In Vitro - Ex Vivo Correlation of Fluticasone Propionate Pharmacokinetic Profiles

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INTRODUCTION

In inhalation drug development there is a need not only for traditional particle characterization data such as size distribution and physicochemical data on the formulations, but also for *in vitro* methods producing data more indicative of subsequent *in vivo* pharmacokinetic behavior. Here we have compared the pharmacokinetic profiles of the low-solubility inhalation steroid fluticasone propionate (FP) in the promising *in vitro* dissolution/absorption method Dissolv*it*® (DS) [1] with the well-established experimental model for lung pharmacokinetic studies, the *ex vivo* isolated perfused and ventilated rat lung (IPL) [2, 3].

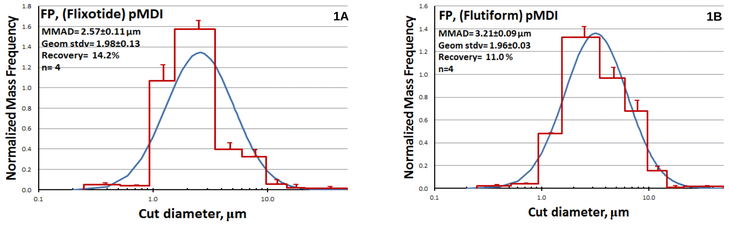

Figure 1. The particle size distributions of the generated aerosols for the pMDI formulations containing FP: **Flixotide (1A)** and **Flutiform (1B).**

METHODS

Two marketed formulations of FP were tested: Flixotide (GSK), (FP, strength 50 µg) and Flutiform (Mundipharma), (FP, strength 250 µg and formoterol strength 10 µg) provided as pressurized metered dose inhalers (pMDIs). The canisters were connected to the US Pharmacopeia Induction Port No 1 of the PreciseInhale® aerosol system and actuated into an air flow of 15 L/min. The aerosols were collected in the PreciseInhale® aerosol holding chamber and immediately dispensed to the Dissolv*it*® and IPL modules at flow rates of 1000 mL/min and 250 mL/min, respectively.

Particle size distributions of the generated aerosols (*Figure 1*) were measured with an 8-stage Marple cascade impactor at a flow rate of 2 L/min.

At the start of the Dissolv*it*® experiment, the aerosol particles deposited on the cover slip (with PreciseInhale®) are brought into contact with the mucus consisting of 1.5% polyethylene oxide [4] and 0.4% L-alphaphosphatidyl choline (Sigma). The mucus simulant had been applied to a polycarbonate membrane corresponding to the basal membrane of the airway mucosa. The mucus simulant together with the polycarbonate membrane constitutes the diffusion barrier. On the other side of the membrane, the blood simulant (perfusate consisting of phosphate buffer with 4% albumin) is streaming. Dissolved particles were absorbed at a perfusate flow rate of 0.4 ml/min. Dissolution was studied by observing particle disappearance using optical microscopy, and by chemical analysis of substance removed by absorption in the flow-past perfusate. The amount of FP retained in the system (comparable with the amount of drug retained in lung tissue in IPL) was analysed at the end of the 2 h experiments.

The IPL was prepared as previously described [2]. Briefly, whole lungs were isolated from female CD IGS (Sprague Dawley) rats (Charles River, Sulzfeld, Germany). The lungs were ventilated with a negative alternating pressure and perfused in single-pass mode with a Krebs-Henseleit buffer containing 4% albumin. For the exposures of the IPL, the FP aerosols actuated via the induction port, were delivered to the lungs (n=3 per formulation) by the PreciseInhale® active dosing system, which calculates in real time the cumulative inhaled aerosol dose. The system automatically terminates the exposure when the inhaled target dose is reached. The liquid propellant evaporated within PreciseInhale® system, and thus did not reach the lung. The perfusate was repeatedly sampled in an automatic fraction collector during a 2 h period post exposure. Thereafter, the lungs and trachea were harvested for analysis of the amount of FP retained in the tissues after the perfusion period.

Analytical quantitation of FP in all samples from the Dissolv*it*® and IPL experiments was performed by LC/MS/MS, with a LLOQ of 100 pg/ml.

RESULTS

The concentration of absorbed FP in the perfusate/blood simulant over time is shown in *Figure 2A (Flixotide)* and *2B (Flutiform)*. In *Figure 2C (Flixotide)* and *2D (Flutiform)* for both models the amount of FP cleared with the perfusate during each sample interval has been expressed as percent of the initially deposited dose clearing per second. This compensates both for the differing deposited doses of the two exposure models and for the difference between the varying perfusate flow rate of the IPL and the constant flow rate of the Dissolv*it*® system. In *Figure 3A (Flixotide)* and *3B (Flutiform)* is shown the amount of FP still retained in the rat lung and the Dissolv*it*® lung simulant model at each sample time, expressed as fraction of the initially deposited dose. The retained FP fraction represents either undissolved particles or dissolved substance not yet absorbed by the perfusate buffer/blood simulant.

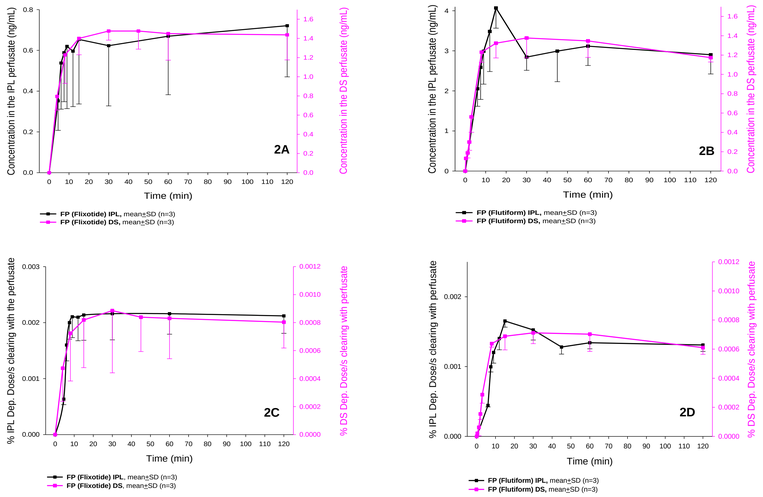

Figure 2. Two different scales are used on the y axes: black for IPL values and pink for Dissolv*it*® values. The concentrations of FP from the two formulations: *Flixotide (A)* and *Flutiform (B)* in the perfusate/blood simulant over time in the IPL (black curves) and the Dissolv*it*® (pink curves). Panels *C (Flixotide)* and *D (Flutiform)* show the percent of deposited doses (IPL black curves and Dissolv*it*® pink curves) clearing with the perfusate per second.

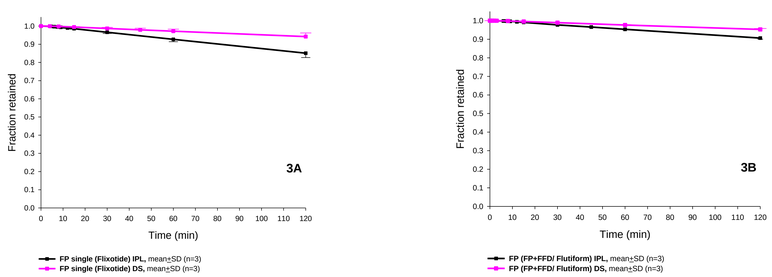

Figure 3. The amount of FP from the two formulations: *Flixotide* (A) and *Flutiform* (B), as normalized to the initially deposited dose, retained in the rat lung (black curves) and in the Dissolv*it*® model (pink curves) over time.

The key exposure and pharmacokinetic parameters for the IPL and Dissolv*it*® system are presented in *Table 1*.

Parameter	FP (Flixotide)		FP (Flutiform)	
	IPL	Dissolv*it*®	IPL	Dissolv*it*®
Exp. duration, (min)	6±1	-	14±1	-
Tot. Deposited dose, (µg)	14.2±1.6	1.26±0.44	120.6±6.9	1.34±0.11
FP Deposited dose, (µg)	14.2±1.6	1.26±0.44	116.4±6.7	1.29±0.12
C_{max}, perfusate, (ng/mL)	0.8±0.2	1.6±0.1	4.1±0.5	1.4±0.1
T_{max}, (min)	knee	45±15	15±-0	40±17
Fraction $perf_{peak}$, (%Dep.dose/s)	0.00225± 0.00034	0.00095± 0.00039	0.00165± 0.00009	0.00074± 0.00006
T_{peak}, (min)	knee	45±15	20±9	40±17
Fraction retained $_{(120\ min)}$	0.85±0.02	0.94±0.02	0.91±0.01	0.95±0.01
$t_{1/2}$, (h)	8.57±1.40	24.81±7.16	13.76±0.97	28.91±0.01

Table 1. Key exposure and pharmacokinetic parameters (± standard deviation) for FP (*Flixotide* and *Flutiform*) in the IPL and Dissolv*it*® system. **C_{max}:** Maximum concentration of drug in the perfusate; **t_{max}:** Time to maximum concentration in the perfusate; **Fraction $perf_{peak}$:** Peak values for the fraction of the deposited dose clearing with the perfusate per second; **t_{peak}:** Time at which Fraction $perf_{peak}$ occurred; **Fraction retained $_{(120\ min)}$:** Fraction of deposited FP dose left in the Dissolv*it*® lung simulant and in the rat lung after the 120 min perfusion period; **$t_{1/2}$:** Estimated half-time of drug clearance with the perfusate after fitting the fraction retained values to a first order decay curve.

DISCUSSION AND CONCLUSIONS

Previously published methods on *in vitro* dissolution testing in inhalation drug development, such as the flow through cell, paddle apparatus and the Franz cell, all give cumulative dissolution/absorption curves [5, 6] which are not easily comparable to *in vivo* pharmacokinetic profiles. In contrast, the Dissolv*it*® *in vitro* dissolution/absorption method [1] has a dynamic flow-past perfusion strategy which generates concentration curves containing c_{max} and t_{max} values. These values can be more readily compared with human clinical pharmacokinetic profiles. Here, the rat IPL, previously shown to be a good pharmacokinetic model [2, 3], was used for comparison.

In Figure 2 it is shown that the profiles in the Dissolv*it*® are very similar to the IPL profiles, especially for Flixotide. For the normalized perfusate clearance values, the curves are also quite similar in shape. The curves on fraction retained (Figure 3) in the IPL and Dissolv*it*® models rank both FP formulations similarly. A large fraction of drug is retained in the air/perfusate barriers of both systems, as expected from the low solubility of the FP formulations. One factor contributing to the faster clearance of Flixotide compared to Flutiform in both exposure models, is likely to be the finer particle size distribution of Flixotide. Clearance with the perfusate from the IPL is faster than from the DS system for both formulations, which is evident both from the steeper declining retention curves (Figure 3) and the nearly doubled normalized perfusate clearance rate of the IPL, as shown in Figures 2C and D. The main reason for this difference is likely to be the thicker 60 µm diffusion barrier of the DS compared to the air/blood barrier of the IPL being dominated by the µm-thick barrier in the alveolar region.

It is evident, however, that Dissolv*it*® can generate pharmacokinetic profiles of FP that resemble those in the rat lung. This indicates that Dissolv*it*® may be a valuable *in vitro* dissolution/absorption method to use for IV-IVC in the development of new and generic drugs.

1. Börjel M, S.R., Gerde P. *The Dissolvit: An In Vitro Evaluation of the Dissolution and Absorption of Three Inhaled Dry Powder Drugs in the Lung*. in *Respiratory Drug Delivery*. 2014. Puerto Rico. 2. Selg, E., F. Acevedo, R. Nybom, B. Blomgren, Å. Ryrfeldt, and P. Gerde, *Delivering Horseradish Peroxidase as a Respirable Powder to the Isolated, Perfused and Ventilated Lung of the Rat: The Pulmonary Disposition of an Inhaled Model Biopharmaceutical*. Journal of Aerosol Medicine and Pulmonary Drug Delivery, 2010. **23**(5): p. 273-284. 3. Selg, E., P. Ewing, F. Acevedo, C.O. Sjoberg, A. Ryrfeldt, and P. Gerde, *Dry powder inhalation exposures of the endotracheally intubated rat lung, ex vivo and in vivo: the pulmonary pharmacokinetics of fluticasone furoate*. J Aerosol Med Pulm Drug Deliv, 2013. **26**(4): p. 181-9. 4. Shah, S., K. Fung, S. Brim, and B.K. Rubin, *An in vitro evaluation of the effectiveness of endotracheal suction catheters*. Chest, 2005. **128**(5): p. 3699-704. 5. May, S., B. Jensen, M. Wolkenhauer, M. Schneider, and C.M. Lehr, *Dissolution techniques for in vitro testing of dry powders for inhalation.* Pharm Res, 2012. **29**(8): p. 2157-66. 6. Salama, R.O., D. Traini, H.K. Chan, and P.M. Young, *Preparation and characterisation of controlled release co-spray dried drug-polymer microparticles for inhalation 2: evaluation of in vitro release profiling methodologies for controlled release respiratory aerosols.* Eur J Pharm Biopharm, 2008. **70**(1): p.145-52.