

The 5-lipoxygenase-activating protein inhibitor, GSK2190915, attenuates the early and late responses to inhaled allergen in mild asthma

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Summary

Background GSK2190915, a potent 5-lipoxygenase-activating protein inhibitor, prevents the synthesis of leukotrienes and 5-oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-EETE).

Objective To assess the effect of GSK2190915 on the allergen-induced asthmatic responses.

Methods Nineteen eligible male subjects with mild asthma were enrolled in and completed this four-centre, double-blind, two-way crossover study (ClinicalTrials.gov NCT00748306). Subjects took GSK2190915 100 mg and placebo orally once daily for 5 days in randomized order. On Day 1 and 4 they had a methacholine challenge, on Day 3 they had an inhaled allergen challenge, and on Days 4 and 6 they had sputum induction.

Results GSK2190915 attenuated the early (0–2 h) and late (4–10 h) asthmatic responses to inhaled allergen compared with placebo. There was a statistically significant attenuation of the early asthmatic response (EAR) by GSK2190915; treatment difference of GSK2190915 vs. placebo for the minimum FEV₁ EAR was 0.408 L (0.205, 0.611). There was a statistically significant attenuation of the late asthmatic response (LAR) by GSK2190915; the treatment difference of GSK2190915 vs. placebo for the minimum FEV₁ LAR was 0.229 L (0.041, 0.417).

There was a statistically significant attenuation of allergen-induced sputum eosinophil count on Day 4 following GSK2190915: mean treatment difference (95% CI) between GSK2190915 and placebo was –9.95% (–18.15%, –1.77%). Compared with placebo, GSK2190915 100 mg reduced median sputum LTB₄ by > 90% on Days 4 and 6. There was no effect on methacholine PC₂₀ post allergen. GSK2190915 was generally well tolerated.

Conclusion and Clinical Relevance GSK2190915 shows potential as a treatment for patients with asthma.

Keywords 5-lipoxygenase-activating protein inhibitor, AM803, bronchial allergen challenge, GSK2190915, leukotriene, sputum eosinophils

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Introduction

The role of leukotrienes in asthma is well documented [1]. Leukotrienes are formed from the metabolism of the membrane phospholipid arachidonic acid (AA) by 5-lipoxygenase (5-LO). 5-lipoxygenase-activating protein (FLAP) binds to 5-LO and enables transfer of AA to 5-LO. AA is subsequently converted to leukotriene A₄

(LTA₄), which may be converted to LTB₄ or the cysteinyl leukotrienes (cysLTs) LTC₄, LTD₄ and then LTE₄. CysLT levels are raised in the lungs of patients with asthma, acting through cysLT1 and cysLT2 receptors to cause bronchoconstriction, mucosal oedema and eosinophilic inflammation [2]. CysLT1 receptors are expressed on various inflammatory and structural cells including eosinophils, neutrophils and airway smooth

muscle cells [3]. Cells which express cysLT₂ receptors include human peripheral basophils [4] and endothelial cells [5]. LTB₄ can promote the chemotaxis and activation of inflammatory cells including neutrophils and lymphocytes, acting through BLT1 and BLT2 receptors [1]. BLT1 receptor expression also occurs in macrophages [6], whereas both BLT1 and BLT2 receptors are expressed in human mast cell line [7].

AA may also be reduced by 5-LO to 5-hydroxyeicosatetraenoic acid (5-HETE). The metabolism of 5-HETE produces 5-oxo-6, 8, 11, 14-eicosatetraenoic acid (5-oxo-EETE), a potent activator of neutrophils and eosinophils [8].

The 5-LO inhibitor, zileuton, is approved for the treatment of asthma, but the doses used in clinical practice only partially inhibit the synthesis of leukotrienes and the therapeutic index is limited by side-effects [1]. CysLT receptor antagonists such as montelukast, pranlukast and zafirlukast antagonize cysLT₁, but not cysLT₂ or the actions of LTB₄ or 5-oxo-EETE. Currently, no drug is available that completely inhibits the actions of all the mediators produced by the 5-LO pathway.

Subjects with asthma when exposed to a relevant allergen experience acute bronchoconstriction, known as the early asthmatic response (EAR), and many also experience a delayed bronchoconstriction, the late asthmatic response (LAR). This natural phenomenon can be replicated in the laboratory using a controlled allergen challenge model. Mast cell activation and subsequent release of spasmogenic mediators, including leukotrienes, occurs during and underlies EAR [9]. Classically, EAR peaks 20–30 min post challenge and lasts 2 h [9]. In 40–60% of cases, EAR is followed by delayed bronchoconstriction, the LAR [10], which is associated with increased influx of inflammatory cells to the central and peripheral airways, most notably airway eosinophils. LAR is also usually associated with airway hyperresponsiveness to bronchoconstrictor stimuli [11]. Leukotriene antagonists [12–15] and FLAP inhibitors [16, 17] have previously been shown to inhibit both the EAR and LAR to inhaled allergen, whereas 5-LO inhibitors [18, 19] and an LTB₄ antagonist [20] have not.

GSK2190915 (previously known as AM803) is a potent FLAP inhibitor currently in development for the treatment of asthma. In rat lungs instilled with A23187 (a calcium ionophore), GSK2190915 significantly reduced the levels of cysLTs and LTB₄ in rat bronchoalveolar lavage fluid [21]. These findings were supported by the results of a clinical investigation in healthy volunteers where GSK2190915 inhibited LTB₄ production in whole blood challenged *ex vivo* with A23187 and the levels of LTE₄ in urine [22].

We examined the efficacy, safety and pharmacodynamics of GSK2190915 in subjects with mild asthma, using an inhaled allergen challenge model.

Material and methods

Study Design

This was a four-centre, randomized, double-blind, placebo-controlled, two-period crossover study (GSK Protocol Number LPA111834, ClinicalTrials.gov NCT00748306) completed in accordance with the International Conference of Harmonization (ICH) Guideline for Good Clinical Practice [23] from 15 December 2008 to 03 September 2009. The Sponsor was responsible for generating the randomization code (using validated GlaxoSmithKline computer software) and assigning each subject to a treatment sequence. All sites (three in the United Kingdom, one in the Netherlands) received Ethics Committee approval. Each subject provided written informed consent before enrolment by the Investigator. There was a screening visit 14–49 days before the first treatment period (Period 1), and a follow-up visit 5–21 days after the second treatment period (Period 2). Treatment periods consisted of once-daily dosing with an oral solution of either GSK2190915 100 mg or matching placebo for five consecutive days (Days 1–5). There was a minimum 14-day washout period between Periods 1 and 2. This was to allow adequate time for recovery from any allergen-induced hyperresponsiveness which can persist for 2 weeks [24, 25] and also exceeded five half-lives of GSK2190915 ($t_{1/2}$ ~20 h). A study schematic is presented in Figure 1. On Days 1 and 4 a methacholine challenge was performed, on Day 3 an inhaled allergen challenge was performed and on Days 4 and 6 sputum induction was performed.

Subjects

Key inclusion criteria were non-smoking men or women of non-childbearing potential (given no reproductive toxicology had been conducted at the time of the study) aged 18–55 years, body mass index 18.5–35.0 kg/m², bronchial asthma requiring only short-acting β -agonists, pre-bronchodilator FEV₁ > 70% of predicted [26], hyperresponsiveness to inhaled methacholine with a provocative concentration of methacholine resulting in a 20% fall in FEV₁ (PC₂₀) of < 8 mg/mL, a minimum number (> 70%) of subjects able to complete sputum induction and provide an adequate sputum sample, positive weal and flare reaction following skin prick test to at least one allergen and an EAR between 0–2 h after allergen challenge and LAR between 4–10 h after allergen challenge.

Key exclusion/continuation criteria were respiratory tract infection and/or asthma exacerbation within 4 weeks of commencing Period 1, history of life-threatening asthma, previous clinical study participation with a new molecular entity within 3 months of first dose,

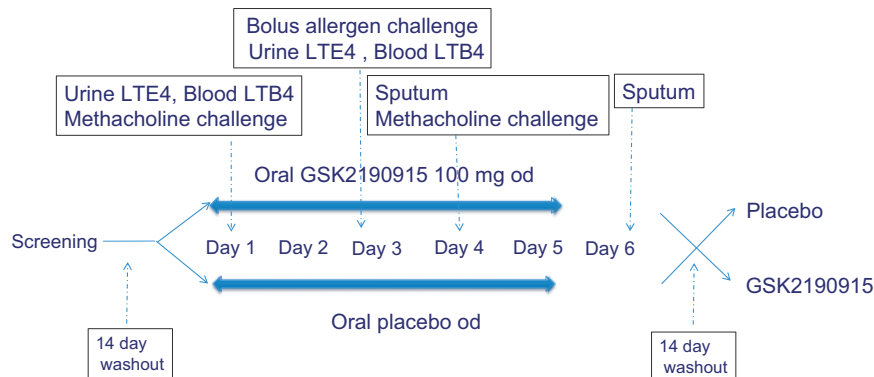


Fig. 1. Study Schematic. On Day 3, an allergen challenge was done 2 h post dose. FEV₁ (independent of challenge) was measured on Days 1 and 3 (pre-dose and 2 h post-dose) and Day 6. Methacholine challenge was completed pre-dose on Days 1 and 4. Induced sputum was collected on Day 4 (pre-dose) and Day 6. Blood was collected on Day 1 (pre-dose) and Day 3 (pre-dose, 1 h post-dose and 12 h post-allergen challenge) for LTB₄ analysis. Urine was collected on Day 1 (pre-dose) and Day 3 (pre-dose, 2 h post-dose, then at 3-hourly intervals during 0–12 h post-allergen challenge) for analysis of LTE₄. Blood was collected on Day 1 (pre-dose and 2 h post-dose) and Day 3 (pre-dose, 0.5, 1, 2, 4, 8 and 24 h post-dose) for measurement of GSK2190915.

allergic rhinitis symptoms, current allergen desensitization therapy, use of medication within 2 weeks of screening (except for short-acting β -agonists and/or up to 4 g/day paracetamol) or use of oral or injectable steroids within 5 weeks of screening or intranasal and/or inhaled steroids within 4 weeks of screening, inability to abstain from short-acting β -agonists for at least 8 h prior to allergen challenge or lung function testing and a difference between PC₂₀ values recorded at screening and pre-dose Day 1 (both Periods) deemed to indicate asthma instability by the investigator.

Procedures and analysis

Allergen challenge. Allergen and saline solutions were administered from a breath-activated dosimeter (Mefar MB3 CE; Mefar, Brescia, Italy). For each solution/concentration, five breaths were taken by slow inspiration from functional residual capacity (FRC) to total lung capacity (TLC) over 3 s each followed by a breath hold for 6 s. FEV₁ was recorded before and after inhaling 0.9% nebulized saline. These values were within 10% of each other before commencing allergen challenge with six-grass mix, house-dust mite or cat hair and dander (all allergens sourced from ALK-Abelló UK, Berkshire, United Kingdom). FEV₁ recorded following saline inhalation was considered baseline for each allergen challenge. Allergen concentrations inhaled at screening were incrementally increased from 250 SQ-U/mL as follows: fourfold where the post-challenge FEV₁ was within 10% of baseline; twofold where the post-challenge FEV₁ differed by 10% and 15% of baseline. The challenge was terminated when a post-challenge FEV₁ decrease $\geq 15\%$ of baseline was observed. Further inhalation of allergen was permitted if the FEV₁ did not decrease $\geq 20\%$ baseline at the subsequent

FEV₁ time point. Allergen concentration inhaled at screening did not exceed 32 000 SQ-U/mL. The cumulative allergen concentrations which caused an EAR at screening were inhaled as a single bolus on Day 3 of both treatment periods. Following final allergen inhalation at screening and Day 3, FEV₁ measurements were recorded at 15, 20, 30, 45 and 60 min, then every 30 min for 10 h. On Day 3, FEV₁ readings were also taken at 5 and 10 min post challenge.

Lung function testing. FEV₁ measurements were recorded body temperature pressure saturated using a dry-wedge bellows volume-displacement spirometer, in accordance with investigational site standard operating procedures.

Methacholine challenge. Methacholine and phenol saline solutions were administered from a breath-activated dosimeter (Mefar MB3 CE; Mefar). For each solution/concentration, five breaths were taken by slow inspiration from FRC to TLC over 3 s each followed by a breath hold for 6 s. FEV₁ measurements recorded before and after inhaling five breaths of nebulized phenol-saline control (sodium chloride 0.9% containing phenol 0.4%, pH 7.0; www.acic.com/europe) were within 10% of each other before proceeding to methacholine inhalation (Provocholine®; Methapharm Europe, Geneva, Switzerland). FEV₁ recorded following phenol-saline inhalation was considered baseline. Methacholine concentration was incrementally increased twofold from a starting concentration of 0.0625 mg/mL. FEV₁ was measured at 60 and 180 s after each inhalation. The challenge was terminated on a fall of FEV₁ $\geq 20\%$ of baseline.

Induced sputum. The highest of three post-bronchodilator (2 puffs of salbutamol 100 μ g via a metered dose

inhaler) FEV₁ measurements was considered baseline for each sputum induction. Nebulized 4.0% hypertonic saline (Steriflex®; Ivex Pharmaceuticals Limited, Larne, United Kingdom) was then inhaled for 5 min before sputum collection. The inhalation cycle was repeated twice unless significant asthma symptoms or a fall in FEV₁ $\geq$ 20% of the post-bronchodilator baseline occurred.

Colour, purulence and blood contamination of sputum specimens were noted. Selected sputum plugs (all solid or dense-looking material selected from the clear salivary fluid) were briefly vortexed and gently agitated (rolling mixer or rocker, 15 min, 25°C), then mixed (> 75 mg) with a volume of Dulbecco's phosphate-buffered saline (D-PBS) eight times the sputum weight (grams). Samples were centrifuged at 790 *g* (4°C, 10 min). Half the supernatant was frozen (–70°C) before ELISA analysis of LTB₄ (lower limit of quantification, LLQ, 11.7 pg/mL) and high-performance liquid chromatography/mass spectrometry (LCMS) of LTC₄, LTD₄ and LTE₄ (LLQ 1 pg/mL). The remaining supernatant was mixed with a volume of 0.2% DTT four times the sputum weight by brief vortex and agitation. A filtered (48–55 µm pore mesh nylon filter pre-wet with PBS) sample was centrifuged at 790 *g* (4°C, 10 min) to precipitate the cells into a soft pellet.

A cytospin slide prepared using the cell pellet resuspended in D-PBS (0.5–1.0 $\times$ 10⁶ cells/mL) was examined with a Neubauer haemocytometer (total and squamous cell counts, cell viability) then stained with DiffQuick or Hema 3. The slide was assessed manually by two trained analysts independent of the study for absolute and percentage cell counts (eosinophils, neutrophils, macrophages, lymphocytes, epithelial cells). Evaluable slides comprised at least 200 non-squamous cells. When the two analysts' absolute counts were not within 10% of each other, a third reader was employed to count the cells. Data from the two readers in closest agreement were used for analysis.

Blood LTB₄ analysis: unstimulated and ex vivo stimulated. Each blood sample collected in a 4.0 mL sodium heparin-coated vacutainer tube was divided into six (250 µL) aliquots. Four aliquots were stimulated with 5 µL 1 mM calcium ionophore solution [50 mM stock of calcium ionophore A23187 (www.sigma-aldrich.com) diluted in 50% Hanks Balanced Salt Solution (HBBS) containing calcium and 50% dimethyl sulfoxide (DMSO)]. The two remaining aliquots were unstimulated (5 µL vehicle control solution of 50% DMSO in HBBS containing calcium only). All six aliquots were incubated (37°C, 30 min), then centrifuged at 350 *g* (4°C, 10 min). Plasma supernatant was stored at –70°C

before ELISA analysis of LTB₄ (LLQ 11.7 pg/mL). The unstimulated samples were subtracted from the stimulated samples to derive the LTB₄ value for a given time point.

Urine LTE₄ analysis. Urine was collected pre-dose on Day 1 and 0–2 h after dosing on Day 3 and 0–3 h, 3–6 h, 6–9 h and 9–12 h after allergen challenge on Day 3. An 8-mL aliquot from each time point was stored at –70°C before LC/MS/MS analysis of LTE₄ (LLQ 1 pg/mg creatinine).

Pharmacokinetics. Blood samples were collected pre-dose on Day 1 and pre-dose and 30 min, 1 h, 2 h, 4 h, 8 h and 24 h after dose on Day 3 in a 3.0 mL K3/K2 EDTA vacuette tube and were centrifuged within a 2-h period of collection at 1500 *g* (4°C, 15 min). The plasma supernatant was stored at –20°C protected from light before analysis using a validated analytical method based on protein precipitation, followed by HPLC/MS/MS. The lower limit of quantification for GSK2190915 was 5 ng/mL using a 25-µL aliquot of plasma with a higher limit of quantification of 5000 ng/mL. The computer systems that were used in this study to acquire and quantify data included analyst version 1.4.2 and SMS2000 version 2.2. The analytical runs met all pre-defined run-acceptance criteria.

Safety and tolerability. Investigators detected adverse events (AEs) using open-ended, non-leading questions, and assessed causality and intensity. Serious adverse events (SAEs) were defined as any medical occurrence that resulted in death, was life-threatening, required hospitalization or resulted in disability or congenital anomaly.

Study endpoints

The primary endpoint was the EAR, defined as the minimum FEV₁ and weighted mean (WM) FEV₁ between 0–2 h after Day 3 allergen challenge. Secondary endpoints were LAR (defined as the minimum FEV₁ and WM FEV₁ between 4 h and 10 h after Day 3 allergen challenge), non-challenge FEV₁ on Days 1, 3 and 6, PC₂₀ on Day 4, sputum, blood and urine biomarkers; pharmacokinetics and treatment-related AEs.

Statistical analysis

Sample size. A sample size of 19 was sufficient to provide at least 90% power to detect a 50% attenuation from the change from pre-dose baseline placebo response for minimum EAR, using a two-sided 5% significance level, assuming a within-subject standard

deviation of 0.40 (L) and a change from baseline of -0.90 (L).

Allergen challenge. The minimum WM and AUC changes from baseline for EAR and LAR after allergen challenge on Day 3 were analysed using mixed-effects models (treatment period, treatment, subject baseline and period baseline as fixed effects, and subject as a random effect). Absolute change from baseline on Day 3 FEV₁ by time was also analysed using the same mixed-effects model above.

Lung function testing. A mixed-model analysis examined the absolute change from pre-dose baseline. The mixed-model analyses for Days 1 and 6 were identical to those used for minimum and weighted mean for EAR and LAR after Day 3 allergen challenge. For Day 3, a repeated-measures analysis was undertaken (treatment period, treatment, subject baseline and period baseline, time, treatment by time and period baseline by time interactions as fixed effects, and subject as a random effect).

Methacholine challenge. Day 1 PC₂₀ was summarized using descriptive statistics. Day 4 PC₂₀ values were log₂ transformed and analysed using a mixed-effects model identical to that used for analysis of minimum and weighted mean EAR and LAR after allergen challenge on Day 3.

Analysis of induced sputum, blood LTB₄ and urine LTE₄. Pharmacodynamic data were summarized using descriptive statistics. LT values below the LLQ were set to half the LLQ and imputed. A mixed-effects model examined total eosinophil count, percentage eosinophils, total leucocytes count (period and treatment as fixed effects, and subject as a random effect).

Pharmacokinetics. The area under the concentration–time curve (AUC) was calculated using the trapezoidal method. The maximum observed concentration (C_{max}) and the time of its occurrence (T_{max}) were obtained directly from the concentration–time data.

Safety and tolerability. Adverse events were listed using descriptive statistics.

Results

Subject demographics

Twenty men aged 20–45 years (mean 29.8 ± 7.8) were randomized and completed this study, as no women met eligibility criteria (Table 1). Nineteen subjects were included in the efficacy analysis; the data from one

Table 1. Demographics of completed subjects. Twenty male subjects were randomized and completed the study

	Total (N = 20)
Age in Years, Mean (SD)	29.8 (7.80)
Sex, n (%)	
Female:	0
Male:	20 (100%)
BMI (kg/m ²), Mean (SD)	24.18 (2.36)
Height (cm), Mean (SD)	176.7 (7.34)
Weight (kg), Mean (SD)	75.53 (9.091)
Ethnicity, n (%)	
Hispanic or Latino:	2 (10%)
Not Hispanic or Latino:	18 (90%)
Race, n (%)	
White	17 (85%)
African American/African Heritage	1 (5%)
American Indian or Alaska Native	1 (5%)
African American/African Heritage & White	1 (5%)

subject being excluded from the efficacy analysis as he experienced symptomatic allergic rhinitis during Period 2.

Allergen challenge

GSK2190915 100 mg showed statistically significant attenuation of the EAR and LAR (minimum and WM FEV₁) on Day 3 (Figure 2, Table 2). Treatment difference of GSK2190915 vs. placebo for the minimum FEV₁ EAR was 0.408 L (0.205, 0.611) and for WM FEV₁ EAR was 0.425 L (0.210, 0.640), which was a 33.3% attenuation of the placebo response for minimum FEV₁ and a 62.6% attenuation of the placebo response for WM FEV₁. Treatment difference of GSK2190915 vs. placebo for the minimum FEV₁ LAR was 0.229 L (0.041, 0.417) and for WM FEV₁ LAR was 0.233 (0.060, 0.386), which was a 15.8% attenuation of the placebo response

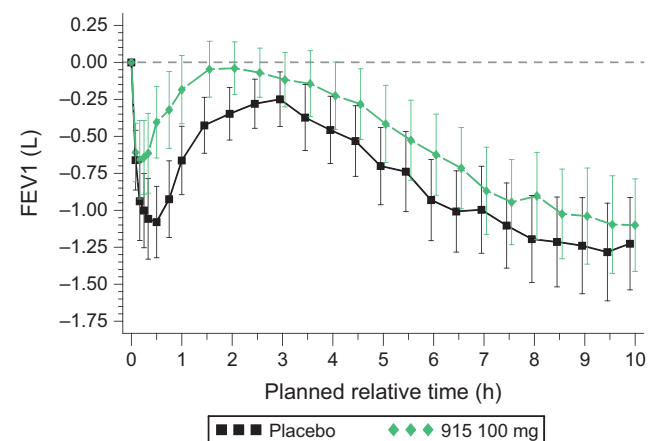


Fig. 2. Adjusted mean absolute change from saline baseline FEV₁ in the 10 h following allergen administration on Day 3.

Table 2. Summary of the statistical analysis of allergen challenge absolute change from saline baseline on day 3. GSK2190915 100 mg showed statistically significant attenuation of the EAR and LAR

Derived Endpoint	Treatment	N	Adjusted Mean		Treatment Difference (vs. Placebo)		P-value
			Estimate	95% CI	Estimate	95% CI	
Minimum EAR (L)	Placebo	19	-1.225	(-1.495, -0.955)			
	915 100 mg	19	-0.817	(-1.087, -0.547)	0.408	(0.205, 0.611)	<0.001
AUC EAR (L.hr)	Placebo	19	-1.364	(-1.769, -0.960)			
	915 100 mg	19	-0.504	(-0.909, -0.100)	0.860	(0.431, 1.289)	<0.001
Weighed Mean EAR (L)	Placebo	19	-0.679	(-0.881, -0.477)			
	915 100 mg	19	-0.254	(-0.456, -0.052)	0.425	(0.210, 0.640)	<0.001
Minimum LAR (L)	Placebo	19	-1.448	(-1.753, -1.144)			
	915 100 mg	19	-1.219	(-1.524, -0.914)	0.229	(0.041, 0.417)	0.02
AUC LAR (L.hr)	Placebo	19	-5.877	(-7.484, -4.269)			
	915 100 mg	19	-4.551	(-6.158, -2.943)	1.326	(0.369, 2.283)	0.01
Weighed Mean LAR (L)	Placebo	19	-0.982	(-1.250, -0.714)			
	915 100 mg	19	-0.759	(-1.027, -0.491)	0.223	(0.060, 0.386)	0.01

EAR, early asthmatic response; late asthmatic response, late asthmatic response.

for minimum FEV₁ and a 22.7% attenuation of the placebo response for WM FEV₁.

Lung function testing

There was no statistical evidence of a treatment difference for non-challenge FEV₁ absolute change from pre-dose Day 1 of the respective treatment period.

Methacholine challenge

There was no evidence of a treatment difference for PC₂₀ post allergen. The Day 4 adjusted mean (95% CI) treatment doubling dose difference was 0.049 mg/mL (-0.725, 0.823). The adjusted geometric means (95% CI) for Day 4 PC₂₀ were 0.521 mg/mL for GSK2190915 (0.307, 0.883) and 0.503 for placebo (0.293, 0.864).

Induced sputum

On Day 4, eight placebo-treated subjects (mean eosinophil count 23%) and seven GSK2190915-treated subjects (mean eosinophil count 13%) provided a sputum sample. There was a statistically significant decrease in percent eosinophils caused by GSK2190915: adjusted mean difference (95% CI) was -9.95 (-18.13, -1.77, *P* = 0.03). Six subjects produced an evaluable sputum sample for cell count on Day 4 of both treatment periods. Mean eosinophil count was 25% following placebo and 15% following GSK2190915 in these six subjects. There was a statistically significant decrease in percent eosinophils caused by GSK2190915: adjusted mean difference (95% CI) was -9.91 (-18.11, -1.71, *P* = 0.03).

Insufficient evaluable samples were available for analysis of cell count on Day 6. More than 90% reduc-

tion in sputum LTB₄ on Days 4 and 6 following treatment with GSK2190915 compared with placebo was evident by comparing median values. The majority of the LTC₄ samples were BLQ (data not shown). There was an apparent reduction in median sputum LTD₄ (Day 4 and 6) and LTE₄ (Day 4) (Table 3).

Ex vivo stimulated blood LTB₄ analysis

Treatment for 3 days with GSK2190915 100 mg suppressed LTB₄, whereas placebo did not. The percentage median change from LTB₄ baseline on Day 3 at 1 h post dose were -93.05 for GSK2190915 and 2.06 for placebo, and on Day 3 at 12 h post allergen challenge was -89.33 for GSK2190915 and 114.52 placebo.

Urine LTE₄ analysis

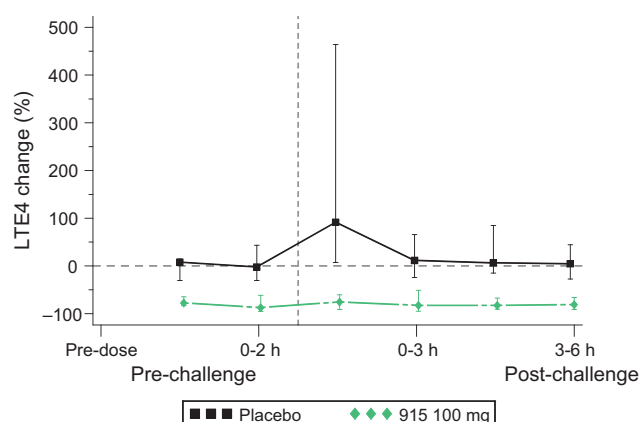
Treatment for 3 days with GSK2190915 100 mg suppressed urine LTE₄, whereas placebo did not. On Day 3, a median suppression of 75% from pre-dose baseline was seen in subjects receiving GSK2190915. Placebo-treated subjects had no obvious change from baseline in LTE₄ on Day 3 at all time points except for 0-3 h after allergen challenge, where a median increase of 90% from baseline in LTE₄ was observed (Figure 3).

Pharmacokinetics

After 3-day treatment with GSK2190915 100 mg, the geometric mean (95% CI) maximum plasma concentration (C_{max}) was 671 ng/mL (494, 910) and area under the concentration-time curve from pre-dose to last time of quantifiable concentration (AUC_{0-∞}) was 9521 ng.h/mL (7143, 12690). Time to maximum plasma

Table 3. Summary statistics of sputum endpoints. For eosinophils range is maximum and minimum and for leukotriene interquartile range

Treatment	N	Number Imputed	Time point	Mean	SD	Median	Range
Eosinophils							
Placebo	8	0	Day 4 Pre-dose	22.99	13.64	27.42	1.7–37.4
	4	0	Day 5 24 h	22.90	22.97	21.78	0.4–47.6
915 100 mg	7	0	Day 4 Pre-dose	12.80	13.02	6.82	0.0–33.2
	5	0	Day 5 24 h	12.16	20.38	4.44	0.4–48.4
LTB₄							
Placebo	11	1	Day 4 pre-dose	621.76	660.29	524.00	199.00–898.00
	11	1	Day 5 24 h	900.99	748.75	837.00	271.00–1524.00
915 100 mg	14	3	Day 4 pre-dose	85.90	181.87	31.15	13.70–54.00
	11	4	Day 5 24 h	120.56	325.14	18.10	5.85–38.60
L TD₄							
Placebo	16	6	Day 4 pre-dose	13.71	17.69	9.27	0.50–21.19
	15	7	Day 5 24 h	7.28	10.33	3.99	0.50–11.72
915 100 mg	18	9	Day 4 pre-dose	24.62	80.52	1.48	0.50–8.460
	15	7	Day 5 24 h	22.95	28.82	1.20	0.50–52.11
LTE₄							
Placebo	16	5	Day 4 pre-dose	25.53	34.67	9.20	0.50–32.69
	15	10	Day 5 24 h	43.00	114.05	0.50	0.50–37.97
915 100 mg	18	12	Day 4 pre-dose	4.24	6.31	0.50	0.50–8.92
	15	10	Day 5 24 h	4.20	10.10	0.50	0.50–3.88

Fig. 3. Urine LTE₄ Median (IQR) percentage change from baseline (Pre-dose Day 1) on Day 3.

concentration ($T_{\max}$) median (range) was 4.0 h (1.1–8.1 h) (Figure 4).

Safety and tolerability

Overall, 29 AEs were reported (10 GSK2190915, 19 placebo) and resolved prior to study completion. A total of 16 AEs (six for GSK2190915, 10 for placebo) were deemed related to treatment. The most frequent treatment-related AE was headache (one GSK2190915, three placebo). All AEs were of mild to moderate intensity, with the exception of one severe episode of pre-syncope (placebo). One episode of rash over the chest and arms, which began on the evening of Day, 4 led to treatment discontinuation (GSK2190915) on Day 5 of Period 1.

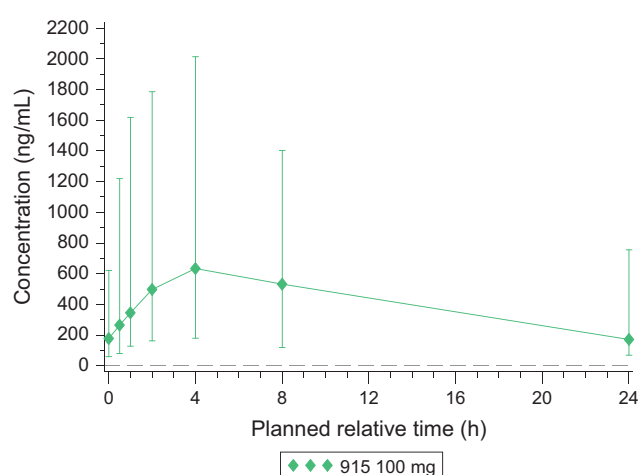


Fig. 4. Plot of Median (Range) Plasma GSK2190915 Concentration on Day 3.

The rash, judged related to treatment, resolved with clobetasol propionate 0.05% topical application, and the subject completed Period 2 (placebo). No other subject interrupted treatment. No SAEs were reported.

Discussion

Once-daily dosing for 3 days with GSK2190915 100 mg significantly inhibited EAR (33.3% of the placebo response for minimum FEV₁; 62.6% attenuation of the placebo response for WM FEV₁) and LAR (15.8% of the placebo response for minimum FEV₁; 22.7% attenuation of the placebo response for WM FEV₁) following allergen challenge. Three-day dosing with GSK2190915

100 mg also significantly decreased the percentage cell count for sputum eosinophils compared with placebo (mean treatment difference -9.95 , 95% CI: -18.15 , -1.77). However, no treatment difference was evident for methacholine PC₂₀.

Stringent cross-study comparisons are difficult for allergen challenge studies as there is considerable variation in the methods used e.g. method of allergen administration, period of measurement of the EAR and LAR and methods for calculating and expressing the EAR and LAR. In an allergen challenge using the same allergen challenge protocol, it has been reported that montelukast compared with placebo caused 42% and 62% inhibition of the EAR minimal FEV₁ and WM FEV₁, respectively, and 17% and 26% inhibition of the LAR minimal FEV₁ and WM FEV₁ respectively [13]. These results are comparable to those observed with GSK2190915 in this study. The effects seen with GSK2190915 are also comparable to those of montelukast and other CysLT antagonists [12, 14, 15] using other allergen protocols and FLAP antagonists [16, 17]. These comparable effects of CysLT antagonists and GSK2190915 in this study are as expected as LTB₄ is not, or only minimally, involved in the EAR or LAR. That LTB₄ is not involved in the EAR and LAR is supported by the fact that 5-LO inhibitors do not attenuate the EAR or LAR [18, 19] despite almost complete suppression of LTB₄ and that an LTB₄ antagonist [20] did not show any effect on EAR or LAR.

FLAP inhibitors prevent synthesis of 5-oxo-EETE, a potent eosinophil chemoattractant [8]. This may result in a greater effect on eosinophil counts compared to leukotriene receptor antagonists. Three-day dosing with GSK2190915 100 mg significantly attenuated sputum eosinophils 24 h after allergen challenge. Published allergen challenge studies with 5-LO and other FLAP inhibitors do not include data on sputum eosinophils [16–19]. The effect of LT receptor antagonists on eosinophils in the allergen challenge model is variable. Montelukast had no effect on bronchoalveolar lavage eosinophils 24 h after allergen challenge following 14-day dosing [14] or on eosinophil increase in sputum 24 h after allergen challenge following 3-day dosing [15]. Following 7-day dosing, montelukast attenuated the increase in sputum eosinophils at 7 h, but not 24 h after allergen [12]; and a single dose of montelukast did not attenuate the allergen-induced increase in eosinophils 7 h after allergen, but did at 24 h [14].

GSK2190915 almost completely suppressed LTB₄ (blood and sputum) and LTE₄ (urine). This is in contrast to 5-LO inhibitors, which appear to cause much less inhibition of LTE₄ than LTB₄ in human [18, 19] and are less potent at inhibiting CysLTs in rat lung than FLAP inhibitors [27]. While the data in human is limited, five FLAP inhibitors and five 5-LO inhibitors were tested in

rat lung [25] with consistent differences between the two classes of compounds suggesting this may be a real class effect. These results suggest that FLAP inhibitors may be better than 5-LO inhibitors for inhibiting CysLTs, particularly in asthmatic patients in whom CysLTs are a trigger. Many of the samples for sputum LTC₄, LTD₄ and LTE₄ were below the LLQ and consequently imputed, including pre-dose samples, making interpretation of the data difficult. However, there were trends to reduction in median LTD₄ and LTE₄. However, the high number of samples below the LLQ especially for LTE₄ meant no meaningful conclusions from this data could be made. The increase in mean LTD₄ following GSK2190915 is likely to be due to artefact, with the data being driven by an extreme outlier.

Hypertonic saline inhalation, which induces release of inflammatory mediators by increasing the osmolarity of the bronchial epithelium, has been widely used as a bronchial challenge agent in asthmatic subjects to induce sputum for biomarker analysis [18, 19]. The difficulty in obtaining sputum from subjects using this procedure is evident in this study, given the low number of subjects able to provide a sample on Days 4 and 6 of both treatment periods.

Three-day dosing with GSK2190915 did not affect the post-allergen challenge methacholine PC₂₀. However, the study was not powered to detect a treatment difference for this endpoint. Interestingly, many studies investigating leukotriene modifiers have not detected a change in PC₂₀ [16, 18, 28]. Three and a half days of dosing with a FLAP inhibitor showed no effect on bronchial hyperresponsiveness to methacholine challenge in subjects with mild asthma [16]. No effect on PC₂₀ in mild asthmatics was evident following a single dose of a 5-LO inhibitor [18]. These findings may suggest that leukotriene modifiers are not suitable for assessment by methacholine challenge.

Short-term administration of GSK2190915 100 mg was generally well tolerated in subjects with mild asthma. There was a report of rash in one subject receiving GSK2190915. Although this AE was deemed treatment-related, the investigator cited alcohol wipes used to clean the skin before the application of the ECG tabs as a possible alternate cause. Alcohol wipes were not used during Period 2 for this subject and there was no rash. This finding is of interest, given reports of rash associated with another FLAP inhibitor [29, 30].

In conclusion, 3 days of once-daily treatment with GSK2190915 showed significant inhibition of the early and late response to inhaled allergen, almost complete suppression of LTE₄ and LTB₄ and was well tolerated in adults with mild asthma. Further clinical studies are warranted to explore the potential of GSK2190915 as a treatment for asthma.

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Conflict of interest

VN is employed by, and holds shares in, GlaxoSmithKline. PS and SK were paid contractors of GSK for this study. ZD, MB, BO and DS were paid by GSK for the clinical conduct of the study. DS has received lecture fees, research grants, consultancy fees and support for conference attendance from various pharmaceutical companies including AstraZeneca, Almirall, Boehringer Ingelheim, Chiesi, Cipla, Forest, GlaxoSmithKline, Merck, Novartis, Nycomed and Roche. BO has been reimbursed by GlaxoSmithKline for attending symposiums and has also received fees for speaking on behalf of GlaxoSmithKline.

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