

Lung Deposition and Absorption of Insulin from Technosphere® Insulin

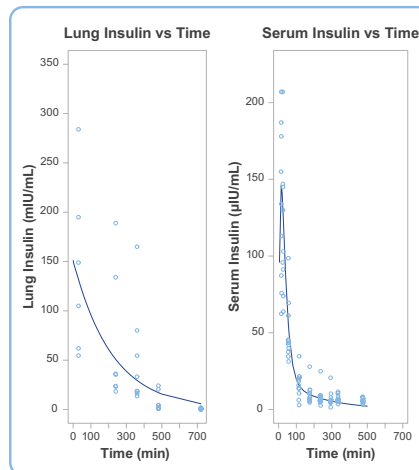
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ABSTRACT

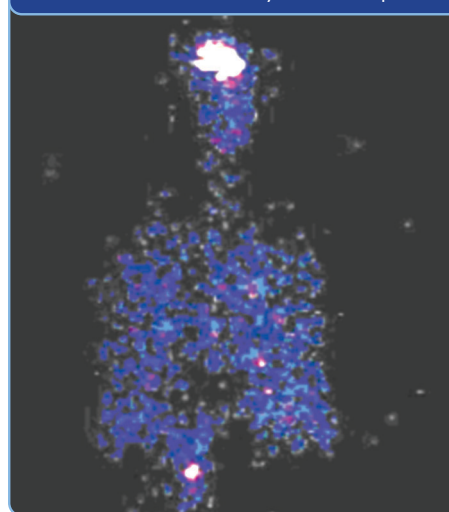
Background and aims: A clinical trial in 12 healthy subjects determined the pulmonary concentrations of insulin and fumaryl diketopiperazine (FDKP) using bronchoalveolar lavage (BAL) before and after administration of a rapid-acting insulin, Technosphere® Insulin (TI) Inhalation Powder. **Materials and methods:** Each subject received a 60 U dose and had two bronchoscopies at 0.5 and 6 h, or 4 and 8 h, or 0.5 h before dosing and 12 h after dosing. At each bronchoscopy, two independent BAL samples were collected. Serum samples for serum insulin, FDKP, and serum C-peptide were collected at baseline and up to 475 minutes post-dose. Urea concentrations in BAL samples and blood urea nitrogen were measured and used for correcting BAL concentrations (see figure below). **Results:** We previously reported a phase 1 trial using ^{99m}Tc-labeled TI inhalation particles. These particles were evenly distributed with approximately 28% to 48% (mean 39.4%) of the ^{99m}Tc TI delivered to the deep lung. In this study, subjects were dosed with 60 U of TI. Concentrations of insulin and FDKP in BAL fluid were corrected for urea BAL to serum concentration ratios. The amount of insulin and FDKP remaining in the lung epithelial lining fluid was highest (21.5 mIU/mL) in the first post-dose BAL samples (0.5 h). By 4 h, levels were 31% of maximum; at 12 h, insulin and FDKP values in BAL were 0.3% and 0.4% of maximum, respectively. Accumulation of insulin and FDKP in the lungs after chronic dosing is unlikely. **Conclusion:** Preliminary pharmacokinetic modeling of insulin (see solid line figure below) suggests a two-compartment lung absorption model, one with fast absorption reflected in the plasma profile of insulin. The other compartment reflects reduced absorption, which is presumably due to proteolysis and mucociliary clearance of insulin.



BACKGROUND AND AIMS

Whenever a substance is introduced into the body, the duration of exposure both in terms of full body exposure and for inhaled medications, the duration of exposure in the lungs are of clinical importance. A study conducted by MannKind Corporation with Technicium^{99m}-labeled TI Inhalation Powder demonstrated the powder was uniformly distributed within the lungs (Figure 1). Using gamma scintigraphy, the estimated percentage delivered to the lungs was approximately 40% of the cartridge load. In order to determine the duration of TI in the lung, MKC-TI-122 was conducted using 60 U of TI Inhalation Powder administered to healthy subjects. At times both predose (to ensure no inherent insulin concentration) and times out to 12 hours post-dose, the concentration of the two major components of TI (insulin and FDKP) were measured in BAL fluid. The urea dilution technique was used to estimate the total amount of these two components within the lungs out to 12 hours after dosing.

Figure 1. Scintigraph of ^{99m}Tc-labeled TI Inhalation Powder Following Inhalation to a Healthy Normal Subject



MATERIALS AND METHODS

This was a Phase 1, open-label, controlled clinical trial to determine the pulmonary concentrations of insulin and FDKP, using BAL before and after administration of TI Inhalation Powder, in up to 13 healthy subjects. The trial consisted of 3 clinic visits: 1 screening visit, 1 treatment visit, and 1 follow-up visit. All subjects were given 60 U of TI and had two BALs performed at:

Cohort 1: 30 minutes and 6 hours after dosing,

Cohort 2: 4 hours and 8 hours after dosing

Cohort 3: 30 minutes before (ie, -30 minutes) and 12 hours after dosing.

In each subject, the bronchoscope was wedged into the right middle lobe of the lung for the first time point and 50 mL of normal saline was instilled and withdrawn to obtain BAL fluid (Sample 1). With the bronchoscope still in place, a second 50 mL of normal saline was instilled and withdrawn (Sample 2); ie, 2 separate BALs were performed on the same lung segment at each collection time. At the second time point, BAL sampling was performed similarly but from the left lower lobe of the subject's lung.

Blood samples for serum insulin and serum C-peptide were collected at treatment, at the following time points: 30 minutes before dose administration, immediately before TI Inhalation Powder administration (t = 0), and 15, 25, 55, 115, 175, 235, 295, 355, and 475 minutes after dose administration. FDKP was analyzed from aliquots of this serum with the exception of the -30 minute sample. Serum samples for urea concentrations were obtained 5 minutes before each bronchoscopy. All serum samples were stored frozen at the clinical site until shipment for analysis. Assay for FDKP in both serum and BAL fluid was the standard validated method (LC-MS/MS) used throughout all MannKind trials. Serum insulin was analyzed by the double antibody CILA method (Roche E170 platform) used for all later stage MannKind trials. Concentrations of insulin in BAL fluid were quantified using Linco human insulin ELISA kit (EZHI-14k) that used a single polyclonal antibody reaction step (reactivity with insulin degradants was not available from the manufacturer).

The amount of insulin and FDKP in the epithelial lung fluid (ELF) at any measured time, was estimated using the following equation modified from Gotfried, et al.¹

$$Conc_{Insulin / FDKP_{ELF}} = Conc_{Insulin / FDKP_{BAL}} \times \frac{Urea_{Blood}}{Urea_{BAL}}$$

The total amount of analyte in the lungs was further estimated using the mean of literature estimates of ELF, 60 mL.^{2,3}

RESULTS

One subject was excluded from the 12-hour BAL analysis prior to analytical analysis of their BAL samples due to site-reported difficulty in obtaining the 12-hour bronchoscopy sample and a new subject was added to provide 4 subjects for all time points. Figure 2 shows the urea corrected concentration in the lungs (mIU of insulin per mL of epithelial lining fluid) as well as the exogenous serum insulin concentration (calculated via C-peptide correction) following the administration of 60 U of TI Inhalation Powder. The PK profile is in agreement with values seen throughout the clinical development program for TI. Table 1 shows the mean BAL results for insulin and Table 2 displays the FDKP results. All predose samples were below the lower limit of quantification (BQL) for insulin (< 2 µIU/mL) and FDKP (< 2.5 ng/mL). The highest concentrations in BAL fluid were observed at the first post-dose time point (30 minutes post-dose). This time point is already after the t_{max} (15 minutes) for both analytes and hence is likely to be an underestimation of the initial analyte load within the lungs. Comparison of the AUC for serum FDKP with the value obtained following IV administration in the clinical study MKC-T-123 would indicate a 40% bioavailability or approximately 7 mg of FDKP getting to the lungs. This is substantially higher than the value reported at 30 minutes (1.7 mg, Table 2) and likely reflects very rapid early concentration dependent absorption from the lungs (ie, within the first 30 minutes after inhalation). By 4 hours after inhalation of TI, the amount of insulin

RESULTS (CONT'D)

and FDKP in the lungs as estimated from the urea-corrected BAL fluid concentrations had dropped to less than 30% and 45% of the respective concentrations obtained at 30 minutes. The clearance from the lungs continues such that by 8 hours approximately 3% of the insulin and 7% of the 30-minute FDKP remains in the lungs. By 12 hours postinhalation of TI, concentrations of insulin and FDKP in recoverable BAL fluid is near the limit of quantification for each of the analytes.

Figure 2. Concentration of Insulin in Epithelial Lung Fluid and Serum (C-peptide Corrected)

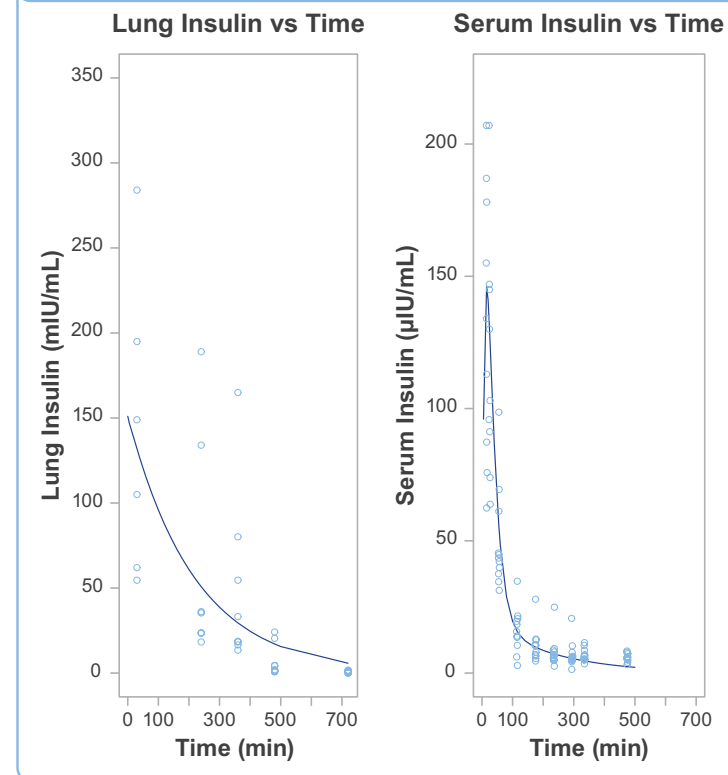


Table 1. Summary of BAL Insulin Concentration in Epithelial Lung Fluid Corrected for Serum Urea/BAL Fluid Urea Ratio (Per Protocol Population)

Parameter	Time Points					
	Within 30 min Before Dose (Cohort 3)	30 min (Cohort 1)	240 min (Cohort 2)	360 min (Cohort 1)	480 min (Cohort 2)	720 min (Cohort 3)
N*	10	8	7	8	8	8
% of Dose		21.5	6.6	5.0	0.73	0.07
Mean (mIU/mL)	BQL	214.7	65.6	50.0	7.30	0.660
Mean (U/lung) ^a		12.9	3.94	3.00	0.438	0.040
CV (%)	NA	74.7	95.5	104	128	100

N* = total number of samples (maximum of 2 samples/subject/time point)
a = assumes 60 mL of EFT

RESULTS (CONT'D)

Table 2. Summary of BAL FDKP Concentration in Epithelial Lung Fluid Corrected for Serum Urea/BAL Fluid Urea Ratio (Per Protocol Population)

Parameter	Time Points					
	Within 30 min Before Dose (Cohort 3)	30 min (Cohort 1)	240 min (Cohort 2)	360 min (Cohort 1)	480 min (Cohort 2)	720 min (Cohort 3)
N*	10	8	7	8	8	8
% of Dose		8.3	3.6	1.0	0.58	0.02
Mean (µg/mL)	BQL	27.7	12.0	3.32	1.93	0.089 ^b
Mean (mg/lung) ^a		1.66	0.72	0.20	0.12	0.004
CV (%)	NA	75.2	101	145	114	95.4

N* = total number of samples (maximum of 2 samples/subject/time point)
a = assumes 60 mL of EFT b = BQL was replaced with Lower Limit of Quantification (LLOQ/2)

CONCLUSIONS

- Greater than 70% of the insulin and 55% of the FDKP that gets to the lungs is cleared from the lungs within 4 hours of administration.
- By 12 hours, the concentration of insulin and FDKP in BAL fluid approaches the limit of detection.
- By 12 hours, the amount of insulin and FDKP extractable from the lungs via BAL is less than 0.1% of the total cartridge load.

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