Bioequivalence and Dose Proportionality of Inhaled Fluticasone Furoate

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Abstract

Fluticasone furoate (FF), a new inhaled corticosteroid delivered via the ELLIPTA dry powder inhaler, is being developed as a once-daily inhaled treatment for asthma. Study 1 was a part-randomised, open-label, four-way crossover, single- and repeat-dose study in healthy subjects (n=36) to assess whether the systemic exposure of FF increased proportionately across different strengths of FF (50 µg, 100 µg and 200 µg) and to determine the absolute bioavailability of FF inhalation powder. Study 2 was a randomised, open-label, replicate, six-way crossover, single-dose study in healthy subjects (n=30) to determine the bioequivalence of FF inhalation powder (single-strip configuration) compared with FF inhalation powder (two-strip configuration) and with fluticasone furoate/vilanterol (FF/VI) inhalation powder. A population pharmacokinetic analysis was also conducted on sparse samples collected from asthma patients in five Phase III studies conducted with FF/VI or FF.

Overall, FF systemic exposure, as measured by single-dose AUC₀-∞, was dose proportional whilst Cₘₚ showed a less than proportional increase. Inhaled absolute bioavailability of FF was 14%. Bioequivalence was not demonstrated for FF single-strip compared with either FF two-strip or FF/VI since the estimated 90% confidence interval for the ratio of adjusted geometric means for AUC and Cₘₚ did not fall completely between 0.8000-1.2500. However, this difference was in line with the higher respirable mass delivered by the batch of single-strip product used in this study.

Although the formal bioequivalence study showed higher exposure with FF single-strip ELLIPTA compared with FF two-strip or FF/VI, the results of the population PK analysis of data in asthmatics show that there is no notable difference in systemic exposure between the FF configurations (single-strip, two-strip or FF/VI). In healthy subjects all treatments were generally safe and no new safety issues were apparent at these supra-therapeutic inhaled doses of FF (up to 1200 µg) and high intravenous FF dose (250 µg).

Keywords: Bioavailability; Bioequivalence; Fluticasone furoate; Healthy subjects; Inhaled; Pharmacokinetics; Proportionality; NCT01669070; NCT01485445

Abbreviations: AE: Adverse Event; AUC₂₈: Area Under the Curve from Zero (Pre-Dose) to the Time of Last Common Measurable Time Point Within Subject; BLQ: Below the Limit of Quantitation; CI: Confidence Interval; Cₘₚ: Maximum Plasma Concentration; COPD: Chronic Obstructive Pulmonary Disease; CV: Coefficient of Variation; DPI: Dry Powder Inhaler; FF: Fluticasone Furoate; FF: Fluticasone Propionate; ICS: Inhaled Corticosteroid; KEDTA: Tri-potassium Ethylene Diamine Tetra-acetic Acid; LABA: Long Acting Beta-Agonist; MAT: Mean Absorption Time; NA: Not Applicable; t'ₘₚ: Time to Maximum Observed Concentration; VI: Vilanterol; VPC: Visual Predictive Check

Introduction

Fluticasone furoate (FF) is a new inhaled corticosteroid (ICS) in development for the treatment of asthma and in combination with vilanterol (VI), a new long-acting beta2 agonist (LABA), for asthma and chronic obstructive pulmonary disease (COPD). FF is structurally distinct from fluticasone propionate (FP) [1,2] and has demonstrated a longer duration of action than FP in vitro [3]. Once-daily dosing of FF is non-inferior to the same total dose given twice daily [4], and once-daily doses of FF 100 µg and 200 µg are effective in improving lung function and asthma symptoms in patients with asthma uncontrolled on low- or mid-dose ICS, with an acceptable safety profile [5-8]. Furthermore, FF 200 µg once-daily has been shown to be non-inferior to FP 500 µg twice-daily on trough FEV₁ over 24 weeks in patients with moderate-to-severe asthma [9].

The pharmacokinetic, pharmacodynamic and safety profiles of FF administered as the FF/VI combination have been described in healthy subjects as well as in patients with asthma and COPD [10-16].

The ELLIPTA™ (trade mark of the GlaxoSmithKline group of companies) dry powder inhaler (DPI) will be used to deliver both FF monotherapy and the FF/VI combination. The ELLIPTA DPI can be used for FF monotherapy (with either single strip [the developed formulation] or as a two-strip configuration [with matched VI placebo in the second strip]), or to deliver the FF/VI combination (as a two-strip configuration [i.e. with FF and VI in separate strips]).

Dose proportionality occurs when the clearance of a drug remains constant and exhibits linear time-independent pharmacokinetics over a range of doses, and can be demonstrated when the observed plasma concentrations increase proportionately to the dose

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administered over the clinically relevant dose range. Understanding that the pharmacokinetics of the investigational product is linear over the concentration range of interest facilitates prediction of the effect of changing dose. Study 1 was performed to demonstrate dose proportionality of FF following single and repeat dose administration of three strengths of FF (50 µg, 100 µg and 200 µg) via ELLIPTA. The absolute bioavailability of FF at the highest single dose administered in the study, 1200 µg (given as six inhalations of FF 200 µg) was also determined. The bioavailability of inhaled FF predominantly represents absorption from the lung as the oral bioavailability of FF from the swallowed portion of the inhaled dose is negligible (approximately 1%) [17].

The two-strip configuration has been used for the majority of FF monotherapy treatment arms in previous studies. Study 2 was conducted to determine the bioequivalence of FF monotherapy (single strip) compared with FF monotherapy (two-strip), and to determine the bioequivalence of FF monotherapy (single strip) compared with FF/Vi, administered via ELLIPTA. Due to the low systemic bioavailability of FF, and consequently the low blood levels after dosing at the clinical dose, it is not possible to assess bioequivalence, dose proportionality or absolute bioavailability at the proposed clinical doses (FF 100 µg, 200 µg). Consequently, in these studies multiple inhalations were administered in order to provide adequate pharmacokinetic data following single dose administration.

The FF pharmacokinetics in subjects with asthma was also evaluated using population pharmacokinetic methods on sparse samples collected from five Phase III studies conducted in subjects with asthma administered FF/Vi or FF (single and two-strip) via ELLIPTA. Configuration (single or two-strip) was included as a potential covariate in the modelling procedure to investigate whether there were differences between the single and two-strip configurations.

Methods

Study design and subjects

Study 1: FF dose proportionality and absolute bioavailability (NCT0115441; NCT01669070): This part-randomised, open-label, four-way crossover, single and repeat-dose study was conducted healthy male or female subjects (n=36) to determine the dose proportionality and absolute bioavailability of fluticasone furoate administered as the single strip configuration ELLIPTA. Subjects were assigned to one of six treatment sequences (ABCD, ACBD, BACD, CBAD) where A=single dose of 300 µg FF (6 inhalations of 50 µg FF) on Day 1, followed by 50 µg FF once daily for 7 days: B=single dose of 600 µg FF (6 inhalations of 100 µg FF) on Day 1, followed by 100 µg FF once daily for 7 days: C=single dose of 1200 µg FF (6 inhalations of 200 µg FF) on Day 1, followed by 200 µg FF once daily for 7 days: D=single intravenous dose of FF 250 µg (1 mL of 250 µg/mL in 100% propylene glycol administered at a constant rate of infusion over 20 minutes using a syringe and pump) that was administered in the final study period for all subjects. The inhaled and intravenous treatment periods had different dosing procedures, sampling requirements, pharmacokinetic sampling time points and treatment administration. Therefore, it was considered more practical to perform the study with all subjects receiving the intravenous treatment in groups to minimise any logistical issues. No significant statistical issues were expected in following this approach.

Study 2: FF Bioequivalence (NCT0115440; NCT01485445): This randomised, open-label, replicate, six-way crossover, single-dose study in healthy male or female subjects (n=30) was conducted to determine the bioequivalence of FF inhalation powder (single strip configuration) compared with FF inhalation powder (two-strip configuration) and compared with fluticasone furoate/vilanterol (FF/Vi) inhalation powder. Subjects were assigned to one of six treatment sequences (ABCA, BACAB, CABAC, CBABC, ACBCA or ACBCAB) where A=FF 400 µg (2 inhalations of 200 µg), administered from ELLIPTA with no second strip (single-strip configuration): B=FF 400 µg (2 inhalations of 200 µg) administered from ELLIPTA with a filled (lactose and magnesium stearate) second strip (two-strip configuration): C=FF/Vi 400/50 µg (2 inhalations of 200/25 µg) administered from ELLIPTA.

Both studies included non-smoking healthy male and female subjects aged 18 to 65 years with body mass index 18.5 to 29.0 kg/m². Exclusion criteria included: treatment with an investigational drug within 30 days or five half-lives prior to the first dose of study treatment; use of prescription or non-prescription drugs and/or dietary supplements within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose of study medication; history of alcohol/drug abuse or dependence within 6 months of the study; subjects who have suffered a lower respiratory tract infection within 4 weeks of the screening visit; electrocardiogram demonstrating corrected QT interval greater than 450 msec at screening. Females who were pregnant or nursing were also excluded.

There was a washout period of at least 7 days but no more than 14 days between treatment periods for both studies. The duration for each subject was approximately 14 weeks and 11 weeks for Study 1 and 2, respectively.

Both healthy subject trials were conducted in compliance with Good Clinical Practice with the ethical principles that have their origins in the Declaration of Helsinki. The investigators obtained institutional review board approvals for the study protocols (Study 1 was conducted at PRA International, Hanzeplein 1 Entrance 23 Groningen Netherlands 9713 GZ, and Study 2 was conducted at Parexel International GmbH, Klinikum Westend, Haus 18, Spandauer Damm 130 14050 Berlin, Germany). All subjects gave their written informed consent before participating in the trial.

Asthma patient population pharmacokinetic analysis

This Phase III, multicentre, randomised, double-blind, parallel-group study was a 24-week study in patients (n=238 randomised) aged ≥ 12 years with moderate-severe persistent asthma to evaluate the efficacy and safety of two strengths of once-daily FF: 100 µg and 200 µg (single-strip ELLIPTA) conducted at 40 centres in 6 countries (Argentina, United States, Chile, Russian Federation, Mexico, France [France did not participate in the PK aspects]).

Pharmacokinetic evaluations

Study 1: Venous blood samples (approximately 2 mL) for analysis of FF plasma concentrations were collected in KEDTA tubes as follows: Inhaled dosing (Days 1 and 9): pre-dose and at 15, 30 and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24 hours and Day 9 only at 36 and 48 hours; Intravenous dosing: pre-dose and at 10, 20, 25, 30 and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours after the start of dosing.

Study 2: Venous blood samples (3 mL) for analysis of FF plasma
concentrations were collected in KEDTA tubes pre-dose and at 15, 30 and 45 minutes, and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 and 36 hours after the start of dosing.

**Asthma study**: Single venous blood samples (4 mL) for analysis of FF plasma concentrations were collected in KEDTA tubes at Weeks 4 and 18 at pre-dose and between 45 and 75 minutes post-dose.

The blood samples for all studies were put on ice until centrifugation at 1500 g for approximately 10 minutes at 4°C. Following centrifugation supernatant plasma was transferred into 1.4 mL matrix screw capped polypropylene tubes and frozen. All samples were stored at -20°C or colder until shipment.

**Analytical methods**

Plasma samples (150 μL aliquot for Study 1 and 500 μL aliquot for Study 2) from both Studies 1 and 2 were analysed for FF by solid phase extraction using [13C2H3]-GW685698 or [13C]-CC118781 as internal standard for Study 1 and 2, respectively, followed by high performance liquid chromatography with tandem mass spectrometry using an Applied Biosystems API-3000 (Applied Biosystems/MDS Sciex, Foster City, USA). A gradient system using 5 mM ammonium formate and applied to the plasma samples. The ion transition for FF was m/z 539 to 313. The validation range for FF was 10-1000 pg/mL. The conditions for stability for FF were that the concentration data were increasing, and the logarithmic trapezoidal rule for intervals where the concentration data were decreasing, and then extrapolated to infinity using λ to obtain the AUC_

**Pharmacokinetic analysis**

Pharmacokinetic analyses of plasma FF concentration-time data from the asthma study was performed with the computer program NONMEM v7.1.2 (ICON Development Solutions) running in the Predictive Modelling Environment (PME), a UNIX server based environment for NONMEM analysis [referred to as the Pop PK Analysis]. The method selected for minimisation was Stochastic Approximation Expectation Maximization (SAEM) with interaction. For purposes of data handling, exploratory diagnostics and simulation included Xpose V4 [18] and R (The R Foundation for Statistical Computing Version 2.10.1 or above). Studies in subjects with asthma (FFA114496 [NCT01431950] [19], HZA106827 [NCT0116513] [20], HZA106829 [NCT01134042] [9], HZA106839 [NCT01018186] [8], HZA106851 [NCT01086410] [14]) were included for the population pharmacokinetic analysis only and no efficacy or safety data for these studies are presented. Given the sparse nature of the sampling in these studies and the high proportion of records reporting FF concentrations below the lower limit of quantification (LLQ; 10 pg/mL) particularly at the lower doses, addition of more extensively sampled concentration-time data from a FF/V1 study in healthy subjects at a higher dose (800/100) and also 200/25 (HZA102936 [21]) was required to achieve an appropriate structural model to describe the data. FF concentration-time data for the following treatments were included: 100 μg FF, 200 μg FF, 100/25 μg FF/V1, 200/25 μg and 800/100 μg. Configuration (FF single-strip, FF two-strip or FF/V1) was included as a variable. As a consequence of the large extent of non-quantifiable data in each dataset it was necessary to use methodology that maximised the likelihood for all the data, treating those data below the LLQ as censored. The data were analysed using the methodology referred to as M3 and requires the use of the F_FLAG option and PHI function available in NONMEM v7 [22].

The population pharmacokinetics of FF has previously been reported for the asthma patient data [23] for studies which used the two-strip configuration. The population PK analysis presented here is based on the earlier analysis but also includes study FFA114496 which used the single-strip configuration and allows for evaluation of configuration to be a potential covariate on FF pharmacokinetics. The covariates considered for evaluation of effects on FF pharmacokinetics in the analysis presented here included population (healthy subjects or subjects with asthma), age, weight, height, sex, ethnicity (Hispanic or Latino/non-Hispanic or Latino), race, BMI, PFEV (FEV1 % predicted), study and configuration (FF single-strip, FF two-strip or FF/V1). Due respectively, of WinNonlin Professional Edition Version 5.2 (Pharsight Corporation, Mountain View, CA, USA). Pharmacokinetic variables for all treatments were calculated as follows: Cmax and tmax were derived directly from plasma concentration-time profiles, the slowest disposition rate constant (λz) was calculated by log-linear regression of the terminal portion of the concentration-time profiles where there were sufficient data, t1/2 was calculated as 0.693/λz, AUC(0-t) was calculated using the linear trapezoidal rule for intervals where the concentration data were decreasing, and then extrapolated to infinity using λ to obtain the AUC(0-∞). In addition, AUC(0-12) and AUC(0-24) were derived in Study 1 for single and repeat dose, respectively and AUC(0-24) was derived for Study 2, where t was the common time of the last quantifiable concentration within a subject across all treatments. For intraindividual treatment only, total plasma clearance and steady-state volume of distribution (Vss) were determined and mean absorption time (MAT) was derived for inhaled treatment in Study 2.

Population PK modelling of concentration-time data from the asthma study was performed with the computer program NONMEM v7.1.2 (ICON Development Solutions) running in the Predictive Modelling Environment (PME), a UNIX server based environment for NONMEM analysis [referred to as the Pop PK Analysis]. The method selected for minimisation was Stochastic Approximation Expectation Maximization (SAEM) with interaction. For purposes of data handling, exploratory diagnostics and simulation included Xpose V4 [18] and R (The R Foundation for Statistical Computing Version 2.10.1 or above). Studies in subjects with asthma (FFA114496 [NCT01431950] [19], HZA106827 [NCT0116513] [20], HZA106829 [NCT01134042] [9], HZA106839 [NCT01018186] [8], HZA106851 [NCT01086410] [14]) were included for the population pharmacokinetic analysis only and no efficacy or safety data for these studies are presented. Given the sparse nature of the sampling in these studies and the high proportion of records reporting FF concentrations below the lower limit of quantification (LLQ; 10 pg/mL) particularly at the lower doses, addition of more extensively sampled concentration-time data from a FF/V1 study in healthy subjects at a higher dose (800/100) and also 200/25 (HZA102936 [21]) was required to achieve an appropriate structural model to describe the data. FF concentration-time data for the following treatments were included: 100 μg FF, 200 μg FF, 100/25 μg FF/V1, 200/25 μg and 800/100 μg. Configuration (FF single-strip, FF two-strip or FF/V1) was included as a variable. As a consequence of the large extent of non-quantifiable data in each dataset it was necessary to use methodology that maximised the likelihood for all the data, treating those data below the LLQ as censored. The data were analysed using the methodology referred to as M3 and requires the use of the F_FLAG option and PHI function available in NONMEM v7 [22].

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to limited numbers of subjects in some of the race categories subjects were grouped and categorised as 'RACE1' as follows: RACE1=1 - White Caucasian; RACE1=2 - East Asian, Japanese and South East Asian; RACE1=3 - African American/African, White Arabic, American Indian/Native Alaskan and Mixed. Plots of inter-subject variability (ETA) versus each covariate were used to select potential covariates for inclusion in the FF PK model. Each potentially significant covariate identified from the plots was individually included on the fixed parameter in the base model to identify significant covariates. Next, all the significant covariates were added to the base model. After the full model had been defined, the significance of each covariate was tested individually by removal one at a time from the full model.

Model evaluation to assess the adequacy of the final model, including the effects of statistically significant covariates was performed using a Visual Predictive Check (VPC) procedure [24]. This procedure was conducted as follows: 1000 replicates of the original dataset were simulated, based on the parameter estimates of the final model, and a 95% prediction interval computed based on the simulated datasets. The observed plasma concentration-time data was plotted on the prediction interval to visually assess the concordance between the simulated and observed data. In addition the observed proportion of data below the limit of quantification (BLQ) was plotted with the model prediction interval for proportion of the BLQ data to visually assess the concordance between the simulated and observed BLQ data.

Safety evaluations

Adverse events (AEs), clinical laboratory tests and vital signs were monitored throughout both of the healthy subject studies. In the healthy subject studies a complete physical examination, including 12-lead electrocardiogram, was done at screening.

Statistical analysis

A hypothesis testing approach was used to assess FF dose proportionality. Dose proportionality was to be concluded if the 90% confidence interval (CI) for the slope from the power model analysis fell within the pre-defined acceptance range (0.84, 1.16) for AUC and for Cmax separately. To assess dose proportionality of FF logarithmically transformed single and repeat dose FF AUC(0-∞), and Cmax data from Study 1 were analysed separately using the power model, fitting log (dose) and period as fixed effects, and individual subject intercept as a random effect. An estimate of slope (with corresponding 90% CI) was calculated.

Analysis of variance (ANOVA) was used as a secondary approach to assess dose proportionality. Following log transformation, single and repeat dose, dose-normalised AUC(0-∞), and Cmax of FF were analysed separately using a mixed effects model, fitting log dose and period as fixed effects, and subject intercept as a random effect. The reference doses for the ANOVA were FF 600 and 1200 µg for single and repeat dose, respectively, as these are comprised of the 100 µg strength, which is anticipated to be the most commonly used clinical strength.

For Study 1, AUC(0-∞) from the inhaled and intravenous formulations was used to estimate absolute bioavailability for inhaled FF. The AUC values were dose-normalised, by dividing by nominal dose, then using log-transformed values, analysed using a mixed effects model. Mixed models were fitted with treatment as a fixed effect and subject as a random effect. A point estimate and associated 90% CI was constructed for the difference of inhalation versus intravenous dosing, then exponentially back-transformed to provide corresponding estimates for the ratio.

To assess dose proportionality of FF loge-transformed, AUC(0-∞), AUC(0-1), and Cmax of FF, were analysed using a mixed effects model with fixed effect terms for period and treatment. Subject was treated as a random effect in the model. Point estimates and associated 90% confidence intervals were constructed for the differences "FF single strip - FF two-strip" and "FF single strip - FF/VI", then exponentially back-transformed to provide point estimates and associated 90% confidence intervals for the treatment ratios. For each comparison of interest, bioequivalence was to be concluded if the 90% confidence intervals for the ratios of all primary endpoints (AUC(0-∞), AUC(0-1), AUC(0-0.5) and Cmax) were completely contained within the range 0.8000 to 1.2500.

Results

Study disposition and demographics

Thirty-six subjects (30 Caucasian, 4 Asian and 2 mixed race) were enrolled into Study 1. There were two withdrawals, one subject withdrew prior to period 2 for personal reasons and was lost to follow up and one subject withdrew prior to period 3 due to an adverse event (upper respiratory tract infection). The subjects (22 males, 14 females) had a mean [range] age of 45-65 [20] years. Ten subjects took concomitant medications during the study. Seven subjects took paracetamol for headache, one subject took paracetamol for sore throat, one subject took aspirin for headache and one subject took paracetamol, codeine and doxycycline for upper respiratory tract infection. None of these medications are considered to be likely to have affected the study outcome.

Thirty subjects (all Caucasian) were enrolled into Study 2. The subjects (15 males, 15 females) had a mean [range] age of 47-64 [25] years. One subject withdrew prior to dosing in period 4 due to a protocol deviation (tested positive for drugs of abuse). Seven subjects took concomitant medications during the study. Six subjects took paracetamol for headache and one subject took multiple medications (heparin fraction sodium salt, ibuprofen, metamizole sodium and pantoprazole) related to treatment of a fractured humerus. None of these medications are considered to be likely to have affected the study outcome.

Pharmacokinetics

Fluticasone Furoate Dose Proportionality: The mean plasma concentration versus time profiles for single and repeat dose FF are shown in Figure 1a and 1b, respectively. Whilst plasma concentrations of FF were generally quantifiable in the majority of subjects up to 48 hours following single doses of FF 600 µg and 1200 µg dosing, and 24 hours following single dose FF 300 µg, data was more limited following repeat doses of FF 50, 100 and 200 µg (Figure 2). On average, the maximum plasma concentrations of FF were achieved at later times (tmax) as the FF dose increased (Table S1).

Following repeat dosing (single inhalation), AUC(0-24) was estimated for 0/35 subjects receiving 50 µg and only 21/35 subjects receiving 100 µg due to the number of non-quantifiable concentrations. Therefore, AUC(0-24) has been used to evaluate dose proportionality for repeat dosing. However, it should be noted, that due to the extent of quantifiable data, AUC(0-4) was only determined for 21/35 subjects at the 50 µg dose and 34/35 subjects at the 100 µg dose (Table S1).

Single dose AUC(0-∞) was dose proportional, however 90% confidence intervals for AUC(0-12) and Cmax slope estimates were not
within the pre-defined acceptance range (0.84, 1.16), indicating lack of dose proportionality. Dose proportionality was not demonstrated for repeat dose as the 90% CI for the slope for AUC(0-4) and C_{max} did not fall between (0.84, 1.16). The estimates of slope suggest less than proportional increases in exposure across the dose range studied, following both single and repeat dosing conditions (Table 1).

Further investigation of dose proportionality using an ANOVA model (data not presented) using FF 600 µg single dose and FF 100 µg repeat dose as the reference treatments showed that the lack of dose proportionality for C_{max} was across all doses, with a less than proportional increase in C_{max} as the dose increased. Consistent with the power model analysis, for single dose AUC(0-12) dose proportionality was demonstrated for each pairwise comparison. The results of the ANOVA for partial AUCs suggest that the lack of dose proportionality seen in the power model is due to the low strength (50 µg repeat dose and 300 µg single dose). The comparisons for AUC between the mid and high strength show little or no deviation from dose proportionality whereas the comparisons between the low and mid strength show less than proportional increase in exposure as the dose increases.

**Fluticasone furoate absolute bioavailability:** For FF, the estimate of absolute bioavailability of inhaled treatment relative to intravenous treatment was 13.9%. The 90% CI ranged from 12.7% to 15.3% (Table S2).

**Fluticasone furoate bioequivalence:** Following administration of FF 400 µg single strip, plasma concentrations of FF were quantifiable in the majority of subjects up to 24 h post-dose (29/30 and 25/29 for Dose 1 and Dose 2, respectively) and in approximately half of subjects at the final time point of 36 h post-dose. Plasma concentrations of FF were also quantifiable in the majority of subjects up to 24 h post-dose following FF 400 µg two-strip (22/30 and 23/29 for Dose 1 and 2, respectively) and FF/VI 400/50 µg, (18/30 and 21/29 for Dose 1 and 2, respectively). Approximately 1/3 of subjects had quantifiable FF plasma concentrations at 36 h post-dose following administration of FF 400 µg two-strip or FF/VI 400/50 µg.

On average the highest FF plasma concentrations, were observed following administration of FF 400 µg single strip in both dosing sessions. The lowest FF plasma concentrations, on average, were observed following administration of FF/VI 400/50 µg for both dosing sessions. Plasma FF concentration time profiles and pharmacokinetic parameters were similar for the two dosing occasions of each treatment (Figure 3 and Table S3).

Bioequivalence was not demonstrated for any of the comparisons of interest since the estimated 90% confidence interval (CI) for the ratio of adjusted geometric means for AUC and C_{max} did not fall completely between 0.8000-1.2500 (Table 2).

**Population PK analysis:** To further investigate the relative bioavailability of the single strip configuration compared with two-strip and FF/VI concentration-time data from clinical studies in subjects with asthma (Figure 4) were subjected to population pharmacokinetic analysis using non-linear mixed effects modelling approach [Pop

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</tr>
</tbody>
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AUC = area under the curve; CI = confidence interval; C_{max} = maximum plasma concentration; FF = fluticasone furoate

Table 1: Results of power model assessment of FF dose proportionality following a single and repeat inhaled administration of FF [dose proportionality Study 1].
PK Analysis. A two compartment linear model, with first order absorption and first order elimination (ADVAN4, TRANS4) was found to describe the FF concentration-time data significantly better than a one-compartment model. Configuration (FF single-strip, FF two-strip or FF/VI) was included as a potential covariate on apparent clearance (CL/F) and peripheral volume of distribution (V3/F). Although this was not a significant covariate, it was retained in the final model to ensure any small differences were accounted for when generating FF systemic exposure. The goodness of fit plot for the final model appears to provide a reasonable prediction of plasma concentrations of FF in the study. The plot for the VPC by dose and configuration (Figure S2) showed that the majority of the data is captured in the prediction interval that captures 90% of the population as indicated by the 5th and 95th percentile boundary indicating that the model was valid for this asthma dataset and that the model adequately describes the proportion (%) of data reported as below the LLQ.

In contrast to the data from the bioequivalence study (Study 2), results of the population PK analysis show no evidence for a difference in FF systemic exposure following the single-strip configuration, compared with data following the two-strip configuration as FF or FF/VI (Table 3). At the FF dose of 200 µg, AUC(0-24) for FF single-strip ELLIPTA (395 pg.h/mL) was, on average, 3% and 8% lower compared with FF two-strip ELLIPTA (405 pg.h/mL) and FF/VI (428 pg.h/mL) with considerable overlap in the 95% CIs. At the lower dose, where data was more censored due to non-quantifiable data, AUC(0-24) for FF single-strip ELLIPTA was, on average, 25% and 23% lower, compared with FF two-strip and FF/VI.

Safety: Although supra-therapeutic inhaled doses of FF and a high intravenous FF dose were used in these two studies all treatments had a generally good safety profile and there were no new safety issues identified from review of the AE reports. No fatal serious adverse events were reported during the studies. One subject experienced an AE during the study which was a ‘humerus fracture’ which occurred following a fall on slippery ground. The event was not considered related to the study medication and the subject continued administration of FF/VI.

For Study 2, a total of 32 adverse event (AE) episodes were reported in 16 subjects (53%) during the study, 16 were reported after administration with FF 400 single-strip, 11 were reported after FF 400 two strip and 5 were reported after FF/VI 400/25. The most frequently reported AEs were headache, reported by seven (23%) subjects in the study, and nasopharyngitis, reported by 4 (13%) subjects (Table S6). Four subjects experienced five AEs that were defined as being of special interest and class related (Table S7). None of these events were considered causally related to study drug.

Discussion

Dose proportionality was demonstrated for FF following single inhaled dosing since AUC(0-24) showed dose proportionality across the strengths. However, a lack of dose proportionality was seen for the partial AUCs and Cmax FF is poorly soluble and has been shown previously to exhibit absorption limited pharmacokinetics [25]. Evaluation of dose proportionality using partial AUCs is confounded by this rate-limited absorption. Incomplete absorption, especially using AUC(0-4) for the repeat dose treatments is reflected in the lack of dose proportionality seen for the partial AUCs. The lack of dose proportionality seen for Cmax has previously been reported following administration of FF/VI [11] and evaluation of dose proportionality using Cmax is also confounded by the rate limited absorption of FF. The less than proportional increases seen in FF systemic exposure are supported by the later T_max values observed at higher doses, reflecting slower rates of absorption as dose increases. Median T_max for single dose administration increased from 15 minutes to 30 minutes to 60 minutes as the dose increased. A very similar increase in T_max was seen for repeat dose administration with median T_max values increasing from 15 minutes to 30 minutes to 45 minutes as the dose increased. FF acts topically in the lung, whilst systemic exposure is related to safety. Consequently, the lack of dose proportionality for FF Cmax would be considered not to impact efficacy. Furthermore, as the results show a less than dose proportional increase in FF Cmax this would also be
considered not to have an adverse impact on safety. Therefore, the lack of dose proportionality for FF $C_{\text{max}}$ is considered to be of no clinical relevance.

On average, the absolute bioavailability for FF 200 µg (1200 µg dose) was estimated to be 14% (90% CI: 13%, 15%) and is similar to that reported for FF (on average 15%) following inhaled administration.

---

**Figure 4:** FF concentrations relative to time of last dose following FF (100 and 200 µg) as single and two-strip configuration and FF/VI (100/25 and 200/25) to subjects with asthma [Pop PK Analysis].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment Comparison</th>
<th>Adjusted Geometric Means</th>
<th>Ratio of Adjusted Geometric Means</th>
<th>90% CI of the Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC_{(0-\infty)}$</td>
<td>FF 400 single-strip / FF 400 two-strip</td>
<td>1144.7 / 889.5</td>
<td>1.29</td>
<td>(1.14, 1.46)</td>
</tr>
<tr>
<td></td>
<td>FF 400 single-strip / FF/VI 400/50</td>
<td>1144.7 / 714.8</td>
<td>1.60</td>
<td>(1.37, 1.87)</td>
</tr>
<tr>
<td>$AUC_{(0-t')}$</td>
<td>FF 400 single-strip / FF 400 two-strip</td>
<td>560.2 / 458.0</td>
<td>1.22</td>
<td>(1.16, 1.29)</td>
</tr>
<tr>
<td></td>
<td>FF 400 single-strip / FF/VI 400/50</td>
<td>560.2 / 401.1</td>
<td>1.40</td>
<td>(1.31, 1.49)</td>
</tr>
<tr>
<td>$C_{\text{max}}$</td>
<td>FF 400 single-strip / FF 400 two-strip</td>
<td>67.84 / 60.08</td>
<td>1.13</td>
<td>(1.07, 1.20)</td>
</tr>
<tr>
<td></td>
<td>FF 400 single-strip / FF/VI 400/50</td>
<td>67.84 / 47.75</td>
<td>1.42</td>
<td>(1.33, 1.52)</td>
</tr>
</tbody>
</table>

$AUC =$ area under the curve; CI = confidence interval; $C_{\text{max}} =$ maximum plasma concentration; FF = fluticasone furoate

Table 2: Results of assessment of FF bioequivalence following single inhaled administration of FF [bioequivalence Study 2].

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (µg)</th>
<th>$n$</th>
<th>$AUC_{(0-24)}$</th>
<th>$95%\ CI$</th>
<th>$C_{\text{max}}$</th>
<th>$95%\ CI$</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF single-strip</td>
<td>100</td>
<td>116</td>
<td>180.7</td>
<td>117.4, 292.0</td>
<td>27.0</td>
<td>15.4, 50.3</td>
</tr>
<tr>
<td>FF two-strip</td>
<td>100</td>
<td>186</td>
<td>240.9</td>
<td>112.5, 546.4</td>
<td>32.2</td>
<td>19.3, 58.0</td>
</tr>
<tr>
<td>FF/VI</td>
<td>100</td>
<td>434</td>
<td>235.0</td>
<td>108.8, 505.4</td>
<td>33.8</td>
<td>19.8, 66.5</td>
</tr>
<tr>
<td>FF single-strip</td>
<td>200</td>
<td>115</td>
<td>394.5</td>
<td>194.4, 917.8</td>
<td>55.1</td>
<td>32.6, 98.2</td>
</tr>
<tr>
<td>FF two-strip</td>
<td>200</td>
<td>161</td>
<td>405.4</td>
<td>169.7, 990.7</td>
<td>65.9</td>
<td>40.3, 125.5</td>
</tr>
<tr>
<td>FF/VI</td>
<td>200</td>
<td>432</td>
<td>427.5</td>
<td>191.7, 930.3</td>
<td>68.2</td>
<td>39.2, 123.7</td>
</tr>
</tbody>
</table>

Table 3: Summary statistics for FF $AUC_{(0-24)}$ and $C_{\text{max}}$ in subjects with asthma by configuration [Pop PK analysis].
as FF/VI [11]. Oral bioavailability is approximately 1% for FF [17] and hence pharmacokinetic data following inhaled dosing represents almost exclusively the dose absorbed from the lung, with negligible contribution to systemic exposure from the swallowed portion of the dose.

Bioequivalence was not demonstrated for FF 400 µg (single-strip) compared with either FF 400 µg (two-strip) or FF/VI 400/50 µg, as the 90% CIs for the true ratios of the adjusted geometric means for AUC and \( C_{\text{max}} \) were not completely contained within the range 0.8000-1.25000, for all primary comparisons of interest. Since AUC \( C_{\text{max}} \) could not be derived for all subjects AUC \( C_{\text{max}} \) was also derived and analysed to assess bioequivalence. AUC \( C_{\text{max}} \) is estimated for all profiles in all subjects and the time of the last quantifiable value \((t')\) was consistent across a subject. Hence AUC \( C_{\text{max}} \) would be considered the most robust estimate of AUC for analysis of bioequivalence in this study. The majority of missing values were in the FF/VI treatment often due to the lower exposure and hence it is not surprising that there is a bias to a slightly higher point estimate for AUC \( C_{\text{max}} \). For AUC \( C_{\text{max}} \), due to the higher systemic exposure the time of the last quantifiable value will be later for the FF single-strip compared to the other treatments, and again it is not surprising that there is a bias to a slightly higher point estimate for this parameter.

Batches of drug for any inhaled treatment must be within an agreed specification on the range of particles that can reach the lung (i.e., the respirable mass, generally taken as particles of <4.5 microns); some batch to batch variability will be seen, but the respirable mass must be within the specified range [26]. The FF single-strip batch used in the bioequivalence study was at the higher end of the range of respirable mass of typical batches and the two-strip configuration (FF or FF/VI) had an FF respirable mass that was in the mid to low end of the range for typical batches. These differences in the respirable mass of the batches of drug used for the single- and two-strip products used in the bioequivalence study resulted in approximately 20% more drug being in the respirable fraction for single-strip FF than for two-strip FF or FF/VI and likely resulted in the lack of bioequivalence seen in the healthy subjects in Study 2.

In the population pharmacokinetic model development configuration (FF single-strip ELLIPTA, FF two-strip ELLIPTA or FF/VI) was included as a potential covariate on apparent clearance (CL/F) and peripheral volume of distribution (\( V_\text{d} /F \)) and although this was not a significant covariate, it was retained in the final model to ensure any small differences were accounted for when generating FF systemic exposure. The results of the population PK analysis of concentration-time data from asthma patients show that there is no evidence for higher systemic exposure with the FF single-strip configuration compared with FF two-strip or FF/VI. Therefore, although the formal bioequivalence study showed higher exposure with FF single-strip ELLIPTA compared with FF two-strip or FF/VI this was attributable to the differences in the respirable mass between the batches used in the bioequivalence study. In contrast the results of the population PK analysis of data in subjects with asthma show that there is no notable difference in FF systemic exposure between the FF configurations (single-strip, two-strip or FF/VI) in the relevant patient population at clinical doses.

Although FF was administered at supratherapeutic doses; there were no apparent dose-related effects and the majority of AEs reported in both studies were classified as mild. There was one SAE reported, however this was not considered related to treatment. The AE profiles seen in the healthy subject studies are similar to those seen previously in healthy subjects where FF was administered alone or in combination with VI [10-13,21].

A potential limitation of these studies was that they were conducted at supra-therapeutic doses of FF in order to produce measurable FF plasma concentrations to provide robust pharmacokinetic data to meet study objectives. However, due to the linear time-independent pharmacokinetic characteristics of FF, results for pharmacokinetic data from higher doses can be interpolated to the clinical doses. Another potential limitation was that Study 1 was conducted in a partly-randomised manner (the inhaled treatments were fully randomised however the intravenous treatment was not randomised). The inhaled and intravenous treatment periods had different dosing procedures, sampling requirements, pharmacokinetic sampling time points and treatment administration. Therefore, it was considered more practical to perform the study with all subjects receiving the intravenous treatment in groups to minimise any logistical issues. No significant statistical issues were expected in following this approach.

Study 2 was conducted as a replicate cross-over design such that each treatment was administered on two separate occasions to each subject. The replicate data for each treatment was reproducible; comparable values for Dose 1 and Dose 2 of each treatment were observed for each primary PK parameter, and there was no overlap of values between treatments. This indicates that the lack of bioequivalence between single-strip FF and two-strip FF or FF/VI was due to true treatment differences, and was not attributable to intra-subject variability.

In conclusion, although the formal bioequivalence study showed higher exposure with FF single-strip ELLIPTA compared with FF two-strip or FF/VI, the results of the population PK analysis of data in subjects with asthma show that there is no notable difference in systemic exposure between the FF configurations (single-strip, two-strip or FF/VI). FF systemic exposure, as measured by AUC \( C_{\text{max}} \) dose proportional whilst \( C_{\text{max}} \) showed a less than proportional increase and the inhaled absolute bioavailability of FF was 14%.

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