiotropium bromide		Placebo	
Parameter	Day 1	Day 15	Day 1	Day 15	Day 1	Day 15
Morning pre-dose FEV_1, L	0.163**	0.143**	0.099*	0.107**	-0.022	-0.043
Morning peak FEV_1, L	0.362**	0.408**	0.314**	0.382**	0.144	0.131

*p<0.05, **p<0.0001 vs placebo.

Figure 2. Change from baseline in FEV_1 on Day 15 (LS mean ±SE; ITT population)



*p<0.001; †p<0.01; §p<0.05.
Baseline value was measured as the average of the two values measured just prior to the first dose administration at Day 1 of each treatment period (time points: -1h and 0h before dosing).
ACL, aclidinium bromide; TIO, tiotropium bromide; PBO, placebo.

- The bronchodilatory effect of aclidinium bromide compared to placebo was sustained over the 24-hour period on Day 15 (Figure 2).
  - Mean change from baseline in FEV_1 values after aclidinium bromide administration were significantly increased over placebo at all time points for Day 1 ($p<0.001$) and Day 15 ($p<0.01$).
  - Following administration of tiotropium bromide, the mean change from baseline in FEV_1 was significantly increased over placebo at Days 1 and 15, except at 22h post-dose.

Safety

- Aclidinium 400 µg BID was generally safe and well tolerated.
- Treatment-emergent AEs (TEAEs) were reported by 3 patients (11%) taking tiotropium bromide, 7 patients (24%) taking aclidinium bromide, and 8 patients (27%) taking placebo.
- The most common AEs were diarrhea (n=2 in the aclidinium group) and COPD exacerbations (n=3 in the placebo group).
- Three withdrawals due to TEAEs occurred during treatment with placebo, including one serious AE (severe COPD exacerbation). The two other withdrawals were due to moderate COPD exacerbation and mild atrial fibrillation. None were considered related to aclidinium treatment.
- Laboratory results and blood pressure did not show any relevant changes during the study.

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 FEV_1 AUC_{12-24}, FEV_1 AUC_{12-24} and FEV_1 AUC_{0-24} 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 FEV_1 AUC_{0-12} and FEV_1 AUC_{0-24} on Day 1 and in normalized FEV_1 AUC_{12-24} on Day 1 and Day 15.
- Treatment with twice-daily aclidinium was safe and well tolerated.

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Aclidinium 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.[1] Improved exercise tolerance and dyspnea, and alleviating lung hyperinflation, are important goals in managing COPD[2]; treatment with bronchodilators, including anticholinergics is associated with improvements in these measures.[3, 4]
- Aclidinium 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 aclidinium 200 µg on exercise endurance and lung hyperinflation in patients with COPD.

Methods

Study Design

- This was a Phase III, randomized, double-blind, placebo-controlled, multicenter trial.
- Following a 2-week run-in phase, patients were randomized (1:1) to receive once-daily aclidinium 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 (FEV_1)/forced vital capacity (FVC) ratio <70%
- FEV_1<80% and ≥30% of predicted
- Functional residual capacity (FRC) ≥120% of predicted
- Current or ex-smokers with a smoking history of ≥10 pack-years
- Baseline dyspnea index (BDI) focal 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) at 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% W_{max} to the point of symptom limitation, from baseline to Week 6

Secondary Endpoints

- Changes in ET from baseline to Day 1 and Week 3
- Changes from baseline in FEV_1, IC, FRC, IC/TLC at trough and 2 hours post-dose at all study visits

Other Outcomes

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

Safety

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

Statistical Analysis

- Analyses of efficacy endpoints were performed on the intent-to-treat population (ITT), defined as patients who received at least one dose of the study medication and ≥1 post-baseline assessment of ET.
- Efficacy endpoints were analyzed using analysis of covariance (ANCOVA).

Results

Baseline Demographics

- A total of 181 patients with moderate to severe COPD were randomized to aclidinium (n=86) or placebo (n=95), and 81 and 78 patients, respectively, completed the study.
- Baseline demographic and clinical characteristics were similar between treatment groups (Table 1).

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

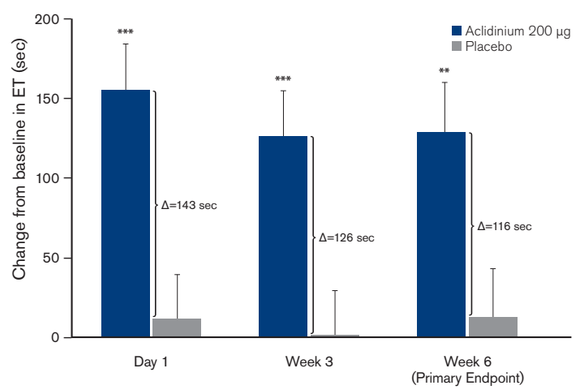
Characteristics	Aclidinium 200 µg (n=86)	Placebo (n=95)
Age, years	64 (10)	66 (8)
Male, n (%)	52 (61)	53 (56)
Caucasian, n (%)	83 (97)	92 (97)
BMI, kg/m²	26.2 (4.6)	26.6 (4.7)
Current smoker, n (%)	38 (44)	31 (33)
Smoking history, pack years	57 (25)	54 (21)
Pulmonary function (spirometry and body plethysmography)		
Pre-bronchodilator FEV_1, L	1.18 (0.44)	1.29 (0.43)
Post-bronchodilator FEV_1, % predicted	49 (11)	52 (14)
Post-bronchodilator FEV_1/FVC ratio, %	44.7 (9.8)	47.4 (9.9)
FRC, L	5.12 (1.23)	4.85 (1.24)
FRC, % predicted	159 (30)	152 (33)
TLC, L	6.85 (1.56)	6.68 (1.47)
IC†, L	1.96 (0.67)	1.97 (0.54)

Data are mean (SD) unless otherwise indicated.
†Measured during the run-in period (Day -10).
BMI, body-mass index, FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; FRC, functional residual capacity; TLC, total lung capacity; IC, inspiratory capacity; SD, standard deviation

Efficacy

- Significant improvements in ET were observed in patients treated with aclidinium as early as the first dose (Day 1) and continued to Week 6 (p≤0.005 vs placebo at all time points; Figure 1).

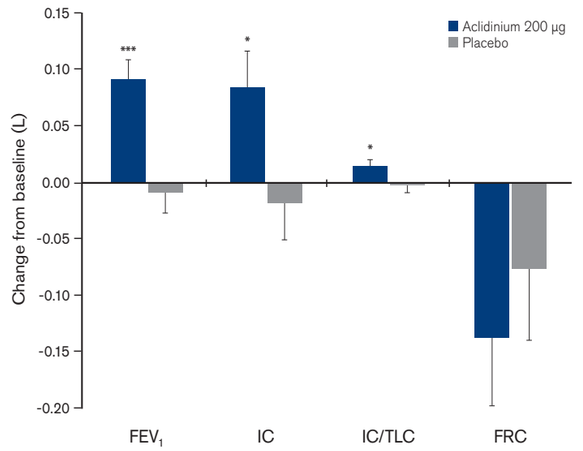
Figure 1. Exercise endurance



Data reported as mean change from baseline±SE.
**p≤0.005, ***p≤0.001 vs placebo
Change in endurance time (ET) measured at 180±10 minutes post-dose. ET was defined as the time from increase in work rate at 75% W_{max} to point of symptom limitation.

- Treatment differences in trough FEV_1, IC, and IC/TLC were significantly higher for aclidinium over placebo at Week 6 (Figure 2); similar results were observed at Week 3.
- Trough FRC showed a trend towards improvement between aclidinium versus placebo at Week 6 but did not reach statistical significance; similar changes were observed at Week 3.

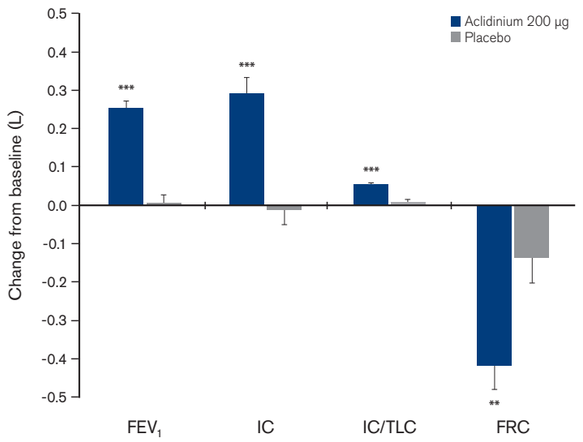
Figure 2. Resting lung function and lung volume at Week 6 (trough)



Data reported as mean change from baseline±SE.
*p<0.05, ***p<0.0001 vs placebo

- Two hours post-administration of aclidinium, changes from baseline in FEV_1, IC, IC/TLC and FRC were also significantly greater in patients treated with aclidinium compared with placebo at Week 6 (Figure 3); similar results were observed at Day 1 and Week 3.
- Significant improvements in dyspnea were observed with aclidinium vs placebo at Weeks 3 and 6, respectively, with placebo-adjusted improvements in mean TDI scores of 1.19 (p=0.005) and 1.71 units (p=0.0004), respectively.

Figure 3. Resting lung function and lung volume at Week 6 (2 hrs post-dose)



Data reported as mean change from baseline±SE.
**p<0.01, ***p<0.0001 vs placebo

- Mean changes from baseline in IC at various time points during cycle ergometry were higher for aclidinium than placebo at Week 6, with significant differences between treatment groups in favor of aclidinium at Day 1 and Week 3 (p<0.05 for both, Table 2).
- Exertional dyspnea at isotime was significantly improved with aclidinium over placebo (>1 Borg unit) on Day 1 and Week 3 (p<0.0001 for both); leg discomfort was also diminished at isotime at Day 1 (p<0.01) and Week 3 (p<0.05), and at peak at Week 6 (p<0.05; Table 2).
- Overall, improvements in exercise measures were apparent as early as Day 1 in patients treated with aclidinium.

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

	Day 1		Week 3		Week 6	
	Aclidinium	Placebo	Aclidinium	Placebo	Aclidinium	Placebo
IC, L						
Rest	0.26 (0.04)***	-0.01 (0.04)	0.24 (0.04)***	0.01 (0.04)	0.13 (0.05)	0.10 (0.06)
Isotime	0.26 (0.04)***	0.02 (0.04)	0.23 (0.04)**	0.06 (0.04)	0.15 (0.05)	0.10 (0.05)
Peak	0.24 (0.04)***	-0.02 (0.04)	0.18 (0.04)*	0.07 (0.04)	0.09 (0.05)	0.08 (0.05)
Dyspnea, Borg						
Rest	-0.03 (0.08)	0.12 (0.08)	0.02 (0.09)	0.15 (0.09)	0.01 (0.07)	-0.01 (0.07)
Isotime	-0.58 (0.22)***	0.56 (0.21)	-0.77 (0.21)***	0.43 (0.21)	-0.59 (0.22)*	0.11 (0.22)
Peak	0.28 (0.20)	0.44 (0.19)	-0.28 (0.22)*	0.37 (0.23)	-0.07 (0.20)	-0.03 (0.21)

Data reported as mean (SE).
*p<0.05, **p<0.01, ***p<0.001 vs placebo; †Day -10 data were used for baseline values if Day -5 data were not available.
IC, inspiratory capacity; ITT, intent-to-treat population; SE, standard error

Safety and Tolerability

- The overall incidence of treatment-emergent AEs (TEAEs) was comparable between the aclidinium group (57.0%) and the placebo group (46.3%); the majority of TEAEs were mild or moderate in severity.
- The most common AEs (reported by >5% of patients) in the aclidinium and placebo groups, respectively, were headache (5.8% and 7.4%), cough (5.8% and 3.2%), upper respiratory tract infection (5.8% and 2.1%), and COPD exacerbation (2.3% and 7.4%).
- No significant changes were seen in vital signs, 12-lead ECGs, or laboratory parameters in either group.

Conclusions

- Treatment with aclidinium 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 aclidinium results in rapid and significant bronchodilation, and reductions in static lung hyperinflation and dyspnea.
- Aclidinium was safe and well tolerated and may be a valuable new option for the treatment of COPD.

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

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Safety and Tolerability Of Aclidinium Administered Intravenously And Absolute Bioavailability Of Inhaled Aclidinium In Healthy Subjects

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Introduction

- Aclidinium bromide is a novel, long-acting muscarinic antagonist currently in Phase III development as a maintenance treatment for COPD.
- Aclidinium bromide exhibits long residence time at M_3 receptors, with a shorter residence time at M_2 receptors, and is rapidly hydrolyzed in human plasma to two inactive metabolites.[1-4]
- Several clinical studies in patients with COPD have demonstrated sustained bronchodilation with aclidinium bromide and a favorable tolerability profile.[5,6] Aclidinium bromide at single doses up to 6000 µg via dry powder inhaler was well tolerated and did not establish the maximum tolerated dose.[2]

Objective

- Part I: To evaluate the safety and tolerability of single ascending doses of IV aclidinium bromide in healthy male subjects.
- Part II: To estimate the absolute bioavailability of aclidinium bromide administered by inhalation via the Genuair® inhaler.

Methods

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 I (n=12) 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 (n=12) 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).
  - Subjects were randomized to 1 of 2 treatment groups (Group C or D) and participated in 2 treatment periods
  - Aclidinium bromide was administered by IV infusion over 5 minutes (Period I) and as a single inhalation (Period II).
- Subjects resided at the clinical research unit from the morning prior to dosing (Day -1) to the morning of Day 3 (48 hours post-dose) of each treatment period. All subjects were assessed via telephone 7 days after study completion.

Table 1. Study design and dosing regimen

A. Part I

	Period I	Period II	Period III
Treatment Group A (n=6)	IV aclidinium bromide 25 µg (n=4), IV placebo (n=2)	IV aclidinium bromide 100 µg (n=4), IV placebo (n=2)	IV aclidinium bromide 300 µg (n=4), IV placebo (n=2)
Treatment Group B (n=6)	IV aclidinium bromide 50 µg (n=4), IV placebo (n=2)	IV aclidinium bromide 200 µg (n=4), IV placebo (n=2)	IV aclidinium bromide 400 µg (n=4), IV placebo (n=2)

B. Part II

	Period I	Period II
Treatment Group C (n=6)	Inhaled aclidinium bromide 200 µg	IV aclidinium bromide 200 µg
Treatment Group D (n=6)	IV aclidinium bromide 200 µg	Inhaled aclidinium bromide 200 µg

All treatment periods were separated by washout periods of ≥7 days.

Study Population

Inclusion Criteria

- Healthy, non-smoking males aged 18 to 45 years, with normal physical examination, vital signs, pulse rate and blood pressure
- BMI between 18 kg/m^2 and 30 kg/m^2

Exclusion Criteria

- Evidence of sensitivity or allergic reaction to muscarinic antagonists
- Clinically significant or relevant cardiovascular conditions, laboratory tests, or electrocardiogram (ECG) parameters
- History of alcohol or substance abuse
- History of orthostatic hypotension, syncope, or vasovagal attacks
- Any clinical condition that may affect the absorption, distribution, metabolism or excretion of aclidinium bromide
- Concomitant medications

Pharmacokinetic Assessments

- Blood samples for pharmacokinetic assessments were taken pre-dose, at 5, 15, 30, 45 min, and at 1, 1.5, 2, 4, 6, 8, 10, 12, 16 (Part II only), 24 and 48 (Part II only) hours post-dose.
- Urine was collected in Part II after inhalation and intravenous administration at pre-dose and at 0-2, 2-4, 4-6, 6-8, 8-12, 12-16, 16-24, 24-36, and 36-48 hours post-dose for measurement of the urinary concentration of aclidinium and its metabolites.
- The lower limit of quantitation (LLOQ) for the plasma measurements was 5 pg/mL for aclidinium bromide, 5 pg/mL for the alcohol metabolite and 100 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 AEs, vital signs, ECGs, clinical laboratory tests, and physical examinations.
- The maximum tolerated dose was defined as the dose that caused function-limiting AEs in at least 50% of subjects, or the dose level that elicited a medically unacceptable, drug-related serious AE in ≥1 subjects.

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 $AUC_{0\text{-}t}$, $AUC_{0\text{-}\infty}$, $AUC_{0\text{-}6h}$, C_{max}, t_{max}, and $t_{1/2}$; C_L and V_z for aclidinium bromide and its two inactive metabolites (acid metabolite and alcohol metabolite); and absolute bioavailability (F) for aclidinium bromide.
- Urinary pharmacokinetic calculations included amount of aclidinium, acid- or alcohol-metabolite excreted in urine, percentage of dose excreted in urine (fe), and renal clearance (CL_R).
- Descriptive statistical analysis of demographic and safety data were performed on the safety population, defined as all subjects who received one dose of aclidinium.

Results

Baseline Characteristics

- A total of 24 subjects were randomized, and 22 completed the study. The mean age (±SD) of all subjects was 29 ± 6.6 (Part I) and 31 ± 8.8 (Part II).
- Baseline demographics and clinical characteristics were similar between treatment groups.

Part I

Plasma Pharmacokinetics Of Aclidinium Following IV Administration

- Maximum plasma concentration (C_{max}) occurred at a median t_{max} of 5-6 minutes (0.08-0.11 hours) after the start of the IV infusion at each dose level (Table 2).
- After C_{max}, plasma levels of aclidinium declined rapidly at all dose levels; most concentrations measured beyond 45 minutes after IV start were close to the LLOQ (5.0 pg/mL).
- Exposure ($AUC_{0\text{-}t}$) and maximum systemic exposure (C_{max}) increased proportionally with increasing dose in the 50 to 300 µg range, and appeared to plateau at the 300 µg IV dose.

Table 2. Mean plasma pharmacokinetic parameters of aclidinium bromide following single IV administration: Part I PK analysis population

Parameter	Aclidinium Bromide (IV) 25 µg (n=3)	50 µg (n=4)	100 µg (n=4)	200 µg (n=4)	300 µg (n=3)	400 µg (n=4)
$AUC_{0\text{-}t}$ (pg.h/mL)	247.5 (37.1)	307.9 (36.0)	857.5 (25.2)	1537.7 (36.8)	2285.8 (21.0)	2365.9 (29.7)
$AUC_{0\text{-}\infty}$ (pg.h/mL)	NC	NC	NC	1545.4 (37.0)	2296.1 (21.1)	2539.2 (30.2)[a]
C_{max} (pg/mL)	1536.3 (36.6)	2009.0 (34.7)	5873.3 (26.8)	10605.9 (32.4)	15363.2 (27.4)	17282.4 (30.4)
t_{max} (h)	0.08 (0.08-0.13)	0.10 (0.10-0.17)	0.11 (0.08-0.15)	0.08 (0.08-0.08)	0.10 (0.08-0.10)	0.08 (0.08-0.08)
$t_{1/2}$ (h)	NC	NC	NC	0.83 (80.7)	1.02 (80.6)	1.35 (92.2)[a]
λ_z (1/h)	NC	NC	NC	1.83 (114.7)	0.98 (57.1)	0.81 (60.2)[a]
CL (L/h)	NC	NC	NC	140.4 (28.4)	135.2 (23.9)	169.7 (35.7)[a]
Vz (L)	NC	NC	NC	140.0 (56.0)	185.3 (66.6)	302.4 (79.1)[a]

Arithmetic mean (CV%) data are presented for all parameters with the exception of t_{max} for which median (min-max) are presented.
IV, intravenous; NC, not calculated; PK, pharmacokinetic
[a]n=3

Plasma Pharmacokinetics Of Acid And Alcohol Metabolites Following IV Aclidinium Administration

- Following IV aclidinium, maximum concentrations of the acid metabolite appeared slightly later (mean t_{max}: 8-15 minutes) than for aclidinium bromide; median t_{max} for the alcohol metabolite was 5-6 minutes (Table 3).
- The mean apparent elimination half-life of the acid metabolite was similar across the 50 to 400 µg IV aclidinium bromide dose range ($t_{1/2}$ range: 3.50-4.22 hours); the elimination half-life across the same range was shorter ($t_{1/2}$ range: 1.10-2.76 hours) for the alcohol metabolite (Table 3).

Table 3. Mean plasma pharmacokinetic parameters of acid and alcohol metabolites: Part I PK analysis population

Parameter	Aclidinium Bromide (IV) 25 µg (n=3)	50 µg (n=4)	100 µg (n=4)	200 µg (n=4)	300 µg (n=3)	400 µg (n=4)
Acid Metabolite						
$AUC_{0\text{-}t}$ (pg.h/mL)	819.7 (16.5)	3215.5 (22.2)	6787.0 (7.1)	14180.9 (19.0)	19453.4 (23.2)	27806.7 (22.9)
$AUC_{0\text{-}\infty}$ (pg.h/mL)	1207.0 (11.0)	3798.5 (19.3)	7463.2 (7.5)	15692.5 (18.0)	21766.1 (21.9)	30510.5 (21.1)
C_{max} (pg/mL)	495.9 (12.4)	1432.4 (16.9)	2662.4 (20.5)	6255.7 (8.7)	8801.4 (24.0)	11218.5 (15.3)
t_{max} (h)	0.25 (0.13-0.25)	0.13 (0.10-0.25)	0.13 (0.10-0.27)	0.25 (0.25-0.25)	0.25 (0.10-0.28)	0.25 (0.25-0.25)
$t_{1/2}$ (h)	2.27 (10.2)	3.50 (27.3)	3.62 (7.8)	3.90 (14.4)	4.04 (12.4)	4.22 (17.0)
λ_z (1/h)	0.31 (9.8)	0.21 (26.4)	0.19 (7.8)	0.18 (14.1)	0.17 (11.6)	0.17 (16.8)
Alcohol Metabolite						
$AUC_{0\text{-}t}$ (pg.h/mL)	137.5 (39.9)	330.5 (28.7)	656.0 (16.3)	1460.3 (14.7)	2156.8 (21.4)	2646.1 (17.8)
$AUC_{0\text{-}\infty}$ (pg.h/mL)	NC	340.2 (28.3)	711.5 (13.6)[a]	1485.1 (14.5)	2180.7 (20.9)	2675.4 (17.8)
C_{max} (pg/mL)	377.8 (32.4)	1090.4 (20.3)	2178.3 (25.0)	4877.7 (16.7)	7244.5 (47.2)	9737.7 (21.7)
t_{max} (h)	0.08 (0.08-0.13)	0.10 (0.10-0.17)	0.11 (0.08-0.15)	0.08 (0.08-0.08)	0.10 (0.08-0.10)	0.08 (0.08-0.08)
$t_{1/2}$ (h)	NC	1.10 (14.2)	2.17 (24.5)[a]	2.79 (17.7)	2.48 (18.0)	2.76 (12.2)
λ_z (1/h)	NC	0.64 (14.8)	0.33 (23.0)[a]	0.25 (18.6)	0.29 (19.0)	0.25 (12.2)

Arithmetic mean (CV%) data are presented for all parameters with the exception of t_{max} for which median (min-max) are presented.
IV, intravenous; NC, not calculated; PK, pharmacokinetic
[a]n=3

Part II

Plasma Pharmacokinetics Of Aclidinium Following Inhalation And IV Administration

- The absolute bioavailability of inhaled aclidinium 200 µg was low at a mean of <5% (Table 4), with values for individual subjects ranging from 1.6% to 9.1%.
- Aclidinium appeared rapidly in plasma following aclidinium 200 µg inhalation, with maximum plasma concentrations occurring at a median t_{max} 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).

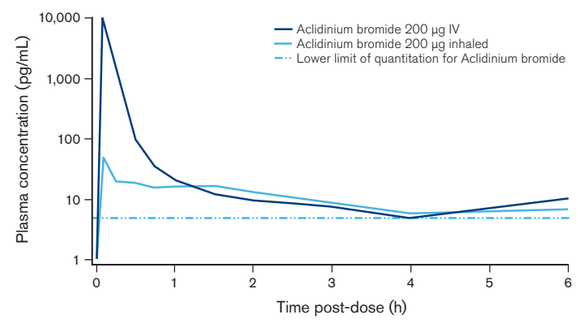
Table 4. Mean plasma and urinary pharmacokinetic parameters of aclidinium bromide: Part II PK analysis population

Parameter	Aclidinium Bromide 200 µg (Inhaled) (n=12)	200 µg (IV) (n=12)
F (%)[a]	4.37 (52.1)	NC
$AUC_{0\text{-}6}$ (pg.h/mL)	71.1 (52.8)	1744.1 (40.4)
$AUC_{0\text{-}\infty}$ (pg.h/mL)	76.8 (43.5)[b]	1816.3 (53.7)[c]
C_{max} (pg/mL)	52.7 (63.8)	9882.4 (39.4)
t_{max} (h)	0.09 (0.05-0.35)	0.10 (0.08-0.13)
$t_{1/2}$ (h)	2.64 (43.8)[b]	0.68 (129.3)[c]
λ_z (1/h)	0.30 (40.0)[b]	2.62 (63.4)[c]
CL (L/h)	NC	146.2 (59.8)[c]
Vz (L)	NC	94.9 (80.9)[c]
Ae (ng)	329.5 (33.7)	5448.0 (19.5)
fe (%)	0.16 (33.7)	2.72 (19.5)
CL_R (mL/min)[a]	87.0 (39.4)	60.3 (43.7)

Arithmetic mean (CV%) data are presented for all parameters with the exception of t_{max}, for which median (min-max) are presented.
Ae, amount of unchanged drug excreted in urine; CL_R, renal clearance; fe, dose excreted in urine; IV, intravenous; NC, not calculated; PK, pharmacokinetic.
[a]Based on $AUC_{0\text{-}t}$; [b]n=5; [c]n=6

- Aclidinium bromide concentrations declined rapidly in plasma following aclidinium inhalation (Figure 1); most concentrations measured beyond ~1 hour were close to the LLOQ.

Figure 1. Mean plasma concentration of aclidinium bromide (semi-logarithmic scale): Part II PK analysis population

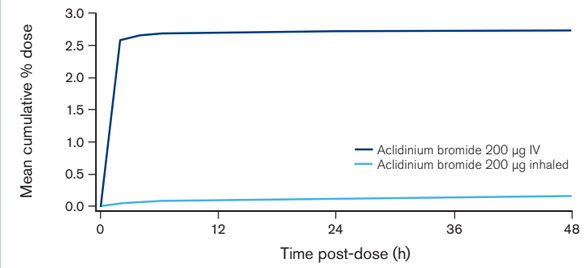


IV, intravenous; PK, pharmacokinetic

Urinary Pharmacokinetics Of Aclidinium Bromide

- The amount of unchanged aclidinium excreted in urine was low when measured up to 48 hours post-dose for both inhaled aclidinium (0.16%) and IV aclidinium (2.72%), a 17-fold difference (Figure 2).
- Excretion of the acid metabolite in urine was moderate when measured up to 48 hours post-dose after inhalation (16%) and IV administration (26%) of aclidinium.
- Excretion of the alcohol metabolite in urine following inhalation of aclidinium was approximately one third that of IV administration (8% vs 25%, respectively).
- Renal clearance of aclidinium and the acid metabolite was similar for the two aclidinium formulations; the alcohol metabolite was 2.5-3.3 times higher following aclidinium inhalation vs IV administration.

Figure 1. Mean cumulative urinary excretion (%) of aclidinium bromide: Part II PK analysis population



PK, pharmacokinetic

Safety

- The MTD was not determined in this study.
- There were no clinically relevant changes in laboratory values, vital signs, or ECG parameters.
- Of the 24 subjects in the safety population, 20 reported 51 TEAEs mild to moderate in intensity; none were serious or led to discontinuation. 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).
- There was no dose relationship in the frequency or kinds of AEs in Part I. The IV dose of aclidinium bromide 200 µg led to a higher incidence of AEs compared to the inhalation dose in Part II (66.6% vs 49.9%).
- Despite the high dose of aclidinium bromide administered in Part I, no anticholinergic AEs were reported.

Conclusions

- Absolute bioavailability of aclidinium was low (<5%) following a single 200 µg inhaled dose.
- Aclidinium appeared rapidly in plasma following inhalation and was rapidly cleared from the body after both inhaled and IV administration.
- Urinary excretion of unchanged aclidinium was very low (0.16% for inhaled and 2.72% 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 pharmacokinetic profile and a low potential for adverse events associated with systemic exposure.

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Pharmacokinetics Of Aclidinium Bromide 200 µg And 400 µg In Young And Elderly Patients With Chronic Obstructive Pulmonary Disease

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Introduction

- Chronic obstructive pulmonary disease (COPD) has an increased prevalence in the elderly population.[1] Elderly patients are also at greater risk of other conditions including kidney failure and renal impairment;[2] therefore, concomitant disorders should be considered in the pharmacologic management of COPD.
- Aclidinium bromide is a novel, long-acting muscarinic antagonist currently in clinical development for the maintenance treatment of COPD.
- In clinical studies, aclidinium has demonstrated long-lasting bronchodilatory activity and a favorable safety profile.[3,4] Moreover, preclinical and clinical studies have shown that aclidinium is rapidly hydrolyzed in human plasma to two major inactive alcohol and acid metabolites, suggesting a reduced potential for systemic side effects.[5,6]
- The aim of this study was to assess the pharmacokinetic profile of aclidinium (200 µg and 400 µg) and its two metabolites after a single dose and at steady state in young and elderly patients with stable moderate to severe COPD.

Methods

Study Design And Treatment

- This was a Phase I, open-label, two-period, multiple-dose study in young (aged 40–59 years) and elderly (aged ⩾70 years) patients with stable moderate to severe COPD.
- Patients received once-daily aclidinium 200 µg (single inhalation) for three days. Following a seven-day washout period, patients then received once-daily aclidinium 400 µg (two consecutive 200 µg inhalations) for three days.
- Aclidinium was administered via the Genuair®* inhaler, a novel, multidose dry powder inhaler.

Assessments

- On Days 1 and 3 of treatment with aclidinium 200 µg and 400 µg, blood samples were obtained pre-dose and at 5, 15, and 30 minutes, and 1, 1.5, 2, 4, 6, 8, 12, 16, and 24 hours post-dose. Blood samples were also taken at 36 and 48 hours post-dose on Day 3.
- Urine samples were collected pre-dose on Day 1; at 0–2, 2–4, 4–6, 6–8, 8–12, and 12–24 hours post-dose on Days 1 and 3; and at 24–36 and 36–48 hours post-dose on Day 3 of treatment with aclidinium 200 µg and 400 µg.
- Determination of aclidinium and its metabolites in plasma and urine was performed by liquid chromatography tandem mass spectrometry. These assays were fully validated, achieving a lower limit of quantification in plasma of 5 pg/mL for aclidinium and the alcohol metabolite, and 100 pg/mL for the acid metabolite. In urine, the lower limit of quantification was 0.2 ng/mL for aclidinium, 0.25 ng/mL for the alcohol metabolite, and 2 ng/mL for the acid metabolite.
- Safety assessments included monitoring of adverse events (AEs), physical examination, vital signs, 12-lead electrocardiograms (ECGs), and laboratory data.

Statistical Analysis

- Pharmacokinetic parameters for aclidinium and its two main metabolites were calculated after a single administration (Day 1) and at steady state (Day 3). Measurements included: area under the concentration time curve from time zero to infinity (AUC), area under the concentration time curve over a 24-hour dosing interval (AUC_{τ}), maximum observed plasma concentration (C_{max}), minimum observed plasma concentration (C_{min}), average plasma concentration at steady state (C_{av}^{ss}), time of maximum observed plasma concentration (t_{max}), apparent plasma terminal elimination half-life ($t_{1/2}$), renal clearance (CL_R), 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 recruited into 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 appeared rapidly in the plasma with a median t_{max} between 10 and 15 minutes; following C_{max} plasma levels of aclidinium declined rapidly with $t_{1/2}$ values between 1 and 3 hours (Table 2). The plasma exposure at the 400 µg inhaled dose was approximately two-fold higher than the 200 µg dose in all patients at both Days 1 and 3 (Table 2).
- The urinary excretion of aclidinium over 24 hours was very low at <0.15% of the dose for young and elderly patients at each dose and day (Table 2). The mean CL_R of aclidinium was greater in young (47 to 51 mL/min) versus elderly (35 to 39 mL/min) patients at each dose and day (Table 2).

Metabolites

- Maximum concentrations of the two metabolites occurred later than aclidinium, with a median t_{max} across both age groups, dose levels, and days between 15 minutes and 1.5 hours for the alcohol metabolite, and 4 hours for the acid metabolite (Table 3). The plasma exposure of the two metabolites was greater than that of aclidinium and was generally greater in elderly compared with young patients for each dose and day (Table 3).
- Urinary excretion over 24 hours was greater for the two metabolites than for aclidinium, ranging from 3.7% to 6.1% of the dose for the alcohol metabolite, and 9.3% to 13.2% of the dose for the acid metabolite, for young and elderly patients across each dose and day (Table 3).

Safety

- There was a low incidence of AEs for both age groups at both dose levels (Table 4). The majority of AEs were of mild to moderate intensity.
- No serious AEs or deaths occurred during the study.
- There were no clinically relevant changes on physical examination, vital signs, ECG parameters, or laboratory assessments across the age and dose groups.

Table 1. Baseline demographic and clinical characteristics

	Young patients (n=12)	Elderly patients (n=12)	All patients (n=24)
Age, mean (SD) years	53 (5.1)	73 (2.9)	63 (10.9)
Male, n (%)	6 (50.0)	10 (83.3)	16 (66.7)
Caucasian, n (%)	12 (100)	12 (100)	24 (100)
BMI, mean (SD) kg/m²	26.0 (4.82)	26.3 (4.05)	26.1 (4.35)
Current smoker, n (%)	10 (83.3)	4 (33.3)	14 (58.3)
Smoking history, mean (SD) pack-years	55.1 (27.90)	69.4 (42.40)	62.3 (35.86)
Pre-bronchodilator FEV_1, mean (SD) L	1.46 (0.57)	1.31 (0.38)	1.39 (0.48)
Post-bronchodilator FEV_1, mean (SD) % of predicted value	55.2 (14.75)	57.0 (13.43)	56.1 (13.83)
Post-bronchodilator FEV_1/FVC ratio, mean (SD) %	47.5 (11.71)	50.2 (12.1)	48.8 (11.77)
Creatinine clearance, mean (SD) mL/min	111.5 (35.3)	79.3 (26.1)	95.4 (34.5)

BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; SD, standard deviation

Figure 1. Mean plasma concentration time profiles following an inhaled dose of aclidinium 200 µg on Days 1 and 3 in young and elderly patients with COPD

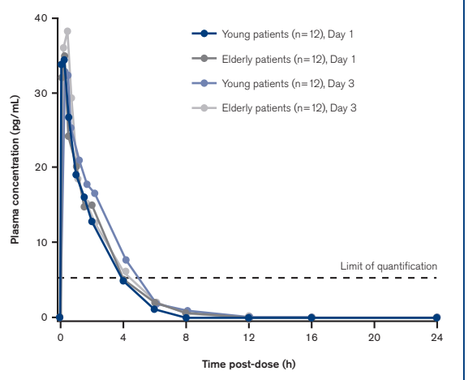

Figure 2. Mean plasma concentration time profiles following an inhaled dose of aclidinium 400 µg on Days 1 and 3 in young and elderly patients with COPD

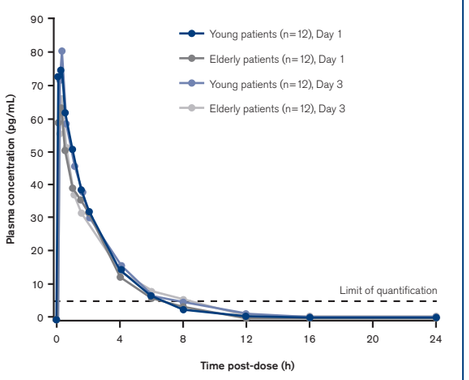

Table 2. Pharmacokinetic parameters for aclidinium after a single dose (Day 1) and at steady state (Day 3) in young and elderly patients with COPD

	Aclidinium dose	Young patients (n=12)		Elderly patients (n=12)	
		Day 1	Day 3	Day 1	Day 3
Plasma					
Day 1 AUC, pg·h/mL	200 µg 400 µg	79.9 (48.6) 193.9 (51.7)	NA NA	97.3 (67.4) 192.6 (48.0)	NA NA
Day 1 AUC_{τ}; Day 3 AUC_{τ}^{ss}, pg·h/mL	200 µg 400 µg	75.4 (49.0) 193.5 (51.4)	103.1 (45.4) 199.6 (36.4)	92.0 (69.5) 171.3 (56.7)	94.7 (37.5) 191.1 (47.4)
Day 1 C_{max}; Day 3 C_{max}^{ss}, pg/mL	200 µg 400 µg	39.0 (46.2) 82.3 (32.0)	37.8 (44.1) 86.1 (31.6)	38.3 (62.4) 71.1 (58.2)	40.1 (56.0) 67.8 (44.7)
Day 2 C_{min}; Day 3 C_{min}^{ss}, pg/mL	200 µg 400 µg	<5.00 (0.00) <5.00 (0.00)	<5.00 (0.00) <5.00 (0.00)	<5.00 (0.00) <5.00 (0.00)	<5.00 (0.00) <5.00 (29.86)
Day 3 C_{av}^{ss}, pg/mL	200 µg 400 µg	NA NA	4.30 (45.40) 8.32 (36.40)	NA NA	3.94 (37.45) 7.96 (47.38)
t_{max}, h	200 µg 400 µg	0.17 (0.08–0.50) 0.17 (0.08–1.00)	0.19 (0.08–1.00) 0.25 (0.08–1.03)	0.25 (0.10–2.00) 0.25 (0.08–1.50)	0.25 (0.08–0.50) 0.25 (0.08–0.50)
$t_{1/2}$, h	200 µg 400 µg	1.71 (37.12) 1.92 (29.03)	2.20 (20.18) 2.31 (32.20)	1.73 (39.16) 1.97 (29.00)	2.26 (29.92) 3.16 (34.38)
Urine					
$fe_{(0\text{-}24h)}$%	200 µg 400 µg	0.10 (46.25) 0.12 (50.37)	0.14 (58.47) 0.13 (47.47)	0.07 (50.01) 0.08 (40.95)	0.09 (54.71) 0.09 (39.24)
CL_R, mL/min	200 µg 400 µg	50.4 (43.2) 47.1 (48.9)	50.6 (56.3) 50.2 (56.6)	37.2 (68.5) 38.9 (47.9)	37.2 (45.5) 34.9 (43.9)

Data reported as mean (coefficient of variation %) except for t_{max} which is reported as median (range)
AUC, area under the concentration time curve from time zero to infinity; AUC_{τ}, area under the concentration time curve over a 24-hour dosing interval; AUC_{τ}^{ss}, area under the concentration time curve over a 24-hour dosing interval at steady state; C_{max}, maximum observed plasma concentration; C_{max}^{ss}, maximum observed plasma concentration at steady state; C_{min}, minimum observed plasma concentration; C_{min}^{ss}, minimum observed plasma concentration at steady state; C_{av}^{ss}, average plasma concentration at steady state; t_{max}, time of maximum observed plasma concentration; $t_{1/2}$, apparent plasma terminal elimination half-life; CL_R, renal clearance; $fe_{(0\text{-}24h)}$, percentage of dose excreted in urine from 0 to 24 hours; NA, not available

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

	Aclidinium dose	Metabolite	Young patients (n=12)		Elderly patients (n=12)	
			Day 1	Day 3	Day 1	Day 3
Plasma						
Day 1 AUC, pg·h/mL	200 µg	Alcohol Acid	209.1 (64.8) 7819.8 (34.4)	NA NA	372.4 (79.9) 9123.8 (38.8)	NA NA
	400 µg	Alcohol Acid	380.5 (63.4) 16530 (40.6)	NA NA	733.8 (61.7) 21734 (29.9)	NA NA
Day 1 AUC_{τ}; Day 3 AUC_{τ}^{ss}, pg·h/mL	200 µg	Alcohol Acid	165.6 (59.7) 7369.8 (35.0)	216.8 (56.7) 8569.5 (52.6)	327.5 (65.2) 8479.9 (38.2)	366.4 (57.1) 11382 (40.8)
	400 µg	Alcohol Acid	382.8 (57.2) 15359 (41.6)	443.0 (41.3) 18196 (40.0)	628.7 (52.3) 20317 (30.2)	742.6 (59.4) 22458 (22.0)
Day 1 C_{max}; Day 3 C_{max}^{ss}, pg/mL	200 µg	Alcohol Acid	34.0 (58.9) 805.9 (36.2)	34.1 (41.5) 844.0 (57.4)	37.6 (46.3) 829.2 (30.4)	41.0 (59.9) 1029.5 (41.1)
	400 µg	Alcohol Acid	66.2 (43.3) 1669.8 (44.7)	61.4 (35.2) 1812.0 (48.5)	79.2 (51.8) 1942.6 (31.0)	80.5 (39.3) 2058.7 (26.3)
Day 2 C_{min}; Day 3 C_{min}^{ss}, pg/mL	200 µg	Alcohol Acid	<5.00 (0.00) <100.00 (33.61)	<5.00 (0.00) <100.00 (55.75)	<5.00 (107.53) <100.00 (58.73)	<5.00 (86.21) 100.18 (48.56)
	400 µg	Alcohol Acid	<5.00 (56.09) 128.20 (58.33)	<5.00 (46.55) 170.43 (43.50)	7.40 (108.72) 176.31 (35.79)	10.70 (105.92) 233.67 (32.66)
Day 3 C_{av}^{ss}, pg/mL	200 µg	Alcohol Acid	NA NA	9.03 (56.73) 357.06 (52.63)	NA NA	15.27 (57.08) 474.24 (40.75)
	400 µg	Alcohol Acid	NA NA	18.46 (41.32) 758.15 (40.03)	NA NA	30.94 (59.44) 935.74 (21.98)
t_{max}, h	200 µg	Alcohol Acid	1.00 (0.25–4.00) 4.00 (1.50–4.02)	0.50 (0.25–2.00) 4.00 (1.50–6.00)	0.50 (0.25–6.00) 4.00 (1.50–6.00)	1.25 (0.25–8.00) 4.00 (2.00–6.00)
	400 µg	Alcohol Acid	0.25 (0.25–2.00) 4.00 (1.50–4.00)	0.25 (0.25–4.00) 4.00 (1.50–6.00)	1.25 (0.25–8.00) 4.00 (2.00–8.00)	1.50 (0.25–6.00) 4.00 (2.00–6.00)
$t_{1/2}$, h	200 µg	Alcohol Acid	4.51 (79.53) 5.35 (28.5)	4.53 (42.24) 6.41 (37.78)	5.83 (41.99) 5.42 (32.50)	7.18 (46.35) 8.69 (54.64)
	400 µg	Alcohol Acid	4.66 (44.41) 5.86 (30.45)	8.16 (50.62) 10.21 (44.22)	7.77 (35.00) 5.43 (17.36)	11.66 (33.13) 11.58 (39.81)
Urine						
$fe_{(0\text{-}24h)}$, %	200 µg	Alcohol Acid	3.71 (31.48) 9.99 (41.86)	5.03 (52.68) 12.01 (60.92)	4.15 (57.04) 9.29 (48.27)	5.52 (58.55) 12.67 (43.08)
	400 µg	Alcohol Acid	4.37 (55.06) 11.48 (43.19)	4.98 (39.23) 12.85 (43.55)	4.69 (52.84) 11.20 (37.94)	6.09 (71.61) 13.16 (38.16)
CL_R, mL/min	200 µg	Alcohol Acid	499.0 (46.2) 23.0 (42.9)	558.9 (57.9) 22.5 (39.6)	279.6 (39.6) 18.2 (29.6)	319.4 (41.6) 20.6 (44.1)
	400 µg	Alcohol Acid	448.9 (42.0) 25.6 (34.1)	454.1 (48.5) 23.9 (30.9)	285.4 (30.8) 18.1 (25.0)	308.0 (34.2) 19.5 (30.3)

Data reported as mean (coefficient of variation %) except for t_{max} which is reported as median (range)
AUC, area under the concentration time curve from time zero to infinity; AUC_{τ}, area under the concentration time curve over a 24-hour dosing interval; AUC_{τ}^{ss}, area under the concentration time curve over a 24-hour dosing interval at steady state; C_{max}, maximum observed plasma concentration; C_{max}^{ss}, maximum observed plasma concentration at steady state; C_{min}, minimum observed plasma concentration; C_{min}^{ss}, minimum observed plasma concentration at steady state; C_{av}^{ss}, average plasma concentration at steady state; t_{max}, time of maximum observed plasma concentration; $t_{1/2}$, apparent plasma terminal elimination half-life; CL_R, renal clearance; $fe_{(0\text{-}24h)}$, percentage of dose excreted in urine from 0 to 24 hours; NA, not available

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

Adverse event (preferred term)	Aclidinium 200 µg		Aclidinium 400 µg	
	Young patients (n=12)	Elderly patients (n=12)	Young patients (n=12)	Elderly patients (n=12)
Any	13	4	13	4
Fatigue	3	2	9	1
Headache	3	1	1	2
Dyspnea	2	0	1	0

Conclusions

- Once-daily doses of aclidinium 200 µg and 400 µg (administered as two consecutive 200 µg inhalations) 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 after administration of 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 receptors and enzymes, including muscarinic receptors.[5]
- These findings suggest that no dose adjustment of aclidinium is required when treating elderly patients with COPD.

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

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Introduction

- Aclidinium bromide is an inhaled, novel, long-acting, muscarinic antagonist currently in Phase III development for the treatment of COPD.
- Preclinical studies have shown aclidinium bromide has longer residence time at M_3 receptors and shorter residence time at M_2 receptors.[1]
- Additionally, aclidinium bromide is rapidly hydrolyzed in human plasma to two inactive metabolites, limiting the potential for systemic exposure.[2-4]
- Clinical trials in patients with COPD have demonstrated sustained bronchodilation and a favorable tolerability profile.[5]

Objective

- To determine the rates and routes of aclidinium elimination and to identify and characterize its metabolites when administered as a single 400 µg intravenous dose to healthy male subjects.

Methods

Study Design

- This was a Phase I, single-center, open-label, mass balance study.
- Subjects (N=12) were randomized (1:1) to receive a single dose of aclidinium bromide as either 400 µg [phenyl-U-^{14}C]–aclidinium bromide or 400 µg [glycolyl-U-^{14}C] –aclidinium bromide; treatments contained approximately 40 µCi of radioactivity administered intravenously over a 5-minute period.
- The hydrolysis of [phenyl-U-^{14}C]–aclidinium bromide produces ^{14}C-labeled alcohol metabolite and unlabeled acid metabolite, whereas hydrolysis of [glycolyl-U-^{14}C] –aclidinium bromide produces ^{14}C-labeled acid metabolite and unlabeled alcohol metabolite. Thus, [phenyl-U-^{14}C]–aclidinium bromide and [glycolyl-U-^{14}C] –aclidinium bromide were administered to two different groups of healthy subjects in order to properly determine rates and routes of elimination.

Study Population

Inclusion Criteria

- Healthy, non-smoking males aged 18 to 45 years with normal physical examination, vital signs, pulse rate, and blood pressure
- BMI between 18 kg/m^2 and 30 kg/m^2

Exclusion Criteria

- Evidence of sensitivity or allergic reaction to muscarinic antagonists
- Clinically significant or relevant cardiovascular conditions, laboratory tests, or electrocardiogram (ECG) parameters
- History of alcohol or substance abuse
- History of orthostatic hypotension, syncope, or vasovagal attacks
- Any clinical condition that could affect the absorption, distribution, metabolism, or excretion of aclidinium
- Concomitant medications within 14 days prior to study drug administration

Assessments

- Blood samples were collected at baseline (pre-dose), 5, 15, 30, and 45 min, at 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48 hours and at 24-hour intervals thereafter up to 168 hours after the start of infusion.
- Urine was collected pre-dose, 0-2, 2-4, 4-6, 6-8, 8-12, 12-16, 16-24, 24-36, and 36-48 hours post-dose and into 24-hour samples thereafter up to 168 hours.
- Feces were collected in 24-hour intervals during 0-168 hours after dosing.
- Blood, plasma, urine, and feces samples were analyzed for radioactivity by HPLC and Liquid Scintillation Counting (LSC).
- Determination of aclidinium and its metabolites in plasma was performed using solid phase extraction (SPE) and liquid chromatography tandem mass spectrometry (LC-MS/MS).
- Safety and tolerability were assessed via adverse events (AEs), vital signs, 12-lead electrocardiograms (ECGs), physical examinations, and clinical laboratory tests.

Statistical Analysis

- Pharmacokinetic parameters were calculated using actual sampling times and were derived using noncompartmental analyses with WinNonlin software (version 4.1 or higher).
- The Pharmacokinetic Analysis Population was comprised of subjects in the Safety Population who received the full dose of study drug and completed the study.
- Rates and routes of aclidinium bromide elimination were assessed along with pharmacokinetic parameters (AUC_{0-t}, C_{max}, t_{max}, and $t_{½}$) for total radioactivity in plasma and whole blood; pharmacokinetic parameters for LAS34850 free acid (acid metabolite) and LAS34823 cation (alcohol metabolite) were assessed in plasma only.
- Because the LC-MS/MS method measured the unlabeled compound rather than the actual concentration of the compound (unlabeled + ^{14}C-labeled), correct analysis of concentrations and pharmacokinetic parameters were only possible for the acid metabolite following 400 µg [phenyl-U-^{14}C]–aclidinium bromide and for the alcohol metabolite following 400 µg [glycolyl-U-^{14}C]–aclidinium bromide.
- Descriptive statistical analysis of demographic and safety data was performed on the Safety Population (all subjects who received one dose of study treatment).

Results

Baseline Demographics

- A total of 12 subjects were randomized and completed the study. The mean age (±SD) of all subjects was 23 ± 4.1.
- Baseline demographics and clinical characteristics were similar between treatment groups.

Pharmacokinetic Parameters

Plasma

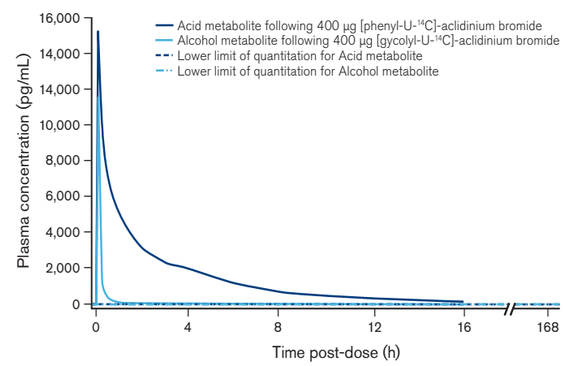
- Following IV administration of 400 mg [phenyl-U-^{14}C]– and [glycolyl-U-^{14}C]–aclidinium bromide, maximum concentrations of aclidinium bromide's two inactive metabolites (acid and alcohol metabolites, respectively) occurred at median t_{max} of approximately 5 minutes (Table 1).
- Plasma levels of the alcohol metabolite were quantifiable (≥5.0 pg/mL) for 16 hours after the start of IV administration; the plasma levels of the acid metabolite were quantifiable (≥100 pg/mL) for 8-16 hours post-dose (Figure 1).

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

	Acid Metabolite	Alcohol Metabolite
AUC_{0-t}, pg·hr/mL	26500 (11.1)	2340.4 (26.9)
C_{max}, pg/mL	14444 (19.4)	11460 (23.5)
t_{max}, h	0.09 (0.08-0.20)	0.08 (0.08-0.10)
$t_{½}$, h	3.41 (13.1)	2.70 (28.2)

Data are arithmetic mean (coefficient of variation, CV%) except for t_{max}, which is median (minimum-maximum).

Figure 1. Plasma concentration-time profile of aclidinium metabolites



- Plasma radioactivity AUC_{0-t} and C_{max} were higher following IV [glycolyl-U-^{14}C]–aclidinium bromide than IV [phenyl-U-^{14}C]–aclidinium bromide (increased 8.2- and 1.7-fold, respectively), indicating the clearance of the acid metabolite is probably slower than the clearance of the alcohol metabolite (Table 2).
- Total radioactivity in plasma was quantifiable (≥0.3 ng.eq/mL) for 12-16 hours following administration of [phenyl-U-^{14}C]–aclidinium bromide and 36-48 hours following [glycolyl-U-^{14}C]–aclidinium bromide.

Whole Blood

- Maximum whole blood concentrations of total radioactivity occurred at a median t_{max} of approximately 5 minutes for [phenyl-U-^{14}C]– and [glycolyl-U-^{14}C]–aclidinium bromide. AUC_{0-t} and C_{max} values for total radioactivity following [glycolyl-U-^{14}C]–aclidinium bromide were 20.3-fold and 2.0-fold higher, respectively, than those seen with [phenyl-U-^{14}C]–aclidinium bromide (Table 2).
- Total radioactivity was quantifiable (≥0.4 ng.eq/mL) for 1-2 hours following administration of [phenyl-U-^{14}C]–aclidinium bromide and 16-24 hours following [glycolyl-U-^{14}C]–aclidinium bromide.

Table 2. Pharmacokinetic parameters for total radioactivity

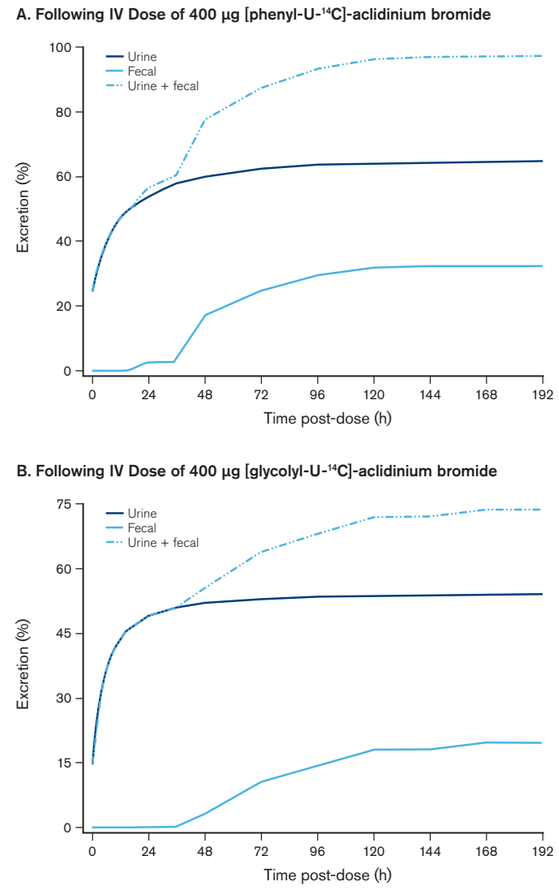
	[Phenyl-U-^{14}C]–aclidinium bromide 400 µg	[Glycolyl-U-^{14}C]–aclidinium bromide 400 µg
Plasma		
AUC_{0-t}, ng eq·h/mL	12.8 (20.3)	105.0 (10.3)
C_{max}, ng eq/mL	24.5 (48.6)	42.2 (18.5)
t_{max}, h	0.09 (0.08-0.20)	0.08 (0.08-0.10)
$t_{½}$, h	8.63 (28.5)	13.3 (22.2)
Whole Blood		
AUC_{0-t}, ng eq·h/mL	2.31 (26.9)	46.9 (14.4)
C_{max}, ng eq/mL	10.8 (53.5)	21.4 (15.1)
t_{max}, h	0.09 (0.08-0.20)	0.08 (0.08-0.10)
$t_{½}$, h	1.19 (82.4)	5.53 (13.3)

Data are arithmetic mean (CV%) except for t_{max}, which is median (minimum-maximum).

Mass Balance

- The predominant route of excretion for both treatments was renal (Figure 2).
- Most [phenyl-U-^{14}C]–associated radioactivity (93%) was recovered in the first 96 hours post-dose. Within 120 hours post-dose, most [glycolyl-U-^{14}C]–associated radioactivity (72%) was recovered (Figure 2).

Figure 2. Mean cumulative excretion of total radioactivity



IV, intravenous

Metabolite Profiles

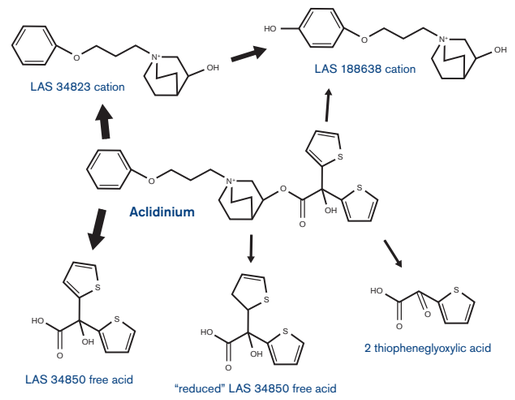
Excreta

- Metabolite profiles were obtained by analyzing urine samples from 0 to 24 hours and feces samples from 0 to 96 hours.
- Following IV [phenyl-U-^{14}C]–aclidinium bromide, 1.2% of the dose was excreted unchanged in urine, 41.8% as the p-hydroxy alcohol metabolite and 32.5% as alcohol metabolite. No other metabolites with a significant amount of radioactivity were observed.
- Following IV [glycolyl-U-^{14}C]–aclidinium bromide, 1.7% of the dose was excreted unchanged in urine, 39.5% as the acid metabolite, and 16.8% in equal parts 2-thiopheneglyoxylic acid, "reduced" LAS34850, and an as-yet-unknown species. No other metabolites with a significant amount of radioactivity were observed.
- The proposed metabolic pathway of aclidinium in healthy male subjects is shown in Figure 3.

Plasma

- The highest plasma concentrations of unchanged aclidinium (1.8-5.5 ng eq/mL), the alcohol metabolite (23.8 ng eq/mL), and the acid metabolite (36.4 ng eq/mL) were observed approximately 5 minutes post-dose. The plasma concentration of p-hydroxy LAS34823 metabolite was low and was only 0.045 ng eq/mL of aclidinium bromide. The "reduced" LAS34850 and the unknown metabolite were not detected in plasma.

Figure 3. The proposed metabolic pathway of aclidinium



Safety

- A total of 2 of 12 subjects reported 3 treatment-emergent AEs (TEAEs), which included infusion site pain and pain in an extremity; none were serious or led to discontinuation. All TEAEs were considered related to [glycolyl-U-^{14}C] treatment and were mild to moderate in intensity.
- No clinically relevant changes in laboratory values, vital signs, or ECG parameters were reported.

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

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In Vitro M_3/M_2 *Kinetic Selectivity Of Aclidinium 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 M_3 receptors. However, this effect is often accompanied by the blockade of other muscarinic receptor subtypes, particularly M_2 receptors.
- Kinetic selectivity for M_3 over M_2 receptors is desirable, since the M_2 subtype can down-regulate parasympathetic nerve activity.[1] Moreover, as blockade of M_2 receptors in cardiac muscle is associated with unwanted cardiovascular effects,[2] 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 residence time at M_3 receptors and a shorter residence time at M_2 receptors.[3] Furthermore, aclidinium has demonstrated a reduced potential for cardiovascular effects compared with tiotropium in animal models,[3,4] which may be due to its relatively faster dissociation rate from M_2 receptors[3] and rapid hydrolysis in plasma.[5]
- The aim of this study was to investigate the effects of glycopyrrolate on M_3 and M_2 *in vitro* systems, and compare the results with previously presented data for aclidinium, using tiotropium and ipratropium as comparators, under similar study conditions.[3,6]

Methods*

Affinity For Human M_1–M_5 Muscarinic Receptors

- The affinity (K_i) of each antagonist for muscarinic M_1–M_5 receptors was evaluated by displacement of 1-[N-methyl-^{3}H] scopolamine (^{3}H-NMS) binding to membrane preparations from cells expressing human muscarinic receptors.
- M_1, M_2, M_3, M_4, and M_5 receptor membrane preparations (protein concentrations 8.1, 10.0, 4.9, 4.5, and 5.0 µg/well, respectively) were incubated at room temperature with ^{3}H-NMS (0.3 nM for M_1 and M_4; 1 nM for M_2, M_3, and M_5) in the presence of a range of antagonist concentrations (10^{-5} to 10^{-14} M) or 1 µM atropine (to measure non-specific binding).
- After a 2- or 6-hour incubation period (M_1–M_4 and M_5, respectively) to ensure that equilibrium was achieved, bound ^{3}H-NMS was separated from free ^{3}H-NMS by rapid vacuum filtration, and radioactivity in the bound fraction was quantified using a scintillation counter.
- K_i values were calculated as described by Cheng and Prusoff.[7]

Dissociation From Human M_2 And M_3 Muscarinic Receptors

- Membranes expressing M_2 and M_3 receptors (final protein concentration 15 µg/mL) were incubated at room temperature with ^{3}H-aclidinium (2.5 nM), ^{3}H-glycopyrrolate (15 and 5 nM for M_2 and M_3, respectively), ^{3}H-tiotropium (2.5 nM), or ^{3}H-ipratropium (10 nM) for 135 minutes. These conditions allowed for the radioligands to reach equilibrium with approximately 90% occupancy of the binding sites.
- Atropine (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 ^{3}H-NMS that remained bound at different time points was determined by removing the free ^{3}H-NMS by rapid vacuum filtration and quantifying radioactivity using a scintillation counter.
- Dissociation half-lives ($t_{1/2}$) were calculated using one-phase exponential decay.[8]

Assessment Of Potency And Duration Of Action At M_2 And M_3 Receptors

M_2 Receptors

- Isolated guinea pig left-atria were suspended in an organ bath at 32°C and electrically stimulated to induce M_2 receptor-mediated contraction. Carbachol was added to the stimulated atria to inhibit electrically induced contractions via the M_2 receptor.
- Potency at M_2 receptors was assessed by adding increasing concentrations of aclidinium, glycopyrrolate, tiotropium, and ipratropium (0.01 to 1000 nM) to the carbachol (1 µM)-treated atria every 5 to 10 minutes.
- To measure duration of action at M_2 receptors, antagonists were added to the carbachol (10 µM)-treated atria at a concentration that inhibited 80% of the maximum carbachol-induced relaxation. When inhibition of tone was stable, the antagonists were washed out and the atria were incubated with carbachol (10 µM) for 240 minutes.

M_3 Receptors

- Isolated guinea pig trachea strips were mounted in a superfusion chamber at 37°C and electrically stimulated to induce M_3 receptor-mediated contraction.
- To assess potency at M_3 receptors, increasing concentrations of aclidinium, glycopyrrolate, tiotropium, and ipratropium (0.01 nM to 1 µM) were infused to the trachea strips every 30 minutes.
- Duration of action at M_3 receptors was examined by incubating stimulated trachea strips with each antagonist at a concentration producing 80–90% inhibition of the electrically stimulated contraction. After 45 minutes, the antagonist was washed out to allow the trachea strips to recover the electrically stimulated contraction.

Analysis

- The concentrations required for 50% inhibition (EC_{50}) of the electrically stimulated contraction (M_3 receptors) and maximum carbachol-induced relaxation (M_2 receptors) were calculated using non-linear regression analysis.
- The time taken to achieve 50% recovery ($t_{1/2}$) of tone (M_3 receptors) was calculated using non-linear regression analysis. The $t_{1/2}$ for recovery of the maximum carbachol-induced relaxation (M_2 receptors) was calculated using one-phase (aclidinium, glycopyrrolate, and tiotropium) or two-phase (ipratropium) exponential decay.

Results*

Affinity For Human M_1–M_5 Muscarinic Receptors

- At equilibrium, aclidinium 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 M_3 versus M_2 receptors; however, the magnitude of the effect was limited (approximately 3-fold; Table 1).

Table 1. Binding affinity of aclidinium, glycopyrrolate, tiotropium, and ipratropium for human M_1, M_2, M_3, M_4, and M_5 receptors

	K_i (nM)				
	M_1	M_2	M_3	M_4	M_5
Aclidinium	0.10 ± 0.00	0.14 ± 0.04	0.14 ± 0.02	0.21 ± 0.04	0.16 ± 0.01
Glycopyrrolate	0.42 ± 0.02	1.77 ± 0.06	0.52 ± 0.04	0.78 ± 0.04	1.29 ± 0.09
Tiotropium	0.13 ± 0.00	0.13 ± 0.04	0.19 ± 0.04	0.30 ± 0.09	0.18 ± 0.06
Ipratropium	1.31 ± 0.15	1.12 ± 0.13	1.24 ± 0.08	1.92 ± 0.18	3.22 ± 0.15

Data are reported as mean ± standard error of the mean; n=3
K_i, affinity

Dissociation From Human M_2 And M_3 Muscarinic Receptors

- Aclidinium dissociated more quickly from M_2 and M_3 receptors compared with tiotropium, and showed slower dissociation from these receptors than glycopyrrolate and ipratropium (Figures 1a and b; Table 2).
- The kinetic selectivity for M_3 over M_2 receptors (M_3/M_2 ratio) was of a similar magnitude for all compounds (Table 2).

Table 2. Dissociation half-lives of ^{3}H-aclidinium, ^{3}H-glycopyrrolate, ^{3}H-tiotropium, and ^{3}H-ipratropium from human M_2 and M_3 receptors

	M_2 $t_{1/2}$ (h)	M_3 $t_{1/2}$ (h)	Relative half-life at M_3 receptor	M_3/M_2 ratio
Aclidinium	4.69 ± 0.29	29.24 ± 0.61	62	6.2
Glycopyrrolate	1.07 ± 0.20	8.10 ± 0.45	17	7.3
Tiotropium	15.11 ± 1.57	62.19 ± 2.96	132	4.1
Ipratropium	0.08 ± 0.01	0.47 ± 0.02	1	5.9

Data are reported as mean ± standard error of the mean; n=3
$t_{1/2}$, dissociation half-life

Assessment Of Potency And Duration Of Action At M_2 And M_3 Receptors

M_2 Receptors

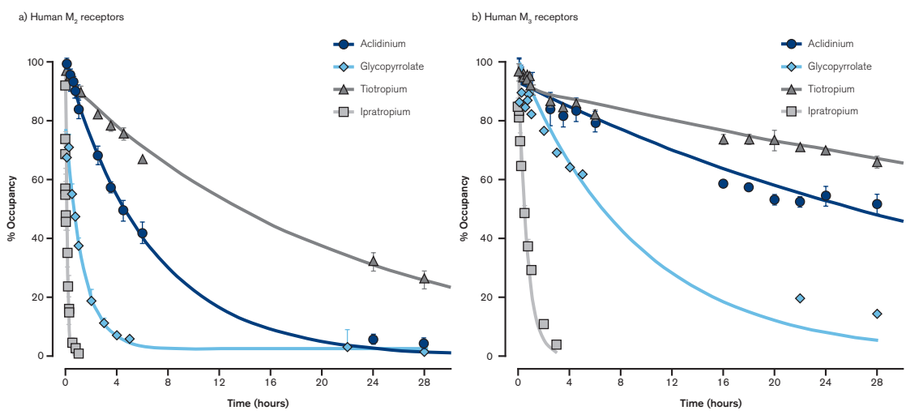
- In the isolated left-atria assay, tiotropium displayed greater potency at M_2 receptors compared with aclidinium, glycopyrrolate, and ipratropium, which all showed similar potency (Table 3).
- Glycopyrrolate had a shorter offset time at left-atria M_2 receptors compared with aclidinium and tiotropium, but a longer offset time compared with ipratropium (Table 3).

Table 3. *In vitro* potency and duration of action of aclidinium, glycopyrrolate, tiotropium, and ipratropium at M_2 receptors (guinea pig atria) and M_3 receptors (guinea pig trachea)

	M_2 receptors		M_3 receptors	
	EC_{50} (nM)[a]	$t_{1/2}$ (min)	EC_{50} (nM)[a]	$t_{1/2}$ (min)
Aclidinium	17.4 ± 1.1	102	5.3 ± 1.6	>480
Glycopyrrolate	17.3 ± 1.2	30	4.2 ± 0.3	>480
Tiotropium	11.8 ± 1.1	184	3.0 ± 0.6	>480
Ipratropium	19.9 ± 1.14	4	3.0 ± 0.4	42

[a]Data are reported as mean ± standard error of the mean; n=3–13
EC_{50}, concentrations required for 50% inhibition; $t_{1/2}$, dissociation half-life

Figure 1. Dissociation of ^{3}H-aclidinium, ^{3}H-glycopyrrolate, ^{3}H-tiotropium, and ^{3}H-ipratropium from human M_2 and M_3 receptors



M_3 Receptors

- All four muscarinic antagonists showed similar potency at M_3 receptors in the isolated trachea strip assay (Table 3).
- Aclidinium, glycopyrrolate, and tiotropium had a similar offset time at M_3 receptors, which was of greater magnitude than that observed for ipratropium (Table 3).

Conclusions

- Aclidinium, glycopyrrolate, tiotropium, and ipratropium are potent muscarinic antagonists that display similar kinetic selectivity for M_3 over M_2 receptors.
- In isolated organ studies, aclidinium and glycopyrrolate have similar relative potency at M_3 and M_2 receptors. Therefore, the slight preference for M_3 versus M_2 receptors observed for glycopyrrolate in the membrane binding experiments does not translate into improved relative potency at M_3 and M_2 receptors compared with aclidinium.
- Compared with the other antagonists tested, tiotropium has the greatest potency at M_2 receptors.
- Aclidinium and tiotropium have a longer dissociation half-life than glycopyrrolate from the M_3 receptor, suggesting a longer bronchodilatory effect in humans.

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Acknowledgements

This study was supported by Almirall S.A., Barcelona, Spain.
*Methods and results for aclidinium, tiotropium, and ipratropium are as previously reported.[3,6]

Almirall Forest Laboratories, Inc.

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In Vivo Studies Of Aclidinium Bromide And Glycopyrrolate To Assess Bronchodilatory Activity And Potential To Induce Dry Mouth

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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 M_3 receptors and a shorter residence time at M_2 receptors.[3] In addition, aclidinium is rapidly hydrolyzed in human plasma to two inactive metabolites. These features suggest aclidinium may provide sustained bronchodilation with a reduced potential for systemic anticholinergic effects.[3,4]
- 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.[3]
  - The second study investigated the effects of either glycopyrrolate or aclidinium on salivation in rats, using tiotropium as a comparator.[5]

Methods*

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

- Aclidinium, glycopyrrolate, tiotropium, 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.
- Bronchoconstriction 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 (EC_{50}) of bronchoconstriction, determined from a dose-response curve constructed using the inhibition values obtained at each of the time points studied. Onset of action (t_{max}) for each compound was defined as the time required to achieve the maximal potency. The duration of action ($t_{1/2}$), defined as time to achieve 50% recovery of a submaximal inhibitory concentration of antagonist, was derived from time-course bronchoconstriction 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 sialorrhea (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. ED_{50} values (the dose of test compound inhibiting pilocarpine-induced salivation in 50% of rats) were calculated by non-linear regression.

Results*

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

- Aclidinium, glycopyrrolate, and ipratropium achieved maximal inhibition of bronchoconstriction at 2 hours post-administration, compared with 4 hours for tiotropium (Table 1).[3]
- All four antagonists showed similar potency at the time of maximal effect (Table 1).
- The duration of action was longer for tiotropium ($t_{1/2}$ = 64 h) and aclidinium ($t_{1/2}$ = 29 h) compared with glycopyrrolate and ipratropium ($t_{1/2}$ = 13 h and $t_{1/2}$ = 8 h, respectively; Figure 1).[3]
- At the concentrations selected, all four compounds achieved an inhibitory effect on bronchoconstriction of 97–98% at 1 hour post-administration (Figure 1).

Study 2: Salivation Studies In Conscious Rats

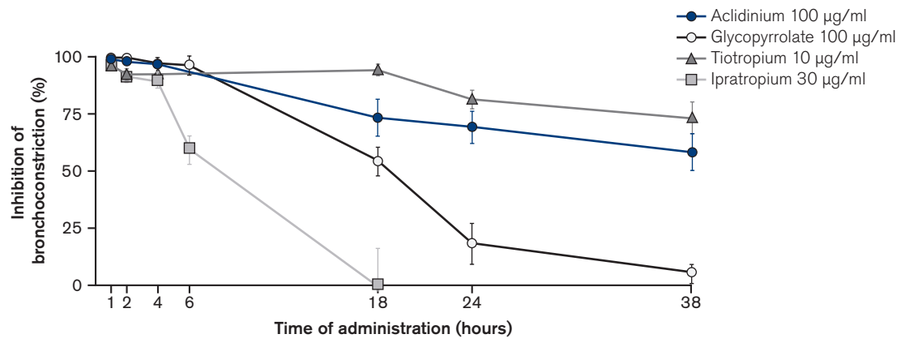
- Aclidinium inhibited pilocarpine-induced salivation to a lesser extent than tiotropium (ED_{50} [µg/kg] = 38 and 0.88, respectively; Figure 2a).[5]
- Inhibition of pilocarpine-induced salivation by glycopyrrolate was very similar to that of tiotropium (ED_{50} [µg/kg] = 0.74 and 0.72, respectively; Figure 2b).

Table 1. Onset of action and potency of aclidinium, glycopyrrolate, tiotropium, and ipratropium in reversing acetylcholine-induced bronchoconstriction in guinea pigs[3]

	EC_{50}, µg/ml (95% CI)				
	1 hour	2 hours	4 hours	18 hours	24 hours
Aclidinium	5.9 (3.7–9.4)	2.5 (1.7–3.5)	2.9 (1.8–4.7)	12.4 (4.1–37.6)	23.1 (9.3–57.3)
Glycopyrrolate	7.2 (4.1–12.8)	3.8 (2.5–5.7)	8.8 (5.2–14.8)	68.7 (39.6–119.2)	242.3 (162.0–362.2)
Tiotropium	2.4 (1.4–3.8)	3.9 (2.0–7.6)	1.4 (0.7–2.5)	1.4 (0.7–2.9)	3.3 (2.0–5.2)
Ipratropium	6.9 (4.0–11.7)	3.4 (1.9–5.9)	7.3 (4.0–13.4)	689.7 (337.1–1411.0)	NA

CI, confidence interval; EC_{50}, concentration required to induce 50% inhibition; NA, not available

Figure 1. Duration of action of aclidinium, glycopyrrolate, tiotropium, and ipratropium in reversing acetylcholine-induced bronchoconstriction in guinea pigs[3]



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

Figure 2a. Effects of aclidinium and tiotropium on pilocarpine-induced salivation in conscious male Wistar rats[5]

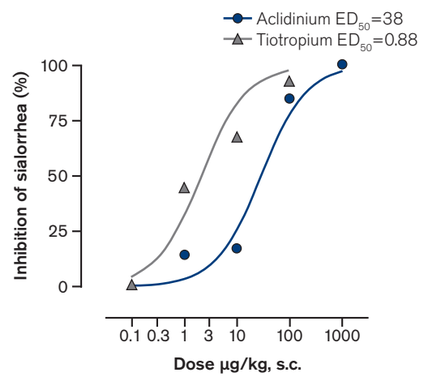
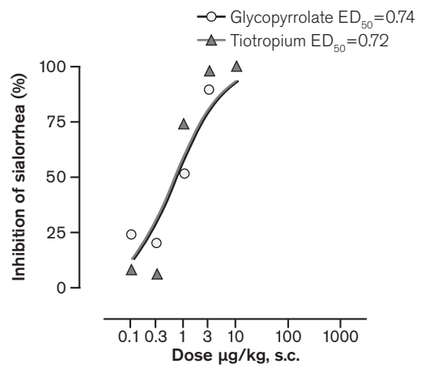


Figure 2b. Effects of glycopyrrolate and tiotropium on pilocarpine-induced salivation in conscious male Wistar rats



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 bronchoconstriction 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.

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Acknowledgements

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*Methods and results for aclidinium, tiotropium, and ipratropium are as previously reported.[3,5]

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Efficacy And Safety Of Single Inhaled Doses Of LAS100977, A Novel Long-Acting β_2-Agonist, In Patients With Persistent Asthma

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Introduction

- LAS100977 is a novel, potent, and selective long-acting β_2-agonist (LABA) 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 β_2-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]
- 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 screening, either alone or in combination with a short- or long-acting β_2-agonist
- A forced expiratory volume in one second (FEV_1) between 60% and 85% of the predicted normal post-bronchodilator value at screening
- FEV_1 reversibility ⩾12% and an absolute increase of at least 200 mL over baseline value following salbutamol inhalation
- Pre-dose FEV_1 of each treatment period within 80%–120% of pre-dose FEV_1 at screening

Exclusion Criteria

- History of smoking during previous 12 months and history of ⩾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 β_2-antagonists

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, patients were randomized 1:1:1:1:1 to LAS100977 once daily (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 medications and ICS treatments.
- LAS100977 doses (5, 10, or 25 µg) were administered in the morning as a single inhaled dose delivered via the Cyclohaler® device. Salmeterol was administered as two inhaled doses, one in the morning and one in the evening, via the Accuhaler® device. Corresponding placebo treatments were administered using the Cyclohaler® and Accuhaler® devices.
- Treatment periods lasted 36 hours, with a minimum seven-day washout period between consecutive treatments.

Assessments

- Lung function tests (spirometry and body plethysmography) were performed at 5, 15, and 30 minutes, and at 1, 2, 3, 4, 6, 8, 12, 14, 23, 24, and 36 hours post-dose.
- For spirometry, FEV_1, forced vital capacity (FVC), peak expiratory flow (PEF), and forced mid-expiratory flow (FEF_{25-75}) were determined.
- For body plethysmography, airway resistance (Raw) and specific airway conductance (sGaw) were measured.
- Safety assessments included adverse events (AEs), 12-lead electrocardiograms (ECGs), physical examinations, laboratory tests, and vital signs.

Study Endpoints

- The primary efficacy endpoint was change from baseline in trough FEV_1 after one day of treatment, expressed as the mean of the 23- and 24-hour post-administration values.
- Secondary endpoints included the change from baseline in FEV_1, FVC, PEF, and FEF_{25-75}, Raw and sGaw, all of which were measured at predetermined time points for 36 hours post-dose.
- Safety and tolerability assessments were analyzed descriptively.

Results

Subjects

- Overall, 25 eligible male patients with asthma were enrolled and randomized to one of the five treatment groups. Baseline patient demographics and other characteristics are shown in Table 1.

Table 1. Baseline demographics and other characteristics (n=25)

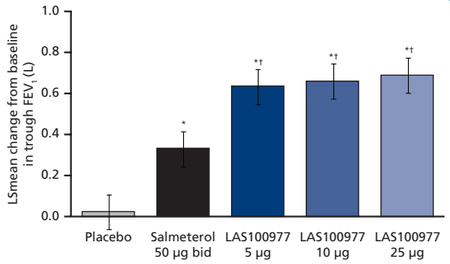
Age, mean (SD) years	44.2 (10.3)
BMI, mean (SD) kg/m²	27.1 (2.5)
Smoking consumption, n (%)	
Non-smokers	20 (80)
Ex-smokers (8–20 cigarettes/day)	5 (20)
Pulse rate, mean (SD) beats/min	62.9 (6.6)
FEV_1, mean (SD)	
Pre-bronchodilator (L)	2.90 (0.45)
Post-bronchodilator (L)	3.61 (0.60)
Percentage of predicted value	73.89 (6.75)
Reversibility* (%)	100

BMI, body mass index; FEV_1, forced expiratory volume in one second; SD, standard deviation
*Reversibility defined as ⩾12% increase in pre-bronchodilator FEV_1 and a minimum absolute increase of 200 mL

Efficacy

- Single inhaled doses of LAS100977 5 µg, 10 µg, and 25 µg induced significant increases in trough FEV_1 compared with placebo and salmeterol (Figure 1). There were no statistically significant differences between the effects of the three different LAS100977 doses on the trough FEV_1.
- At 5 minutes post-dose, LAS100977 (5 µg, 10 µg, and 25 µg) significantly improved lung function compared with placebo ($p<0.0001$) and salmeterol ($p<0.05$), and demonstrated a rapid onset of action from 0–60 minutes post-dose (Table 2 and Figure 2).
- At all doses tested, LAS100977 provided sustained bronchodilation for the entire duration of the study period. Increases in FEV_1 were observed at all time points and were significantly greater than placebo ($p<0.0001$) and salmeterol 50 µg bid ($p<0.05$; Figure 3).

Figure 1. LSmean change (SE) from baseline in trough FEV_1 (L) after one day of treatment



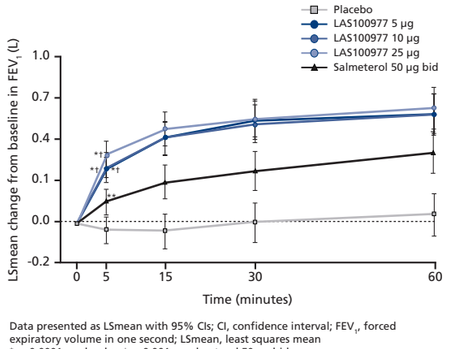
FEV_1, forced expiratory volume in one second; LSmean, least squares mean; SE, standard error
*$p<0.0001$ vs placebo; †$p<0.0001$ vs salmeterol 50 µg bid

Table 2. LSmean change (SE) from baseline in key spirometry measurements (L) 5 minutes post-dose

	Placebo (n=24)	Salmeterol 50 µg bid (n=24)	LAS100977 5 µg (n=24)	LAS100977 10 µg (n=24)	LAS100977 25 µg (n=24)
FEV_1	-0.032 (0.040)	0.135 (0.040)*†	0.338 (0.040)*†	0.367 (0.040)*†	0.434 (0.040)*†
FVC	-0.052 (0.045)	0.103 (0.045)	0.214 (0.045)*††	0.218 (0.045)*††	0.277 (0.045)*††
PEF	-0.069 (0.115)	0.194 (0.116)	0.771 (0.115)*†	0.785 (0.115)*†	0.968 (0.112)*†
FEF_{25-75}	0.035 (0.048)	0.105 (0.048)*†	0.335 (0.048)*†	0.381 (0.048)*†	0.485 (0.048)*†

FEF_{25-75}, forced mid-expiratory flow; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; LSmean, least squares mean; PEF, peak expiratory flow; SE, standard error
*$p<0.0001$ vs placebo; †$p<0.001$ vs placebo; *†$p<0.001$ vs salmeterol 50 µg bid; ††$p<0.05$ vs salmeterol 50 µg bid

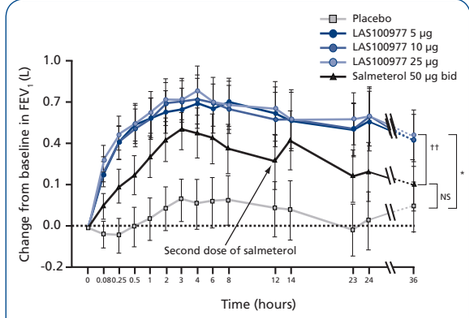
Figure 2. LSmean change (95% CI) from baseline in FEV_1 (L) 0–60 minutes post-dose



Data presented as LSmean with 95% CIs; CI, confidence interval; FEV_1, forced expiratory volume in one second; LSmean, least squares mean
*$p<0.0001$ vs placebo; †$p<0.001$ vs salmeterol 50 µg bid

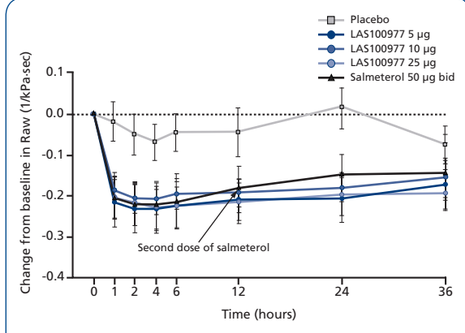
- All LAS100977 doses decreased mean values of Raw from baseline as early as 1 hour post-dose and at all remaining time points post-dose (Figure 4).
- Compared with baseline, mean values of sGaw increased with all LAS100977 doses as early as 1 hour post-dose and at all remaining time points post-dose (Figure 5).
- There were no apparent dose-response relationships for LAS100977 on either airway resistance or airway conductance.

Figure 3. Change from baseline in FEV_1 (L) over 36 hours



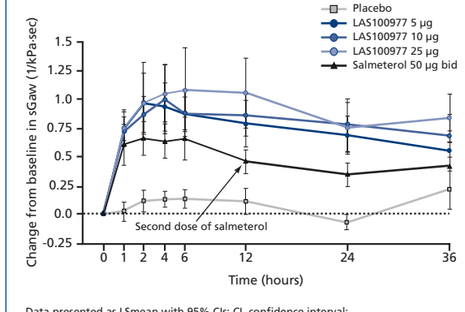
Data presented as LSmean with 95% CIs; CI, confidence interval; FEV_1, forced expiratory volume in one second; LSmean, least squares mean; NS, not significant
*$p<0.0001$ (LAS100977 [all doses] vs placebo at all time points);
††$p<0.05$ (LAS100977 [all doses] vs salmeterol at all time points)

Figure 4. Change from baseline in airway resistance (Raw) over 36 hours



Data presented as LSmean with 95% CIs; CI, confidence interval; LSmean, least squares mean

Figure 5. Change from baseline in specific airway conductance (sGaw) over 36 hours



Data presented as LSmean with 95% CIs; CI, confidence interval; LSmean, least squares mean

Safety And Tolerability

- A total of 66 treatment-emergent AEs (TEAEs) were reported in the LAS100977 5 µg (n=8; 20% of subjects), 10 µg (n=12; 36% of subjects), 25 µg (n=40; 68% of subjects), salmeterol (n=2; 8% of subjects), and placebo (n=4; 16% of subjects) groups.
- The most frequently reported drug-related TEAEs were tremor (17 episodes in 10 patients), restlessness (8 episodes in 6 patients) and nervousness (4 episodes in 4 patients), which were exclusively reported after the two highest doses of LAS100977 (10 µg and 25 µg).
- No patients were withdrawn from the study due to AEs, and no deaths occurred. All AEs were resolved by the end of the study.
- LAS100977 had no clinically relevant effects on physical examination, laboratory data, vital signs, or ECG outcomes. A non-clinically relevant increase in pulse and heart rate was observed at the two higher doses (10 µg and 25 µg).

Conclusions

- **LAS100977 showed potent, rapid, and long-acting bronchodilatory effect at all doses and has 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.**

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Almirall

Forest Laboratories, Inc.

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Single Doses Of LAS100977, A Novel Long-Acting β_2-Agonist, Show High Activity And Long Duration In Healthy Subjects

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Introduction

- LAS100977 is a novel, potent, and selective long-acting β_2-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 β_2-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/m^2.
- 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 3:1 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®).

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 (AUC_{0-24}) 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.

Results

Subjects

- A total of 48 subjects were enrolled, all of whom completed the study.
- Baseline demographic and other characteristics are shown in Table 1.

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

Age, mean (SD) years	48 (7.6)
BMI, mean (SD) kg/m^2	25.5 (2.3)
Smoking history, n (%)	
Non-smokers	29 (60.4)
Current smokers, ≤5 cigarettes/day	19 (39.6)
Systolic blood pressure, mean (SD) mmHg	129.7 (9.8)
Diastolic blood pressure, mean (SD) mmHg	75.4 (7.9)
Pulse rate, mean (SD) beats/min	64.1 (11.0)
Heart rate, mean (SD) beats/min	63 (10.1)

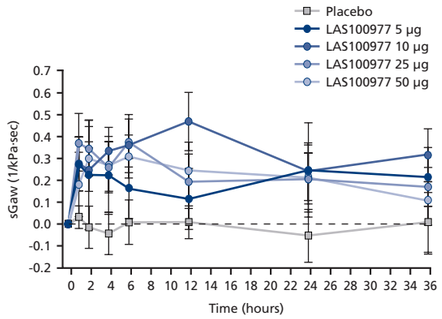
BMI, body mass index; SD, standard deviation

Effect On Airway Caliber

sGaw

- At 24 hours post-dose, all LAS100977 doses increased sGaw mean values by 0.208 to 0.247 1/kPa·sec compared with baseline (Figure 1). In contrast, the sGaw mean value for placebo remained essentially unchanged from baseline at 24 hours post-dose (Figure 1).
- Compared with placebo, sGaw mean values were higher with all LAS100977 doses at all time points over 36 hours post-dose (Figure 1).

Figure 1. Specific airway conductance (sGaw): change from baseline over 36 hours



Data are mean and 95% confidence interval

- sGAW AUC_{0-24} was increased with all doses of LAS100977 compared with placebo (Table 2).

Table 2. Mean (SD) normalized AUC_{0-24} for sGaw and Raw

	Placebo (n=12)	LAS100977 5 µg (n=6)	LAS100977 10 µg (n=12)	LAS100977 25 µg (n=12)	LAS100977 50 µg (n=6)
sGaw	0.960 (0.260)	1.293 (0.296)	1.121 (0.200)	1.146 (0.179)	1.230 (0.206)
Raw	0.291 (0.079)	0.197 (0.033)	0.245 (0.063)	0.240 (0.061)	0.198 (0.064)

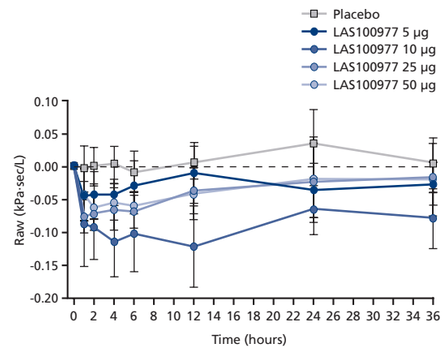
AUC, area under the curve; Raw, airway resistance; SD, standard deviation; sGaw, specific airway conductance

- There was no apparent dose-response relationship for LAS100977 on airway conductance, which may be due to low sample size.

Raw

- All LAS100977 doses decreased Raw mean values from baseline at all time points over 36 hours post-dose (Figure 2).

Figure 2. Airway resistance (Raw): change from baseline over 36 hours



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- At 24 hours post-dose, all LAS100977 doses decreased Raw 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 AUC_{0-24} versus placebo (Table 2).
- No clear dose-response relationship was observed for LAS100977 on airway resistance.

Safety And Tolerability

- A total of 16 AEs were reported in the LAS100977 10 µg (n=2; 2/12 subjects), 25 µg (n=5; 4/12 subjects), and 50 µg (n=7; 4/6 subjects), and placebo (n=2; 2/12 subjects) groups. No AEs occurred with the 5 µg dose of LAS100977.
- The only AEs after LAS100977 administration were palpitations (n=8; 8/36 subjects), tremor (n=4; 4/36 subjects), nausea (n=1; 1/36 subjects), and asthenia (n=1; 1/36 subjects). All AEs in the LAS100977 groups were of mild intensity, except for one case of moderate palpitations that was reported in the 50 µg dose group.
- There were no deaths, serious AEs, or withdrawals due to AEs observed during the study.
- Single doses of LAS100977 did not result in any clinically relevant changes on physical examination, laboratory data, or vital signs. The 5 µg and 10 µg doses had no clinically relevant effects on pulse rate; however, a slight increase in pulse rate was observed with the 25 µg and 50 µg doses, but this was not clinically relevant. Apart from an increase in heart rate observed with the two highest doses, serial ECG recordings revealed normal findings throughout 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

1. Aparici M, Gomez-Angelats M, Vilella D, et al. The *in vitro* pharmacological profile of LAS100977 – a potent, selective and long-acting beta-2 receptor agonist. Abstract 5161 presented at the American Thoracic Society International Conference, New Orleans, Louisiana, USA, May 14-19, 2010.
2. Miralpeix M, Gomez-Angelats M, Aparici M, et al. LAS100977, a novel beta-2 receptor agonist, with a longer duration of action and more favorable safety margin than salmeterol in anesthetized dogs. Abstract 5282 presented at the American Thoracic Society International Conference, New Orleans, Louisiana, USA, May 14-19, 2010.

Acknowledgements

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Almirall

Forest Laboratories, Inc.

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The *In Vitro* Pharmacological Profile Of LAS100977 – A Potent, Selective, And Long-Acting Beta-2 Receptor Agonist

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[1]Almirall, R&D Centre, Barcelona, Spain; [2]Departament de Farmacologia, Facultat de Medicina i Odontologia, Universitat de València, València, Spain

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- LAS100977 is a novel β_2-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 $\beta_1/\beta_2/\beta_3$-Adrenergic Receptor Affinity

- Radioligand displacement binding studies for human β_1- and β_2-adrenergic receptors were performed in Sf9 cell membrane preparations expressing the recombinant human β_1- and β_2-adrenergic receptors. Membranes were suspended in assay buffer and incubated with the β-adrenergic blocker ^{3}H-CGP12177 (0.14 nM) and different concentrations of the compounds. Non-specific binding was measured in the presence of 1 µM propanolol.
- β_3-adrenergic receptor radioligand displacement binding was studied using human SK-N-MC neurotumor cells. The growth and membrane preparation of these cells has been previously described.[1] To facilitate the selective binding to β_3-adrenergic receptors, membranes were incubated in 1 nM ^{125}I-CYP ((-)-3-[^{125}I] Iodocyanopindolol) and 0.3 µM CGP20712A, a β_1-antagonist, and different concentrations of the compounds. Non-specific binding was determined in the presence of 100 µM alprenolol.
- Binding reactions were terminated by filtration and washing, and residual radioactivity was then measured. The concentration at which each compound inhibited 50% of the total binding (IC_{50}) was calculated by non-linear regression analysis using SAS.

Functional β_1-Adrenergic Activity In Rat Left Atria

- Left atria were dissected from euthanized male Wistar rats, suspended in an organ bath containing Krebs Henseleit solution and connected to a force transducer. After a stabilization period, preparations were electrically stimulated at a frequency of 1 Hz and cumulative concentration-response curves were performed for each compound.
- β_1-adrenergic activity was expressed as the concentration of agonist required to induce 50% of the maximum contraction produced by isoprenaline 1 µM (EC_{50}).

Functional β_2-Adrenergic Activity In Guinea Pig Trachea

- Tracheas were excised 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).
- β_2-adrenergic activity was expressed as the concentration of agonist required to induce 50% of the maximum relaxation induced by isoprenaline 0.1 µM (EC_{50}).

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 University Clinic Hospital (Valencia, Spain) and informed consent was obtained from all patients. Bronchial rings were dissected from lung tissue as described by Cortijo et al[2] 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 (EC_{50}).
- Onset and duration of action were determined by applying an EC_{50} of LABA in preparations at ST and following subsequent changes in tone for up to 14–15 hours. Onset was defined as the time spanning from agonist addition to the attainment of 50% of the maximal relaxation produced by the concentration added. Duration of action was defined as the time spanning from agonist washout to the attainment of 50% recovery from the relaxation produced by the concentration added.

Results

Affinity And Selectivity For Human β-Adrenergic Receptor Subtypes

- In cell lines expressing human β-adrenergic receptors, LAS100977 had the highest affinity for the β_2-receptor compared with salmeterol, formoterol, and indacaterol (Table 1).
- LAS100977 demonstrated higher selectivity for human β_2-receptor (β_1/β_2 binding affinity ratio) than formoterol and indacaterol and lower than salmeterol (Table 1).

Table 1. Affinity and selectivity of LAS100977 and reference compounds for human β-adrenergic receptors

	Affinity (IC_{50}, nM)			Selectivity
	β_1	β_2	β_3	β_1/β_2 ratio
LAS100977	36.2	0.6	3001.2	65
Salmeterol	1781	2.7	5996	667
Formoterol	710.6	25.7	>10000	28
Indacaterol	135.6	34.0	3931.3	4

IC_{50} values are presented as mean (n=2–7)

Functional β_1/β_2-Adrenergic Receptor Selectivity

- In isolated guinea pig tracheal rings, LAS100977 exhibited the most potent relaxant activity of all the compounds tested, demonstrating 60-, 40-, and 3-fold more relaxant potency than salmeterol, indacaterol, and formoterol, respectively (Table 2).
- LAS100977 showed a functional β_1/β_2 selectivity 5 times and 10 times higher than formoterol and indacaterol, respectively (Table 2).

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	Potency (EC_{50}, nM)		Selectivity
	β_2	β_1	β_1/β_2 ratio
LAS100977	0.02	215	10,750
Salmeterol	1.2	>10000	>8,333
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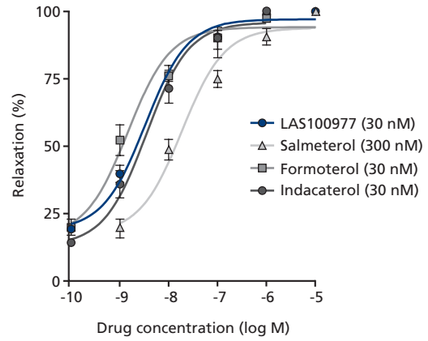
EC_{50}, concentration required to achieve 50% of the maximum effect. Data correspond to 2–3 independent experiments

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

- LAS100977 exhibited a relaxant potency in human bronchi in the nanomolar range (1.5 nM), being 8-fold greater than salmeterol (11 nM) and comparable to that of formoterol (0.6 nM) and indacaterol (3 nM) (Figure 1).
- LAS100977 showed a faster onset than that observed for salmeterol and indacaterol, and slower than formoterol in human bronchi (Figure 2 and Table 3).
- In human bronchi, the duration of action of LAS100977 was longer than that recorded for salmeterol, formoterol, and indacaterol (Table 3).

Figure 1. Relaxation-response curves of LAS100977 and reference compounds in isolated human bronchi



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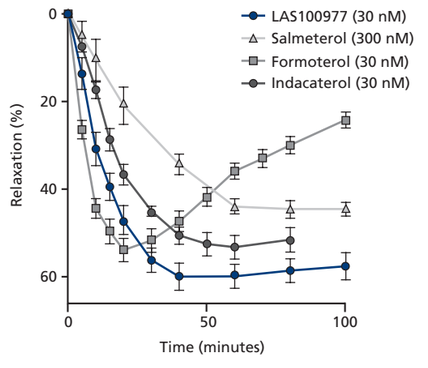

Data reported as mean ± standard error of the mean for 5–12 experiments from 3–5 patient samples

Table 3. Onset and duration of action of LAS100977 and reference compounds in isolated human bronchi

	Concentration tested (nM)	Onset $t_{½}$ (minutes)	Duration of action $t_{½}$ (minutes)
LAS100977	30	10 ± 2	699 ± 77
Salmeterol	300	19 ± 5	230 ± 55
Formoterol	30	6 ± 1	76 ± 14
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Onset and duration of action times are presented as mean ± standard error of the mean

Conclusions

- **The results of this study show that LAS100977 is a potent β_2-adrenergic agonist.**
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References

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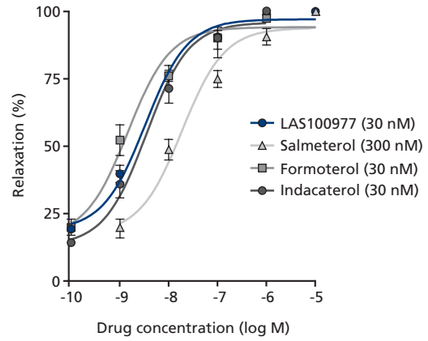

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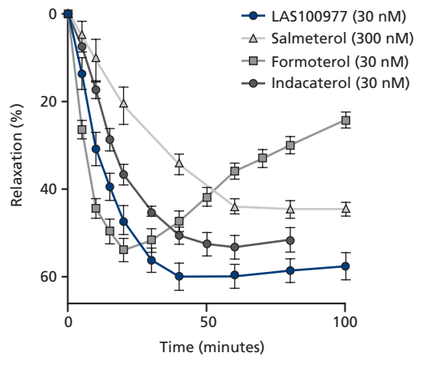

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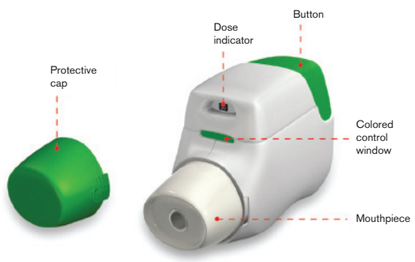
Stability Of Aclidinium Bromide Inhalation Powder (200 µg Per Dose) Delivered From The Genuair® Inhaler

Kathrin Block, Sonja Folger, Beatrix Fyrnys ▪ 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.

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



- 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 administration.[1,2]
- 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.[3]
- In order to obtain marketing authorization for an inhalation drug product, the amount of active drug dispensed must be within the range of 80–120% of the nominal dose.[4,5] In addition, as both drug stability and inhaler performance can be affected by environmental conditions, the stability of the finished drug product following storage at both 25°C/60% relative humidity (RH) and 40°C/75% RH must be demonstrated.[4,5]
- The aim of this study was therefore to evaluate the effects of temperature and humidity on the stability of aclidinium 200 µg delivered using the Genuair® inhaler.

Methods

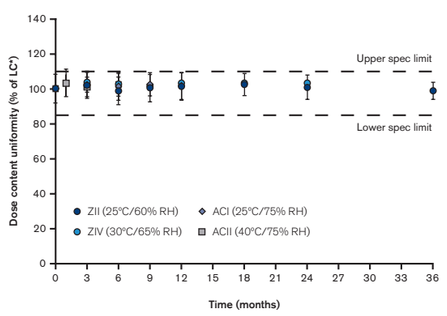
- Three pilot-scale (PS) and three laboratory-scale (LS) batches of aclidinium were stored for up to two and three years, respectively, under the following conditions:
  - Climatic zone II: 25°C/60% RH
  - Climatic zone IV: 30°C/65% RH
  - Accelerated conditions I: 25°C/75% RH
  - Accelerated conditions II: 40°C/75% RH.
- 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.
- All investigations were performed according to the European Pharmacopoeia, United States Pharmacopeia, 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 [n=6–57] or PS [n=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 <9.5% for all batches and storage conditions.

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



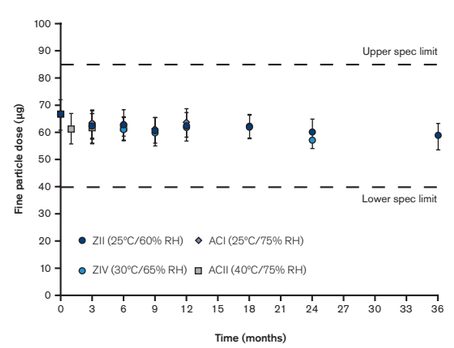
*Percentage of the label claim, defined as 181 µg aclidinium bromide
AC, accelerated conditions; LC, label claim; RH, relative humidity; SD, standard deviation; Z, climatic zone

- There was also no change in the mean fine particle dose (LS [n=6–57] or PS [n=45–300] 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:
  - ≤0.9% for LS batches
  - Below the limit of quantification (0.03% 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:
  - 0.24–1.62% for LS batches (Table 2)
  - ≤0.2% for PS batches (data not shown).

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



AC, accelerated conditions; RH, relative humidity; SD, standard deviation; Z, climatic zone

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

	Sample time points (months)								
	0 (initial dose)	1	3	6	9	12	18	24	36
LS batch									
ZII (25°C/60% RH)									
Delivered dose (µg)	178.4±13.0	–	180.0±17.0	168.9±11.8	167.4±12.2	173.6±15.6	178.8±11.6	182.7±14.9	179.1±8.3
Fine particle dose (µg)	64.4±2.7	–	55.5±3.2	57.0±4.1	60.5±4.5	60.1±6.3	63.1±3.3	57.8±5.5	58.9±4.8
ACI (25°C/75% RH)									
Delivered dose (µg)	178.4±13.0	–	179.9±11.2	171.8±8.8	178.4±11.8	171.0±11.6	–	–	–
Fine particle dose (µg)	64.4±2.7	–	59.7±2.6	58.3±1.7	56.2±3.9	59.8±4.6	–	–	–
ACII (40°C/75% RH)									
Delivered dose (µg)	178.4±13.0	173.5±7.9	178.2±11.8	174.9±11.3	–	–	–	–	–
Fine particle dose (µg)	64.4±2.7	57.3±3.9	55.9±3.8	56.7±4.0	–	–	–	–	–
PS batch									
ZII (25°C/60% RH)									
Delivered dose (µg)	182.8±14.9	–	187.3±11.1	185.4±12.1	186.6±11.5	186.9±12.5	187.7±10.3	182.6±11.7	–
Fine particle dose (µg)	67.4±6.0	–	64.2±4.7	63.5±5.6	60.6±5.4	63.0±4.8	61.6±4.7	60.7±5.3	–
ZIV (30°C/65% RH)									
Delivered dose (µg)	182.8±14.9	–	188.0±9.9	186.1±10.5	184.3±10.1	187.2±11.1	187.2±10.9	187.1±11.5	–
Fine particle dose (µg)	67.4±6.0	–	63.1±5.3	61.0±4.8	59.8±4.3	61.8±4.4	62.1±4.5	57.1±6.2	–
ACI (25°C/75% RH)									
Delivered dose (µg)	182.8±14.9	–	186.3±14.3	186.0±14.6	188.0±11.1	186.0±12.8	–	–	–
Fine particle dose (µg)	67.4±6.0	–	64.4±5.4	63.8±3.0	63.0±3.3	64.6±4.9	–	–	–
ACII (40°C/75% RH)									
Delivered dose (µg)	182.8±14.9	188.7±13.9	184.5±8.9	187.5±10.3	–	–	–	–	–
Fine particle dose (µg)	67.4±6.0	61.5±5.1	62.3±5.5	62.1±4.1	–	–	–	–	–

– = not planned
AC, accelerated conditions; LS, laboratory scale; PS, pilot scale; RH, relative humidity; SD, standard deviation; Z, climatic zone

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

	Sample time points (months)							
Total sum of impurities (%)	0 (initial dose)	3	6	9	12	18	24	36
ZII (25°C/60% RH) LS1; LS2; LS3	0.93; 0.82; 0.24	0.84; 0.97; 0.28	0.91; 1.05; 0.33	1.00; 1.12; 0.34	0.95; 1.12; 0.37	1.14; 1.26; 0.39	1.03; 1.31; 0.41	1.16; 1.43; 0.44
ACI (25°C/75% RH) LS1; LS2; LS3	0.93; 0.82; 0.24	0.84; 1.02; 0.28	0.92; 1.17; 0.32	1.09; 1.25; 0.34	1.02; 1.20; 0.37	– ; – ; –	– ; – ; –	– ; – ; –
ACII (40°C/75% RH) LS1; LS2; LS3	0.93; 0.82; 0.24	1.01; 1.42; 0.38	1.23; 1.62; 0.48	– ; – ; –	– ; – ; –	– ; – ; –	– ; – ; –	– ; – ; –

– = not planned
AC, accelerated conditions; RH, relative humidity; Z, climatic zone

Conclusions

- 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 fulfils 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.

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Acknowledgements

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

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

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The Genuair® Inhaler: A Reliable Device Technology In Inhalation Therapy

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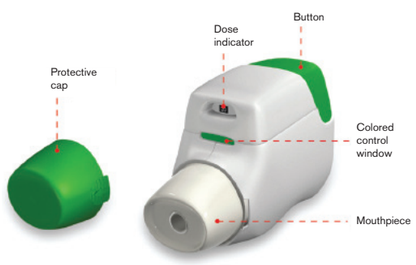
Introduction

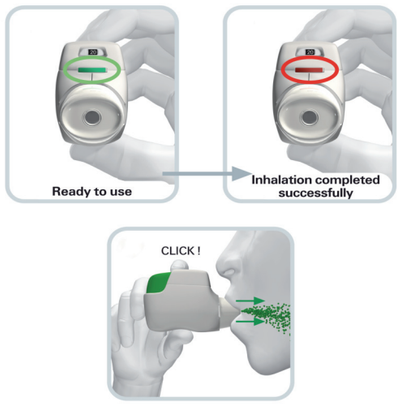
- The Genuair®* inhaler (Figure 1) is a novel, breath-actuated, multidose, dry powder inhaler designed for the effective delivery of various types of inhaled drugs,[1] including long-acting muscarinic antagonists such as aclidinium bromide.

Figure 1. The Genuair® inhaler: design and features



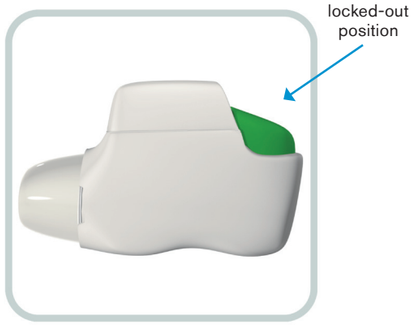
- This inhaler incorporates a new design of the dispersion set (mouthpiece and cyclone unit) which enhances fluid and particle dynamics to ensure effective deagglomeration of the inhalation powder into a suitable aerosol, even at a low inhalation flow rate and volume.
- Features of this inhaler include:
  - 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 (Figure 2)

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



  - A flow-rate trigger threshold mechanism that prevents accidental double-dosing
  - A lock-out mechanism that prevents further use when the inhaler is empty (Figure 3).

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



- 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/g using a vibrating sieve device with empty inhalers or inhalers loaded with empty cartridges.
- 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 <55 L/min required to activate trigger mechanism).
- To test the colored control window, dose indicator, and lock-out mechanism, each inhaler was actuated at a flow rate corresponding to a pressure drop of 4 kPa (~65 L/min flow rate; 2 L inhalation volume) over the entire inhaler life (30–38 dosages). An aerodynamic test system with a mass flow meter, a differential pressure sensor, a flow-control valve, and vacuum pumps was used.
- The function of the trigger threshold mechanism was tested by determining the air-flow rate required to reset the control window using the aerodynamic test system described above.

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 >25,000 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.

Results

Reliability Under Thermal And Mechanical Stress

- The Genuair® inhaler met all performance requirements measured under various thermal and mechanical stress treatments (Table 1).

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

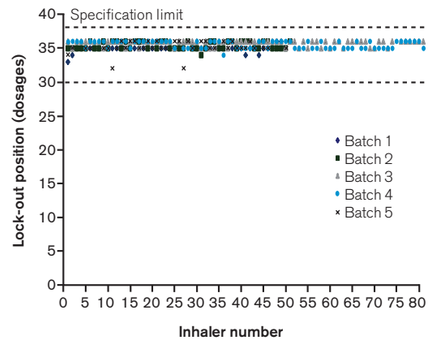
	Cold storage (2–8°C) for 3 days (n=6)	Cold storage (2–8°C) for 8 days (n=6)	Storage at 40°C and 75% relative humidity for 3 days (n=6)	Storage at 40°C and 75% relative humidity for 8 days (n=6)	Hot storage (60°C) for 1 hour (n=6)	Hot storage (60°C) for 6 hours (n=6)	Vibration of empty inhalers for 1 hour (n=6)	Vibration of inhalers loaded with empty cartridges for 1 hour (n=6)
Fastening of the slide cover and mouthpiece	√	√	√	√	√	√	√	√
Function of the colored control window	√	√	√	√	√	√	√	√
Function of double-dosing prevention	√	√	√	√	√	√	√	√
Function of dose indicator mechanism	√	√	√	√	√	√	√	√
Function of the lock-out mechanism	√	√	√	√	√	√	√	√
Function of the trigger threshold mechanism	√	√	√	√	√	√	√	√

Conformation (√) or non-conformation (×) to the defined technical specification is indicated above

Reliability Of The Lock-Out And Trigger Threshold Mechanisms

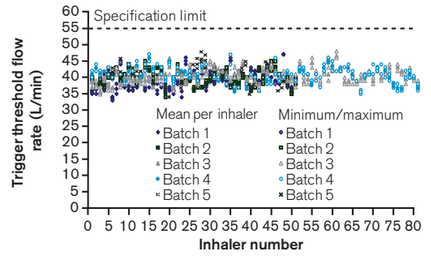
- The lock-out mechanism worked correctly on all inhalers, blocking the inhaler securely within the specified range of 30 to 38 doses (Figure 4).

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



- For all inhalers, the trigger threshold was reached within the specified flow rate (<55 L/min; Figure 5).
- The mean trigger threshold flow rate across batches ranged from 39 to 41 L/min.

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



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.[2]
- 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.

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Acknowledgements

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Almirall Forest Laboratories, Inc.

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Impact Of Different Inhalation Volumes On The Aerodynamics Of Aclidinium Bromide Delivered Through The Genuair® Inhaler

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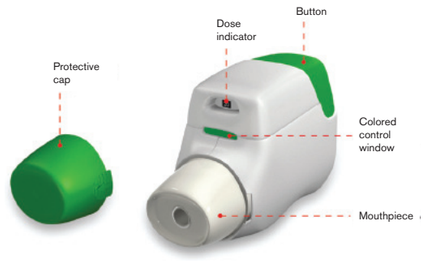
Introduction

- 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[1] and 4 liters,[2] 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.[3]

Figure 1. Design and features of the Genuair® inhaler



- The Genuair® inhaler has demonstrated highly reproducible dose delivery for a range of inhalation formulations,[4] thus confirming the high aerodynamic precision of this inhaler observed in *in vitro* studies.[5]
- Aclidinium bromide administered via the Genuair® inhaler is currently in clinical development for the maintenance treatment of patients with COPD.
- The aim of this study was to evaluate whether differences in inhalation volume affect the dose content uniformity and fine particle dose of aclidinium delivered using the Genuair® inhaler. The stability of the aerodynamic performance and aclidinium particle size distribution, including fine particle dose, was also assessed.

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 aclidinium using the Genuair® inhaler.
- Two formulations (50 µg and 400 µg) of aclidinium were tested at a pressure drop of 4 kPa using identical flow rates (~65 L/min) through a sample collection tube and an Andersen Cascade Impactor, respectively.
- Solutions were analyzed using an isocratic high performance liquid chromatography system with ultraviolet detection and a C18 column.
- Different flow rates of 25, 35, 45, 55, ~65 (4 kPa), 75, 85, and 90–95 L/min were also evaluated using both inhalation volumes on three pilot-scale (PS) batches of aclidinium 200 µg; samples were taken from three consecutive dosages using two inhalers for each batch.
- Stability was assessed by repeating aerodynamic assessments on three PS and three laboratory-scale (LS) batches of aclidinium 200 µg, which were stored for two and three years, respectively, under various stability conditions (25–40°C and 60–75% relative humidity).
- 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.
- All investigations were performed according to the European Pharmacopoeia, United States Pharmacopeia, and the Food and Drug Administration requirements.

Results

Effect Of Inhalation Volume On Aclidinium Dose Content Uniformity

- The mean total dose and fine particle dose of aclidinium (50 µg and 400 µg) were consistent for both inhalation volumes (Figure 2).
- Mean aerodynamic particle size distribution was not affected by inhalation volume for both the 50 µg and 400 µg formulations, with comparable stage-to-stage profiles obtained for both inhalation volumes tested (Figure 3).

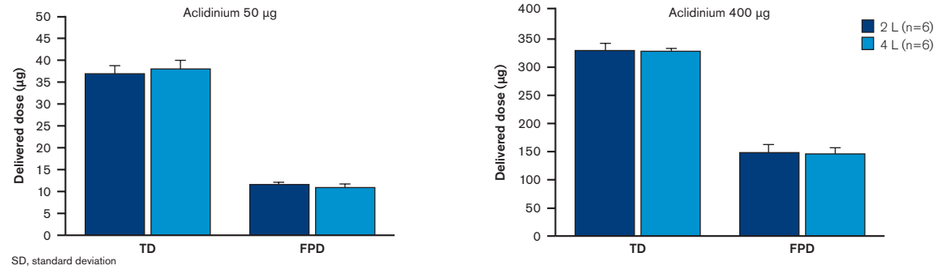
Effect Of Flow Rate And Inhalation Volume On Aclidinium Dose Content Uniformity

- The mean total dose and fine particle dose of PS batches of aclidinium (200 µg) at various flow rates were not significantly affected by the different inhalation volumes (Figure 4).
  - With the exception of the 25 L/min flow rate and 2 L inhalation volume, the mean total dose remained within ±15% of the LC dose of 181 µg for all flow rates and inhalation volumes
  - With the exception of the 25 L/min flow rate (2 L and 4 L inhalation volumes), the mean fine particle dose delivered remained within specification limits (40–85 µg) for all flow rates and inhalation volumes.

Effect Of Storage On Aclidinium Dose Content Uniformity

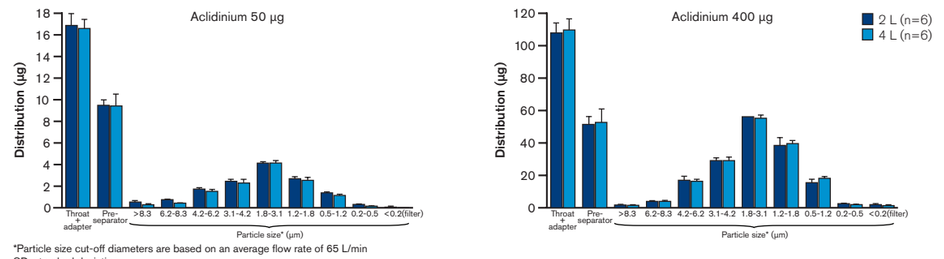
- The mean total dose and fine particle dose of PS and LS batches of aclidinium 200 µg were consistent for both inhalation volumes following storage under various stability conditions for up to three years (Figure 5).
  - The mean total dose and fine particle dose remained within specification limits (total dose: ±15% of the LC dose of 181 µg; fine particle dose: 40–85 µg) for all batches and storage conditions.

Figure 2. Effect of inhalation volume on mean (±SD) total dose (TD) and fine particle dose (FPD) of aclidinium delivered with the Genuair® inhaler



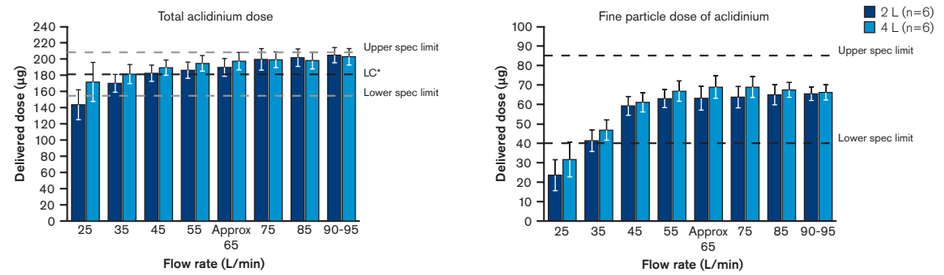
SD, standard deviation

Figure 3. Effect of inhalation volume on mean (±SD) particle size distribution of aclidinium delivered with the Genuair® inhaler



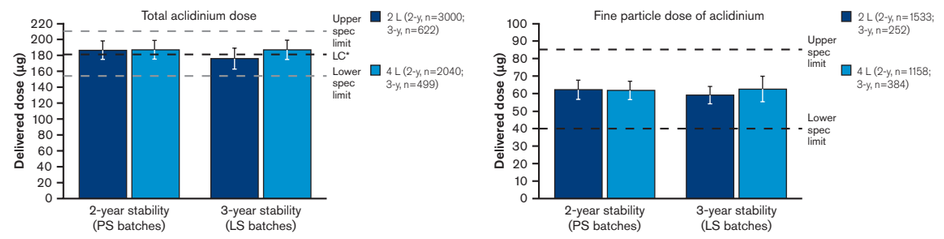
*Particle size cut-off diameters are based on an average flow rate of 65 L/min
SD, standard deviation

Figure 4. Effect of flow rate on mean (±SD) total dose and fine particle dose of aclidinium 200 µg delivered with the Genuair® inhaler using 2 L and 4 L inhalation volumes



*LC dose is 181 µg; LC, label claim; SD, standard deviation

Figure 5. Effect of inhalation volume on mean (±SD) total dose and fine particle dose of aclidinium 200 µg delivered with the Genuair® inhaler following storage of up to three years



*LC dose is 181 µg; LC, label claim; SD, standard deviation

Conclusions

- Administration of aclidinium inhalation powder with the Genuair® 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 aclidinium at different inhalation volumes.
- This MDPI therefore fulfils the regulatory requirements of both the US and EU and does not require adjustments of aclidinium inhalation powder used based on inhalation volume.

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