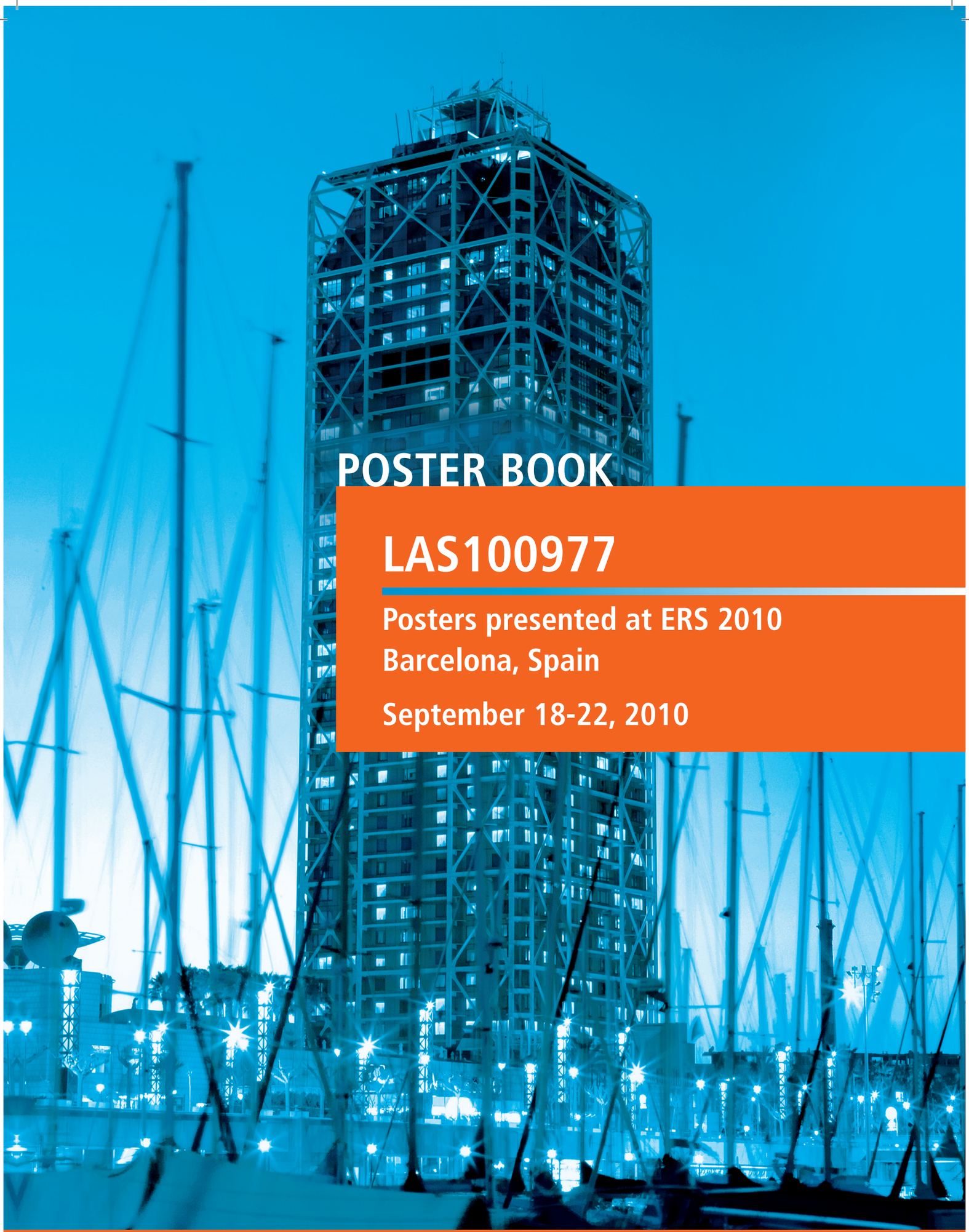


POSTER BOOK

LAS100977

Posters presented at ERS 2010
Barcelona, Spain

September 18-22, 2010

Contents

Clinical posters

Preclinical posters

LAS100977, a novel, selective, long-acting β_2-agonist, improves airway conductance and reduces airway resistance in healthy subjects

Wolfgang Timmer,[1] Èric Massana,[2] Eulàlia Jiménez,[2] Beatriz Seoane,[2] Gonzalo de Miquel,[2] Sandrine Ruiz[2]

[1]Clinical Research Services Mannheim GmbH, Germany; [2]Almirall, R&D Centre, Barcelona, Spain

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 an inhaled corticosteroid.
- *In vitro* studies have shown LAS100977 to be a potent and selective β_2-receptor agonist, with rapid onset and long duration of action.[1] In addition, *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 in humans, was to determine the activity, safety and tolerability of different doses of LAS100977 in healthy subjects.

Methods

Subjects

- All subjects were healthy Caucasian males, aged 18–45 years, with a body mass index of 18.5–30 kg/m^2.
- Concomitant medications were not permitted.

Study design

- This was a Phase I, randomised, parallel, single-blind, placebo-controlled, single-centre, dose-escalation study.
- Within 14 days of a screening visit, subjects were randomised to receive single doses of LAS100977 (5 µg, 10 µg, 25 µg or 50 µg) or placebo. The randomisation was 3:1 for LAS100977 versus placebo at each dose.
- LAS100977 and matching placebo were provided as dry powder in hard capsules and were administered in the morning by inhalation via Cyclohaler®.

Assessments

- All pharmacodynamic assessments were of an exploratory nature (descriptive statistics only).
  - Airway resistance (Raw) was assessed 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).
  - Normalised area under the curve between 0 and 24 hours post dose (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

- In total, 48 subjects were enrolled and 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)

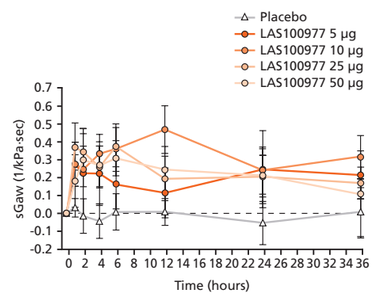
BMI, body mass index; SD, standard deviation

Effect on airway calibre

sGaw

- At 24 hours post-dose, all doses of LAS100977 had increased mean sGaw values by 0.208 to 0.247 1/kPa·sec compared with baseline (Figure 1). In contrast, mean sGaw values for placebo remained essentially unchanged from baseline to 24 hours post-dose (Figure 1).
- Compared with placebo, mean sGaw was higher with all LAS100977 doses at all time points up to 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} increased with all doses of LAS100977 compared with placebo (Table 2).

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

	Placebo	LAS100977			
	(n=12)	5 µg (n=6)	10 µg (n=12)	25 µg (n=12)	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)

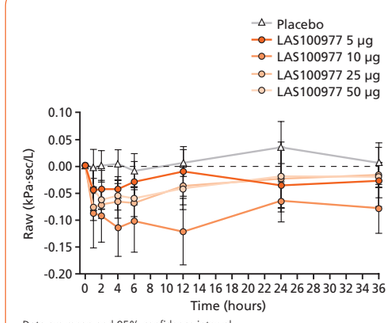
AUC, area under the curve; Raw, airway resistance; SD, standard deviation; sGaw, specific airway conductance

- No apparent dose-response relationship was observed for LAS100977 and sGAW.

Raw

- At the doses tested, LAS100977 decreased mean Raw values from baseline at all time points from 0 to 36 hours post-dose (Figure 2).

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



Data are mean and 95% confidence interval

- At 24 hours post-dose, all doses of LAS100977 decreased mean Raw by -0.018 to -0.067 kPa·sec/L versus baseline. In subjects receiving placebo, there was no improvement in Raw at 24 hours post-dose compared with baseline.
- Mean Raw values were lower with all LAS100977 doses compared with placebo at all time points from 0–36 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 LAS100977 5 µg.
- The AEs reported 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). AEs in the LAS100977 groups were of mild intensity, except for one case of moderate palpitations reported in the 50 µg 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. LAS100977 5 µg and 10 µg 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 a modest increase in heart rate observed with the two highest doses, serial ECG recordings were normal 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. Am J Respir Crit Care Med 2010; 181: A5675 (abstract).
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. Am J Respir Crit Care Med 2010; 181: A5675 (abstract).

Acknowledgements

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

Poster presented at the European Respiratory Society Annual Congress, Barcelona, Spain, 18-22 September 2010

Abstract

LAS100977, a novel, selective, long-acting β_2-agonist, improves airway conductance and reduces airway resistance in healthy subjects

Wolfgang Timmer,[1] Èric Massana,[2] Eulàlia Jiménez,[2] Beatriz Seoane,[2] Gonzalo de Miquel,[2] Sandrine Ruiz[2]

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Introduction

Preclinical studies have shown LAS100977 to be a potent and selective long-acting β_2-agonist with a rapid onset of action. This study explored the effect of single doses of LAS100977 on specific conductance (sGaw) and resistance (Raw) of the airways in healthy subjects.

Methods

This was a single-blind, placebo-controlled, dose-escalation study. In total, 48 healthy male subjects were randomised to receive single doses of LAS100977 (5 µg, 10 µg, 25 µg and 50 µg) or placebo. sGaw and Raw were assessed pre-dose and up to 36 h post-dose. The pharmacokinetics and safety of LAS100977 were also assessed.

Results

At 24 h post-dose, LAS100977 (all doses) increased sGaw, compared with pre-dose (0.208 to 0.247 1/kPa.sec); placebo decreased sGaw (-0.056 1/kPa.sec). In addition, for all doses of LAS100977, Raw decreased (-0.018 to -0.067 kPa.sec/L) 24 h post-dose compared with pre-dose. In subjects receiving placebo, Raw increased (0.035 kPa.sec/L) 24 h post-dose compared with baseline. There was no apparent dose-response relationship of LAS100977 on airway conductance. The most common treatment-emergent adverse events were mild to moderate palpitations and tremor occurring in 16.7% and 8.3% of patients, respectively. There was no increase in mean heart rate at 5 µg and 10 µg doses although a slight increase was observed at the two higher doses.

Conclusion

LAS100977 increased airway conductance and decreased airway resistance over the dose range studied (5–50 µg). LAS100977 was well-tolerated at all doses tested and no safety concerns arose.

Efficacy and safety of single doses of inhaled LAS100977 in patients with mild to moderate asthma

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Introduction

- LAS100977 is a novel, potent and selective long-acting β_2-agonist (LABA) being developed as a once-daily treatment of asthma, in combination with an inhaled corticosteroid (ICS).
- LAS100977 displays high potency and selectivity at β_2-receptors, with a rapid onset and long duration of action *in vitro*.[1] In addition, *in vivo* studies in dogs suggest that LAS100977 provides 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

- 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
- 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
- Hospitalisation 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, randomised, 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 randomised 1:1:1:1:1 to LAS100977 once daily (5, 10 or 25 µg), salmeterol 50 µg twice daily or placebo. Ongoing asthma medications were withdrawn during the run-in period, with the exception of rescue medications and ICS treatments.
- LAS100977 (5, 10 or 25 µg) was administered in the morning as a single inhaled dose via the Cyclohaler®. Salmeterol was administered as two inhaled doses, one in the morning and one in the evening, via the Accuhaler®. Corresponding placebo treatments were administered using the Cyclohaler® and Accuhaler®.
- 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.
- Spirometry assessments included FEV_1, forced vital capacity (FVC), peak expiratory flow (PEF) and forced mid-expiratory flow (FEF_{25-75}).
- Airway resistance (Raw) and specific airway conductance (sGaw) were assessed using body plethysmography.
- Safety assessments included adverse events (AEs), 12-lead electrocardiograms (ECGs), physical examinations, laboratory tests and vital signs.

Study endpoints

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

Results

Subjects

- In total, 25 patients were enrolled and randomised 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)

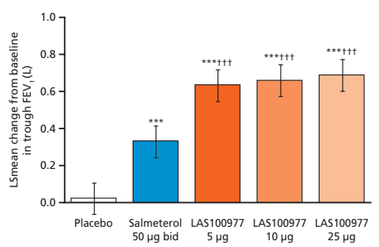
Age, mean (SD) years	44.2 (10.3)
BMI, mean (SD) kg/m²	27.1 (2.5)
Smoking consumption, n (%)	
Non-smokers	20 (80)
Ex-smokers (8–20 cigarettes/day)	5 (20)
Pulse rate, mean (SD) beats/min	62.9 (6.6)
FEV_1, mean (SD)	
Pre-bronchodilator (L)	2.90 (0.45)
Post-bronchodilator (L)	3.61 (0.60)
Percentage of predicted 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 trough FEV_1 for the three LAS100977 doses.
- 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 to 60 minutes post-dose (Table 2 and Figure 2).
- At all doses tested, LAS100977 provided sustained bronchodilation throughout 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



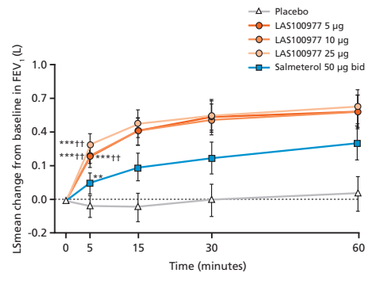
FEV_1, forced expiratory volume in one second; LSmean, least squares mean; SE, standard error
***p<0.0001 vs placebo; †††p<0.0001 vs salmeterol 50 µg bid

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)***†

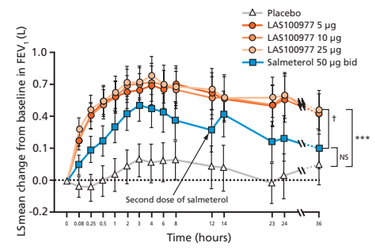
FEF_{25-75}, forced mid-expiratory flow; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; LSmean, least squares mean; PEF, peak expiratory flow; SE, standard error
***p<0.0001 vs placebo; **p<0.001 vs placebo; †p<0.001 vs salmeterol 50 µg bid; †p<0.05 vs salmeterol 50 µg bid

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 placebo; ††p<0.001 vs salmeterol 50 µg bid

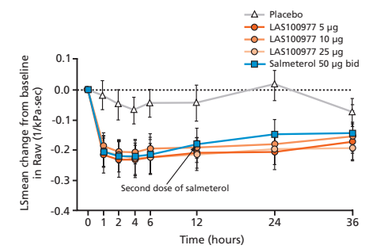
Figure 3. LSmean change from baseline (95% CI) in FEV_1 (L) over 36 hours



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)

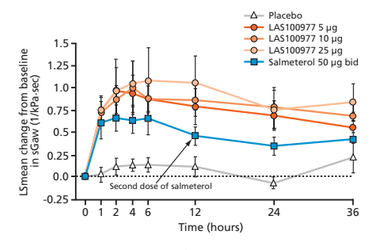
- All LAS100977 doses decreased mean Raw from baseline as early as 1 hour post-dose and at all remaining time points post-dose (Figure 4).
- Compared with baseline, mean sGaw increased with all LAS100977 doses as early as 1 hour post-dose and at all remaining time points post-dose (Figure 5).
- There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

Figure 4. LSmean change from baseline (95% CI) in airway resistance (Raw) over 36 hours



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

Figure 5. LSmean change (95% CI) 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 withdrew from the study due to AEs, and no deaths occurred. All AEs 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 with LAS100977 (10 µg and 25 µg).

Conclusions

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

References

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. Am J Respir Crit Care Med 2010; 181: A5675 (abstract).
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. Am J Respir Crit Care Med 2010; 181: A5676 (abstract).
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Poster presented at the European Respiratory Society Annual Congress, Barcelona, Spain, 18-22 September 2010

Abstract

Efficacy and safety of single doses of inhaled LAS100977 in patients with mild to moderate asthma

Jutta Beier,[1] Rainard Fuhr,[2] Èric Massana,[3] Eulàlia Jiménez,[3] Beatriz Seoane,[3] Gonzalo de Miquel,[3] Sandrine Ruiz[3]

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Introduction

LAS100977 is a novel, long-acting β_2-agonist in clinical development for the treatment of asthma and COPD. This study investigated the efficacy and safety of single doses of LAS100977 in patients with mild to moderate asthma.

Methods

This was a double-blind, double-dummy, placebo and active-control, cross-over study. In total, 25 male adult patients with persistent asthma were randomised to receive single doses of LAS100977 (5, 10 and 25 µg QD), salmeterol 50 µg BID or placebo on separate days. Serial spirometry was performed at pre-dose to 36 h post-dose; outcomes included mean change from baseline in trough FEV_1, FVC and PEF. Body plethysmography parameters were also assessed. Safety assessments included adverse event reports.

Results

For all doses studied, LAS100977 significantly increased the change from baseline in trough FEV_1, FVC and PEF, compared with salmeterol 50 µg BID (Table). LAS100977 showed a rapid onset of effect with significant improvements in lung function at 5 min post-dose, compared with placebo and salmeterol 50 µg BID. LAS100977 had a similar safety profile to placebo. At the two higher doses, there was a small but non-significant increase in the number of class-associated AEs.

Table. Change from baseline in key spirometry assessments (per-protocol population)

Change from baseline [LS mean (SE)]	Placebo (n=24)	Salmeterol 50 µg BID (n=24)	LAS100977 5 µg QD (n=23)	LAS100977 10 µg QD (n=24)	LAS100977 25 µg QD (n=24)
Trough FEV_1 (L)	0.024 (0.086)	0.333 (0.087)***	0.636 (0.087)***,†††	0.660 (0.087)***,†††	0.689 (0.086)***,†††
Trough FVC (L)	0.017 (0.096)	0.258 (0.096)***	0.400 (0.096)***,††	0.427 (0.096)***,††	0.441 (0.096)***,††
Trough PEF (L)	-0.246 (0.189)	0.628 (0.190)***	1.393 (0.190)***,†††	1.464 (0.189)***,†††	1.610 (0.189)***,†††

***p<0.0001 vs placebo ; †††p<0.0001 vs salmeterol 50 µg BID; ††p<0.001 vs salmeterol 50 µg BID.
LSmean: least squares mean; SE: standard error; FEV_1: forced expiratory volume in 1 second; FVC: forced vital capacity; PEF: peak expiratory flow

Conclusion

LAS100977 (5–25 µg) once-daily was a potent, fast and long-acting bronchodilator in patients with mild to moderate asthma. LAS100977 5 µg and 10 µg were generally safe and well-tolerated.

LAS100977 is a novel, potent β_2-agonist with rapid onset and sustained duration of action *in vitro*

Mònica Aparici,[1] Mireia Gómez-Angelats,[1] Dolors Vilella,[1] Julio Cortijo,[2] Esteban Morcillo,[2] Carla Carcasona,[1] Amadeu Gavaldà,[1] Jordi Beleta,[1] Carlos Puig,[1] Hamish Ryder,[1] Montserrat Miralpeix[1]

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Introduction

- LAS100977 is a novel β_2-receptor agonist currently being developed as a treatment for asthma, in combination with an inhaled corticosteroid.
- This study investigated the human β-adrenergic receptor-binding profile of LAS100977 *in vitro*. Additionally, the potency, onset and duration of action of LAS100977 in human bronchi was compared with 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 agonists. 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), 0.3 μM CGP20712A, a β_1-antagonist, and different concentrations of the agonists. Non-specific binding was determined in the presence of 100 μM alprenolol.
- Binding reactions were stopped by filtration and washing and the residual radioactivity was measured. The concentration of agonist that inhibited 50% of the total binding (IC_{50}) was calculated for each compound by non-linear regression analysis.

Functional β_1-adrenergic activity in rat left-atria

- Left atria were dissected from euthanised male Wistar rats, suspended in an organ bath containing Krebs Henseleit solution and connected to a force transducer. After stabilisation, 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 (EC_{50}) produced by isoprenaline 1 μM.

Functional β_2-adrenergic activity in guinea pig trachea

- Tracheas were excised from euthanised male Dunkin Hartley guinea pigs, dissected into rings and suspended in an organ bath containing Krebs Henseleit solution, connected to a force transducer. Cumulative relaxation-response curves were performed for each agonist 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 (EC_{50}) induced by isoprenaline 0.1 μM.

Potency, onset and duration of action in human bronchi

- Human bronchial tissue was obtained from patients with no prior history of asthma who were undergoing surgery for lung carcinoma. 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 an organ bath.
- Potency was determined 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 (EC_{50}) induced by theophylline 3 mM.
- 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 taken from the addition of agonist to reach 50% maximal relaxation produced by the concentration added. Duration of action was defined as the time taken from agonist washout to reach 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-receptors (β_1/β_2 binding affinity ratio) than formoterol and indacaterol but 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
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-fold and 10-fold greater than formoterol and indacaterol, respectively (Table 2).

Table 2. Functional β-adrenergic activity and selectivity in rat left-atria (β_1) and guinea pig trachea (β_2)

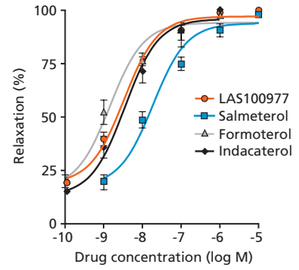
	Potency (EC_{50}, nM)		Selectivity
	β_2	β_1	β_1/β_2 ratio
LAS100977	0.02	215	10,750
Salmeterol	1.2	>10,000	>8,333
Formoterol	0.07	133	1,900
Indacaterol	0.8	809	1,011

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 similar to that of formoterol (0.6 nM) and indacaterol (3 nM) (Figure 1).

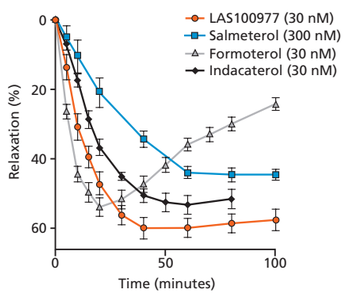
Figure 1. Relaxation-response curves of LAS100977 and reference compounds in isolated human bronchi



EC_{50}, concentration required to achieve 50% of the maximum effect. Data reported as mean ± standard error for 9–18 experiments from 4–9 patient samples

- In human bronchi, LAS100977 showed a faster onset than salmeterol and indacaterol and slower than formoterol (Figure 2 and Table 3).

Figure 2. Onset of action of LAS100977 and reference compounds in isolated human bronchi



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

- The duration of action of LAS100977 in human bronchi was longer than that of salmeterol, formoterol and indacaterol (Table 3).

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
Indacaterol	30	14 ± 1	449 ± 62

Onset and duration of action times are presented as mean ± standard error of the mean; $t_{½}$, half-life

Conclusions

- **The results of this study show that LAS100977 is a potent β_2-adrenergic agonist.**
- **LAS100977 is a selective β_2-adrenergic agonist with a β_1/β_2 selectivity ratio similar to salmeterol but superior to indacaterol and formoterol.**
- **LAS100977 has a rapid onset and sustained duration of action in isolated human bronchial tissue, and is similar to indacaterol.**

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Acknowledgements

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Poster presented at the European Respiratory Society Annual Congress, Barcelona, Spain, 18-22 September 2010

Abstract

LAS100977 is a novel, potent β_2-agonist with rapid onset and sustained duration of action *in vitro*

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Introduction

LAS100977 is a novel, long-acting β_2-agonist in clinical development for the treatment of asthma and COPD. This study assessed the selectivity, potency, rate of onset and duration of action (DoA) of LAS100977 *in vitro*.

Methods

Receptor affinity was assessed using radioligand displacement assays in cells expressing human β_1-β_3 adrenergic receptors. Potency, onset and DoA were assessed in isolated human bronchi. Cumulative concentration-response curves for relaxation defined the potency of each compound, expressed as EC_{50}. Relaxation was induced and after wash-out, changes were measured for up to 15 h. Onset (time to achieve 50% maximal relaxation) and DoA (time to achieve 50% recovery of maximal relaxant effect) were determined. β_2- and β_1-adrenergic selectivity was measured in isolated guinea-pig trachea and rat atria, respectively. Formoterol, salmeterol and indacaterol were the comparators.

Results

LAS100977 showed the highest affinity for β_2-receptors (0.6 nM) vs comparators. The potency of LAS100977 in isolated human bronchi was 1.5 nM, 10-fold greater than salmeterol and similar to formoterol and indacaterol. Onset of LAS100977 (10 min) was faster than salmeterol (19 min) and indacaterol (14 min) and slower than formoterol (6 min). LAS100977 DoA (670 min) was longer than formoterol (77 min) and salmeterol (230 min) and similar to indacaterol (450 min). LAS100977 exhibited greater selectivity than formoterol and indacaterol in binding and tissue-function studies (64-fold and 10750-fold, respectively).

Conclusion

LAS100977 is a potent and selective β_2-agonist with a rapid onset and long DoA *in vitro*. These data may suggest a sustained bronchodilator effect in man.

LAS100977, a novel β_2-agonist, has a longer duration of action and a more favourable therapeutic index than salmeterol in anaesthetised dogs

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Introduction

- LAS100977 is a novel, inhaled, long-acting β_2-agonist (LABA) currently being developed as a treatment for asthma, in combination with an inhaled corticosteroid.
- *In vitro* studies have shown LAS100977 to be a potent and selective β_2-adrenergic agonist, with rapid onset and sustained duration of action.[1]
- In this study, the bronchodilatory potency and duration of action of aerosolised LAS100977 was investigated in dogs and compared with salmeterol. The effect of LAS100977 and salmeterol on heart rate was also measured in order to determine the therapeutic index (TI) of each LABA.

Methods

Animals

- Male Beagle dogs (9–19 kg) were housed at 15–21°C, 40–70% humidity, under a 12-hour light/dark cycle, and fed a maintenance diet with free access to water. All experiments were carried out with the approval of the Animal Ethical Committee of Almirall.

Inhibition of bronchoconstriction and heart rate assessment in Beagle dogs (therapeutic index calculation)

- Animals were prepared prior to the assay according to the method of Konzett and Rossler,[2] modified by Misawa et al.[3] Fasted male Beagle dogs were anaesthetised using sodium pentobarbital (35 mg/kg i.v. plus a permanent infusion of 6 mg/kg/hour i.v.) administered through the cephalic vein. The trachea was cannulated, and the animals were artificially ventilated with room air at a constant pressure of 10 cm of H_2O with a respiratory rate of 14 strokes/minute and a tidal volume of 15 to 20 mL/kg.
- Responses of the bronchial musculature to ventilation overflow were continuously measured by a pneumotachograph (Biopac Systems) as an index of airway resistance.
- Heart rates were monitored continuously throughout the experiment by attaching electrocardiogram (ECG) leads to record the DII derivative of the ECG.
- Bronchoconstriction was induced by acetylcholine (ACh, 5 µg/kg i.v.), administered as a bolus. Two similar ACh basal responses were required before the test compounds were administered for one minute using an ultrasonic nebuliser (Mumed Ltd, London, UK). ACh was administered again 10 and 20 minutes after administration of the agonists. Inhibition of bronchoconstriction was calculated at 20 minutes post-dose. Potency was defined as the dose of agonist required to inhibit 50% (ID_{50}) of the bronchoconstriction induced by ACh.
- For each agonist, the TI was defined as the ratio between the dose required to increase the heart rate by 5% (ED_5), measured 20 minutes post-dose, and the bronchoprotective ID_{50} effect.

Duration of action in Beagle dogs

- Fasted male Beagle dogs were anaesthetised using propofol (6 mg/kg i.v. plus a permanent infusion of 0.6–0.7 mg/kg/minute i.v.), administered through the cephalic vein. The animals were connected to an artificial respirator and mechanically ventilated.
- Inhibition of bronchoconstriction at 20 minutes, 3, 6 and 24 hours after compound administration, and heart rate assessment, were determined as described above. After each assessment, animals were disconnected from the respirator and allowed to regain consciousness.

Statistical analysis

- Statistical analysis was carried out using analysis of variance (ANOVA) and post-hoc testing, including Bonferroni correction.

Results

Inhibition of bronchoconstriction and heart rate assessment in Beagle dogs (therapeutic index calculation)

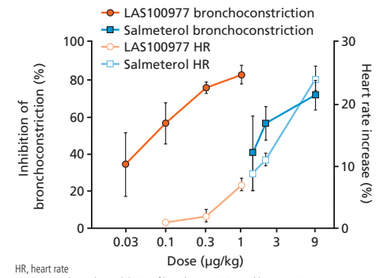
- LAS100977 inhibited ACh-induced bronchoconstriction approximately 27-fold greater than salmeterol (ID_{50} = 0.07 µg/kg and 1.9 µg/kg, respectively; Table 1 and Figure 1).

Table 1. Therapeutic index of LAS100977 and salmeterol in Beagle dogs

	ID_{50} (µg/kg)	ED_5 (µg/kg)	Therapeutic index (ED_5/ID_{50})
LAS100977	0.072	0.59	8
Salmeterol	1.93	0.86	0.4

ED_5, dose required to increase heart rate by 5% (µg/kg); ID_{50}, dose required to inhibit 50% of maximal bronchoconstriction (µg/kg)

Figure 1. Effects of LAS100977 and salmeterol on ACh-induced bronchoconstriction and heart rate in Beagle dogs



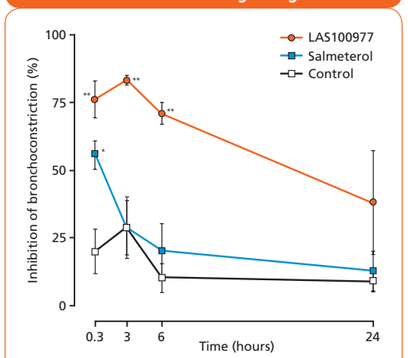
HR, heart rate
n=2–4 animals per dose. Inhibition of bronchoconstriction and heart rate increase presented as mean ± standard error of the mean

- The doses of LAS100977 and salmeterol required to increase heart rate by 5% were similar (ED_5 = 0.59 µg/kg and 0.86 µg/kg, respectively) (Table 1).
- The TI for LAS100977 is 20-fold greater than salmeterol (Table 1 and Figure 1).

Duration of action in Beagle dogs

- LAS100977 1 µg/kg showed a sustained inhibition of bronchoconstriction up to 6 hours (29% loss of effect 6 hours post-dose), whereas salmeterol 9 µg/kg showed a 50% loss of effect at 3 hours post-dose (Figure 2, Table 2).

Figure 2. Duration of action of aerosolised LAS100977 and salmeterol in Ach-induced bronchoconstriction in Beagle dogs



n=2–5 animals per dose. Inhibition expressed as mean ± standard error of the mean.
Statistical analysis: two-way analysis of variance and Bonferroni post-test in comparison with control group
*p<0.01 vs control; **p<0.001 vs control

- At 24 hours post-dose, the loss of bronchoprotection was 2-fold for LAS100977 and 5-fold for salmeterol (Figure 2, Table 2). These differences were not statistically significant compared with placebo.
- At all doses investigated, LAS100977 produced a modest, albeit non-significant, increase in heart rate (4%), whereas salmeterol significantly increased heart rate (26%; p<0.01 vs control) (Table 2).

Table 2. Heart rate increases and inhibition of Ach-induced bronchoconstriction by LAS100977 and salmeterol in Beagle dogs

			Inhibition of bronchoconstriction (%)			
		Heart rate increase post-dose (%)	0.3 hours	3 hours	6 hours	24 hours
LAS100977	1 µg/kg	4 ± 2	76 ± 7**	83 ± 2**	71 ± 4**	38 ± 19
Salmeterol	9 µg/kg	26 ± 2*	56 ± 5*	28 ± 12	20 ± 10	12 ± 8
Control	-	-2 ± 5	20 ± 8	29 ± 10	10 ± 5	9 ± 4

n=2–5 animals per dose. Inhibition expressed as mean ± standard error of the mean
Statistical analysis: two-way ANOVA and Bonferroni post-test in comparison with control group
*p<0.01 vs control; **p<0.001 vs control

Conclusions

- **LAS100977 inhibits ACh-induced bronchoconstriction in anaesthetised dogs more potently and with a longer duration of action than salmeterol.**
- **The TI of LAS100977 in dogs is significantly higher than salmeterol, which suggests that there may be a reduced potential for cardiac side effects in humans.**

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Acknowledgements

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Poster presented at the European Respiratory Society Annual Congress, Barcelona, Spain, 18-22 September 2010

Abstract

LAS100977, a novel β_2-agonist, has a longer duration of action and a more favourable therapeutic index than salmeterol in anaesthetised dogs

Montserrat Miralpeix, Mireia Gómez-Angelats, Mònica Aparici, Marisa Viñals, Jordi Beleta, Amadeu Gavaldà, Carlos Puig, Hamish Ryder, Jesús Llenas, Jordi Gras

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Introduction

LAS100977 is a novel, long-acting β_2-agonist in Phase II clinical development for the treatment of asthma and COPD. Here we report the potency and duration of action (DoA) of aerosolised LAS100977 and salmeterol on inhibition of bronchoconstriction in dogs. The effect of each drug on heart rate was also assessed to determine the therapeutic index (TI) of each compound.

Methods

Transient bronchospasm was induced by acetylcholine (Ach, 5 µg/kg i.v.) in anaesthetised Beagle dogs. Aerosolised β_2-agonists were administered via an ultrasonic nebuliser. ID_{50} (dose that inhibited bronchospasm by 50%) and ED_5 (dose that increased heart rate by 5%) were calculated for each drug 20 min post-dose. TI was defined as the ratio between ED_5 and ID_{50}. For DoA assays, compounds were administered at submaximal doses, and inhibition of bronchospasm was assessed at 20 min and 3, 6 and 24 h post-dose.

Results

LAS100977 was 27-fold more potent in inhibiting the Ach-induced bronchoconstriction than salmeterol (ID_{50} = 0.07 and 1.9 µg/kg, respectively). The TIs of LAS100977 and salmeterol were 8 and 0.4, respectively. At submaximal doses, LAS100977 had a longer anti-bronchoconstrictive effect than salmeterol (2-fold vs 5-fold loss of effect at 24 h) and, unlike salmeterol, did not show any significant effect on heart rate (4% and 26% increases for LAS100977 [1 µg/kg] and salmeterol [9 µg/kg], respectively).

Conclusion

In anaesthetised dogs, LAS100977 inhibits bronchoconstriction more potently and with a longer DoA than salmeterol. LAS100977 also has a higher TI than salmeterol, suggesting a reduced potential for cardiac side effects in man.