The Pharmacokinetics of Fluticasone Furoate and Vilanterol Following Single Inhaled Administration in Combination and Intravenous Administration of Individual Components in Healthy Subjects

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Abstract

Fluticasone furoate (FF)/vilanterol (VI), a novel inhaled corticosteroid/long-acting β₂-agonist combination, is being developed as a once-daily inhaled treatment for asthma and chronic obstructive pulmonary disease. Two studies described here assess FF dose proportionality and VI equivalence across the clinical strengths of FF/VI and the absolute bioavailability of the components administered as FF/VI in combination via the dry powder inhaler (DPI) intended for commercial use. Study 1 (NCT01213849) was a randomized, open-label, three-way crossover, single-dose study in healthy subjects designed to assess whether the systemic exposure of FF increased proportionately and VI systemic exposure was constant across different strength combinations of FF/VI (four inhalations of FF/VI: 50/25 µg, 100/25 µg and 200/25 µg). Study 2 (NCT01299558) was an open-label, non-randomized, three-way crossover, single-dose study in healthy subjects conducted to determine the absolute bioavailability of FF/VI inhalation powder. Both FF and VI have high plasma clearance and extensive distribution into tissues. Overall, FF systemic exposure, as measured by AUC_{0-t'}, was dose proportional over the 200-800 µg FF dose range. The less than dose proportional increase seen for FF C_{max} is likely due to rate limited absorption from the lung. FF acts topically in the lung, whilst systemic exposure is related to safety. Consequently, the lack of dose proportionality for FF C_{max} would be considered not to impact efficacy. Equivalence of VI exposure across the three FF/VI dosage strengths was demonstrated for AUC_{0-t'}, and C_{max}. Following a single inhaled dose of FF/VI administered via DPI the absolute bioavailabilities of FF and VI were estimated to be 15% (90% confidence interval [CI]: 13%, 18%) and 27% (90% CI: 22%, 35%), respectively. FF showed longer retention in the lung than VI following inhaled administration, with the time for 90% of the total to be absorbed from the lung being on average 35.2 hours and 3.8 hours, respectively.

Keywords: Healthy subjects; Pharmacokinetics; Fluticasone furoate; Vilanterol; Inhaled; Bioavailability; Proportionality

Abbreviations: AE: Adverse Event; ANOVA: Analysis of Variance; AUC_{0-t'}: Area Under the Curve From Zero (Pre-dose) to the Time of Last Common Measurable Time Point Within a Subject; BMI: Body Mass Index; CI: Confidence Interval; C_{max}: Maximum Plasma Concentration; COPD: Chronic Obstructive Pulmonary Disease; CV: Coefficient of Variation; DPI: Dry Powder Inhaler; FF: Fluticasone Furoate; kₐ: Absorption Rate Constant; KEDTA: Tri-Potassium Ethylene Diamine Tetra-Acetic Acid; MAT: Mean Absorption Time; NA: Not Applicable; t_{1/2,abs}: Absorption Half-Life; t_{max}: Time to Maximum Observed Concentration; t_{1/2}: Half-Life; t_{90%}: Time to 90% to be Absorbed; VI: Vilanterol; Vₐ: Volume of Distribution at Steady-State; λ₁: Disposition Rate Constant.

Introduction

Fluticasone furoate (FF; GW685698), a novel glucocorticoid, and vilanterol (VI; GW642444M), a potent, inhaled, long-acting, β₂-receptor agonist, are currently under development in combination for use as a once-daily inhaled treatment for asthma and chronic obstructive pulmonary disease (COPD). In addition, FF is being developed as a monotherapy product for asthma and VI is being developed as a monotherapy product and in combination with a novel, long-acting muscarinic antagonist for the treatment of COPD. The pharmacokinetic, pharmacodynamic and safety profiles of the FF/VI combination have been described in healthy subjects as well as in patients with asthma and COPD [1,2]. The FF/VI combination has shown favourable safety and tolerability profiles in these subjects [1-3] with little evidence of effects of clinical concern that have previously been reported for inhaled corticosteroids (ICSs; decreased serum cortisol) [4,5] or long-acting, β₂-receptor agonists (hypokalaemia, hyperglycaemia and tachycardia) [6,7]. At clinical doses these effects were not generally seen (apart from tachycardia [3]). In addition, once-daily administration of FF/VI was effective at improving lung function in patients with COPD [8-10] or asthma [11,12]. In phase 3 clinical trials VI was administered as a fixed dose of 25 µg in combination with doses of 50 µg, 100 µg or 200 µg of FF in COPD, or at doses of 100 µg or 200 µg in asthma.

Previous published data on the pharmacokinetics of FF or VI were obtained following administration of early development formulations/
DPIs. The two studies described here assessed FF dose proportionality and VI equivalence across the clinical strengths of FF/VI and the absolute bioavailability of the components administered as FF/VI in combination via the dry powder inhaler (DPI) [13] intended for commercial use.

Study 1 was performed to demonstrate dose proportionality of FF and equivalence of VI following single-dose administration of three strengths of FF/VI (50/25 µg, 100/25 µg and 200/25 µg) via the DPI. 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 administered over the clinically relevant dose range. Understanding that the pharmacokinetics of the investigational products are linear over the concentration range of interest facilitates prediction of the effect of changing dose. As VI is given as a fixed dose it was important to understand that the pharmacokinetics were equivalent when administered from the three FF/VI clinical strengths being evaluated in phase 3 clinical trials.

Study 2 was performed to determine the absolute bioavailability of both FF and VI when delivered in combination from the DPI, and to characterize the pharmacokinetics of FF and VI following inhaled administration of FF/VI and intravenous administration of FF and VI given separately. The bioavailability of both inhaled FF and VI predominantly represents absorption from the lung as the oral bioavailability of both FF and VI from the swallowed portion of the inhaled dose is negligible (approximately 1%) [14,15]. Use of analytical deconvolution techniques on data from this study allowed the rate and duration of the input (absorption) of FF and VI into the systemic circulation after inhalation in combination to be assessed over the entire absorption period.

Due to the low systemic bioavailability of FF and VI, and consequently the low blood levels after dosing at the clinical dose, it is not possible to assess dose proportionality or absolute bioavailability at the proposed clinical doses (FF/VI 100/25 µg, 200/25 µg). Consequently, in these studies four inhalations of the FF/VI 50/25 µg, 100/25 µg and 200/25 µg strengths were administered (total doses of FF/VI 200/100 µg, 400/100 µg and 800/100 µg, respectively) in order to provide adequate pharmacokinetic data.

Methods
Study design and subjects

**Study 1:** FF dose proportionality and VI equivalence (HZA102932; NCT01213849): This randomized, open-label, three-way crossover, single-dose study was designed to assess whether the systemic exposure of FF increased proportionately across different strength combinations of FF/VI and whether VI systemic exposure was constant across the different strengths. Healthy male or female subjects (n=24) participated in three treatment periods as follows: Study 1, and from period 1 of Study 2, was separated approximately 8 weeks for each subject.

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Both studies included non-smoking healthy male and female subjects aged 18 to 64 years with a 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 (t½s) 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 was a potential enzyme inducer) or 5 t½s (whichever was longer) prior to the first dose of study medication; history of alcohol/drug abuse or dependence within 12 months of the study; subjects who had suffered a lower respiratory tract infection within 4 weeks of the screening visit; electrocardiogram demonstrating corrected QT interval greater than 450 ms at screening. Females who were pregnant or nursing were also excluded.

Both studies 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 Hammersmith Medicines Research Unit, Park Royal, London, UK, and Study 2 was conducted at GlaxoSmithKline Medicines Research Unit, Prince of Wales Hospital, Randwick, NSW, Australia). All subjects gave their written informed consent before participating in the trial.

Pharmacokinetic evaluations

**Study 1:** Venous blood samples (4 mL) for analysis of plasma drug concentrations were collected in KEDTA tubes pre-dose and at 5, 10, 20, 30 and 45 minutes, and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 32 and 48 hours after the start of dosing.

**Study 2:** FF and VI absolute bioavailability (HZA102934; NCT01299558): This open-label, non-randomized, three-way crossover, single-dose study was conducted to determine the absolute bioavailability of FF/VI inhalation powder. Healthy male or female subjects (n=16) participated in three treatment periods as follows: treatment period 1: single dose of FF/VI 800/100 µg (four inhalations of FF/VI 200/25 µg via DPI), treatment period 2: 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) and treatment period 3: single intravenous dose of VI 55 µg (75 mL of 110 µg/150 mL in saline administered at a constant rate of infusion over 1 hour using a syringe and pump). There was a washout period of at least 7 days but no more than 14 days between treatment periods. The duration of the study was approximately 9 weeks for each subject.

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**Study 2:** Venous blood samples (approximately 4 mL for each dosing) for analysis of plasma drug concentrations were collected in KEDTA tubes as follows: pre-dose and at 5, 10, 15, 20, 30 and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 32, 48 and 48 hours after the start of dosing.

**Study 2:** Venous blood samples (approximately 4 mL for each dosing) for analysis of plasma drug concentrations were collected in KEDTA tubes as follows: pre-dose and at 5, 10, 15, 20, 30 and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 32, 48 and 48 hours after the start of dosing.
equally into two separate 1.4 mL matrix screw-capped polypropylene tubes and frozen, one for each analyte (FF and VI). For the remaining treatment periods in Study 2 supernatant plasma was transferred into 1.4 mL matrix screw-capped polypropylene tubes and frozen. All samples were stored at −70°C or colder until shipment.

**Analytical methods**

Worldwide Bioanalysis, Drug Metabolism and Pharmacokinetics, GlaxoSmithKline analyzed plasma samples (150 µL aliquots) from both studies for FF by solid phase extraction using ([1,4C]H2]-GW685698 as internal standard) followed by high performance liquid chromatography with tandem mass spectrometry using an Applied Biosystems API-5000 (Applied Biosystems/MDS Sciex, Foster City, USA). A gradient system using 5 mM ammonium formate and methanol was run with column AC50×2.1 mm, C18.3 µm, Hichrom Ltd running at 45°C. The ion transition for FF was m/z 539 to 313. The validation range of the assay was 10-1000 pg/mL for FF. Interbatch precision was ≤13.1% coefficient of variation (CV) over the assay range; the lower limit of quantification for FF was 10 pg/mL. For FF the conditions for stability for freeze/thaw, matrix, processed extract and long-term stability in frozen matrix were 5 freeze-thaw cycles at −20°C/ambient temperature, 24 hours at ambient temperature, 72 hours at ambient temperature and 290 days at −20°C, respectively.

Worldwide Bioanalysis, Drug Metabolism and Pharmacokinetics, GlaxoSmithKline analyzed plasma samples (200 µL aliquots) from both studies for VI by solid phase extraction using ([1,4C]H2]-GW642444 as internal standard) followed by high performance liquid chromatography with tandem mass spectrometry using an Applied Biosystems API-5000. A gradient system using 10 mM ammonium formate containing 0.1% formic acid and acetonitrile containing 0.1% formic acid was run with column 50×2.1 mm internal diameter Hypersil Gold, 3 µm, Thermo Scientific running at 50°C. The ion transition for VI was m/z 486 to 159. The validation range of the assay was 10-100,000 pg/mL for VI. Interbatch precision was ≤14.4% CV over the assay range; the lower limit of quantification for VI was 10 pg/mL. For VI the conditions for stability for freeze/thaw, matrix, processed extract and long-term stability in frozen matrix were 5 freeze-thaw cycles at −80°C/ambient temperature, 24 hours at ambient temperature, 72 hours at ambient temperature and 290 days at −80°C, respectively.

Where reported concentrations were above the higher limit of quantification the plasma samples were diluted, as appropriate, prior to analysis to provide concentrations within the validated range. Quality control results from these studies met the acceptance criteria of no more than one third of the quality control results deviating from the nominal concentration by more than 15%, with at least one quality control result acceptable at each concentration. In addition, incurred sample reproducibility was conducted on 11-14% of the samples for each analyte and study. Results showed that 66.7% or more of the incurred sample results were within the limits of ±20% of the mean of the reanalysis result and its corresponding original result, confirming bioanalytical reproducibility in incurred human plasma samples.

**Pharmacokinetic analysis**

Pharmacokinetic analyses of plasma FF and VI concentration-time data following inhaled and intravenous administrations were conducted using non-compartmental Model 200 (for extravascular administration) and Model 202 (for constant infusion), respectively, of WinNonlin Professional Edition Version 5.2 (Pharsight Corporation, Mountain View, CA, USA). Pharmacokinetic variables for all treatments were calculated as follows: maximum plasma concentration (Cmax) and time to maximum observed concentration (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, t½ was calculated as 0.693/λz, AUC(0–t) was calculated using the linear trapezoidal rule for intervals where the concentration data were increasing, and the logarithmic trapezoidal rule for intervals where the concentration data were decreasing, and then extrapolated to infinity using λz to obtain the AUC[∞]. In addition, AUC(τ) was derived, where τ was the common time of the last quantifiable concentration for an analyte, within a subject across all treatments. For intravenous treatment only, total plasma clearance and steady-state volume of distribution (Vss) were also determined.

For Study 2 only, inhalation data for FF and VI were subjected to deconvolution analysis using WinNonlin Professional Edition [Version 5.2]. The micro-constants describing distribution and elimination were obtained by fitting an appropriate infusion model to the time-concentration data following intravenous dosing of FF and VI using WinNonlin Pro. The absorption rate constant (ka) was obtained by fitting a mono-exponential function to the percent remaining to be absorbed versus time data visually assessed to lie on the linear portion of the semi-logarithmic plots. The absorption half-life (t½,abs) was calculated as the ratio of loge2/ka and this was used to calculate the time for 90% to be absorbed (T90).

**Safety evaluations**

A complete physical examination, including 12-lead electrocardiogram, was conducted at screening. Adverse events (AEs), clinical laboratory tests and vital signs were monitored throughout each of the studies.

**Statistical analysis**

All statistical analyses were performed using SAS version 9.1.3 (SAS Institute Inc. Cary, NC) on a UNIX platform. 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 for AUC (0.84, 1.16) and for Cmax (0.74, 1.26), separately. To assess dose proportionality of FF loge-transformed FF AUC(0–t) and Cmax data from Study 1 were analyzed 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 was used as a secondary approach to assess dose proportionality. Following loge-transformation, dose-normalized AUC(0–t) and Cmax of FF were analyzed separately using a mixed-effects model, fitting dose and period as fixed effects, and subject intercept as a random effect. The reference dose for the analysis of variance (ANOVA) was 400/100 µg as this is comprised of the 100/25 µg strength, which is anticipated to be the most commonly used clinical strength. Point estimates and associated 90% CIs for the differences 200/100–400/100 µg and 800/100–400/100 µg were constructed using the residual variance. These point estimates and CIs were then exponentially back-transformed to provide point estimates and associated 90% CIs for the ratios 200/100:400/100 µg and 800/100:400/100 µg.
A test of the equivalence of VI exposure (log-transformed AUC\textsubscript{0–\text{t'}} and log-transformed C\textsubscript{\text{max}}) across the three dosage strengths from Study 1 was carried out by an assessment of the slope using the same regression model as used to test for dose proportionality of FF. Log(dose) was fitted in the model, as a continuous variable, using the FF dose. Equivalence across dosage strengths of VI exposure was to be concluded if the 90% CI for the slope fell within the pre-specified acceptance equivalence range on the log-scale for AUC (–0.223, 0.223) and for C\text{max} (–0.357, 0.357).

For Study 2, AUC\textsubscript{0–\text{t}} from the inhaled and intravenous formulations was used to estimate absolute bioavailability for both inhaled FF and VI. The AUC values were dose-normalized, by dividing by nominal dose, then using loge-transformed values, analyzed using a mixed-effects model for each analyte. Mixed models were fitted with treatment as a fixed effect and subject as a random effect. A point estimate and associated 90% CI was calculated for the difference of inhalation versus intravenous dosing. The point estimate and associated 90% CI was then back-transformed to provide a point estimate and 90% CI for the absolute bioavailability.

Results

Study disposition and demographics

Twenty-four subjects (16 Caucasian, 7 African–American/African, 1 mixed-race) were enrolled into Study 1 and all completed the study. The subjects (8 males, 16 females) had a mean age of 34 [range 18-65] years and body mass index (BMI) of 24.7 [20.0-28.9] kg/m\textsuperscript{2}. Five subjects took concomitant medications during the study. Three subjects took paracetamol (acetaminophen) for headache, 1 subject took ibuprofen and 1 subject took paracetamol and phenylephrine hydrochloride for minor ailments during the study. None of these medications are considered to be likely to have affected the study outcome.

Sixteen subjects (15 Caucasian, 1 Native Hawaiian/Pacific Islander) were enrolled into Study 2 and all completed the study. The subjects (11 males, 5 females) had a mean age of 25 [range 21-40] years and BMI of 23.7 [18.8-28.5] kg/m\textsuperscript{2}. Five subjects took paracetamol and 1 subject took paracetamol and phenylephrine hydrochloride for minor ailments during the study. None of these medications are considered to be likely to have affected the study outcome.

Pharmacokinetics

FF dose proportionality: The mean plasma concentration versus time profiles for FF is shown in (Figure 1a). Whilst plasma concentrations of FF were quantifiable in all subjects up to 48 hours following FF/VI 800/100 µg, and 15 of 23 subjects up to 32 hours following 400/100 µg, data were more limited at 200/100 µg, with quantifiable concentration in only half the subjects by 10 hours post-dose. On average, the maximum plasma concentrations of FF were achieved at later times (t\text{max}) as the FF dose increased (Table 1: median t\text{max}: FF/VI 200/100 µg 5 minutes, FF/VI 400/100 µg 10 minutes and FF/VI 800/100 µg 60 minutes). Overall exposure, as measured by AUC\textsubscript{0–\text{t'}} was dose-proportional, while peak exposure (C\text{max}) increased in a less than proportional manner over the 200–800 µg dose range (Table 2). For AUC\textsubscript{0–\text{t'}} the 90% CI for the slope was completely contained within the pre-defined acceptance range (0.84, 1.16), while for C\text{max}, the 90% CI for the slope was not within the pre-defined acceptance range (0.74, 1.26). Further investigation of dose proportionality using an ANOVA model (not presented), using FF/VI 400/100 µg as the reference treatment, showed that the lack of dose proportionality for C\text{max} was across all doses, with a less than proportional increase in C\text{max} as the dose increased. For AUC\textsubscript{0–\text{t'}} the ANOVA results supported the power model.

Vilanterol equivalence: The mean plasma concentration versus time profiles for VI is shown in (Figure 1b). Mean C\text{max} was observed at 6-9 minutes post-dose for all treatments (Table 1). The decline from C\text{max} occurred in a triphasic manner with concentrations in the majority of subjects falling below the limit of quantification by 12 hours post-dose (Figure 1b).

Equivalence of VI exposure across the three FF/VI dosage strengths was demonstrated as the 90% CIs for the slope of AUC\textsubscript{0–\text{t'}} and C\text{max} were
The Pharmacokinetics of Fluticasone Furoate and Vilanterol Following Single Inhaled Administration in Combination and Intravenous Administration of Individual Components in Healthy Subjects. J Bioequiv Availab 5: 165-173. doi:10.4172/jbb.1000153

### Table 1: Pharmacokinetic parameters of FF and VI following inhaled administration of FF/VI (200/100 µg, 400/100 µg and 800/100 µg; dose proportionality Study 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FF</th>
<th>CV (%)</th>
<th>VI</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC&lt;sub&gt;0-t'&lt;/sub&gt; (pg•h/mL) (geometric mean [95% CI])</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF/VI 200/100 µg</td>
<td>163.0 (122.7, 216.6)</td>
<td>76</td>
<td>393.6 (343.5, 451.0)</td>
<td>29</td>
</tr>
<tr>
<td>FF/VI 400/100 µg</td>
<td>292.8 (227.1, 377.4)</td>
<td>66</td>
<td>403.7 (357.6, 455.7)</td>
<td>25</td>
</tr>
<tr>
<td>FF/VI 800/100 µg</td>
<td>575.8 (436.2, 760.2)</td>
<td>74</td>
<td>410.0 (363.6, 462.3)</td>
<td>27</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (pg/mL) (geometric mean [95% CI])</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF/VI 200/100 µg</td>
<td>53.6 (46.6, 61.7)</td>
<td>34</td>
<td>516.6 (450.8, 592.1)</td>
<td>28</td>
</tr>
<tr>
<td>FF/VI 400/100 µg</td>
<td>64.8 (57.2, 73.4)</td>
<td>30</td>
<td>564.9 (512.8, 622.2)</td>
<td>22</td>
</tr>
<tr>
<td>FF/VI 800/100 µg</td>
<td>105.0 (92.2, 119.5)</td>
<td>31</td>
<td>557.7 (501.0, 620.8)</td>
<td>23</td>
</tr>
<tr>
<td>t&lt;sub&gt;max&lt;/sub&gt; (h) (median [range])</td>
<td>0.08 (0.08-1.50)</td>
<td>NA</td>
<td>0.115 (0.08-0.23)</td>
<td>NA</td>
</tr>
<tr>
<td>FF/VI 400/100 µg</td>
<td>0.17 (0.08-2.00)</td>
<td>NA</td>
<td>0.100 (0.08-0.18)</td>
<td>NA</td>
</tr>
<tr>
<td>FF/VI 800/100 µg</td>
<td>1.00 (0.08-4.00)</td>
<td>NA</td>
<td>0.150 (0.08-0.17)</td>
<td>NA</td>
</tr>
<tr>
<td>t&lt;sub&gt;'&lt;/sub&gt; (h) (median [range])</td>
<td>8.00 (3.00-32.00)</td>
<td>NA</td>
<td>10.00 (3.00-12.00)</td>
<td>NA</td>
</tr>
</tbody>
</table>

### Table 2: Results of power model assessment of FF dose proportionality and VI equivalence following a single inhaled administration of FF/VI (200/100 µg, 400/100 µg and 800/100 µg; dose proportionality Study 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Point Estimate</th>
<th>90% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>FF dose proportionality</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t'&lt;/sub&gt; (pg•h/mL)</td>
<td>Log (dose)</td>
<td>0.910 (0.843, 0.978)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (pg/mL)</td>
<td>Log (dose)</td>
<td>0.484 (0.400, 0.569)</td>
</tr>
<tr>
<td>VI equivalence</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0-t'&lt;/sub&gt; (pg•h/mL)</td>
<td>Log (dose)</td>
<td>0.029 (~0.018, 0.077)</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt; (pg/mL)</td>
<td>Log (dose)</td>
<td>0.055 (~0.010, 0.121)</td>
</tr>
</tbody>
</table>

### Results:

- **Absorption pharmacokinetics of inhaled FF and VI:** Peak FF plasma concentrations (<i>t</i><sub>max</sub>) were observed on average 1 hour post-dose and VI plasma concentrations were observed 10 minutes post-dose following inhaled FF/VI (Table 3). To characterize the absorption profile of FF and VI following inhaled FF/VI, concentration-time data for FF and VI following inhaled administration were subjected to deconvolution analysis.

- The estimate of apparent 1/2 of inhaled FF was longer than after intravenous dosing (Table 3). The absorption of FF was prolonged and essentially complete by 24 hours post-dose (Figure 3a). A summary of the FF absorption parameters, mean absorption time (MAT), T90 and t½, abs are presented in Table 4.

- In contrast to FF, the absorption of VI was essentially complete by 3 hours post-dose (Figure 3b) while the VI t½ was similar for inhaled and intravenous administration (Table 3). A summary of the VI absorption parameters, MAT, T90 and t½, abs are presented in Table 4.

### Safety

Although supra-therapeutic inhaled doses of FF/VI and high concentrations were found to be generally safe, additional studies are needed to fully evaluate the safety profile of FF/VI at high doses.
intravenous FF and VI doses were used in these two studies all treatments had a generally good safety profile and were well tolerated. All enrolled subjects (24 in Study 1, 16 in Study 2) successfully completed all doses as planned. No non-fatal or fatal serious AEs were reported during the study and no subjects were withdrawn. In both studies all AEs were considered by the investigators to be of mild or moderate intensity; there were no AEs of severe intensity. There was no individual electrocardiogram or vital signs that were considered to be
Clinically significant by the investigator. No individual safety laboratory value was reported as an AE or showed trends of concern.

For Study 1, a total of 36 AE episodes were reported during the study, 15 were reported after FF/VI 200/100 µg, 11 were reported after FF/VI 400/100 µg and 10 were reported after FF/VI 800/100 µg. There was no increase in total or specific AE frequency with increasing FF dose. Palpitations and headache were the most frequent AEs, reported in 7 (29%) and 5 (21%) subjects, respectively. Neither of these AEs appeared to be dose-dependent. The incidence of palpitations was not consistent across the three treatments despite the fact that they contained the same VI dose (25% 200/100 µg; 4% 400/100 µg; 8% 800/100 µg).

For Study 2, the highest incidence of AEs reported in two or more subjects was seen after a single intravenous dose of VI 55 µg predominantly due to palpitations (38%), which occurred at the end of the infusion and were of short duration. For inhaled FF/VI 800/100 µg upper respiratory tract infection was the highest incidence reported in two or more subjects (13%), whilst for FF 250 µg intravenous dose headache was the most frequent AE (19%).

Discussion

Both FF and VI exhibit multiphasic distribution and elimination profiles in plasma following inhaled administration in combination or intravenous administration of the individual components. Following inhaled administration in combination, VI absorption was very rapid with maximum plasma concentrations observed within 10 minutes of dosing. FF absorption was generally slower. On average, the absolute bioavailability for FF and VI were estimated to be 15% and 27%, respectively. Oral bioavailability is approximately 1% for both FF and VI [14,15] and hence pharmacokinetic data from inhaled dosing represents almost exclusively the dose absorbed from the lung, with no contribution to systemic exposure from the swallowed portion of the dose. Data from in vitro performance data, including cascade impaction, show the fine particle mass for FF and VI for FF/VI 200/25 µg inhalation powder delivered via the DPI to be 21.8% and 31.2%, respectively. This is considered to be representative of the respirable portion of the inhaled dose and therefore suggests that the majority of drug that is likely to be delivered to the lung reaches the systemic circulation. This can be inferred because the values of absolute bioavailability for both molecules approached the respective percentages of the total nominal inhaled doses that were fine particle mass.

The intravenous pharmacokinetics of FF and VI showed both molecules to have high plasma clearance (geometric mean values of 65.4 L/hour and 108 L/hour, respectively) which was similar to liver blood flow (87 L/hour) for a 70 kg man [16]. Vss (geometric mean values of 661 L and 165 L, respectively) was greater than that quoted for total body water (42 L) for a 70 kg man [16], indicating extensive distribution into tissues. The intravenous pharmacokinetic data for FF and VI presented are consistent with previous data [14,15,17].

Overall FF systemic exposure, as measured by AUC_{(0-t')}, was dose-proportional as the 90% CI for the slope was completely contained within the pre-defined acceptance range (0.84, 1.16). For FF C_{max}, the 90% CI for the slope was not within the pre-defined acceptance range (0.74, 1.26) and the ANOVA results showed this to be a less than proportional increase in Cmax over the 200-800 µg FF dose.

Table 4: Summary of FF and VI absorption pharmacokinetic parameters following inhaled administration of FF/VI 800/100 µg (absolute bioavailability Study 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>FF Geometric Mean (95% CI)</th>
<th>VI Geometric Mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAT (h)</td>
<td>10.53 (8.52, 13.01)</td>
<td>0.659 (0.286, 1.517)</td>
</tr>
<tr>
<td>T90 (h)</td>
<td>35.2 (32.0, 38.7)</td>
<td>3.83 (2.64, 5.57)</td>
</tr>
<tr>
<td>t_{1/2},abs (h)</td>
<td>8.76 (7.82, 9.81)</td>
<td>1.074 (0.775, 1.489)</td>
</tr>
</tbody>
</table>

CI = confidence interval; FF = fluticasone furoate; MAT = mean absorption time; t_{1/2,abs} = absorption half-life; T90 = time for 90% to be absorbed; VI = vilanterol.
range. This lack of dose proportionality for FF $C_{\text{max}}$ is likely due to rate limited absorption from the lung, which is well characterized for FF [17]. This is supported by tmax being observed at later times as the FF dose increased. FF acts topicaly in the lung, whilst systemic exposure is related to safety. Consequently, the lack of dose proportionality for FF $C_{\text{max}}$ 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 seen for FF $C_{\text{max}}$ is considered to be of no clinical relevance particularly as the overall extent of exposure, as measured by AU$C_{(0-t)}$, was dose proportional. Equivalence of VI exposure across the three FF/VI dosage strengths was demonstrated, as the 90% CIs for the slope of AU$C_{(0-t)}$ and $C_{\text{max}}$ were completely contained within the pre-specified log-scale equivalence ranges.

The results showed that the estimate of apparent FF $t\frac{1}{2}$ following single-dose, inhaled administration of FF/VI was notably longer than that seen following intravenous dosing, whereas for VI the apparent $t\frac{1}{2}$ values were similar following the two routes. This suggests that FF is exhibiting absorption rate-limited pharmacokinetics and that the apparent $t\frac{1}{2}$ is an estimate of absorption rate following inhaled administration. Use of analytical deconvolution techniques allowed the rate and duration of the input (absorption) of FF and VI into the systemic circulation after inhalation to be assessed over the entire absorption period. Following inhaled FF/VI administration FF showed considerably longer retention in the lung than VI, with T90s on average, of 35.2 hours and 3.8 hours, respectively. The absorption pharmacokinetic parameters for FF following inhaled administration of FF/VI are very similar to those previously observed using different formulations administered from a different DPI [17]. Hence, the absorption rate-limiting pharmacokinetics of FF is an inherent characteristic of the molecule and independent of formulation and inhaler.

A potential limitation of these studies was that they were conducted at supra-therapeutic doses of FF/VI in order to produce measurable FF and VI plasma concentrations and provide robust pharmacokinetic data to meet study objectives. However, due to the linear time-independent pharmacokinetic characteristics of both FF and VI, results for pharmacokinetic data from higher doses can be interpolated to the clinical doses. Another potential limitation was that Study 2 was conducted in a non-randomized manner, for several logistical reasons. Each treatment period had different dosing procedures, sampling requirements, and pharmacokinetic sampling time points, and the infusion preparation and administration requirements for the two intravenous treatments also differed. Therefore, it was considered more practical to perform the study with a fixed treatment sequence, with all subjects receiving the same treatments in groups to minimize any logistical issues. No significant statistical issues were expected in following this approach.

Conclusion

Both FF and VI have high plasma clearance and extensive distribution into tissues. Following a single inhaled dose of FF/VI administered via DPI, the absolute bioavailabilities for FF and VI were estimated to be 15% and 27%, respectively. Overall FF systemic exposure, as measured by AU$C_{(0-t)}$, was dose-proportional over the 200-800 µg FF dose range. The less than dose proportional increase seen for FF $C_{\text{max}}$ is likely due to rate limited absorption from the lung. FF acts topically in the lung, whilst systemic exposure is related to safety. Consequently, the lack of dose proportionality for FF $C_{\text{max}}$ would be considered not to adversely impact efficacy or safety. Equivalence of VI exposure across the three FF/VI dosage strengths was demonstrated.

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