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Randomized trial of allergen-induced asthmatic response in smokers and non-smokers: effects of inhaled corticosteroids

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Experimental
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Summary

Background It is thought that asthmatics who smoke cigarettes respond less well to inhaled corticosteroid (ICS) therapy than asthmatics who do not smoke.

Objective To evaluate the effects of smoking on allergen-induced airway responses in asthmatics treated with ICS.

Methods Randomized, double-blind, crossover study evaluating twice daily fluticasone propionate (FP) 100 µg, FP 500 µg and placebo, for 7 days, on allergen-induced asthmatic responses in 18 non-smoking and 17 smoking atopic asthmatics (NCT01400906). At 1 h post-morning dose on Day 6, forced expiratory volume in 1 sec (FEV₁) was measured up to 10 h post-challenge. Exhaled nitric oxide (eNO), induced sputum cell counts, and responsiveness to methacholine were assessed the following day.

Results The late asthmatic response (LAR) was suppressed by FP in smokers and non-smokers; with placebo, the LAR was also attenuated in smokers versus non-smokers (adjusted mean minimum change in FEV₁ (L) over 4–10 h [95% CI] in non-smokers: placebo –1.01 [1.31, 0.70], FP 100 µg –0.38 [0.54, 0.22], FP 500 µg –0.35 [0.54–0.22]; and in smokers: placebo –0.63 [0.84, 0.43]; FP 100 µg –0.44 [0.65, 0.23]; FP 500 µg –0.46 [0.59–0.32]). The Early AR was suppressed by FP treatment in non-smokers, but was not impacted in smokers. The reduction in methacholine hyperresponsiveness after FP was greater in non-smokers (1.5- and twofold doubling dose difference from placebo after FP 100 µg and FP 500 µg) than smokers (1.0 and 1.3 difference, respectively). Allergen-induced increases in eNO and sputum eosinophils were lower in smokers than non-smokers and were suppressed in both groups by FP.

Conclusion and Clinical Relevance Allergen-induced LARs were of a similar amplitude in both smoking and non-smoking atopic asthmatics at the end of ICS treatment, but attenuation of the LAR in smokers was only partly associated with ICS treatment. The marked attenuation of the LAR observed in smokers in the absence of ICS treatment is a novel observation.

Keywords allergen challenge, asthma, atopic, cigarette smoking, inhaled corticosteroids, late asthmatic response

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Introduction

Increased morbidity and mortality from asthma has been shown in patients with asthma who smoke cigarettes compared with non-smokers [1]. This is evidenced by more severe respiratory symptoms and asthma exacerbations [2, 3], a greater need for general practitioner visits and rescue medication use [4], increased hospitalizations due to asthma [5], an accelerated

decline in lung function [6, 7] and a greater risk of death following severe asthma attack [8]. Studies also suggest that asthmatics who smoke respond less well to oral [9] and inhaled corticosteroid (ICS) therapy [10, 11] than do non-smokers, and this lack of responsiveness appears to be ICS dose-dependent [12]. In a responder analysis of the Gaining Optimal Asthma control (GOAL) study, clinical improvements in all patients treated with ICS or ICS plus long-acting beta2-agonist (LABA) were observed,

irrespective of smoking history, but the probability of having well-controlled asthma at the end of treatment was significantly greater in never smokers compared with current smokers and former smokers [11]. This was true for both treatment regimens, although smokers were shown to benefit more from treatment with ICS/LABA compared with ICS alone. A benefit of ICS/LABA treatment over ICS alone in improving airway responsiveness to methacholine has also been reported in patients with asthma who smoke [13].

There are few published studies describing the pattern of airway inflammation in asthmatics who smoke [1]. One study in patients with mild asthma reported higher total induced sputum cell count and sputum interleukin (IL)-8 and reduced sputum eosinophil count in smoking versus non-smoking asthmatics [14]. Other studies in mild asthma evaluating exhaled nitric oxide (eNO) as a marker of eosinophilic airway inflammation have shown reduced eNO in smoking compared with non-smoking asthmatics [15, 16]. Most recently, a reduced proportion of sputum eosinophils and lower eNO levels were reported in current smokers versus never and ex-smokers in severe asthma [17]. A study looking at allergen challenge in atopic asthmatic patients showed a similar response in allergen-induced airway bronchoconstriction and induced sputum eosinophil numbers in smokers and non-smokers, although compared with the non-smokers, the smokers did not develop allergen-induced methacholine airway hyperresponsiveness [18]. This study did not however assess the effect of ICS in these groups. Despite the evidence that asthmatics smokers respond less well to ICS, the effect of smoking status on the attenuation by ICS of the early and late asthmatic response has not been studied. The aim of the current study was to evaluate the effects of cigarette smoking on allergen-induced airway responses in asthmatic subjects treated with fluticasone propionate (FP) 100 and 500 µg twice daily (bd) compared with placebo.

Methods

Study design

This was a randomized, double-blind, three-way crossover study undertaken at two sites: the Clinic of Immuno-allergology, Centre Hospitalier Universitaire (CHU) Brugmann (ULB), Brussels, Belgium, and Hammersmith Medicines Research Ltd, London, UK, between July 2011 and December 2012 (GSK Protocol RES114748; NCT01400906). After a screening visit 15–30 days before the first treatment period, subjects each received FP 100 µg bd, FP 500 µg bd and matching placebo via a dry powder inhaler device for 7 days, with a minimum 14-day washout between treatments.

Healthy smoking and non-smoking asthmatics were randomized to one of six crossover treatment sequences in accordance with a randomization schedule generated by the sponsor using validated internal software (RandAll; GSK, London, UK). Randomization was stratified by smoking status to ensure that there were equal numbers of smoking and non-smoking subjects on each sequence. All treatments looked identical, to ensure that both investigators and subjects were blinded to treatment.

The first dose of each treatment was administered in the research units; treatment was then self-administered twice daily at home from Day 1 (evening) to Day 5, and again administered in the unit on days 6 (morning and evening) and Day 7 (morning dose only).

During each treatment period, allergen challenge testing was carried out on Day 6 approximately 1 h after the morning dose. Methacholine challenge followed by sputum induction was carried out pre-morning dose on Day 1 and from 1 h post-dose on Day 7, and eNO tests were carried out pre-morning dose on days 1 and 6, and 1 h post-dose on Day 7 of treatment. The primary endpoint of the study was the effect of smoking status on the attenuation by ICS compared with placebo of the allergen-induced late asthmatic response (LAR).

Subjects

Male or female subjects aged 18–55 years with a documented history of atopic asthma for at least 6 months requiring treatment with only short-acting inhaled β_2 -agonist (SABA) therapy, a pre-bronchodilator forced expiratory volume in 1 s (FEV_1) > 70% of predicted normal and body (weight $\geq$ 50 kg) mass index of 18.5–35 kg/m² were eligible. All subjects had to have a positive skin prick test (wheal reaction of $\geq$ 3 mm relative to negative control) to at least one allergen [house dust mite (HDM), grass pollen or cat hair using Soluprick™, ALK Abello UK Ltd, Hungerford, Berkshire, UK]. Demonstration of both an early asthmatic response (EAR) and LAR at the screening inhaled allergen challenge test and a provocative concentration of methacholine resulting in a 20% fall in FEV_1 (PC_{20} methacholine) of < 8 mg/mL were also required. Subjects were either current smokers with a minimum 5 pack-years history, or non-smokers including never smokers and ex-smokers who had stopped smoking more than 6 months prior and had never exceeded 5 pack-years at any time. For inclusion, non-smoking subjects required urinary cotinine levels $\leq$ 500 ng/mL.

Subjects were excluded if they had: a respiratory tract infection and/or asthma exacerbation within the last 4 weeks, current symptoms of hay fever, medications other than as needed SABA, or had received systemic corticosteroids within the last 5 weeks, intranasal

or ICS within 4 weeks, or antihistamines or any other asthma treatments within 2 weeks of the screening visit.

All subjects gave written informed consent, and the study was approved by the National Research Ethics Service, Brent, London, UK, and the Ethics Committee at the CHU Brugmann, Brussels, Belgium.

Study assessments

Inhaled allergen challenge test. Allergen challenge was carried out with the allergen which elicited the largest cutaneous response on skin prick testing, and using the 5-breath dosimeter technique [19]. A starting dose of 250 SQ-U/mL was used followed by increasing doses of allergen until a fall in FEV₁ of $\geq 20\%$ from the post-saline value was achieved. After the last inhalation of allergen, FEV₁ was measured at 15, 20, 30 and 45 min and then every 30 min up to 10 h post-challenge. The EAR was defined as a fall in FEV₁ of $\geq 20\%$ from the post-saline value, on at least one occasion, between 5 and 30 min after the final concentration of allergen; the LAR was defined as a fall in FEV₁ of $\geq 15\%$ from post-saline, on at least three occasions (at least two consecutively), between 4 and 10 h after the final concentration of allergen. Allergen challenges on Day 6 of each treatment period were carried out using a bolus dose of allergen calculated as the cumulative dose of allergen that had produced a 20% fall in FEV₁ at the screening challenge.

Subjects were asked to refrain from using SABA for 8 h and caffeine-containing products for 12 h before any challenge, and up to 10 h post-allergen challenge.

Further details of the allergen challenge method are given in the online supplement.

Methacholine challenge test. Methacholine challenge was carried out using Provocholine™ (Methapharm Inc, Brantford, Canada) and the 5-breath dosimeter technique [19]. After inhalation of saline, doubling concentrations of Provocholine were inhaled from a starting dose of 0.0625 mg/mL until a fall in FEV₁ of $\geq 20\%$ post-saline was recorded or the maximum allowed dose of 16 mg/mL had been given. FEV₁ was recorded at 60 and 180 sec after each concentration. Results were expressed as the provocative concentration of methacholine causing a 20% fall in FEV₁ (PC₂₀). The methacholine PC₂₀ on Day 1 of each treatment period was required to be within 2 doubling dilutions of the screening PC₂₀ in order for treatment to commence.

Spirometry. Spirometry additional to that recorded during allergen and methacholine challenges was carried out at screening, pre-dose and 2 h post-dose on Day 1 of treatment, and pre-dose on days 6 and 7 of

treatment. Day 1 pre-dose FEV₁ was required to be within 10% of screening FEV₁ in order to commence treatment.

Exhaled nitric oxide. Exhaled nitric oxide (eNO) was measured using an Aerocrine Niox Flex, NIOX™ analyser (Aerocrine AB, Solna, Sweden). During a single exhalation at 50 mL/s maintaining a pressure of 20 mbar over ambient pressure, measurements were taken during the plateau phase. Triplicate readings were recorded at each time point, and the average value was used.

Induced sputum collection and processing. Induced sputum was collected after inhalation of 4% hypertonic saline (three 5 min inhalations) using a standard collection protocol [20]. At the investigator's discretion, 3% saline could be used after the first inhalation if the subject experienced a $\geq 15\%$ fall in FEV₁. Sputum samples were processed at each site, and cytospin slides were prepared for cell counting. Cell counting and phenotyping was performed at a central laboratory (US EPA Human Studies Building; UNC Chapel Hill, NC, 27599-7310, USA).

Safety. Vital signs (heart rate and blood pressure), 12-lead electrocardiogram (ECG) and routine blood and urine analyses were measured at the screening visit, and vital signs and blood/urine analyses were carried out pre-dose on Day 1 of each treatment period. Vital signs were also recorded on Day 6 and Day 7 of each treatment period. Adverse events were collected throughout the study.

Statistical analysis. As the primary aim of the study was based on the estimation of the effect of smoking status on the allergen-induced LAR, and no formal hypothesis was tested, the sample size of 16 evaluable smoking and non-smoking subjects was based on feasibility. However, precision estimates around the differences of interest were estimated. With 16 evaluable subjects in each population, and assuming a within-subject standard deviation of 0.270 L, it was estimated that the 95% confidence interval (CI) for the difference between smokers and non-smokers would be within approximately ± 0.27 L of the point estimate. The 95% CI for the differences versus placebo in each population was estimated to be within approximately ± 0.19 L of the point estimate.

The absolute change in post-allergen challenge FEV₁ from saline baseline (on Day 6) was analysed using repeated measures. A mixed-effects ANCOVA model was fitted to the change from saline (maximum value on day 6 from 2, 3 and 4 min reading) values for all time points 0–10 h, excluding saline (5 mins–10 h on

day 6), with covariates: period, smoking status, treatment, subject-level baseline period-level baseline, time, and interactions: treatment by time, period-level baseline by time, smoking status by treatment by time and smoking status by treatment. The following endpoints were analysed to assess EAR and LAR:

- Minimum and Weighted Mean EAR: the minimum and weighted mean FEV₁ values, respectively, between 0 and 2 h post-allergen challenge.
- Minimum and Weighted Mean LAR: the minimum and weighted mean FEV₁ values, respectively, between 4 and 10 h post-allergen challenge.

Methacholine challenge PC₂₀ data (Day 7 of treatment) were log₂-transformed and analysed using a mixed-effects ANCOVA model, with covariates: period, smoking status, treatment, subject-level baseline period-level baseline and interaction: smoking status by treatment. The data for eNO were also log-transformed and summarized.

A separate mixed-effects model was fitted on log-transformed total eosinophil count 10⁴/mL and log-transformed total neutrophil count 10⁴/mL on Day 7 with covariates: period, smoking status, treatment, subject-level baseline period-level baseline and interaction: smoking status by treatment.

Results

Study population

Thirty-six subjects were enrolled and randomized to study treatment; one subject was categorized as a screen failure after randomization and was withdrawn before receiving treatment. The remaining 35 subjects, 18 non-smokers and 17 smokers, all completed the study. Nineteen subjects were enrolled in the UK site and 16 at the Belgian site. There was a predominance of males (67%) in the non-smoking group and a predominance of females (71%) in the smoking group (Table 1). The smokers were slightly less responsive to methacholine

than the non-smokers (PC₂₀ 1.63 and 1.27 mg/mL, respectively). There were no other observed differences in baseline characteristics between groups.

Eight subjects did not completely meet the criteria for EAR and LAR definition at screening allergen challenge. Of those subjects, five were judged to have demonstrated good bronchoconstrictor responses [21], one had an EAR of 19.8% fall in FEV₁ and two had a LAR marked by a fall in FEV₁ of 14%. All subjects were included in the primary analysis; a sensitivity *post hoc* analysis was also undertaken with these subjects excluded, and no impact on the results was seen. (Details given in the online supplement; Table S1, Figure S1).

Allergen-induced asthmatic response

Bronchoconstriction. The LAR was suppressed by both doses of FP in both the smoking and non-smoking populations (Fig. 1, Table 2). The EAR was also suppressed by FP treatment in the non-smoking group, but EAR was not impacted in the smokers. During placebo treatment, a significant attenuation in LAR was observed in the smokers compared with the non-smokers (Fig. 1 and Fig. 2), and that difference was reflected in treatment differences relative to placebo which were therefore larger for the non-smoking group (Table 2, Figure S2 online supplement). A difference in asthmatic response between smokers and non-smokers was also observed at the screening challenge (Fig. 2).

As it is recognized that the type of allergen used as the challenge agent can influence the amplitude of the LAR, the LAR amplitude was also analysed *post hoc* by removing the six subjects who were challenged with grass pollen. The results were consistent with the overall analysis (Figure S3 online supplement). Thus, the LAR difference observed between smokers and non-smokers was not attributable to the nature of the allergen used in the challenge and to which the subject was sensitized.

Methacholine PC₂₀. Bronchial hyper-responsiveness, assessed by methacholine PC₂₀ approximately 24 h

Table 1. Subject characteristics

	Non-smokers (N = 18)	Smokers (N = 17)
Age in years, mean (range)	30 (21–47)	30 (19–48)
Gender, males/females	12/6	5/12
BMI (kg/m ²), mean (range)	24.8 (20.4–34.4)	26.3 (21.3–32.7)
FEV ₁ (L), mean (range)	3.39 (2.60–4.57)	3.75 (2.66–4.74)
FEV ₁ per cent of predicted (%), mean (range)	95.2 (78.3–114.1)	98.9 (73.9–121.0)
Cigarette pack-years, median (range)	0.0 (0–0.1)*	8.0 (5–18)
Allergen extract inhaled Cat/HDM/Grass	1/15/2	2/9/6
Cumulative allergen dose (SQ-U/mL), mean (range)	13 000 (250–53 250)	14426.5 (250–53250)
Methacholine PC ₂₀ (mg/mL), mean (range)	1.27 (0.4–4.85)	1.63 (0.09–6.2)

*One subject was a former smoker who had not smoked for 8 years, all other non-smokers were never smokers

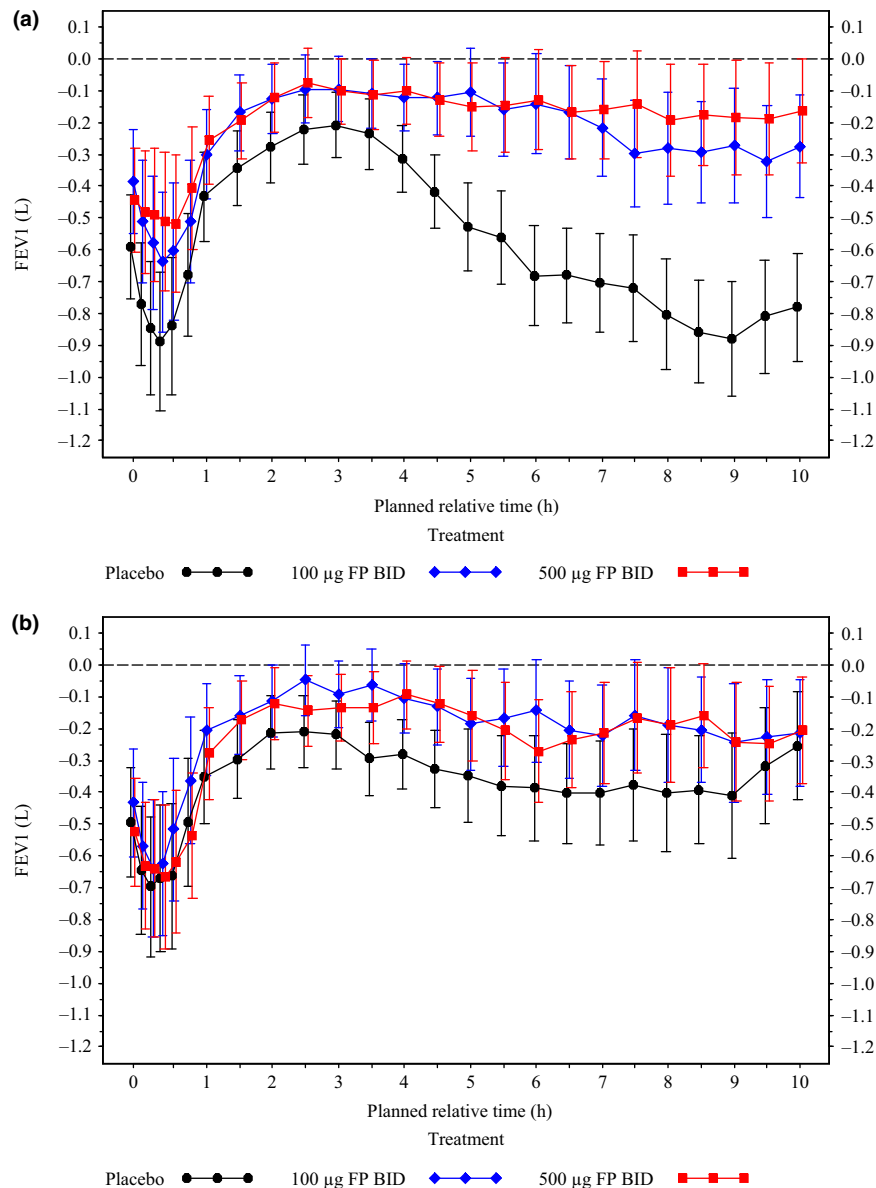


Fig. 1. Allergen challenge time course of adjusted mean change in post-saline FEV₁ (95% CI) on Day 6 in a) non-smokers and b) smokers. FP: fluticasone propionate; FEV₁: forced expiratory volume in 1 s.

after allergen challenge, was significantly reduced by both doses of FP relative to placebo in both the smoking and non-smoking groups (Fig. 3). The level of bronchoprotection was higher in the non-smoking group [adjusted mean difference from placebo (95% CL) for FP 100 µg bd: 1.533 (0.854, 2.213) and FP 500 µg bd: 2.016 (1.353, 2.679)] compared with the smoking group [FP 100 µg bd: 1.092 (0.393, 1.790) and FP 500 µg bd: 1.314 (0.611, 2.017)].

Exhaled nitric oxide. Geometric mean eNO was lower in smokers compared with non-smokers at baseline measured pre-dose on Day 1 of each treatment, and the increase in eNO following allergen challenge, as

observed during placebo, was suppressed by treatment with both doses of FP in both the smoking and non-smoking groups (Table S2 online supplement).

Sputum inflammatory cells. Only 50% of the sputum data were evaluable. Sputum eosinophil count increased after allergen challenge in both smokers and non-smokers, but the increase was higher in the non-smoking group (Fig. 4). Relative to placebo, FP treatment resulted in a greater suppression of the increased eosinophil count in the non-smokers (adjusted mean difference from placebo [95% CL] for FP 100 µg bd: 0.272 [0.027, 2.787] and FP 500 µg bd: 0.0088 [0.007, 1.114]) compared with the smokers (adjusted mean difference

Table 2. Asthmatic bronchoconstrictor responses on Day 6 allergen challenge by treatment arm in smokers and non-smokers

Response: change from post-saline on Day 6	Non-smokers			Smokers		
	Placebo	FP 100 µg bd	FP 500 µg bd	Placebo	FP 100 µg bd	FP 500 µg bd
EAR (0–2 h)						
Minimum FEV ₁ (L)						
Adjusted mean	–0.98	–0.75	–0.68	–0.80	–0.76	–0.82
(95% CI)	(–1.22, –0.74)	(–0.96, –0.53)	(–0.96, –0.54)	(–1.00, –0.60)	(–1.00, –0.53)	(–1.01, –0.63)
Difference from placebo	–	0.24	0.31	–	0.03	–0.02
(95% CI)		(0.05, 0.43)	(0.12, 0.50)		(–0.17, 0.23)	(–0.22, 0.18)
Weighted mean FEV ₁ (L)						
Adjusted mean	–0.51	–0.33	–0.30	–0.45	–0.32	–0.39
(95% CI)	(–0.68, –0.34)	(–0.46, –0.21)	(–0.47, –0.12)	(–0.60, –0.29)	(–0.45, –0.19)	(–0.50, –0.28)
Difference from placebo		0.18	0.22		0.12	0.06
(95% CI)		(0.07, 0.30)	(0.10, 0.34)		(–0.00, 0.24)	(–0.06, 0.18)
LAR (4–10 h)						
Minimum FEV ₁ (L)						
Adjusted mean	–1.01	–0.38	–0.35	–0.63	–0.44	–0.46
(95% CI)	(–1.31, –0.71)	(–0.54, –0.22)	(–0.51, –0.20)	(–0.84, –0.43)	(–0.65, –0.23)	(–0.59, –0.32)
Difference from placebo		0.64	0.66		0.17	0.16
(95% CI)		(0.44, 0.84)	(0.46, 0.86)		(–0.04, 0.38)	(–0.05, 0.37)
Weighted mean FEV ₁ (L)						
Adjusted mean	–0.66	–0.19	–0.13	–0.40	–0.22	–0.26
(95% CI)	(–0.89, –0.44)	(–0.31, –0.06)	(–0.24, –0.03)	(–0.58, –0.23)	(–0.38, –0.05)	(–0.32, –0.13)
Difference from placebo		0.48	0.53		0.18	0.17
(95% CI)		(0.33, 0.63)	(0.38, 0.68)		(0.02, 0.33)	(0.01, 0.32)

EAR, early asthmatic response, FEV₁, forced expiratory volume in 1 s; LAR, late asthmatic response.

from placebo [95% CL] for FP 100 µg bd: 1.287 [0.076, 21.766] and FP 500 µg bd: 1.546 [0.007, 345.623]]. These data were variable as shown by the large confidence intervals.

Sputum neutrophil count showed similarly large variability and did not differ between groups pre- and post-challenge. (Figure S4, online supplement).

Safety

There were no clinically relevant differences in the frequency of adverse events across treatment groups. The most frequently reported event in all groups was headache (placebo: 3 [9%]; FP 100 µg bd 2 [6%]; FP 500 µg bd 3 [9%]). There were also no clinically relevant changes in vital signs during any treatment.

Discussion

This is the first study to evaluate the effects of smoking status on the efficacy of an ICS in the allergen-induced asthmatic response. At the end of treatment with ICS, the amplitude of the LAR was similar in ICS-treated smokers compared with the non-smokers. However, a novel finding of this study is that the LAR was significantly reduced in smokers compared with non-smokers even in the absence of ICS treatment. This effect was

shown consistently, during placebo treatment and at the screening challenge. The attenuation of LAR in the smoking group was unanticipated and was accompanied by a reduction in eosinophilic inflammation after allergen challenge, as shown by lower eNO level responses and sputum eosinophil count following challenge. Smoking was also associated with a reduced bronchial hyperresponsiveness to methacholine at baseline. The EAR showed very little attenuation in the smoking group compared with the non-smoking group. Relatively, therefore ICS appeared less effective in suppressing the EAR in the smokers although the placebo EAR was not as marked in smokers compared to non-smokers.

Whilst previous clinical studies have reported that ICS is less effective in smokers than in non-smokers [10–12], our results showed that the amplitude of the LAR was similar in ICS-treated smokers compared with non-smokers, in this atopic population. It appears that the attenuation of the LAR in smokers was only partly associated with ICS treatment; the finding of a reduced LAR in smokers compared with non-smokers in the absence of ICS treatment suggests that smoking itself is associated with attenuation of the allergic eosinophilic-related responses. This is consistent with data from some studies in animal models. Significant reductions in ovalbumin-induced bronchoconstriction, responsiveness

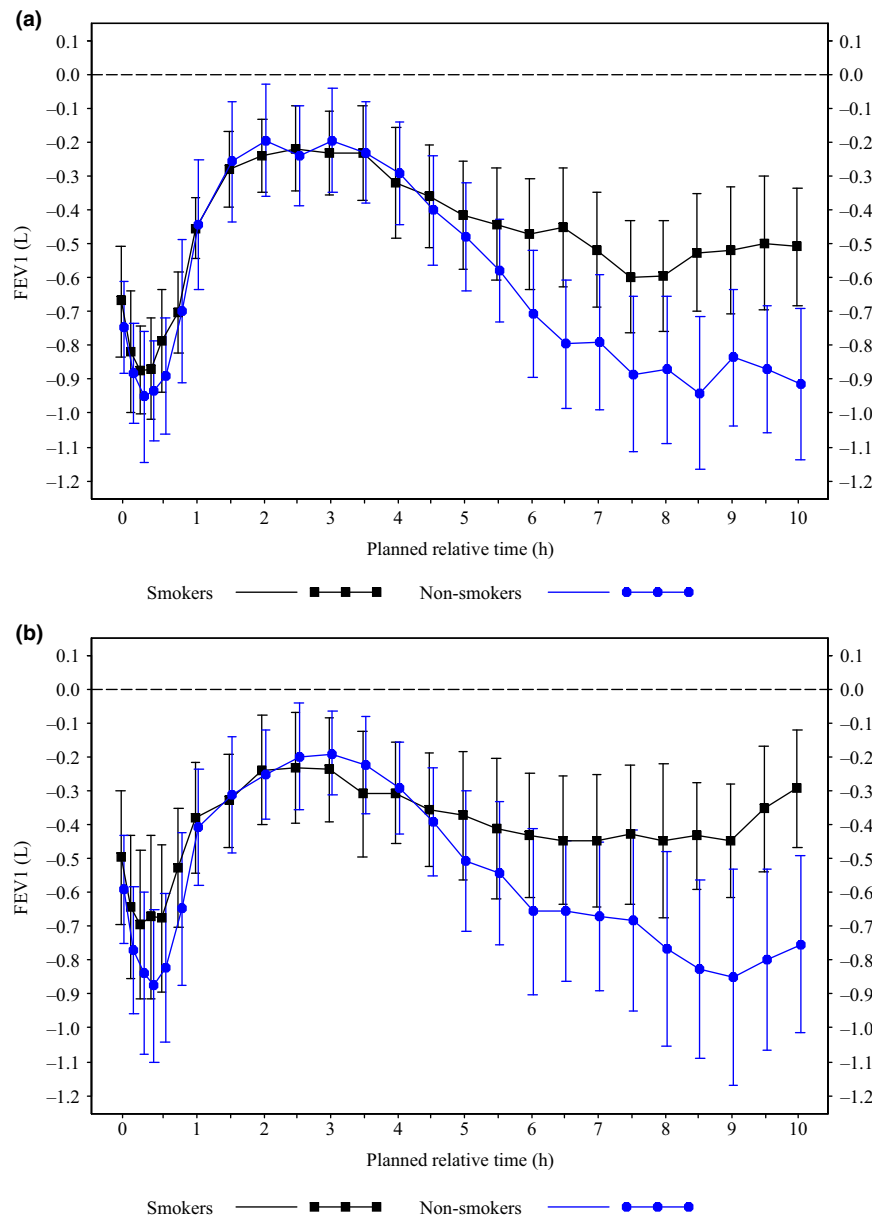


Fig. 2. Allergen challenge time course of mean change in post-saline FEV₁ (95% CI) at (a) Screening and on (b) Day 6 in the placebo group in non-smokers and smokers.

to methacholine and eosinophil numbers in lung lavage fluid [22], as well as a reduced inflammatory response [23, 24], have been reported in sensitized mice exposed to mainstream smoke, compared with 'non-smoking' mice. However, our findings differ from those reported by Meghji *et al.* who evaluated allergen-induced responses in a mild asthmatic population and reported the late phase asthmatic responses to be similar in smokers and non-smokers (mean maximum fall in FEV₁ in LAR, $16.3 \pm 4.3\%$ in non-smokers and $12.9 \pm 7.2\%$ in smokers) [18]. There was also no difference in allergen-induced increases in sputum eosinophils, and a lack of allergen-induced methacholine

airway hyper-responsiveness in their smoking population. This disparity between findings may perhaps be related to differences in study populations. The asthmatic population that smoked and reported by Meghji *et al.* was generally a much lighter smoking group with a median smoking pack-years of 1.2 and only three of 13 smokers having ≥ 5.0 (range 0.8–35) pack-years history, compared with a median pack-years of 8 with all smokers having pack-years ≥ 5.0 (range 5–16) in our population.

Positive effects of ICS treatment on eosinophil count and bronchial hyper-responsiveness in both smokers and non-smokers was shown, but with a greater

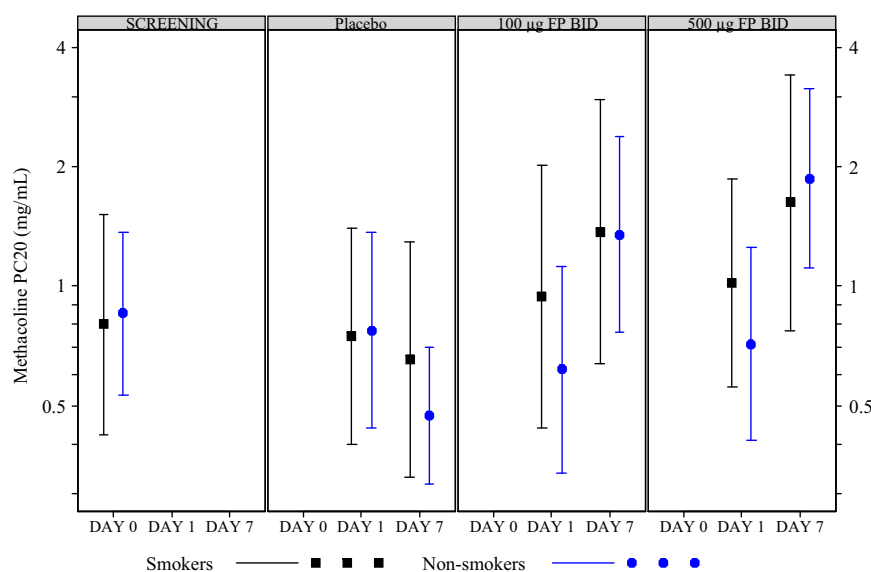


Fig. 3. Adjusted treatment difference of methacholine PC₂₀ (mg/mL) on Day 7 of treatment. FP: fluticasone propionate.

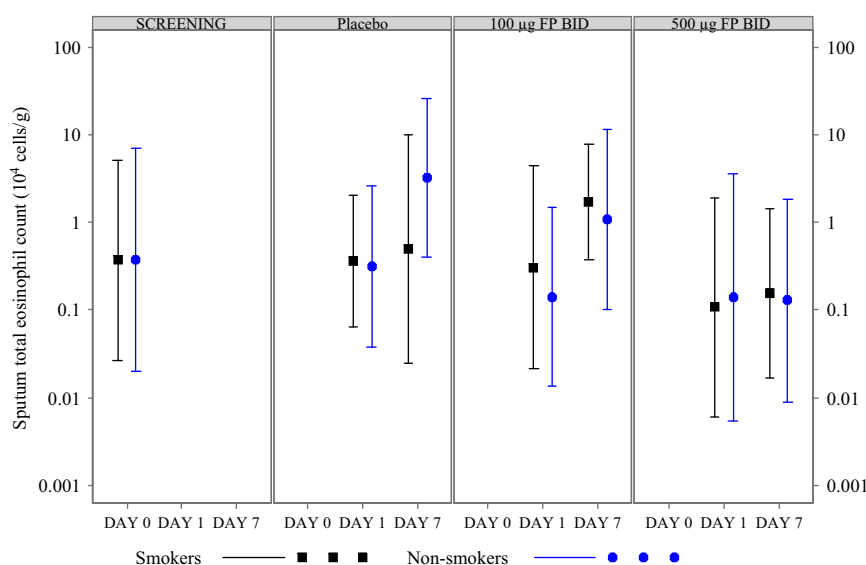


Fig. 4. Mean (95% CI) sputum eosinophil count before and after treatment. FP: fluticasone propionate.

magnitude of effect shown in non-smokers. This is consistent with the pattern of findings reported in the GOAL study for individual clinical outcomes [11]. Clearie *et al.* reported no improvement in methacholine PC₂₀ in smokers following treatment with ICS, but showed a clear benefit of ICS/LABA therapy in their smoking population which they hypothesized was associated with smooth muscle stabilization in association with LABA treatment [13]. Of note, this study and other studies that have reported corticosteroid insensitivity in asthmatic smokers, have studied patients with a longer smoking pack year history than the patients in our study and the study by Meghji [10, 12, 18], and

therefore, it is possible that patients with a longer pack year smoking history may have shown less response to ICS than demonstrated in the current study.

The decreased eNO levels observed in smokers compared with non-smokers in this study agree with results reported in other studies in both mild [15, 16] and severe [17] asthma. Rouhos *et al.* made this observation in atopic but not non-atopic asthmatics, highlighting the fact that findings in populations with atopic asthma may not be generalizable to all smoking asthmatics [15]. In our study, although eNO levels were suppressed in smokers, an increase in eNO levels following allergen challenge was observed in this group which was

suppressed by ICS treatment. Our finding of a reduced sputum eosinophil count in current smokers versus non-smokers is also consistent with that reported by Thomson *et al.* in severe asthmatics [17].

The immunological pathogenesis of atopic asthma is thought to be related to activation of T-helper (Th) 2 cells in response to aeroallergens, initiating an inflammatory response involving mast cell activation, release of mediators, eosinophil recruitment and culminating in the characteristic early and late asthmatic responses [25]. It has been suggested that this response may be altered in asthmatics who smoke, the smoking phenotype being characterized by a predominance of activated macrophages and neutrophils in sputum. This might result in a reduced influence of Th2 and biasing towards Th1 responses, fewer B cells, lower levels of Interleukins (IL)-4 and IL-5 and consequently fewer and less activated eosinophils [25]. The clinical outcomes reported in this study lend some support to this idea although sputum neutrophil count was not markedly higher in the smoking group than in the non-smoking group.

Cigarette smoke contains many compounds [26, 27]; two that might influence the Th2/Th1 balance are nicotine and bacterial endotoxin (lipopolysaccharide (LPS)). Nicotine has been shown to suppress both eosinophil recruitment and Th2 cytokine/chemokine responses in rat models of allergen challenge [28], and nicotinic receptor agonists have been in development for treatment of inflammation-based diseases [29]. McSharry *et al.* reported increased sputum endotoxin levels in association with an impaired lung function response to oral corticosteroids in asthmatic patients [30]. They also reported a significant difference in blood (but not sputum) endotoxin concentration among smokers compared to non-smokers. In addition, studies evaluating the effects of LPS exposure in allergen-sensitized mice have also suggested that different levels of LPS exposure result in different asthma phenotypes with high LPS exposure being characterized by a more prominent Th1 response [31], airway hyperresponsiveness and non-eosinophilic inflammation [32]. In a house dust mite allergen-evoked asthma murine model, the concurrent administration of LPS resulted in a clear inhibition of house dust mite-induced Th2 lung inflammation, as reflected by a dose-dependent reduction in eosinophil influx, mucus production and Th2 cytokine release, whilst increasing neutrophil recruitment [33]. As one cigarette contains significant levels of LPS and it is estimated that up to 1% can survive combustion and is inhaled [34], it is plausible that recurrent exposure to inhaled LPS in a habitual smoker may be responsible for modulating the local immune response in the lung. This is supported by a segmental allergen challenge study showing that endotoxin contamination of allergen extract caused bronchial

neutrophilia, rather than eosinophilia, following allergen challenge in humans [35].

It is estimated that the prevalence of active cigarette smoking in adults with asthma is approximately 25% [1, 36]. Compared with non-smokers with asthma, active smokers are reported to have more severe asthma symptoms [2, 3], accelerated decline in lung function [6, 7], and a reduced response to corticosteroid therapy [10, 11]. Despite this, asthmatics who smoke cigarettes are generally excluded from the majority of clinical studies assessing asthma treatments and that includes allergen challenge studies and most Phase III development programmes. Therefore, one of the limitations of this study is that the subject sample size was estimated from previous allergen challenge studies in non-smokers. Thus, the unexpected attenuation of the LAR in smokers suggests that more subjects may be needed in studies that include smokers, to fully evaluate drugs with potential anti-inflammatory effects in asthma, particularly atopic asthma. Another limitation of this study was the gender imbalance in the smoking and non-smoking groups, with a predominance of males in the non-smoking group and females in the smoking group, and it is possible that this could have influenced the differences in LAR in the smoking and non-smoking groups. Data on the differences in LAR amplitude in male and female asthmatics are however not reported in the literature. Finally, the poor quality of the sputum cytology samples with only half the samples being completely evaluable was another limiting factor of this study. As a consequence, the sputum data were highly variable, showing that sputum cytology remains a difficult endpoint to interpret in clinical studies, even when undertaken by highly experienced centres. It is also acknowledged that the results for eNO and bronchial responsiveness were quite variable; however, these data together with that for sputum eosinophils showed trends which supported the main findings with LAR, giving some confidence in the robustness of these findings.

In conclusion, this study showed that allergen-induced late asthmatic responses were of a similar amplitude in both smoking and non-smoking atopic asthmatics at the end of ICS treatment, but that attenuation of the LAR in smokers was only partly associated with ICS treatment. The marked attenuation of the LAR observed in smokers in the absence of ICS treatment is a novel observation.

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Conflict of interest

Funding for this study was provided by GSK (NCT01400906). All listed authors meet the criteria for authorship set forth by the International Committee for

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Allergen challenge time course of mean change in post-saline FEV₁ (95% CI) on Day 6 of a) placebo, b) FP 100µg bd and c) FP 500 µg bd, in non-smokers and smokers excluding 8 protocol violators

Figure S2. Adjusted mean treatment differences (95% CI) in post-saline FEV₁ by time on Day 6 following treatment with a) FP 100µg bd and b) FP 500 µg bd

Figure S3. Adjusted mean allergen challenge time course of change in post-saline FEV₁ (95% CI) on Day 6 of a) placebo, b) FP 100µg bd and c) FP 500 µg bd in non-smokers and smokers excluding subjects challenged with grass pollen

Figure S4. Adjusted Geometric Means (95% CI) of sputum neutrophil cell count post allergen challenge on Day 7

Table S1. Details of screening allergen challenge protocol violators

Table S2. Summary of log_e transformed exhaled No data (ppb)