

A Randomized, Crossover Study to Investigate the Pharmacokinetics and Safety of Inhaled Fluticasone Furoate and Umeclidinium, Administered Separately and in Combination via Dry Powder Inhaler in Healthy Adult Volunteers

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ABSTRACT

Introduction: A combination of fluticasone furoate (FF) and umeclidinium (UMEC) has been considered for development for the treatment of asthma. The primary objectives were to investigate the plasma and urine pharmacokinetics (PK) of FF/UMEC in combination compared with FF and UMEC monotherapies.

Methods: This randomized, double-blind, three-period crossover, single-center study in healthy volunteers assessed the PK of FF 400 mcg and UMEC 500 mcg administered separately and in combination (four inhalations of FF/UMEC 100/125 mcg, FF 100 mcg, or

UMEC 125 mcg) via dual-strip dry powder inhaler. Subjects were randomized based on codes generated using a validated computerized system (Randall, GlaxoSmithKline).

Results: Eighteen subjects were enrolled; 17 received all three scheduled doses of study medication. Plasma FF and UMEC concentrations peaked at 0.5 and 0.08 h post-dose, respectively, for FF/UMEC and the monotherapies. FF and UMEC co-administration resulted in slightly lower or similar systemic exposure for both drugs versus the monotherapies. In post hoc sensitivity analyses (performed because two subjects administered inhalations incorrectly), the ratio of adjusted geometric means (maximum plasma concentration and area under the curve) was closer to unity than in the planned analyses. Cumulative urinary UMEC excretion (A_e) was similar for FF/UMEC and UMEC. Post hoc sensitivity analyses on A_{e0-24} suggested a small carryover effect but results were similar to those of the population as a whole. Urinary UMEC excretion following FF/UMEC was low (~1.5% over 24 h) and unlikely to have impacted upon PK comparisons. Three adverse events were

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reported; none were severe or led to withdrawal. There were no clinically significant effects on electrocardiogram, vital sign, or laboratory parameters.

Conclusion: Fluticasone furoate and umeclidinium co-administration was well tolerated and was not associated with meaningful changes in systemic or urinary PK versus the monotherapies.

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Keywords: Co-administration; Inhaled corticosteroid; Muscarinic antagonist; Pharmacokinetics; Plasma; Urine

INTRODUCTION

Many patients with asthma remain symptomatic despite the availability of treatments and are at risk of asthma exacerbations [1]. For patients who do not achieve optimal control on an inhaled corticosteroid (ICS) alone, treatment guidelines recommend increasing the ICS dose or adding a long-acting β -agonist (LABA) [2]. Recent evidence has suggested that the addition of a long-acting muscarinic antagonist (LAMA) to ICS therapy may also be beneficial in asthma, with reported improvements in symptoms and/or lung function, an extended time to first asthma exacerbation, and a reduced risk of severe exacerbations [3–5].

A combination of fluticasone furoate (FF) and umeclidinium (UMEC) has been considered for development for the treatment of asthma. FF is an ICS that exhibits prolonged absorption from the lungs into systemic circulation compared with fluticasone propionate, indicating a longer retention period in respiratory tissues [6]. FF has been associated with improvements in lung function and is well tolerated in patients with asthma [7, 8]. An

inhaled combination of FF and the LABA vilanterol (VI) is approved for the regular treatment of asthma in adults and adolescents, and for the symptomatic treatment of adults with chronic obstructive pulmonary disease (COPD) [9].

Umeclidinium is a quinuclidine-based quaternary LAMA, and is a potent anticholinergic with slow functional reversibility at the human M_3 receptor [10]. UMEC has been studied as a monotherapy for the treatment of COPD, with improvements observed in lung function, dyspnea, and health status compared with placebo [11, 12]. UMEC/VI is approved as a combination treatment for the once-daily, long-term maintenance treatment of airflow obstruction in patients with COPD [13].

Extensive data characterizing the safety, pharmacology, and pharmacokinetics (PK) of FF and UMEC were generated in studies in healthy volunteers and patients with asthma and COPD as part of the development program for FF/VI and UMEC/VI [14–18]. To apply these data to guide the development of the FF/UMEC combination, it is necessary to characterize any pharmaceutical and/or PK interactions that result when two active components are administered simultaneously through a single device. The objective of this study was to assess the plasma PK, safety, and tolerability of FF and UMEC when co-administered in healthy volunteers, in comparison with the same treatments as monotherapies. The urine PK of UMEC was also assessed.

METHODS

Study Design

This was a randomized, double-blind, single-dose, three-period crossover study

[GlaxoSmithKline study ILA116524; ClinicalTrials.gov #NCT01725685 (November 2012 to January 2013)], conducted at a single study center in the United States (Baltimore, MD, USA).

The study was approved by an Institutional Review Board (IRB; Aspire IRB, California, USA) and was in accordance with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Good Clinical Practice guidelines, all applicable subject privacy requirements and ethical principles outlined in the Declaration of Helsinki, 2008 [19, 20]. Written, informed consent was obtained from each subject before study procedures were performed.

Subjects

Eligible subjects were: 18–65 years of age (inclusive); had a body mass index of 19–33 kg/m² (inclusive); had an average QTcF <450 ms; had alanine aminotransferase, alkaline phosphatase and bilirubin levels $\leq 1.5 \times$ upper limit of normal; were current non-smokers who had not used tobacco products in the 6-month period before screening; and had a pack history of ≤ 10 pack-years.

Subjects were excluded if they: were of childbearing potential (unless practicing acceptable birth control methods); had a history of respiratory disease in the last 10 years; had a chronic history of liver disease and positive pre-study hepatitis B surface antigen or positive hepatitis C antibody result within the 3 months prior to screening; had a history of smoking or regular use of tobacco or nicotine-containing products within the 6 months prior to screening; or had a disease preventing use of anticholinergic medications

that would pose a safety risk in the opinion of the investigator.

Procedures

Subjects attended a screening visit up to 28 days prior to the first treatment dose. Eligible subjects were randomized 1:1:1:1:1:1 to one of six possible sequences of three treatments [four inhalations of FF/UMEC 100/125 mcg, FF 100 mcg, or UMEC 125 mcg (total inhaled dose FF/UMEC 400/500 mcg, FF 400 mcg, or UMEC 500 mcg)], separated by 7- to 10-day washout periods. The PK profiles of FF and UMEC cannot be fully characterized at the proposed therapeutic doses (FF 200 mcg and UMEC 125–250 mcg), due to the low systemic bioavailability following inhalation. Higher doses were therefore selected for investigation in this study to fully characterize the systemic PK profiles following inhalation, and to enable clearer detection of any potential carryover effect. Previous studies have shown FF and UMEC to be well tolerated in patients with asthma and COPD at doses comparable with those selected in the present study [21, 22]. All treatments were self-administered once in the morning via dry powder inhaler, following training on how to correctly use the inhaler. Subjects attended a follow-up visit 7–14 days after the last dose in Treatment Period 3 (Fig. 1).

The randomization code was generated using a validated computerized system (Randall, GlaxoSmithKline).

Outcomes and Assessments

Sample Collection and Analysis Methods

Blood samples (3 mL into K3 EDTA tubes) were obtained via an in-dwelling catheter or by direct venipuncture and were collected pre-dose and at 5, 15, 30, and 45 min, and 1, 1.5, 2, 3, 4, 6, 8,

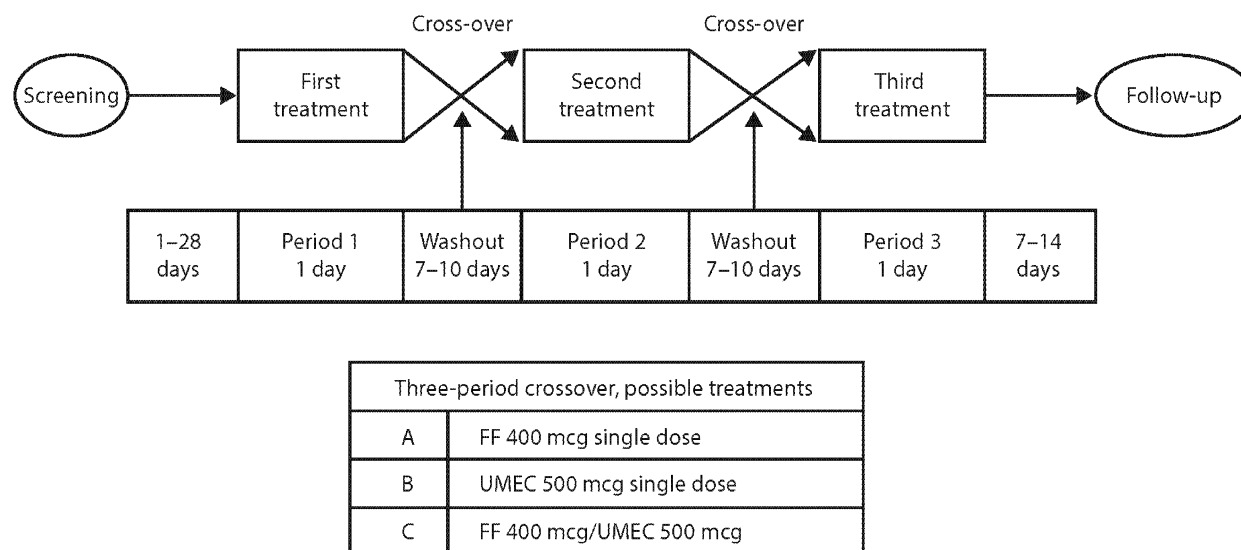


Fig. 1 Study design. *FF* fluticasone furoate, *UMEC* umeclidinium

12, and 24 h post-dose on Day 1 of each treatment period. On collection, samples underwent centrifugation at 1500g or 3000 rpm for 10 min at 4 °C. The supernatant was placed in polypropylene tubes and stored at –20 °C within 1 h of collection.

Urine samples for UMEC PK assessments were taken pre-dose, and between 0–6, 6–8, 8–12, 12–16, and 16–24 h post-dose on Day 1 of each treatment period. The pre-dose control sample (20 mL) and the post-dose urine samples (10 mL) were stored and frozen at –20 °C in 30-mL collection tubes.

Urine samples were not assessed for FF, as urinary excretion accounts for only approximately 1% and 2% of orally and intravenously administered FF doses, respectively, and elimination is primarily via the fecal route [23].

Plasma samples for FF (150 µL) and UMEC (250 µL), and urine samples for UMEC (50 µL) were analyzed by solid phase extraction followed by liquid chromatography with tandem mass spectrometric detection (York Bioanalytical Solutions, York, UK). The

analytical column used for analysis of UMEC was an Acquity BEH C18 [50 × 2.1 mm, 1.7 µm (Waters, Milford, MA, USA)], and an A:B gradient was used, where A was 10% acetonitrile in water and B was 10% water in acetonitrile (both containing 0.05% trifluoroacetic acid).

Quality control (QC) samples, prepared at three different analyte concentrations and stored with study samples, were analyzed with each batch of samples against separately prepared calibration standards. For the analysis to be acceptable, no more than one-third of the QC results were to deviate from the nominal concentration by more than 15% and at least 50% of the results from each QC concentration should be within 15% of nominal. The applicable analytical runs met all predefined run acceptance criteria. Further details on the analytical methods are described in the online electronic supplementary material.

In plasma FF analyses, the lower limit of quantification (LLQ) was 10.0 pg/mL. In plasma and urine UMEC analyses, the LLQ was 10.0 pg/mL. In the plasma FF and UMEC analyses, any

measurements that fell below the limit of quantification were imputed as zero, with two exceptions: if a single non-quantifiable (NQ) measurement was obtained between two measureable concentrations, a null value was used for concentration–time profiles and the calculation of summary statistics (i.e., the NQ value was classed as ‘missing data’); if trailing NQ values were observed (consecutive NQ values in the tail), then null values were used for concentration–time profiles, and the value was imputed as zero for the calculation of summary statistics.

PK Analysis

Plasma PK parameters for FF and UMEC, and urine PK parameters for UMEC were derived using non-compartmental analysis with WinNonlin 5.2.1.

The key plasma PK endpoints were: maximum plasma concentration ($C_{\max}$), area under the concentration–time curve (AUC_{0-t}), $AUC_{0-t'}$ where t' was the time at which AUC was calculable for all subjects, time of maximum observed concentration ($t_{\max}$), and the time of last measurable concentration (t_{last}). An assessment of $AUC_{0-\infty}$, plasma elimination half-life ($t_{1/2}$), elimination rate constant (λ_z), apparent clearance (CL/F), and apparent volume of distribution (V/F) was planned if sufficient data were available.

The key urine PK endpoints were: the cumulative amount of UMEC excreted in urine (A_e) and the percent of UMEC dose excreted. An evaluation of urine half-life ($t_{1/2}$) and renal clearance (CL_r) was planned if sufficient data were available.

Safety Assessments

Safety assessments included the incidence of adverse events (AEs), clinical laboratory measurements, vital signs, and

electrocardiogram (ECG) evaluations. ECG and vital sign assessments were performed at screening, pre-dose, Day 2, and 7–14 days after the last dose. Hematology, clinical chemistry, and urinalysis parameters were assessed at screening, Day –1, and Day 2. Cotinine assessments were performed at Screening and at Day –1 in each treatment period. AEs and serious AEs (SAEs) were collected throughout the study and were recorded on case report forms.

Statistical Analyses

The sample size calculation was primarily based on an estimation approach and feasibility. Data from previous studies in healthy volunteers (GlaxoSmithKline, data on file) were used to obtain estimates of variability in FF 400 mcg alone and UMEC 500 mcg alone. Based on the most conservative within-subject standard deviation of 0.254, and a sample size of 18 subjects, this equated to a half-width of the 90% confidence interval (CI) of 0.143. This, when back-transformed, provided a half-width of the 90% CI around the ratio of the geometric means of approximately $\pm 16\%$ corresponding to a lower and upper confidence limits of 0.87 and 1.15, assuming a true ratio of 1 (no difference between the treatments).

For FF and UMEC plasma $C_{\max}$ and AUC, and urine PK parameter (A_e) analyses, mixed-effects models on the log-transformed parameters were fitted with treatment and period as fixed effects and subject as a random effect. Point estimates and their associated 90% CIs were constructed for the difference between the combination and each monotherapy; the point estimates and their associated 90% CIs were back-transformed to provide point estimates and 90% CIs for the ratio of the combination to each monotherapy.

The final analyses were performed after all subjects had completed the study. All displays

were generated in a Harmonization of Analysis and Reporting Program environment, using SAS[®] version 9.1.3 (SAS Institute Inc., Cary, NC, USA) on a UNIX platform.

RESULTS

Forty-three subjects were screened, of which 18 were randomized. The reasons for screening failure were related to drug screening ($n = 4$), ECG assessments ($n = 4$), vital sign assessments ($n = 3$), medical history ($n = 2$), clinical laboratory assessments ($n = 1$), body mass index ($n = 1$), and other reasons ($n = 1$). One subject passed the screening stage but failed the pre-dose drug screen. Eight subjects passed the screening stage but were not randomized as the cohorts were complete. Of the 18 subjects enrolled and randomized, 17 received all three scheduled doses of study medication. One subject tested positive for cotinine upon Treatment Period 3 admission and was withdrawn from the study at the investigator's discretion. The majority of subjects were male (78%), of African-American/African heritage (72%), and had a mean age of 41.3 years (Table 1).

Planned PK Analyses

All data in the PK population [subjects for whom a PK sample was obtained and analyzed ($N = 18$)] were included in the PK analysis. All pre-dose plasma FF and UMEC concentrations were non-quantifiable, indicating no carryover effects between dosing periods. The mean plasma PK concentration–time profiles of FF/UMEC 400/500 mcg, FF, and UMEC in the PK population are shown in Fig. 2a.

The median (range) $t_{\max}$ of FF was 0.5 (0.08–1.53) h for FF/UMEC and 0.5 (0.08–1.00) h for FF monotherapy. The median (range) t_{last} for FF was similar for FF/UMEC [23.98

Table 1 Subject demographic and baseline characteristics

Demographics	Total ($n = 18$)
Mean age, years (SD; range)	41.30 (12.91; 19–61)
Sex, n (%)	
Female	4 (22)
Male	14 (78)
Mean BMI, kg/m ² (SD; range)	28.78 (3.88; 19.40–33.00)
Ethnicity, n (%)	
Hispanic or Latino	2 (11)
Not Hispanic or Latino	16 (89)
Race, n (%)	
African-American/African	13 (72)
American-Indian or Alaskan native	0 (0)
Asian	0 (0)
Native Hawaiian or other Pacific Islander	0 (0)
White	5 (28)

BMI body mass index, SD standard deviation

(2.00–24.05) h] and FF monotherapy [24.01 (23.92–24.13) h].

Umeclidinium was rapidly absorbed following inhalation, with a median (range) $t_{\max}$ of 0.08 (0.05–0.13) h and 0.08 (0.07–0.10) h for FF/UMEC combination therapy and UMEC monotherapy, respectively. The median (range) t_{last} for UMEC was similar for FF/UMEC [23.98 (7.98–24.05) h] and UMEC monotherapy [24.00 (4.07–24.08) h].

For FF, t' (the time at which AUC was assessable for all subjects) was 2 h. In the FF analysis, $C_{\max}$, AUC_{0-2} , and AUC_{0-t} were reduced when comparing FF/UMEC with FF monotherapy [ratio of adjusted geometric means: $C_{\max}$, 0.94 (90% CI 0.75–1.17); AUC_{0-2} , 0.92 (90% CI 0.79–1.07); AUC_{0-t} , 0.72 (90% CI 0.52–0.98)]. For $C_{\max}$ and AUC_{0-t} , the

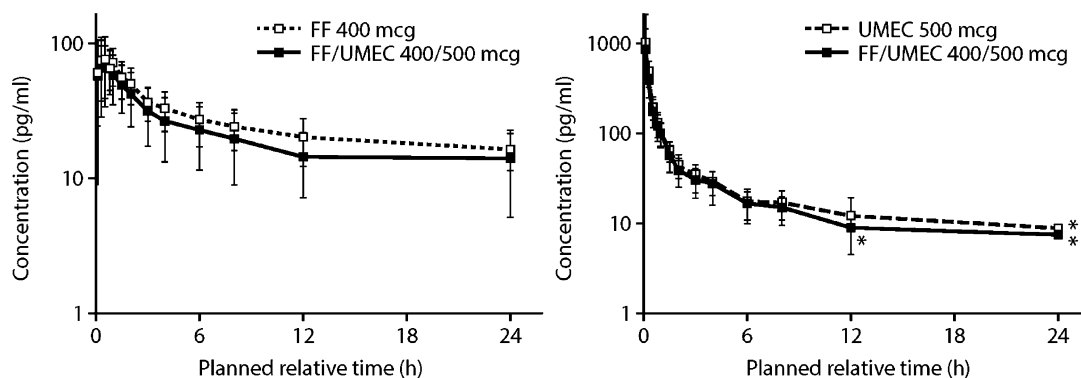
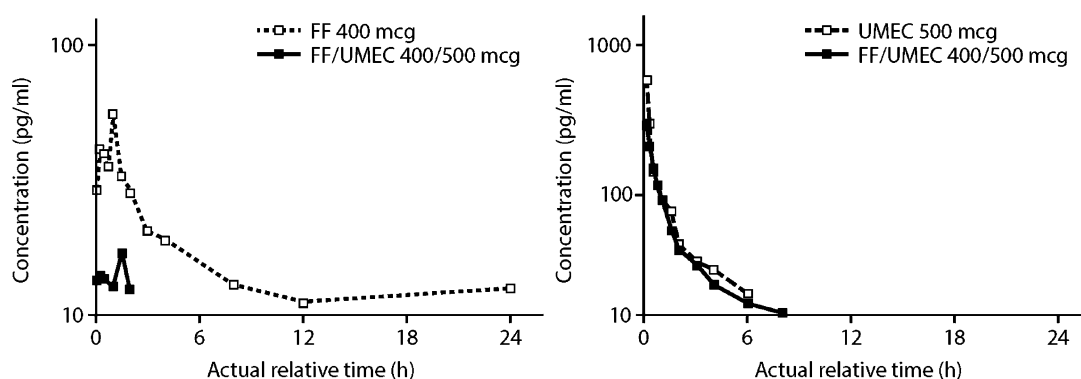
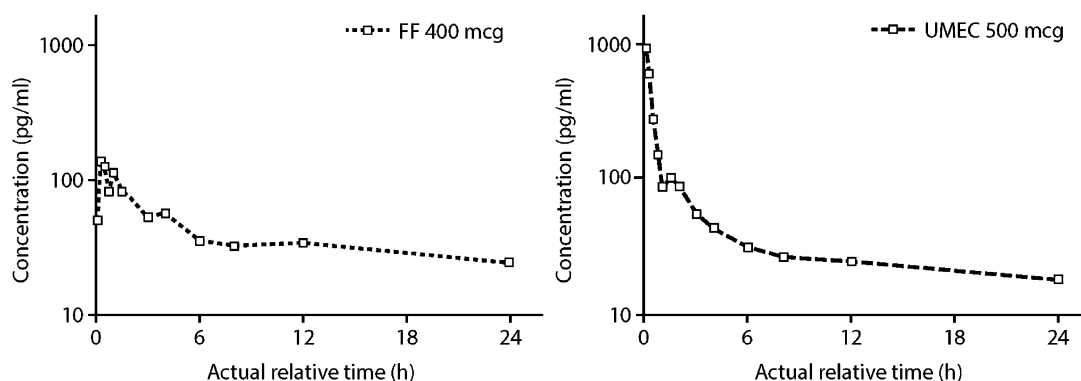
a PK population**b** Subject 107[†]**c** Subject 117[†]

Fig. 2 Mean ($\pm$ SD) plasma FF and UMEC concentration–time profiles in the PK population (**a**) and the individual profiles for the two subjects excluded in sensitivity analyses (**b**, **c**). *AUC* area under the concentration–time curve, *C_{max}* maximum plasma concentration, *FF* fluticasone furoate, *LLQ* lower limit of quantification, *PK* pharmacokinetic, *SD* standard deviation, *UMEC* umecclidinium. * SD not calculated as $>30\%$ of values were imputed due to the presence of concentration values below the LLQ. [†] Subject 107 was excluded in sensitivity

analyses due to a lower exposure to FF following FF/UMEC that was inconsistent with exposure to FF monotherapy. As *AUC* and *C_{max}* values were calculable from the data, the imputation method was not applied. Subject 117 was excluded due to inconsistent exposure across the three treatment periods. When randomized to FF/UMEC 400/500 mcg in Treatment Period 1, FF and UMEC plasma concentrations were below the LLQ (10 pg/mL) at each measurement, and therefore values were imputed as zero

inter-subject variability following FF/UMEC was high (99% and 162%, respectively).

For UMEC, t' was 4 h. For both $C_{\max}$ and AUC_{0-4} , the point estimate of the adjusted geometric mean was lower for FF/UMEC than for FF or UMEC as monotherapy [ratio of adjusted geometric means: $C_{\max}$, 0.88 (90% CI 0.71–1.09); AUC_{0-4} , 0.93 (90% CI 0.82–1.06)]. For $C_{\max}$, the inter-subject variability was moderate (50%) for UMEC monotherapy and high (197%) after FF/UMEC. For AUC_{0-4} and AUC_{0-t} , the inter-subject variability for UMEC alone and FF/UMEC was low-to-moderate (23–52%).

Post Hoc PK Sensitivity Analyses

During Treatment period 1, one subject (Subject 107) did not take deep inhalations for all four doses of study medication, and this was documented as a protocol deviation. The FF $C_{\max}$ for Subject 107 was 12.1 pg/mL after FF/UMEC in this period, which was inconsistent with the subject's exposure to FF monotherapy in Treatment Period 3 (60.5 pg/mL) when no protocol deviation was noted. FF concentrations following FF/UMEC administration in Treatment Period 1 were measureable only at 5 min–2 h post-dose for Subject 107. In comparison, FF concentrations following administration of FF alone were quantifiable up to 24 h in the overall population, with a range of FF $C_{\max}$ from 36.3 to 157.3 pg/mL. There were no apparent differences in UMEC plasma concentrations following FF/UMEC and UMEC monotherapy in Subject 107 (Fig. 2b). A second subject (Subject 117) exhibited inconsistent exposure across the three treatment periods, although there was no evidence of protocol deviation (e.g., inhaler misuse). When randomized to FF/UMEC 400/500 mcg in Treatment Period 1,

the concentration of FF was <10 pg/mL, compared with a value of 145.2 pg/mL in Treatment Period 3 with FF 400 mcg. UMEC plasma concentrations were also below the LLQ at each measurement in Treatment Period 1, but were quantifiable in Treatment Periods 2 with UMEC 500 mcg alone (Fig. 2c).

A post hoc sensitivity analysis was performed which excluded the data for Subject 107 and 117. For $C_{\max}$, AUC_{0-2} , and AUC_{0-t} , the adjusted geometric mean ratios for FF/UMEC compared with FF monotherapy were closer to unity, demonstrating reduced intra-subject variability compared with the planned analyses [i.e., 1.03 (90% CI 0.84–1.26), 0.98 (90% CI 0.86–1.12), and 0.85 (90% CI 0.70–1.05), respectively; Table 2]. In UMEC post hoc sensitivity analyses, $C_{\max}$ and AUC_{0-4} were lower with FF/UMEC compared with UMEC monotherapy [ratio of adjusted geometric means: $C_{\max}$, 0.92 (90% CI 0.73–1.15); AUC_{0-4} , 0.95 (90% CI 0.83–1.10)], and were closer to unity compared with the planned analyses (Table 2).

Urine Analyses

In the planned urine UMEC PK analyses, a small amount of UMEC was excreted over 24 h following inhalation of FF/UMEC (1.502%; range 0.715–2.535) and UMEC (1.627%; range 0.986–2.679). Ae_{0-24} was similar for FF/UMEC and UMEC monotherapy [ratio of adjusted geometric means: 0.99 (90% CI 0.87–1.12)]. The inter-subject variability was similar for FF/UMEC and UMEC monotherapy (34% and 32%, respectively).

A post hoc sensitivity analysis for FF/UMEC compared with UMEC monotherapy that excluded data for the two previously identified subjects demonstrated similar results for Ae_{0-24} [ratio of adjusted geometric means: 1.02 (90% CI 0.89–1.17); Table 3]. As quantifiable levels of

Table 2 Summary of statistical analysis of derived plasma UMEC and FF PK parameters

Parameter	Treatment	Arithmetic mean (n)	Standard deviation	Comparison	Adjusted geometric mean (combination)	Adjusted geometric mean (mono)	GMR (Test:reference)	90% CI for GMR
Derived plasma FF PK parameters								
Planned analysis								
$C_{\max}$ (pg/mL)	FF 400 mcg	89.08 (18)	33.96	FF/UMEC 400/500 mcg	77.71	83.05	0.94	0.75–1.17
	FF/UMEC 400/500 mcg	82.23 (18)	40.90	vs FF 400 mcg				
AUC_{0-2} (pg h/mL)	FF 400 mcg	123.96 (18)	41.82	FF/UMEC 400/500 mcg	107.99	117.78	0.92	0.79–1.07
	FF/UMEC 400/500 mcg	115.14 (17)	38.19	vs FF 400 mcg				
$AUC_{0-\tau}$ (pg h/mL)	FF 400 mcg	613.41 (18)	178.22	FF/UMEC 400/500 mcg	422.89	591.18	0.72	0.52–0.98
	FF/UMEC 400/500 mcg	485.18 (18)	238.14	vs FF 400 mcg				
Sensitivity analysis ^a								
$C_{\max}$ (pg/mL)	NA	NA	NA	FF/UMEC 400/500 mcg vs FF 400 mcg	85.12	82.74	1.03	0.84–1.26
AUC_{0-2} (pg h/mL)	NA	NA	NA	FF/UMEC 400/500 mcg vs FF 400 mcg	115.80	117.76	0.98	0.86–1.12
$AUC_{0-\tau}$ (pg h/mL)	NA	NA	NA	FF/UMEC 400/500 mcg vs FF 400 mcg	504.99	592.10	0.85	0.70–1.05

Table 2 continued

Parameter	Treatment	Arithmetic mean (n)	Standard deviation	Comparison	Adjusted geometric mean (combination)	Adjusted geometric mean (mono)	GMR (Test:reference)	90% CI for GMR
Derived plasma UMEC PK parameters								
Planned analysis								
$C_{\max}$ (pg/mL)	UMEC 500 mcg	975.9 (17)	425.94	FF/UMEC 400/500 mcg vs UMEC 500 mcg	786.84	892.72	0.88	0.71–1.09
	FF/UMEC 400/500 mcg	804.5 (18)	332.07					
AUC_{0-4} (pg h/mL)	UMEC 500 mcg	435.1 (17)	116.51	FF/UMEC 400/500 mcg vs UMEC 500 mcg	393.06	422.24	0.93	0.82–1.06
	FF/UMEC 400/500 mcg	402.7 (17)	81.12					
Sensitivity analysis ^a								
$C_{\max}$ (pg/mL)	NA	NA	NA	FF/UMEC 400/500 mcg vs UMEC 500 mcg	847.00	921.44	0.92	0.73–1.15
AUC_{0-4} (pg h/mL)	NA	NA	NA	FF/UMEC 400/500 mcg vs UMEC 500 mcg	404.89	425.73	0.95	0.83–1.10

AUC area under the curve, CI confidence interval, $C_{\max}$ maximum plasma concentration, FF fluticasone furoate, GMR geometric mean ratio, NA not available, PK pharmacokinetics, $UMEC$ umedidinium

^a Sensitivity analyses were conducted to exclude the data for two subjects. One subject was observed to be administering inhalations incorrectly and another subject did not have quantifiable levels of both UMEC and FF when randomized to FF/UMEC 400/500 mcg in Treatment Period 1

Table 3 Summary of statistical analysis of derived urine UMEC PK parameters [Ae_{0-24} (mcg), FF/UMEC 400/500 mcg versus UMEC 500 mcg]

	Adjusted geometric mean (FF/UMEC 400/500 mcg)	Adjusted geometric mean (UMEC 500 mcg)	Ratio (Test:reference) ^a	Two-sided 90% CI for ratio
Planned analysis	7.93	8.05	0.99	0.87–1.12
Sensitivity analysis of PK population excluding 2 subjects	8.12	7.95	1.02	0.89–1.17

Ae urinary recovery of unchanged drug, *CI* confidence interval, *FF* fluticasone furoate, *PK* pharmacokinetic, *UMEC* umecclidinium

^a Test, FF/UMEC 400/500 mcg; Reference, UMEC 500 mcg

Table 4 AEs (all subjects)

Preferred term	Number of subjects, <i>n</i> (%)			
	FF/UMEC 400/500 mcg (<i>n</i> = 18)	FF 400 mcg (<i>n</i> = 18)	UMEC 500 mcg (<i>n</i> = 17)	Total (<i>n</i> = 18)
Any event	1 (6)	2 (11)	0 (0)	3 (17)
Headache	0 (0)	1 (6)	0 (0)	1 (6)
Dysmenorrhea	0 (0)	1 (6)	0 (0)	1 (6)
Oropharyngeal pain	1 (6)	0 (0)	0 (0)	1 (6)

AE Adverse event, *FF* Fluticasone furoate, *UMEC* Umeclidinium

UMEC were observed in the majority of pre-dose urine samples at Treatment Periods 2 and 3, a separate post hoc sensitivity analysis was performed on the statistical analysis of UMEC Ae_{0-24} to account for the possible carryover effects (as detailed in the online electronic supplementary material), and similar results were obtained to the planned analysis on the full PK population.

Safety

Three subjects reported three on-treatment AEs (FF/UMEC, *n* = 1; FF, *n* = 2; Table 4); two events were considered mild (dysmenorrhea and oropharyngeal pain) and one was considered

moderate (headache). Oropharyngeal pain (with FF/UMEC) and headache (with FF) were considered by the investigator to be study drug related. None of the AEs led to withdrawal from the study and no fatal or non-fatal SAEs were reported.

There were no clinically significant changes in mean laboratory values, vital sign values, or ECG values from pre-dose to 24-h post-dose for any of the three treatments.

DISCUSSION

The objectives of this single-dose, randomized, crossover study were to evaluate the PK, safety, and tolerability of FF/UMEC

co-administration via dry powder inhaler, compared with the constituent monotherapies. The results demonstrated that combined administration of FF and UMEC provided slightly lower or similar systemic exposure for each component compared with administration alone. The plasma concentration–time profiles for the combination therapy and monotherapies were superimposed for both FF and UMEC. However, an assessment of bioequivalence was not an objective of the study, and could not be evaluated as the study was not statistically powered for such an assessment.

Post hoc sensitivity analyses excluding two subjects with inconsistent PK data confirmed the findings from the planned analyses. The considerable difference in FF AUC_{0-t} between FF/UMEC and FF in the planned analysis was due to the two subjects who had inconsistent PK for FF. The high inter-subject variability for UMEC C_{max} , and FF C_{max} , and AUC_{0-t} following FF/UMEC can also be explained by the inconsistent PK exposure in one of these subjects, and was confirmed by the post hoc analyses.

An additional sensitivity analysis of UMEC urine PK was performed due to the identification of UMEC in the majority of pre-dose urine samples at Treatment Periods 2 and 3, which suggested a small carryover effect for UMEC. Findings from the sensitivity analysis were consistent with the primary analysis, indicating a similar proportion of UMEC was excreted in urine following FF/UMEC or UMEC monotherapy. The relatively small amount of UMEC excreted from urine following the inhaled dose of FF/UMEC was similar to that reported in single-dose studies of UMEC 250 and 350 mcg [24], and was considered unlikely by the authors to impact upon the PK comparisons.

The present study had a relatively short sample collection period of 24 h, therefore a number of PK parameters, including $AUC_{0-\infty}$ and $t_{1/2}$, could not be accurately determined. However, the PK characteristics of FF and UMEC have been extensively studied, and the data presented here are broadly consistent with these studies; in healthy volunteers, repeat-dose inhaled FF 200 mcg and 800 mcg had a plasma t_{max} of 30–45 min (similar to the t_{max} of 30 min reported here) and a $t_{1/2}$ of 25.5–32.5 h [25]. Single-dose studies of UMEC and VI co-administration, and UMEC 10–350 mcg demonstrated rapid UMEC absorption (t_{max} 5–15 min) [16, 22]. A $t_{1/2}$ of 1.56 h and 1.78 h has been reported for UMEC 500 mcg alone and UMEC/VI 500/50 mcg, respectively [16], while UMEC 10–350 mcg has demonstrated a mean urine $t_{1/2}$ of 6–10 h [24]. In contrast, a repeat-dose study of UMEC 750–1000 mcg demonstrated a longer estimated geometric mean plasma elimination $t_{1/2}$ and urine $t_{1/2}$ of 26.3–27.7 h and 25.1–34.9 h, respectively [24]. As the PK characteristics of FF and UMEC have been studied at length in their respective development programs, it was considered inappropriate to extend the sample collection period for additional PK analyses such as $AUC_{0-\infty}$; the PK parameters that could not be assessed in the present study would be unlikely to have provided further findings of note.

The majority of subjects in the present study were African-American/African, although this was not specified in the study plan. A previous study has demonstrated higher FF systemic exposure following inhaled dosing in East Asian subjects compared with Caucasian subjects [25], although this was not associated with a clinically significant effect on serum cortisol, and therefore dose adjustment in East Asian subjects was deemed unnecessary. However, there were insufficient data to draw conclusions regarding

differences in systemic exposure between African-American/African and Caucasian subjects in the present study. A limitation of the present study is the lack of Hispanic/Latino representation, as differences in systemic exposure compared with subjects of other ethnicities could not be investigated. However, further data will be collected in larger, Phase III studies.

The impact of patient demographics and baseline characteristics (including gender and ethnicity) on UMEC systemic exposure has been studied using population PK modeling, based on pooled PK data from two large Phase III studies [26]. The results demonstrated no significant impact of gender or ethnicity on UMEC PK following inhalation of UMEC monotherapy, or the combination of UMEC with the LABA VI. The effect of covariates on systemic exposure could not be assessed in the present Phase I study because of the small sample size, and therefore further PK data will be collected during future Phase III studies. A population PK modeling approach will then be used to investigate the effect of potential covariates, including gender and ethnicity on the systemic exposure of FF and UMEC following their co-administration.

The results of the present study are important for the future development of FF/UMEC, as historically β -agonists have been considered as more effective bronchodilators than anti-muscarinic agents in asthma [2]. More recently, the addition of the LAMA, tiotropium, to an inhaled glucocorticoid has been shown to improve symptoms and lung function in patients with inadequately controlled asthma [3]. As such, there is renewed interest in the use of antimuscarinics in the treatment of asthma, particularly in severe asthma and as add-on therapy to ICS/LABA treatment. Healthy subjects were selected in the present study to determine whether the PK of either FF or UMEC

is different when administered in combination compared with monotherapy in patients with healthy lung function. Further studies are planned to confirm and extend these findings in patients with asthma.

CONCLUSIONS

The results of the present study indicate that the PK of FF/UMEC combination therapy is broadly similar to that of the respective monotherapies, with no evidence of pharmacological interaction. No safety concerns were raised following a high-dose combination of FF (400 mcg) and UMEC (500 mcg). Further development of FF/UMEC is warranted based on these findings, to evaluate the efficacy of FF/UMEC and to fully establish the safety profile of this combination. The results of the present study provide a rationale for utilizing clinical pharmacology data from previous studies of FF or UMEC for the development of FF/UMEC as a combination therapy.

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Conflict of interest. Shuying Yang is an employee of GlaxoSmithKline and holds GlaxoSmithKline stock options. Laurie Lee is an employee of GlaxoSmithKline and holds GlaxoSmithKline stock options. Stephen Mallett is an employee of GlaxoSmithKline and holds GlaxoSmithKline stock options. Jonathan Ayer is an employee of GlaxoSmithKline and holds GlaxoSmithKline stock options. Allen Wolstenholme is an employee of GlaxoSmithKline and holds GlaxoSmithKline stock options. Steven Pascoe is an employee of GlaxoSmithKline and holds GlaxoSmithKline stock options.

Compliance with ethics guidelines. This study was approved by an appropriate ethics committee, and was therefore performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All subjects gave their informed consent prior to their inclusion in the study.

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- A combination of fluticasone furoate (FF) and umeclidinium (UMEC) has been considered for development for the treatment of asthma.
- The primary objectives were to investigate the plasma and urine pharmacokinetics of FF/UMEC in combination compared with FF and UMEC monotherapies.
- The combined administration of FF and UMEC provided slightly lower or similar systemic exposure for each component compared with administration of either component as monotherapy.
- This provides a rationale for utilizing clinical pharmacology data from previous studies of FF or UMEC for the development of FF/UMEC as a combination therapy.
- Further development of FF/UMEC as combination therapy is warranted based on these findings.

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A Randomized, Crossover Study to Investigate the Pharmacokinetics and Safety of Inhaled Fluticasone Furoate and Umeclidinium, Administered Separately and in Combination via Dry Powder Inhaler in Healthy Adult Volunteers

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Online Resource 1

Sample collection and analysis methods

In plasma FF analyses, [$^{13}\text{C}_2\text{H}_3$]-FF was used as an internal standard and the ion transition was m/z 539.2–313 for FF and m/z 543.2–313 for [$^{13}\text{C}_2\text{H}_3$]-FF. The analytical column used for analysis of FF was a Poroshell C18 (50 x 3.0 mm, 2.7 μm [Agilent, Berks, UK]), and an A:B gradient was used, where A was 10% methanol in water and B was 10% water in methanol (both containing 0.2% formic acid and 0.2% 1M ammonium formate). The validation range of the assay was 10–1000 pg/mL. The lower limit of quantification (LLQ) was 10.0 pg/mL, the within-run precision was $\leq 10.2\%$ (mean intra-run bias -8.0–1.2%) and the between-run precision was $\leq 9.8\%$ (mean inter-run bias -8.0–1.3%).

In plasma and urine UMEC analyses [$^{13}\text{C}_{12}$]-UMEC was used as an internal standard, and the LLQ was 10.0 pg/mL. The ion transition was m/z 428.4–124.1 for UMEC and m/z 441.4–96.1 for [$^{13}\text{C}_{12}$]-UMEC. In UMEC plasma analyses, the validation range of the assay was 10–2000 pg/mL. The within-run precision was $\leq 8.0\%$ (mean intra-run bias -9.9–1.3%), and the between-run precision was $\leq 13.0\%$ (mean inter-run bias -10.0–1.3%). In UMEC urine analyses, the validation range was 10–5000 pg/mL. The within-run precision was $\leq 6.9\%$ (15.8% at LLQ, mean intra-run bias -2.7–0.9%), and the between-run precision was $\leq 7.3\%$ (16.5% at LLQ, mean inter-run bias -3.0–0.9%).

Statistical analyses

The sample size calculation was primarily based on feasibility. No formal sample size calculations were performed and an estimation approach was adopted. Data from previous studies in healthy volunteers (GSK data on file) were used to obtain estimates of variability in FF 400 alone and UMEC 500 alone, to estimate the width of the confidence interval (CI) for the ratio between treatments. Based on the most conservative within subject standard deviation (SD) of 0.254, and a sample size of 18 subjects, this equated to a half width of the 90% CI of 0.143. This, when back-transformed, provided a half-width of the 90% CI around the ratio of the geometric means of approximately $\pm 16\%$ corresponding to a lower and upper confidence limit of 0.87 and 1.15, assuming a true ratio of 1 (no difference between the treatments).

Sensitivity analyses due to quantifiable pre-dose UMEC concentrations in urine

Two separate sensitivity analyses were conducted on the statistical analysis of UMEC Ae_{0-24} due to the detection of quantifiable pre-dose concentrations.

In the first approach, summaries of urine PK parameters using descriptive statistics and the statistical analysis of Ae_{0-24} were repeated with all treatment periods with quantifiable pre-dose UMEC

concentrations removed. The statistical analysis was reduced to a parallel-group analysis as the dataset contained only one treatment period per subject (instead of two treatment periods per subject in the case of the planned analysis), and therefore the mixed effects model was fitted without subject as random effect. After excluding all treatment periods with quantifiable pre-dose UMEC concentrations, there was a 12% decrease in Ae_{0-24} for FF/UMEC compared with UMEC alone (Online Resource 2), which compared with a 1% decrease in the planned analysis. However, the sensitivity analysis was based on a reduced sample size, as reflected by the wider 90% CI. Due to the reduced sample size, the power of the statistical analysis was reduced compared with the planned statistical analysis of Ae_{0-24} .

The second approach fitted a non-linear mixed effects model to the planned full dataset (UMEC urine PK data from the UMEC combination and monotherapy treatment periods) plus UMEC urine PK data collected from the Treatment Period 2 involving FF monotherapy. Subject was fitted as a random effect acting directly on the mean. The exponentiated estimates for FF/UMEC 400/500 and UMEC 500 with 95% CI, and the ratio of combination to monotherapy with 90% CI, adjusting for carryover effects were obtained. This allowed for estimation of the treatment ratio of FF/UMEC to UMEC after adjusting for first- and second-order carryover effects of UMEC. As the analysis utilized all available PK parameter data the power was not reduced. After adjustment, there was a 2% decrease for FF/UMEC compared with UMEC alone. These results are in line with the data from the planned analysis, and suggest that any carryover effect of UMEC was small (Online Resource 2).

Online Resource 2

Summary of statistical analysis of derived urine UMEC PK parameters (Ae_{0-24} [mcg], FF/UMEC 400/500 vs UMEC 500)

	Adjusted geometric mean (FF/UMEC 400/500 mcg)	Adjusted geometric mean (UMEC 500 mcg)	Ratio ¹ (Test:reference)	Two-sided 90% CI for ratio
Planned analysis	7.93	8.05	0.99	0.87–1.12
Sensitivity analysis excluding treatment periods with quantifiable concentrations at pre-dose	6.72	7.68	0.88	0.66–1.16
Sensitivity analysis adjusting for first- and second-order carryover effects in a non-linear mixed model	7.59	7.73	0.98	0.85–1.11

1. Test, FF/UMEC 400/500 mcg; Reference, UMEC 500 mcg.

Ae, urinary recovery of unchanged drug; CI, confidence interval; FF, fluticasone furoate;

PK, pharmacokinetic; UMEC, umeclidinium.