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ORIGINAL RESEARCH ARTICLE

Use of Propranolol Blockade to Explore the Pharmacology of GSK961081, a Bi-Functional Bronchodilator, in Healthy Volunteers: Results from Two Randomized Trials

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Abstract

Purpose The objective of this study was to explore the pharmacology of GSK961081, a bi-functional bronchodilator, in healthy volunteers.

Methods Two randomized, double-blind, placebo-controlled studies were conducted. Following optimization of the propranolol dosing regimen (study 1), we conducted a five-period crossover study (study 2) in which subjects received the following treatments: dry powder inhaler (DPI) GSK961081 400 µg + oral placebo, DPI GSK961081 1,200 µg + oral placebo, DPI GSK961081 400 µg + oral propranolol 80 mg, DPI GSK961081 1,200 µg + oral propranolol 80 mg and DPI and oral placebo. GSK961081 (or inhaled placebo) was dosed at 0 h. Propranolol (or oral placebo) was dosed at –8, –2, 4, 10, and 16 h. The primary endpoint for both studies was bronchodilation, measured by specific airway conductance (sGaw), which was assessed at 0, 1, 4, 7, 12, 22, and 24 h in study 2. Tolerability and pharmacokinetics were secondary endpoints.

Results Studies 1 and 2 enrolled 18 and 23 subjects, respectively. In study 2, bronchodilation was seen for 24 h following GSK961081 400 and 1,200 µg. In the presence of β₂ blockade, GSK961081 1,200 µg demonstrated bronchodilation in the first 4 h after dosing (treatment difference from placebo at 1 h: 1.206; 90 % confidence interval [CI] 1.126–1.292; and at 4 h: 1.124; 90 % CI 1.078–1.173)

but not at 7 h onwards. In the presence of β₂ blockade, GSK961081 400 µg demonstrated bronchodilation in the first 1 h after dosing (treatment difference from placebo: 1.193; 90 % CI 1.117–1.274), but not at 4 h onwards. Adverse events were reported for 21 (study 1) and 15 subjects (study 2); none were serious, and there were no deaths.

Conclusion The duration of bronchodilation as a result of receiving the muscarinic antagonist component alone was shorter than that from the muscarinic antagonist β₂ agonist combination. Removing the β₂ agonist component may underestimate the contribution of the muscarinic antagonist component to the bronchodilation of the combination.

Key Points

GSK961081 is a novel bi-functional molecule that combines muscarinic antagonism (MA) and β₂ agonism (BA) in a single molecule (MABA).

Bronchodilation following inhaled β₂ agonist and anti-muscarinic agents can be measured by specific airway conductance (sGaw) in healthy volunteers. We used this endpoint, in the presence and absence of propranolol, to explore the pharmacology of GSK961081.

The duration of bronchodilation following GSK961081 from the muscarinic antagonist component alone was shorter than that from the MABA combination. However, removing the β₂ component may underestimate the contribution of the muscarinic antagonist component to the bronchodilation of the combination.

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1 Introduction

Inhaled bronchodilators are the mainstay of the symptomatic treatment of chronic obstructive pulmonary disease (COPD), and both long-acting β_2 agonists (LABAs) and long-acting muscarinic antagonists (LAMAs) are frequently prescribed as maintenance therapy. A combination of these agents can provide greater efficacy for patients who remain symptomatic on LABA or LAMA monotherapy, and a number of studies have demonstrated a superior bronchodilation effect with combined LABA and LAMA compared with the individual agents alone [1–6].

GSK961081 is a novel bi-functional molecule (or dual pharmacophore) that combines muscarinic antagonism (MA) and β_2 agonism (BA) in a single molecule (MABA). Pre-clinical data showed GSK961081 to be a potent functional antagonist of muscarinic receptors, as well as a potent, selective, and full agonist at the β_2 adrenoceptor, which produced significant and sustained bronchoprotection that was significantly greater than that with either of the MA or BA components alone [7, 8]. Clinically, GSK961081 at 400 and 1,200 μg once daily for 2 weeks demonstrated sustained bronchodilation similar to a combination of tiotropium 18 μg once daily plus salmeterol 50 μg twice daily but with a more rapid onset of action in patients with moderate COPD [9]. Additionally, a dose of 400 μg once a day for 28 days resulted in a forced expiratory volume in 1 s (FEV_1) of 215 (139–291 at 95 % confidence interval) mL greater than placebo in patients with moderate and severe COPD [10]. It may be necessary to understand the relative contribution of the β_2 agonist versus anti-muscarinic components of such a molecule in humans. One way to do that is to block one of the components. Bronchodilation following inhaled β_2 agonist and anti-muscarinic agents can be measured by specific airway conductance (sGaw) in healthy volunteers [11–16]. Inhibition of β_2 agonist-mediated bronchodilation (as measured by sGaw) in healthy volunteers by the non-selective β -blocker propranolol has been reported previously [17–21]. However, published studies have generally looked at the effect of a single dose of propranolol on a β_2 agonist over a relatively short (a few hours) period of time, have been small, and have used various doses of propranolol and β_2 agonist with various degrees of inhibition of bronchodilation. Propranolol alone does not affect sGaw in healthy volunteers [19]. However, there were no published data on the effects of β blockade on sGaw following an inhaled anti-muscarinic alone or the combination of inhaled β_2 agonist and anti-muscarinic.

We therefore conducted and report two studies. Study 1 was conducted to confirm a dosing regimen of the β antagonist propranolol, which prevents the increase in

sGaw to a β_2 agonist over 24 h and had acceptable tolerability. It was also to confirm that the β antagonist propranolol did not prevent an increase in sGaw in response to an anti-muscarinic and also to characterize the effect on sGaw of a combination of an anti-muscarinic and β_2 agonist and the effect of propranolol on the combination. Study 2 reports bronchodilation following the administration of GSK961081 in healthy volunteers in the presence and absence of propranolol.

2 Methods

2.1 Subjects

Healthy adult male or female (study 1 only, as reproductive toxicology had not been completed for GSK961081 at the time of study) subjects aged 18–50 years inclusive with a body mass index (BMI) within the range 19–29.9 kg/m^2 were included. Subjects were required to be non-smokers for at least 6 months before the study, have no history of respiratory disease, and have an $\text{FEV}_1 \geq 80$ % predicted and a $\text{FEV}_1/\text{forced vital capacity (FVC)}$ ratio ≥ 0.7 . Subjects were also required to have an increase in sGaw of ≥ 15 % over pre-dose baseline within 2 h of administration of salbutamol 600 μg by metered dose inhaler (MDI) at screening or in the 3 months before screening and have an increase in sGaw of ≥ 25 % over pre-dose baseline within 2 h following ipratropium bromide 40 μg at screening or in the 3 months before screening.

2.2 Study Design

Study 1 was a randomized, double-blind (with respect to propranolol administration), open (with respect to bronchodilator administration), six-period crossover design (GlaxoSmithKline [GSK], London, UK, study number: MAB114954; www.clinicaltrials.gov: NCT00549120) conducted at Parexel Clinical Research Unit (CRU), UK, between 10 September 2007 and 22 October 2007. At study period 1, subjects received propranolol 80 mg (five doses at 6-h intervals) or placebo (five doses at 6-h intervals). At study periods 2, 3, and 4, subjects received each of the following three treatments in randomized order: propranolol 80 mg (five doses at 6-h intervals) + MDI salbutamol 600 μg (four doses at 6-h intervals); placebo (five doses at 6-h intervals) + MDI salbutamol 600 μg (four doses at 6-h intervals); or propranolol 80 mg (five doses at 6-h intervals) or placebo (five doses at 6-h intervals) (whichever treatment was not taken in session 1). At study periods 5 and 6, subjects received propranolol 80 mg (two doses at 6-h intervals) + MDI ipratropium 40 μg (two doses at 6-h

intervals) + MDI salbutamol 600 µg (one dose); and placebo (two doses at 6-h intervals) + MDI ipratropium 40 µg (two doses at 6-h intervals) + MDI salbutamol 600 µg (one dose). In study periods 1–4, propranolol was dosed at 0, 6, 12, 18, and 24 h and salbutamol was dosed at 2, 8, 14, and 20 h. In study periods 5 and 6, propranolol was dosed at 0 and 6 h, ipratropium at 2 and 8 h, and salbutamol at 8 h. Subjects were randomized to treatment sequences in accordance with the randomization schedule generated by Clinical Pharmacology Statistics and Programming (GlaxoSmithKline), using validated software (RandAll; GlaxoSmithKline). The placebo tablets (white in color) did not exactly match the propranolol tablets (pink in color), and the blind was maintained by blindfolding the subjects for propranolol or placebo administration.

Study 2 was a double-blind, placebo-controlled, five-period crossover design (GSK study number: MAB110553; www.clinicaltrials.gov: NCT00687700) conducted at Parxel CRU, UK, between 10 March 2008 and 27 May 2008. Subjects received each of the five treatments in randomized order: dry powder inhaler (DPI) GSK961081 400 µg + oral placebo, DPI GSK961081 1,200 µg + oral placebo, DPI GSK961081 400 µg + oral propranolol 80 mg, DPI GSK961081 1,200 µg + oral propranolol 80 mg, and inhaled DPI and oral placebo. GSK961081 (or inhaled placebo) was dosed at 0 h. Propranolol (or oral placebo) was dosed at –8, –2, 4, 10, and 16 h. Subjects were randomized to treatment sequences in accordance with the randomization schedule generated by Discovery Biometrics, prior to the start of the study, using validated software (RandAll). The placebo tablets (white in color) did not exactly match the propranolol tablets (pink in color), and tablets were over-encapsulated to maintain the blind.

Both studies included a washout of at least 7 days between treatments, and subjects attended a follow-up visit for safety approximately 1 week after completion of the study periods. The study protocols and informed consent were approved by the relevant ethics committees. Written informed consent was obtained from each subject before enrollment.

2.3 Pharmacodynamic Assessment (Primary Endpoint, Both Studies)

Three sGaw measurements, using whole body plethysmography, were performed at each of the following time points and the mean used in the data analysis. In study 1, sGaw was measured at 0, 3, 6, 9, 15, and 26 h during study periods 1–4 and at 0, 3, 6, and 9 h during study periods 5 and 6. In study 2, sGaw was measured at 0, 1, 4, 7, 12, 22, and 24 h.

2.4 Pharmacokinetic Assessment (Secondary Endpoint, Both Studies)

2.4.1 Propranolol

Blood (2 mL) for the determination of plasma propranolol concentrations were collected pre-dose, 3, 4, 6, 9, 15, 26, and 28 h post-dose in study 1, and –8, –2, 4, 10, 16, and 24 h post-GSK961081 dose in study 2. Plasma samples were analyzed for propranolol using a validated analytical method based on turbulent flow extraction, followed by TFC-LC-MS/MS analysis. The lower limit of quantification (LLQ) for propranolol was 1 ng/mL, using a 20-µL aliquot of human plasma with a higher limit of quantification (HLQ) of 250 ng/mL.

2.4.2 GSK961081 (Study 2 Only)

Blood samples (2 mL) for the determination of plasma concentrations of GSK961081 (study 2) were collected pre-GSK961081 dose and 0.5, 1, 2, and 4 h post-dose. Human plasma samples were analyzed for GSK961081 using a validated analytical method based on protein precipitation, followed by HPLC/MS/MS analysis. The LLQ for GSK961081 was 25 pg/mL using a 50-µL aliquot of human plasma with a HLQ of 25,000 pg/mL. The computer systems that were used on this study to acquire and quantify data included Analyst Version 1.4.1 and SMS2000 versions 1.6 and 2.0.

Quality control (QC) samples for both the propranolol and the GSK961081 assays were prepared at three different analyte concentrations and stored with study samples, and were analyzed with each batch of samples against separately prepared calibration standards. For the analyses to be acceptable, no more than one-third of the total QC results and no more than one-half of the results from each concentration level were to deviate from the nominal concentration by more than 15 %. The applicable analytical runs met all predefined run acceptance criteria.

2.4.3 Safety (Secondary Endpoint, Both Studies)

Safety laboratory tests were conducted at screening visit and follow-up for both studies and also 32 h after each inhaled dose for study 2. 12-lead electrocardiogram (ECG), blood pressure, and heart rate were collected frequently throughout both studies. Glucose and potassium were also collected frequently during study 2. Adverse events (AEs) were collected.

2.5 Dose Rationale

2.5.1 GSK961081

Doses of 400 and 1,200 μg were selected as these were studied in a 14-day efficacy study in patients with COPD; these doses gave comparable efficacy [9].

2.5.2 Salbutamol

A dose of salbutamol 600 μg 6 hourly (four doses) was selected to maintain β -agonist activity over the 24-h period. Whilst lower doses would give effective bronchodilation [12], it was important to test the effectiveness of the propranolol regimen at higher than effective bronchodilator doses.

2.5.3 Ipratropium

Ipratropium 40 μg was selected as this dose has been shown to have effective bronchodilation [13] in healthy volunteers. It was also important to use a dose that was at the lower end of the effective dose range.

2.5.4 Propranolol

For study 1, a dose of propranolol 80 mg 6 hourly (five doses in periods 1–4 and two doses in periods 5 and 6) was selected. Using published propranolol information on its pharmacokinetics in humans [22] and its in vitro affinity for the β_2 receptor ($\text{pA}_2 = 9.4$, competitive antagonist [23]), various propranolol dosing scenarios were simulated using Berkeley Madonna version 8.3.14 to determine a dosing regimen to maintain plasma propranolol concentrations above the in vitro predicted 90 % effective concentration (EC_{90}). The final propranolol dosing regimen selection was based on clinical safety experience [24] and the simulated scenarios, and allowed for the dosing interval of propranolol to be increased from once every 6 h to once every 8 h if tolerability issues arose.

For study 2, a dose of 80 mg propranolol 6 hourly (five doses) was selected. The dose selection was based on the results of study 1, where plasma propranolol concentrations were above the in vitro predicted EC_{90} (~ 10.53 ng/mL) in all but 2 % of samples, indicating that adequate propranolol levels were achieved (Fig. 1). In addition, a population pharmacokinetic model was fitted to the study 1 propranolol data to evaluate how many propranolol doses to administer prior to GSK91081 and to determine whether four rather than five propranolol doses would be sufficient. It was determined by simulation that administering two propranolol doses prior to GSK961081 and three propranolol doses post GSK961081 would maintain the lower

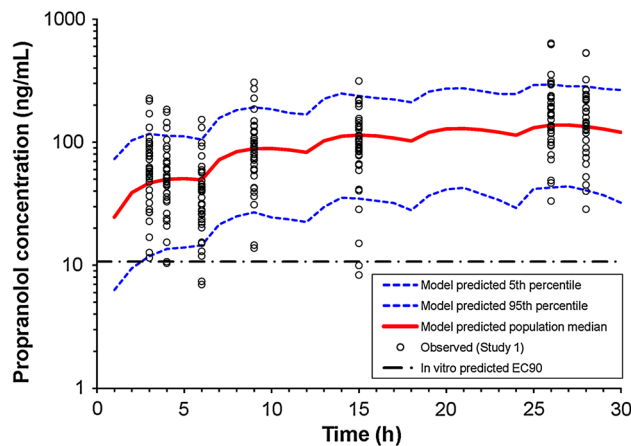


Fig. 1 Observed propranolol concentrations and model prediction (Study 1). EC_{90} 90 % effective concentration

bound of the model predicted plasma propranolol concentration profile above the in vitro predicted EC_{90} over the duration under investigation (Fig. 1). This was also determined by simulation for a propranolol dose of 80 mg, thus enabling dose modification if tolerability issues arose.

2.6 Statistical Methods

2.6.1 Pharmacodynamics

In study 1, a total of 18 subjects were randomized, with the aim that 16 subjects would complete the study with evaluable data; 16 evaluable subjects was calculated to provide approximately 90 % power to detect the non-inferiority at 5 % significance level, assuming within- and between-subject standard deviations of 0.124 and 0.146 for sGaw, respectively. Serial sGaw data were analyzed using mixed effects modeling, following a natural logarithmic transformation. Non-inferiority tests were performed. The upper limit of 90 % confidence interval (CI) of the treatment ratio was compared with 1.15 to assess the non-inferiority of propranolol + salbutamol versus placebo. The lower limit of CI of the treatment ratio was compared with 0.87 to assess the non-inferiority of propranolol + ipratropium versus placebo + ipratropium and propranolol + salbutamol + ipratropium versus placebo + salbutamol + ipratropium.

For study 2, a total of 23 subjects were randomized, with the aim that 20 subjects would complete the study with evaluable data. No formal sample size calculation was conducted, as no formal hypothesis was to be tested; the focus was on estimation. The primary endpoint, serial sGaw, was analyzed, following a natural logarithmic transformation, using a mixed effects model. The model fitted treatment, time, period, and treatment by time as fixed effects; subject-level baseline, period-level baseline,

and period-level baseline by time as continuous covariates; and subject as a random effect. Comparisons were made between GSK961081 + propranolol and placebo treatment groups with the two different GSK961081 doses and at various time points. Point estimates and corresponding 90 % CIs were constructed for the estimated differences.

2.6.2 Pharmacokinetics

Propranolol: In study 1, plasma propranolol concentration-time data were analyzed by non-compartmental methods using WinNonlin 4.1 Software (Pharsight, Mountain View, CA, USA) to determine, for the first propranolol dosing interval, the area under the curve from time 0 to 6 h (AUC_{τ}), peak concentration (C_{max}), and time to reach peak (t_{max}). In addition, for study 1, a one-compartment model with oral absorption was fitted to the propranolol concentration-time data for all dosing intervals using the software NONMEM VI.

GSK961081: In study 2, plasma GSK961081 concentration-time data were analyzed by non-compartmental methods using WinNonlin 4.1 Software to determine the AUC to last quantifiable concentration (AUC_{τ}), C_{max} , t_{max} , and time of last quantifiable concentration (t_{last}).

3 Results

3.1 Subject Disposition

Demographic and baseline characteristics are summarized in Table 1. Eight male and ten female healthy volunteers, mean age 29.6 years (range 21–46) and mean BMI 23.7 kg/m² (range 19.6–28.9) were randomized and completed study 1. Study 2 enrolled 23 healthy male volunteers, mean age 32.0 years (range 20–49) and mean BMI 24.7 kg/m² (range 20.3–29.9). A total of 21 subjects completed the study; two subjects were withdrawn due to protocol violations (one positive result for drugs of abuse, one positive for cotinine).

3.2 Pharmacodynamic Results

In study 1, propranolol alone did not affect sGaw (Fig. 2a; Table 2). Inhalation of salbutamol 600 µg resulted in an expected increase in sGaw (Fig. 2a; Table 2). No significant bronchodilation was observed at the last time point after inhalation of salbutamol, probably because the final sGaw measurement was taken too long after the last salbutamol dose. Propranolol prevented the increase in sGaw in response to salbutamol 600 µg (Fig. 2a); sGaw following propranolol + salbutamol was non-inferior to placebo (Table 2).

In study 1, propranolol did not prevent the sGaw response to ipratropium 40 µg (Fig. 2b); sGaw following propranolol + ipratropium was non-inferior to placebo + ipratropium (Table 2). Addition of salbutamol 600 µg to ipratropium 40 µg showed a small trend to increased sGaw compared with when salbutamol 600 µg was added to ipratropium 40 µg in the presence of propranolol (Fig. 2b: 9-h time point), but the comparison showed that salbutamol + ipratropium + propranolol was non-inferior to salbutamol + ipratropium (Table 2).

In study 2, bronchodilation was seen for 24 h following a single dose of both 400 and 1,200 µg GSK961081 (Fig. 3). In the presence of effective β_2 blockade, GSK961081 1,200 µg demonstrated bronchodilation in the first 4 h after dosing (treatment difference from placebo at 1 h: 1.206 [90 % CI 1.126–1.292] and 4 h: 1.124 [90 % CI 1.078–1.173]) but not at 7 h onwards (Table 3). In the presence of effective β_2 blockade, GSK961081 400 µg demonstrated bronchodilation in the first 1 h after dosing (treatment difference from placebo: 1.193 [90 % CI 1.117–1.274]), but not at 4 h onwards (Table 3).

3.3 Pharmacokinetic Results

3.3.1 Propranolol

In study 1, plasma propranolol concentrations were above the in vitro predicted EC_{90} (~10.53 ng/mL) in all but 2 % of samples. There was no apparent difference in exposure to propranolol when administered alone or in combination with either salbutamol or ipratropium and salbutamol (Table 4). The propranolol population pharmacokinetic model gave a reasonable fit to the observed data (Fig. 1) and model predicted AUC_{τ} , C_{max} , and t_{max} were comparable to the non-compartmental analysis results (data not shown).

In study 2, plasma propranolol concentrations were above the in vitro predicted EC_{90} (~10.53 ng/mL) in all but 0.87 % of samples, all of which were prior to GSK961081 administration.

3.3.2 GSK961081

In study 2, plasma GSK961081 systemic exposure (C_{max} and $AUC_{(0-t)}$) was increased by approximately 60 % in the presence of propranolol (Table 5). In the absence of propranolol, systemic exposure to GSK961081 was in line with systemic exposure previously achieved in healthy volunteers at similar dose levels. Propranolol was not administered in the absence of GSK961081 in this study; however, comparable trough propranolol levels were achieved in study 1 after the first dose of propranolol 80 mg (data not shown).

Table 1 Summary of baseline and demographic characteristics (studies 1 and 2)

Characteristics	Study 1 (MAB104954) <i>N</i> = 18	Study 2 (MAB110553) <i>N</i> = 23
Age, years	29.6 (21–46)	32.0 (20–49)
Male	8 (44)	23 (100)
Ethnicity		
Hispanic/Latino	1 (6)	2 (9)
Not Hispanic/Latino	17 (94)	21 (91)
Race		
African American/African heritage	3 (17)	1 (4)
Asian–Central/South Asian heritage	2 (11)	2 (9)
Asian–East Asian heritage	0 (0)	1 (4)
Asian–Japanese heritage	1 (6)	0 (0)
Asian–South East Asian heritage	0 (0)	2 (9)
Native Hawaiian or other Pacific Islander	1 (6)	0 (0)
White–White Caucasian/European heritage	10 (56)	17 (74)
Mixed race	1 (6)	0 (0)
Height, cm	172.7 (161–191)	173.3 (157–193)
Weight, kg	70.8 (56.1–86.3)	74.23 (60–93)
BMI, kg/m ²	23.67 (19.6–28.9)	24.70 (20.3–29.6)

Data are presented as mean (range) or n (%) unless otherwise indicated

BMI body-mass index

Table 2 Summary of statistical analysis of log-transformed specific airway resistance (1/KPa*s) data (study 1)

Treatment	<i>H</i>	<i>N</i>	Adjusted geometric mean		Treatment ratio	
			Estimate	SE logs	Estimate	90 % CI
PRO + SAL (vs. PL)	3	18	0.824	0.0249	1.069	1.012–1.130
	6	18	0.778	0.0311	0.966	0.900–1.037
	9	18	0.841	0.0330	1.025	0.951–1.106
	15	18	0.780	0.0355	1.043	0.961–1.131
	26	18	0.762	0.0257	0.961	0.907–1.018
PL + SAL (vs. PL)	3	18	0.965	0.0249	1.253	1.185–1.325
	6	18	0.932	0.0311	1.157	1.078–1.243
	9	18	1.045	0.0329	1.273	1.180–1.374
	15	18	0.990	0.0354	1.322	1.218–1.435
	26	18	0.808	0.0261	1.020	0.962–1.082
PRO only (vs. PL)	3	18	0.753	0.0270	0.978	0.925–1.034
	6	18	0.772	0.0330	0.959	0.893–1.030
	9	18	0.808	0.0347	0.984	0.912–1.062
	15	18	0.748	0.0371	0.999	0.920–1.085
	26	18	0.767	0.0278	0.968	0.913–1.027
PRO + IPR (vs. PL + IPR)	3	18	0.901	0.0278	0.973	0.934–1.014
PRO + IPR + SAL (vs. PL + IPR + SAL)	9	18	0.905	0.0316	0.926	0.876–0.978

CI confidence interval, *H* hour, *IPR* ipratropium, *PL* placebo, *PRO* propranolol, *SAL* salbutamol, *SE* standard error

3.4 Safety Results

In study 1, a total of 15 subjects experienced AEs. The most frequently reported AEs were headache (21 reports),

tremor (15), and dizziness (13 reports). All AEs were rated as being mild or moderate in intensity, and no serious AEs were reported. One subject (salbutamol + placebo) missed one dose of propranolol due to an increase in corrected QT

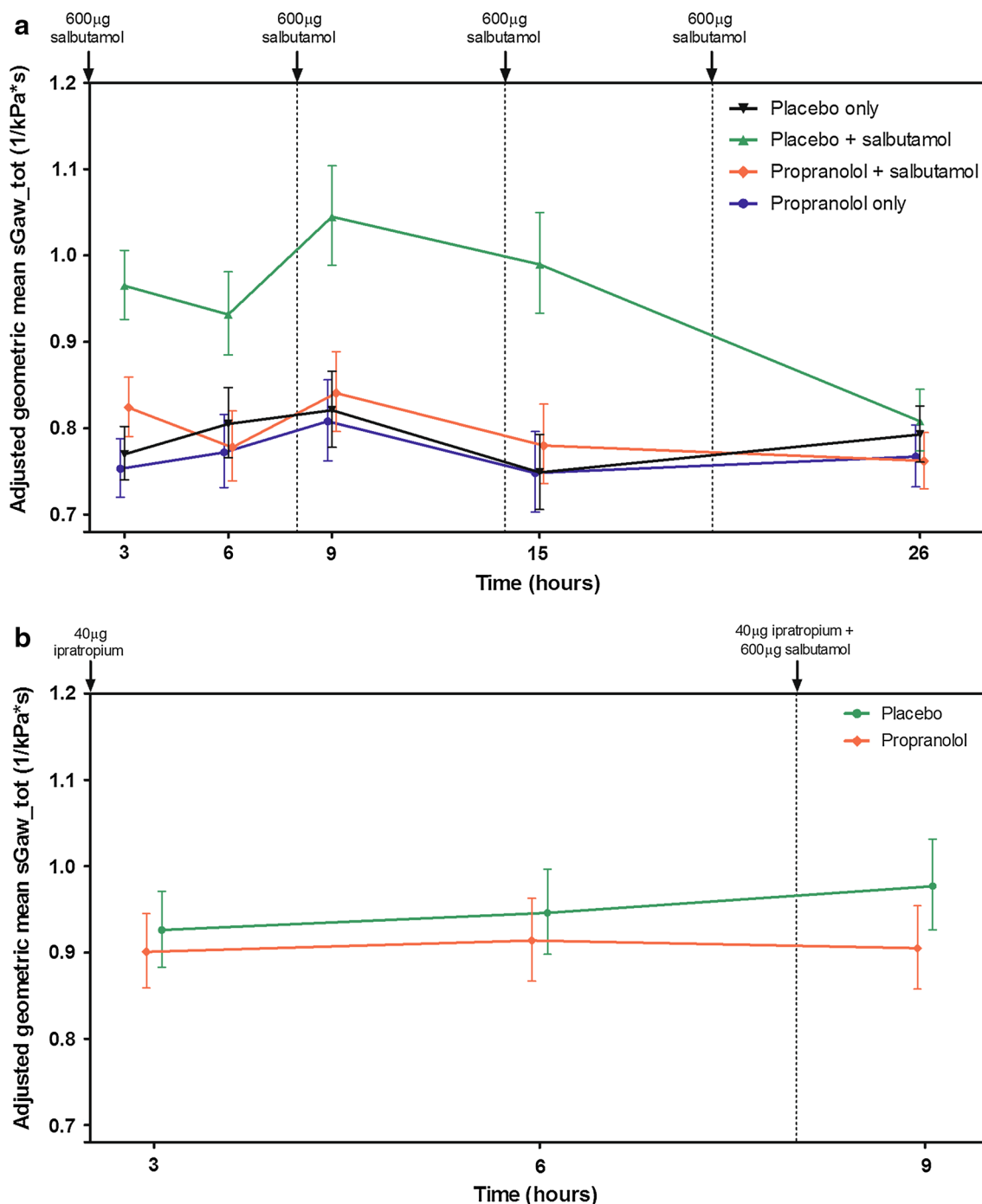


Fig. 2 a sGaw following placebo alone, propranolol alone, salbutamol alone, and propranolol + salbutamol (study 1). **b** sGaw following propranolol + ipratropium and propranolol + ipratropium + salbutamol (study 1). *sGaw* specific airway conductance

interval (QTc) of potential clinical concern to the investigator. There were no clinically important findings in other safety assessments.

In study 2, a total of 21 subjects experienced AEs. The most frequently reported AEs were dysgeusia (24 reports), dizziness (seven reports), and headache (six reports). All AEs were rated as being mild or moderate in intensity, and no serious AEs were reported. One subject (GSK961081

1,200 µg + propranolol) had a PR interval of 224 at 6 h after the first dose of propranolol and therefore did not have further doses of propranolol. The efficacy data for this subject were not included in the analysis for this treatment. There were no clinically important findings in other safety assessments. Potassium and glucose data were similar at all time points (pre-dose, 1, 4, and 24 h) across all treatments, including placebo.

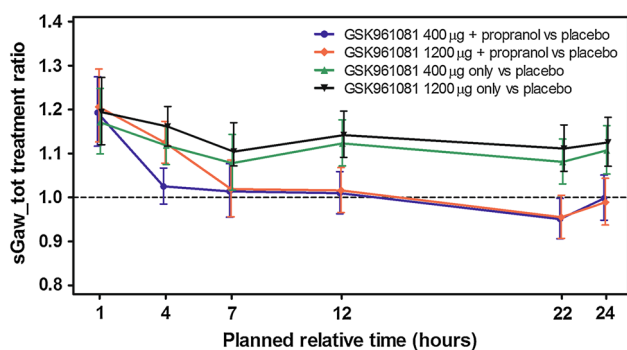


Fig. 3 Estimated treatment ratios (and 90 % CIs) sGaw versus placebo (study 2). *CI* confidence interval, *sGaw* specific airway conductance

4 Discussion

In study 1, inhalation of salbutamol 600 µg (+ placebo) resulted in the expected bronchodilation. At the final time point for sGaw assessment, 6 h after the last dose of salbutamol, there was no increase in sGaw. This is because the final sGaw measurement was taken too long after the

last salbutamol dose. Our data are consistent with published data showing that sGaw 6 h after salbutamol is the same as after placebo [14]. The propranolol regimen used prevented the increase in sGaw in response to salbutamol 600 µg, but did not prevent the sGaw response to ipratropium 40 µg. Therefore, we have demonstrated a propranolol dosing regimen that was effective in preventing bronchodilation to a supra-therapeutic dose of β_2 agonist, but that did not prevent the bronchodilation to a low dose of anti-muscarinic.

This is the first study where the effect of combined salbutamol and ipratropium on sGaw has been explored in healthy volunteers. Whilst there was a trend to an increase in sGaw when salbutamol was added to ipratropium, this assessment does not appear to be sensitive enough to detect the benefit of the two mechanisms in healthy volunteers. This is in contrast to this endpoint in COPD patients, where a single dose of salmeterol 50 µg (+ fluticasone propionate 500 µg) + tiotropium 18 µg was significantly more effective than tiotropium or salmeterol (+ fluticasone) alone in improving post-dose sGaw [25].

Table 3 Summary of statistical analysis of log-transformed specific airway resistance (1/KPa*s) data (study 2)

Treatment	H	N	Adjusted geometric mean		Treatment ratio (vs. Placebo)	
			Estimate	SE logs	Estimate	90 % CI
081 400 µg + PRO	1	23	1.176	0.0291	1.193	1.117–1.274
	4	23	1.050	0.0195	1.025	0.985–1.067
	7	23	1.044	0.0269	1.014	0.955–1.077
	12	23	0.983	0.0223	1.010	0.963–1.059
	22	23	0.923	0.0226	0.951	0.906–0.998
	24	22	0.985	0.0239	0.998	0.948–1.051
081 1,200 µg + PRO	1	22	1.189	0.0309	1.206	1.126–1.292
	4	22	1.151	0.0207	1.124	1.078–1.173
	7	22	1.049	0.0286	1.019	0.956–1.085
	12	22	0.989	0.0236	1.016	0.966–1.068
	22	22	0.927	0.0240	0.955	0.907–1.005
	24	22	0.976	0.0249	0.989	0.938–1.044
081 400 µg only	1	23	1.154	0.0292	1.171	1.099–1.248
	4	23	1.145	0.0196	1.118	1.075–1.162
	7	23	1.110	0.0270	1.078	1.017–1.143
	12	23	1.093	0.0224	1.123	1.072–1.176
	22	23	1.050	0.227	1.081	1.031–1.133
	24	23	1.092	0.0235	1.107	1.053–1.163
081 1,200 µg only	1	23	1.177	0.0292	1.194	1.120–1.273
	4	23	1.190	0.0196	1.161	1.117–1.207
	7	23	1.136	0.0270	1.104	1.041–1.170
	12	23	1.112	0.0224	1.142	1.091–1.196
	22	23	1.079	0.0227	1.111	1.060–1.165
	24	23	1.110	0.0236	1.125	1.071–1.182

CI confidence interval, *H* hour, *PRO* propranolol, *SE* standard error, *081* GSK961081

Table 4 Plasma propranolol pharmacokinetic parameters (study 1)

Treatment	N/n	C_{max}^a (ng/mL)	AUC_t^a (ng.h/mL)	t_{max}^b (h)
PRO only	18/18	55.12 (38.84–78.21)	201.5 (142.2–285.6)	3.05 (3.00–4.00)
PRO + SAL	18/18	63.97 (45.96–89.04)	234.0 (166.3–329.2)	3.05 (3.02–5.90)
PRO + IPR + SAL	18/18	69.78 (52.04–93.55)	258.6 (191.7–348.8)	3.07 (3.00–4.00)

AUC_t area under the concentration time curve from time 0 to 6 h, CI confidence interval, C_{max} maximum observed plasma concentration, IPR ipratropium, PRO propranolol, SAL salbutamol, t_{max} time to maximum observed concentration

^a Geometric mean (95 % CI)

^b Median (range)

Table 5 Plasma GSK961081 pharmacokinetic parameters (study 2)

Treatment	N/n	C_{max}^a (pg/mL)	$AUC_{(0-t)}^a$ (pg.h/mL)	t_{max}^b (h)	t_{last}^b (h)
081 400 μ g + PRO	23/23	165,236 (147,292–185,367)	204,816 (178,161–235,459)	0.50 (0.43–1.12)	2.02 (0.98–3.88)
081 1,200 μ g + PRO	23/23	528,172 (464,459–600,624)	870,949 (768,076–987,602)	0.98 (0.47–1.08)	3.83 (2.02–3.88)
081 400 μ g only	23/23	101,052 (89,546–114,036)	121,249 (97,867–150,218)	0.95 (0.45–1.12)	2.02 (0.97–3.98)
081 1,200 μ g only	23/23	307,291 (264,677–356,765)	585,333 (502,799–681,416)	0.98 (0.48–1.07)	3.85 (2.02–3.90)

AUC area under the concentration–time curve, CI confidence interval, C_{max} maximum observed plasma concentration, PRO propranolol, t_{max} time to maximum observed concentration, 081 GSK961081

^a Geometric mean (95 % CI)

^b Median (range)

In study 2, bronchodilation was seen for 24 h following a single dose of both 400 and 1,200 μ g GSK961081. In the presence of effective β_2 blockade, GSK961081 1,200 μ g demonstrated bronchodilation in the first 4 h after dosing but not at 7 h onwards, whilst GSK961081 400 μ g demonstrated bronchodilation in the first 1 h after dosing, but not at 4 h onwards. Therefore, in this model, the MA alone bronchodilation is of shorter duration than the bronchodilation achieved with the MA/BA activity combined.

This assessment may underestimate the MA contribution to the MA/BA activity combined. Amplification of bronchodilation achieved by one component by the other component is believed to contribute to the benefit of combined anti-muscarinic and β_2 agonist [26]. Investigative studies have suggested that the enhanced MABA activity compared with the single ligands may be due to crosstalk between the engaged M3 and β_2 receptors [27]. Crosstalk between the receptors could be reduced or abolished by blocking the β receptor as has been done in the reported clinical studies.

Therefore, removing the BA component completely may underestimate the contribution of the MA component to the bronchodilation of the combination by removing the potential for amplification of one mechanism by the other. In addition, the response in healthy volunteers may not be the same as in COPD patients. In COPD patients, salbutamol and ipratropium have been added at 1, 12, and 24 h following single doses of GSK961081 400 and 1,200 μ g [28]. In that study, the additional bronchodilation following

salbutamol and ipratropium inhalation was similar, and this indirect assessment may indicate that the activities of the MA and BA components are of a similar magnitude. A further limitation is that the study looked at single-dose GSK961081 only; investigating the response after repeat dosing would also be of interest.

In both studies, all dosing regimens were well tolerated. There were no serious AEs, and all AEs reported were consistent with the known effects of propranolol and salbutamol or the AEs previously reported with GSK961081 [9, 10, 28].

Pharmacokinetic modeling was used to inform propranolol dose selection for the two studies. The propranolol dosing regimen (five doses of propranolol 80 mg at 6-h intervals) was safe and well tolerated and shown to provide plasma propranolol concentrations above the in vitro predicted EC_{90} throughout the treatment session. This propranolol dosing regimen was effective at preventing the increase in sGaw in response to salbutamol 600 μ g. However, it is possible that propranolol did not completely block the effects of the BA component of the MABA, and some of the bronchodilation seen following MABA in the presence of propranolol is due to residual BA effect. There was no apparent difference in exposure to propranolol when administered alone or in combination with either salbutamol or ipratropium and salbutamol. GSK961081 systemic exposure (C_{max} and AUC_t) was increased by approximately 60 % in the presence of propranolol. In vitro data suggest that the difference is unlikely to be a result of

metabolic interaction or plasma protein-binding displacement. Propranolol is a cytochrome P450 (CYP)1A2, CYP2D6, and CYP2C19 substrate with a minor route (~15%) via direct glucuronidation; whereas that of GSK961081 appears to be by direct glucuronidation and a CYP3A4 substrate. A postulated reason for the difference is that GSK961081 apparent clearance is decreased as a result of a decrease in liver blood flow in the presence of propranolol (β_2 adrenoceptor mediated).

In conclusion, in this healthy volunteer model, the duration of bronchodilation from the MA component alone was of shorter duration than that achieved from the MA/BA combination.

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Ethics statement The study protocols (GSK study numbers MAB104954 and MAB110553; NCT00687700 and NCT00549120) and informed consent were approved by the relevant ethics committees. Written informed consent was obtained from each subject before enrolment.

Contributions and Conflict of interest Authors' statement V Norris and C Ambery contributed to study design, oversaw the study conduct, contributed to and approved the final statistical analysis plan, and interpreted the data. V Norris lead the development of the manuscript and C Ambery reviewed and contributed to all drafts; both authors approved the final version. V Norris and C Ambery are employed by, and hold stocks and shares in, GSK.

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