Inhalation Pharmacokinetics of a Respirable Formulation of a Selective PDE4 Inhibitor, CHF6001, in Three Exposure Models: Isolated Perfused Lung, Intratracheally Intubated, and Nose-Only Exposed Rat

Inhalation Sciences

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INTRODUCTION

In the drug discovery/early development phase of a compound to be administered as dry powder (DPI), it is difficult to obtain pharmacokinetic (PK) or pharmacodynamic (PD) data from preclinical models. A powder formulation is frequently not available and compounds are administered to the lung as solution/suspension using excipients that may alter the drug pharmacokinetics. For that reason, an inhalation exposure platform (PreciseInhale™) was developed to allow respirable powder aerosol exposure, with only small amount of substance, to a number of clinically relevant preclinical models from *in vitro* to *in vivo (Figure 1)*.

In the current study, the same respirable aerosol formulation was administered with the PreciseInhale to a ventilated and perfused rat lung *ex vivo* (IPL), and to rats *in vivo* by intratracheal- (INT) and nose-only (N-O) inhalation method. The substance chosen was the Chiesi compound CHF6001 (MW = 687), which is a PDE4 inhibitor under investigation for the treatment of asthma and chronic obstructive pulmonary disease (COPD) *(Figure 2)*.

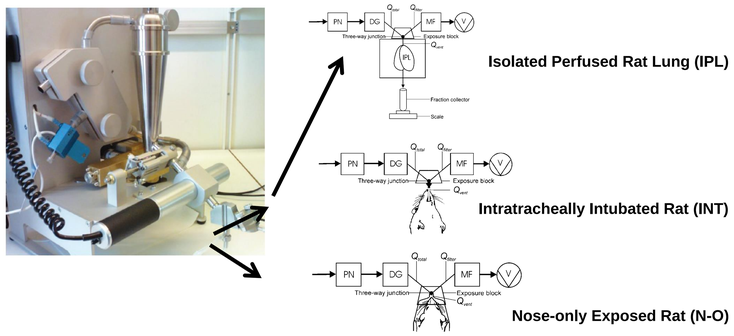

Figure 1. The PreciseInhale system with the three pre-clinical exposure modules outlined. The cone-shaped aerosol holding chamber has a volume of 300 mL.

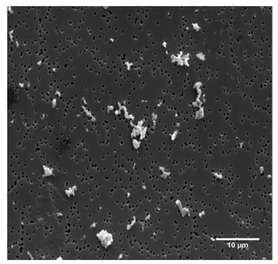

Figure 2. A SEM picture of the aerosolized CHF6001.

METHODS

- **Aerosol generation:** The PreciseInhale platform uses compressed air (up to 160 bar) to produce small volumes (300 mL) of concentrated aerosol from a few milligrams of micronized API, to be immediately dispensed to one exposure subject at a time *(Figure 1)*. During exposure, the inhaled dose is controlled by monitoring both aerosol concentration and ventilation rate of the exposed subject. Prior to exposure, the signal from a Casella Microdust Pro aerosol concentration instrument was calibrated against the inhaled mass of test substance, with the aim to directly perform target dosing in the animals without using range-finding exposures.

- **The IPL model:** Whole lungs isolated from SD rats (n=6) were ventilated with a negative alternating pressure and perfused with albumin buffer in single-pass mode. Lung perfusate was repeatedly sampled for 120 min after administration of the target lung deposited dose of 10 and 100 µg. The lung and trachea were separately collected after the end of the perfusion period.

- **The INT model:** SD rats were intratracheally intubated with a stainless steel catheter under injection anaesthesia (Hypnorm+Midazolam i.p.). Plasma PK was assessed by repeated blood sampling from femoral vein catheterized animals (n=4) after administration of the target deposited lung dose of 10 µg. In parallel, plasma and lung profile was assessed in non-catheterized animals (n=3 for each time point) after administration of two different target deposited lung doses: 10 and 50 µg. Plasma and lung profiles were compared with the levels obtained after intratracheal administration of CHF6001 as DPI by PennCentury™ device.

- **The N-O model:** SD rats were placed awake into restrainer tubes, and then connected to the PreciseInhale platform. Plasma PK was assessed by repeated blood sampling from femoral vein catheterized animals (n=4) after administration of the target deposited lung dose of 10 µg. In parallel, plasma and lung profile was assessed in non-catheterized animals (n=3 for each time point) after administration of the target deposited lung dose of 10 µg.

- **Powder administration by PennCentury™:** SD rats (n = 3 for each time point) were anaesthetized by gas anesthesia (sevoflurane) and CHF6001 DPI 1 µmol/kg was administered by intratracheal route using PennCentury device. After dosing, at fixed time points, animals were sacrificed and lung, trachea and plasma samples were collected to assess compound levels.

- **Quantification of CHF6001 and data analysis**: The concentration in all samples of perfusate, plasma, and tissues was determined using liquid chromatography/mass spectrometry (4000QTRAP MS, Applied Biosystem, coupled to Agilent HPLC 1200 Series). The pharmacokinetic parameters were calculated by non compartmental analysis.

Table 1.

Target Dose (µg)	Deposited Dose IPL		Deposited Dose INT		Deposited Dose N-O		Deposited Dose PennCentury CHF6001 DPI 1 µmol/kg[#]	
	µg in Lung	% in Trachea	µg in Lung	% in Trachea	µg in Lung	% in Trachea	µg in Lung	% in Trachea
10	19.4±9.3	3.4	8.47±1.3	4.2	12.4±6.3	4.4	17.4±6.2	57.4
50	—	—	39.2±11.7	4.6	—	—		
100	141±24	10.9	—	—	—	—		

Tracheal deposition is given in % of total lung deposition (trachea + lung)

[#] Corresponding to 20.6 µg/lung, if 10%,deposited dose is assumed

RESULTS

- Respirable aerosols were generated with a mass median aerodynamic diameter (MMAD) of 2.6 ± 0.2 (GSD = 2.1) µm *(Figure 3)*.
- Since the substance was inhaled as a highly respirable aerosol, the average fraction of substance deposited in the trachea and extrapulmonary bronchi was approximately 5% of total deposition below the larynx, in good agreement with theoretical airway deposition models (MPPD).
- Cmax and Tmax values were consistent among the IPL and INT exposure models, whereas systemic Cmax value for the N-O model was higher than for the lung-only exposure models (INT and IPL). However, it must be remembered that during nose-only inhalation, the substantial deposition of the aerosol in the nasal airways may also contribute to absorption into systemic circulation. The similar levels of Cmax obtained in the two related, but differently perfused, exposure models (INT and IPL) was also seen for the low-soluble steroid fluticasone furoate [Selg et al., 2012].
- The half-life of CHF6001 from the lungs ranged between 12 and 16.4 hours in all three exposure models *(Table 2)*.

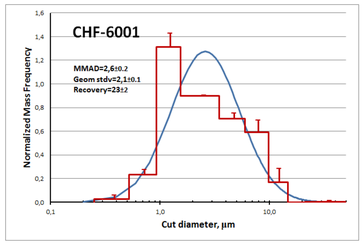

Figure 3. The aerodynamic particle size distribution of CHF6001.

Table 2.

Target Dose Parameter	IPL		INT		N-O
	10 µg	100 µg	10 µg	50 µg	10 µg
t_{max}, plasma (min)	3.0(3-3)	7.5(6-7.5)	5	5	5
C_{max}, plasma (ng/mL)	1.84±0.13	15.4±2.1	1.8	8.5	6.6
$t_{1/2}$, lung (h)	14.2±5.2	13.5±1.0	16.4	14.0	12
AUC, lung*	—	—	76.4	393.0	95.9
AUC, plasma**	—	—	2.5	32.4	8.9

For tmax median and range were considered, instead of mean and standard deviation;
*(h*µg/g) 0-24 h, **(h*ng/mL) 0-24h.

- AUClung was proportional between the two dose levels of the INT model and was in line with the dose level of the N-O model *(Table 2)*.
- Plasma AUC was higher for the N-O in comparison to INT administration, due to the possible absorption of the swallowed compound, which is much higher after N-O dosing.
- The average standard deviation in deposited doses among replicate exposures of this formulation was fairly high (± 26%). However, this precision is acceptable in lung deposition experiments, and the ability to get the expected amounts in the lung with reasonable accuracy is by far the greatest advantage.
- Total consumption of CHF6001 for aerosol characterization and lung exposures was, respectively, 49 mg for the IPL exposures (6 lungs) and 252 mg for the INT and N-O exposures (59 lungs).
- CHF6001 lung and plasma profiles in the INT model are consistent with the levels obtained after PennCentury administration of the DPI formulation; lung levels in the INT model are characterized by a lower variability, due to the greater reproducibility of administration via inhalation.

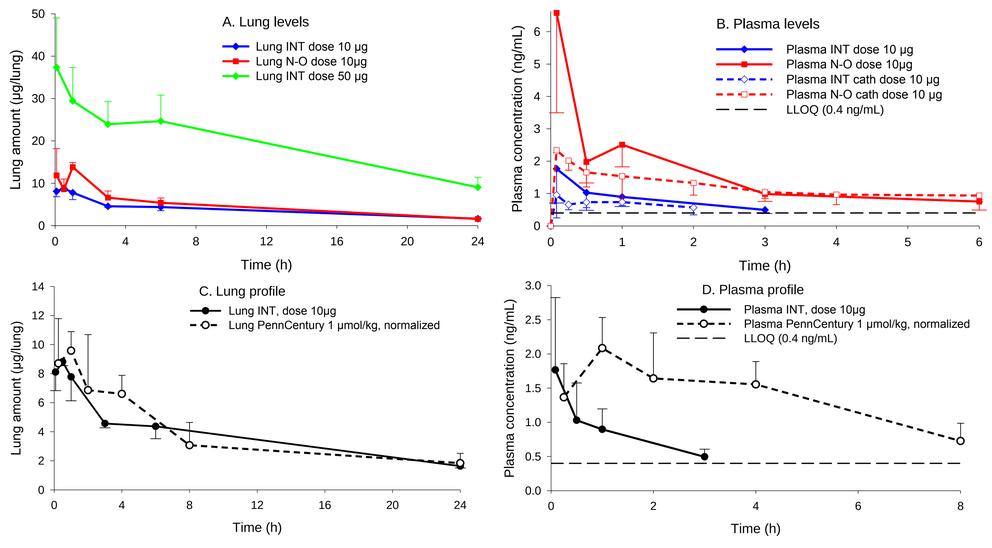

Figure 4. The pharmacokinetic data of the CHF6001 in the INT and N-O in vivo models. **A** and **B**: Lung and plasma levels obtained after administration with PreciseInhale in the N-O and INT model; **C** and **D**: Lung and plasma profiles obtained after administration with PreciseInhale and PennCentury

CONCLUSIONS

●The IPL and INT models together can be favorably used to study in detail the pharmacokinetics of inhaled compounds, in particular more slowly absorbed substances, where the lower perfusion rate of the IPL compared to the in vivo lung (INT) is not likely to make their pulmonary absorption rates differ significantly. However, for substances absorbing really fast in the lungs, the slower perfusion rate of the IPL may underestimate the absorption rate compared to the in vivo INT model.

●The PreciseInhale system enabled a suitable control of the dose delivered to the lung in all the exposure systems used, with very low amount deposited in trachea after dosing, making the system a useful tool for pharmacokinetic evaluation of inhaled compounds. This is a clear advantage in drug discovery/early development phase, where due to the poor available amount of compound, DPI formulations are unfeasible and accordingly the pharmacokinetic analysis of compound inhaled as powder becomes challenging.