Evaluating Lung Exposure and Clearance of an Inhaled Drug in Dogs by HPLC-MS/MS Analysis of Tissue or Lavage Fluid and MALDI-MSI of Lung Sections

Graham R Paul[1] and Peter S Marshall[2]

1 Toxicology and Biometabolism (In Vitro - In Vivo Translation), GlaxoSmithKline, Ware, Hertfordshire, UK
2 Ex Vivo Bioimaging UK (In Vitro - In Vivo Translation), GlaxoSmithKline, Stevenage, Hertfordshire, UK

Creation Date: 19-Dec-2018

Introduction

Blood concentrations of drugs after inhaled administration are often cited but drug persistence or location within the lung are rarely characterised [1] and their toxic potency may be associated with differences in particle clearance [2]. Non-terminal sampling of bronchoalveolar lavage fluid (BALF) or lung biopsies may provide a means of monitoring drug-lung doses of animals with repeated administration and for potential translation to clinical studies. The aims of this experiment were to investigate accumulation and persistence of an inhaled drug of low solubility (37 µg/mL in simulated lung fluid) and membrane permeability (7 nm/sec) in the lungs of dogs using high performance liquid chromatography mass spectrometry (HPLC MS/MS) and matrix assisted laser desorption/ionization-mass spectrometry imaging (MALDI MSI), and to consider the utility of BALF and biopsy sized lung tissue samples for assessing lung exposure.

Methods

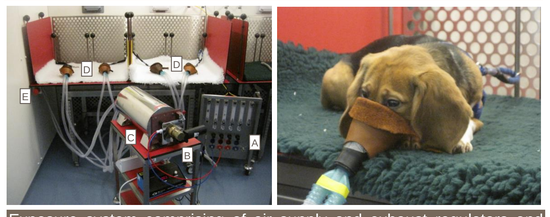
Male beagle dogs (n=2/group; 6 groups) were exposed once or for 14 days, 1 hour daily, to an aerosol (5 L/min/dog; Table 1) generated from micronised drug in lactose using a Wright dust feed [3] and mask-based exposure system (Figure 1) [4]. A clinically relevant dose of 16 µg/kg was selected (800 µg in humans scaled for species related differences in lung deposition [5, 6] and a dose of 4000 µg/kg was selected as a toxicologically relevant dose (unpublished data). Plasma was sampled 0, 1, 3, 4, 8 and 23 hours post exposure on Days 1 and 14. Immediately after a single dose and 24 hours after the 14th dose, dogs (n=2/dose/regimen) were euthanized and sampled for BALF and lung tissue. Additional dogs administered a single or repeated dose of 4000 µg/kg (n=2/regimen) were euthanized and sampled for BALF and lung after an off-dose period of 14 days.

Tab.1: Estimated inhaled doses, aerosol characterisation and toxicokinetic data in dogs

Treatment period	Inhaled dose (µg/kg)		Aerosol characterisation		Toxicokinetic parameters (plasma)		
	Target [A]	Estimated achieved [B]	Concentration (µg/L)	MMAD (GSD) [C]	AUC_{0-t} [D] (ng.h/mL)	C_{max} [D] (ng/mL)	T_{max} [E] (h)
Single dose	16	21.6	0.83	2.0 µm (2.6)	NC [NC, NC]	0.200 [0.171, 0.229]	1.0 [1.0, 1.0]
14 days		17.0	0.65		0.980 [1.78, NC]	0.512 [0.733, 0.291]	1.0 [1.0, 1.0]
Single dose	4000	4209	169	1.5 µm (2.6)	195 [136 - 270]	32.7 [24.8 - 41.2]	2.0 [1.0 - 2.0]
14 days		4220	163		164 [136 - 220]	23.5 [20.3 - 26.1]	2.0 [2.0 - 2.0]

A. Based on body weight of 10kg and inhalation exposure period of 60 minutes/day.
B. Inhaled dose calculated for a 60-minute exposure period and assuming 100% respirability [8].
C. MMAD = Mass Median Aerodynamic Diameter; GSD = Geometric Standard Deviation.
D. Dose-normalised for sampling occasion and corrected for overall group mean (Days 1-14); individual animal values in parentheses. NC = AUC_{0-t} not calculable (insufficient quantifiable data at 16 µg/kg).
E. T_{max} values (median) relative to the start of the 1-hour inhalation exposure period.

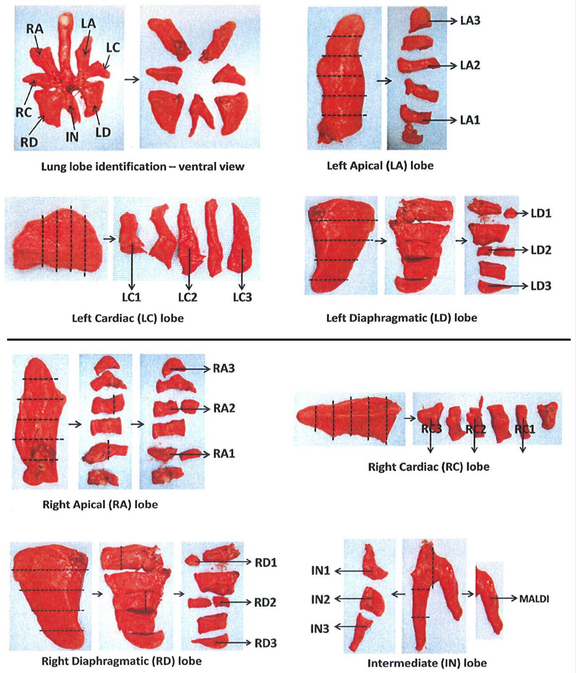
Fig.1: Snout-only inhalation exposure of dogs using a mask-based exposure system



Exposure system comprising of air supply and exhaust regulators and filtration (A), aerosol generator (B), mixing chamber (C), masks (D) and aerosol sampling port (E). With sufficient training, dogs remain calm and require minimal restraint; a harness is attached to a pole at the rear of the 'dosing table' but the dog wearing a mask can otherwise move freely.

Lung was sampled (0.5-1.0g) proximally, centrally and distally to the main airway of each lobe for homogenization and HPLC-MS/MS analysis of drug concentration; biopsy-sized samples (2-5 mg) were taken from adjacent tissue (single dose; n=1/group). The intermediate lung lobe (n=1/group) was flushed with phosphate buffered saline for HPLC-MS/MS analysis of BALF (3x 15mL, pooled for each dog) or sectioned for drug homogenate analysis and MALDI-MSI [7].

Fig.2: Sampling of lung tissue for drug-concentration analysis by HPLC-MS/MS and MALDI-MSI



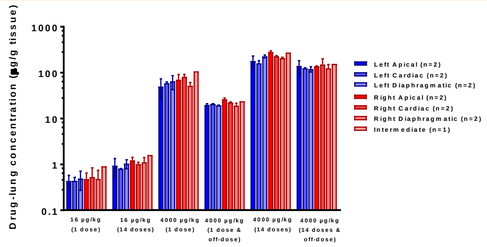
Samples taken proximally (1), centrally (2) and distally (3) to the main airway for homogenization and HPLC-MS/MS analysis of drug; biopsy-sized samples (2-5mg) taken from adjacent tissue. The intermediate lung lobe was either sampled for BALF (3x 15mL washes) or taken for drug homogenate analysis and MALDI-MSI (Marshall et al., 2010).

Results and discussion

There was no notable difference in systemic exposure (AUC_{0-t}; area under drug concentration-time curve) from Days 1 to 14 in dogs administered 4000 µg/kg (Table 1). A paucity of quantifiable data precluded calculation of AUC_{0-t} at the low dose of 16 µg/kg.

Drug-lung distribution was relatively even across lobes. Accumulation at 14 days of treatment was more pronounced at 4000 µg/kg (Figure 3). Mean lung concentrations 24 hours after the last dose were 2.0 and 3.3-fold higher than that immediately after a single dose of 16 or 4000 µg/kg respectively, indicating drug clearance from lungs during the 14-day treatment period. Drug-lung homogenate concentrations for dogs inhaling 4000 µg/kg and maintained off-dose for 14 days indicated drug persistence after cessation of treatment. After the respective off-dose periods, mean drug concentrations were 32% of the post single dose value and 65% of the post repeat dose value indicating ongoing drug clearance throughout the dose administration and off-dose periods.

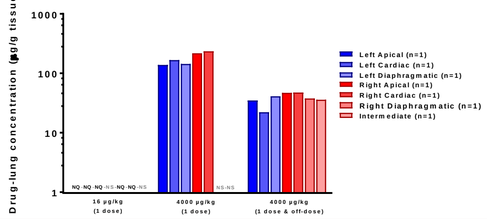
Fig.3: Drug concentrations in homogenate (µg/g lung) after inhaled administration of 16 or 4000 µg/kg to dogs



Drug distribution shown across lung lobes after single or repeated inhaled administration and after a 14-day off-dose period at the high dose

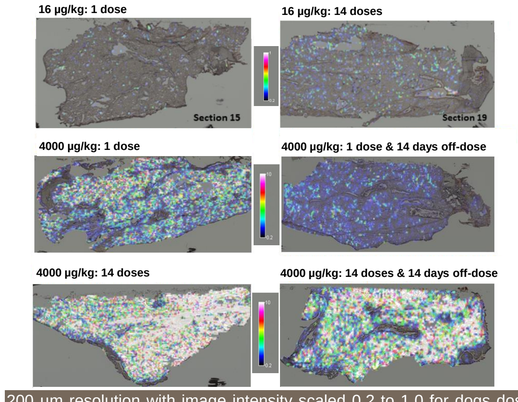
At 4000 µg/kg, mean drug concentrations in biopsy samples were 2 to 3-fold higher than corresponding lung homogenate samples, indicating analysis of small focal samples overestimated drug-lung concentrations in this experiment. Drug was not quantifiable in biopsy samples of dogs inhaling 16 µg/kg, reflecting the small mass of tissue sampled and hence a greater dilution during homogenisation of tissue compared to that for the larger samples (500 1000 mg). Nevertheless, the data show a potential for using biopsy samples to monitor trends in lung dose.

Fig.4: Drug-lung tissue concentrations in biopsy-sized samples of dogs after a single dose of 16 or 4000 µg/kg



Drug concentrations were non-quantifiable (NQ; <20 ng/mL using 25µL aliquot). NS = no biopsy sample taken from lobe.

Fig.5: MALDI-MSI 16 and 4000 µg/kg: representative ion density maps for sections of the intermediate lung lobe



200 µm resolution with image intensity scaled 0.2 to 1.0 for dogs dosed 16 µg/kg and scaled 0.2 to 10 for dogs dosed 4000 µg/kg.

Differences in drug masses recovered in BALF (data not presented) between groups were inconsistent with trends seen for lung homogenate (Figure 3) and biopsy (Figure 4) samples, suggesting BALF analysis *per se* was unreliable as a surrogate for estimating lung dose. High drug concentrations were evident in cells harvested from BALF but could not be quantified (preliminary results exceeded 100 ng/mL, the upper limit of quantification, but sample consumption precluded dilution for reanalysis).

Drug-lung tissue distribution was uniform throughout the sections of intermediate lobe examined by MALDI-MSI (Figure 5). For dogs administered 4000 µg/kg, image intensity for each treatment regimen indicated drug accumulation (with an increased signal intensity associated with major airways) after repeated administration and evidence of clearance after the off-dose period. The spatial resolution (30µm) was insufficient to permit assessment of intracellular distribution.

Conclusions

Drug distribution determined by HPLC-MS/MS of tissue homogenate and MALDI-MSI was even across lung lobes of dogs inhaling 16 or 4000 µg/kg, consistent with deposition of respirable particles.

The drug-lung accumulation with repeated administration and partial clearance after an off-dose period reflected the low aqueous solubility and membrane permeability of the molecule [4].

Drug mass in BALF, relative to tissue and biopsy samples, suggested bronchoalveolar lavage per se was not useful for estimating lung dose.

Biopsy-sized samples overestimated drug-lung concentrations but nevertheless showed a potential application for monitoring trends in lung concentration at toxicologically relevant doses. However, the HPLC-MS/MS method was insufficiently sensitive to quantify drug in biopsies at a clinically relevant dose.

Acknowledgements

The authors thank Navin Sonahee (formerly of GSK-DMPK) for HPLC-MS/MS analysis of lung and BALF samples for drug concentration.

References

1. Patton JS, Brain JD, Davies LA, Fiegel J, Gumbleton M, Kim K-J, Sakagami M, Vanbever R, Ehrhardt C: The particle has landed - characterizing the fate of inhaled pharmaceuticals. *Journal of Aerosol Medicine and Pulmonary Drug Delivery* 2010, 23:S71-S87.
2. Pauluhn J: Inhalation toxicology: methodological and regulatory challenges. Experimental and Toxicologic Pathology 2008, 60:111-124.
3. Wright B: A new dust-feed mechanism. *Journal of Scientific Instruments* 1950, 27:12.
4. Paul GR: Defining dosimetry and implications for aerosol presentation for non-clinical development of respiratory drugs. *Doctoral dissertation, Cardiff School of Pharmacy and Pharmaceutical Science, Cardiff University* 2017.
5. Degeorge JJ, Ahn CH, Andrews PA, Brower ME, Choi YS, Chun MY, Du T, Lee-Ham DY, McGuinn WD, Pei L: Considerations for toxicology studies of respiratory drug products. *Regulatory Toxicology and Pharmacology* 1997, 25:189-193.
6. Jones DR, Baldrick P: Association of Inhalation Toxicologists' (AIT) review of regulatory aspects for inhalation toxicology studies. *Inhalation Toxicology* 2013, 25:84-90.
7. Marshall P, Toteu-Djomte V, Bareille P, Perry H, Brown G, Baumert M, Biggadike K: Correlation of skin blanching and percutaneous absorption for glucocorticoid receptor agonists by matrix-assisted laser desorption ionization mass spectrometry imaging and liquid extraction surface analysis with nanoelectrospray ionization mass spectrometry. *Analytical chemistry* 2010, 82:7787-7794.
8. Alexander DJ, Collins CJ, Coombs DW, Gilkison IS, Hardy CJ, Healey G, Karantabias G, Johnson N, Karlsson A, Kilgour JD: Association of Inhalation Toxicologists (AIT) working party recommendation for standard delivered dose calculation and expression in non-clinical aerosol inhalation toxicology studies with pharmaceuticals. *Inhalation Toxicology* 2008, 20:1179-1189.

All animal studies were ethically reviewed and performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals