GLYCEMIC PROFILE IN RATS AFTER PULMONARY ADMINISTRATION OF AN INSULIN DRY POWDER

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INTRODUCTION

Afrezza® (Mannkind, US), is a powder for inhalation containing a 18% of recombinant human insulin and it is available in different IU strength. Afrezza® has to be stored at 2-8°C and it must be used within 72 hours from blister opening. A pure insulin pulmonary powder produced by spray drying (Ins_SD) was developed and patented by the Food and Drug Department, University of Parma [1,2]. In this powder, the percentage of decomposition products (A21, ORP and HMWP) was found to be below the USP limits (dotted line) for the 6 months of the investigation when the powder was filled in Quali-V®-I capsules, sealed in triflex blister and stored at room temperature (25°C-60% RH) [3].

The aim of this study was to investigate the chemical stability and respirability of an insulin spray-dried powder (Ins_SD) loaded in blister-packed capsules. The in vitro respirability of Ins_SD was compared to one of commercial products, Afrezza®. Finally, the glycemic plasma profile in rats was measured after pulmonary insufflation of Ins_SD and Afrezza® at a dose of 10 IU/Kg

EXPERIMENTAL METHODS

Ins_SD was prepared by spray drying using a mini Spray Dryer Büchi B-290 (Büchi®, CH). HPMC capsules size 3 (Quali-V®-I, Qualicaps Europe, ES) were semi-automatically filled with 2 mg of Ins_SD powder. The *in vitro* respirability was assessed using the Next Generation Impactor (NGI) (Copley Scientific, UK) and RS01® high resistance inhaler (Plastiape, Italy) to aerosolize the formulation. The *in vivo* study was conducted in male Wistar rats (Charles River, LC, Italy) and the glycemic plasma profile (Glucose Oxidase Werfen) was determined after pulmonary insufflation of Ins_SD and Afrezza® powders. Ins_SD and Afrezza® were blended with mannitol spray dried (ins at 4% w/w) to achieve a mass of powder sufficient to be loaded in the device. The insulin powders were intratracheally (IT) administered (n=9) using a powder device DP-4 insufflator_TM (Penn-Century, Inc, US). For SC administration insulin was dissolved in 0.01 N HCl/saline 1:9 (solution pH= 4.7). Blood samples and BALF were collected at different time points after peptide administration and the pharmacokinetic profile (ELISA assay) and local inflammation of the powder were evaluated.

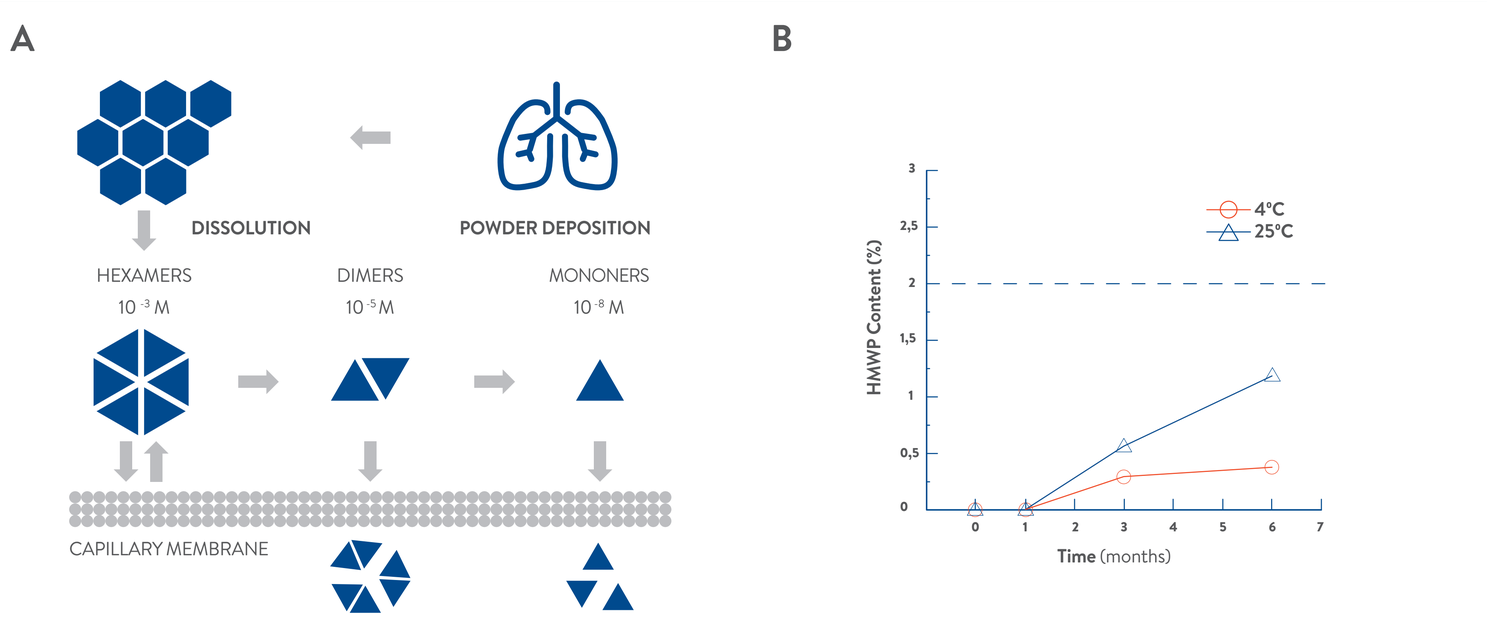

Figure 1.
A. The schematic process of powder dissolution, hexamers dissociation and monomers absorption across capillary membrane is reported.

B. HMWP content of insulin spray-dried powders INS_SD stored at room temperature (25 °C, 60% RH) for six months (mean ± standard deviation, n = 3). USP limits are represented by the dotted lines.

RESULTS

RESPIRABILITY	PHARMACOKINETIC -PHARMACODYNAMIC PROFILE	INFLAMMATORY EVALUATION

Table 1. Aerodynamic parameters of Ins_SD and Afrezza® powder (8 IU cartridge) (n=3).

	Metered Dose (mg)	Emitted Dose (mg)	MMAD (µm)	FPD (mg)	FPF (%)
Ins_SD	1.62 ± 0.08	1.50 ± 0.05	0.89 ± 0.09	1.37 ± 0.04	91.5 ± 5.9
Afrezza®	0.64 ± 0.00	0.60 ±0.00	3.19 ± 0.03	0.41 ± 0.01	68.3 ± 0.6

Ins_SD powder showed a high respirability, considering that the delivered dose was >95% and the FPF<5 µm of 91%. Afrezza® powder, tested for comparison, was successfully emitted from its Gen2 device (more than 95%) and FPF was 68.3%.

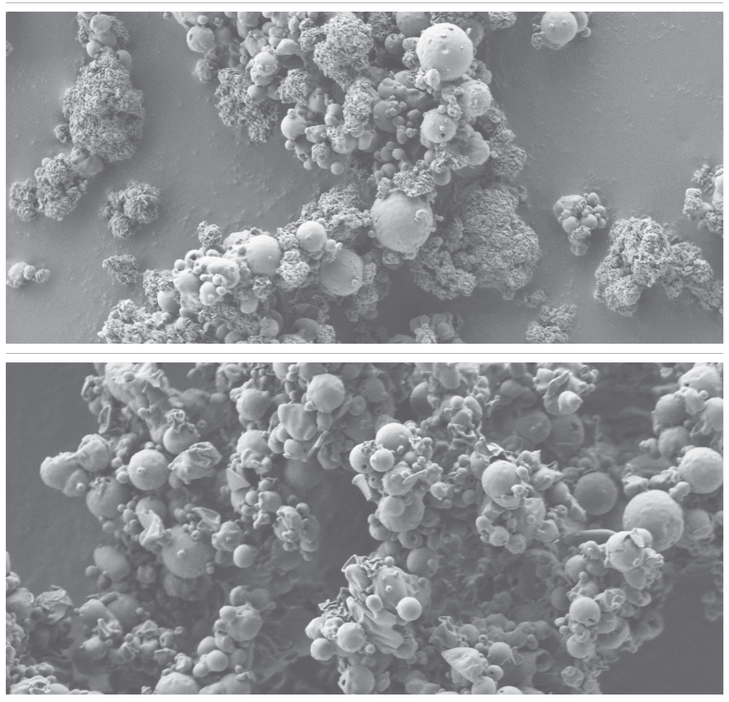

Figure 2. Scanning electron microscopy pictures of Afrezza® -mannitol (**A**) and Ins_SD-mannitol (**B**) powder blends.

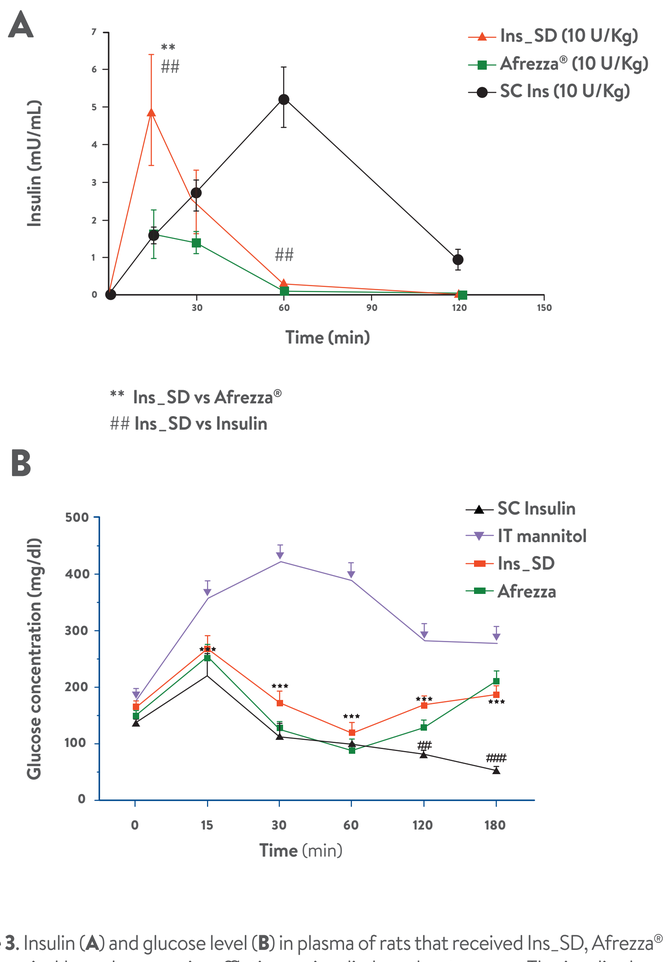

Figure 3. Insulin (**A**) and glucose level (**B**) in plasma of rats that received Ins_SD, Afrezza® and mannitol by pulmonary insufflation or insulin by subcutaneous. The insulin dose administered was 10 IU/Kg per animal. Data are expressed as mean ± SD (n=9).
•••P<0.001, two-way Anova, Bonferroni post test.
##P<0.01; ### P<0.001 two-way Anova, Bonferroni post test.

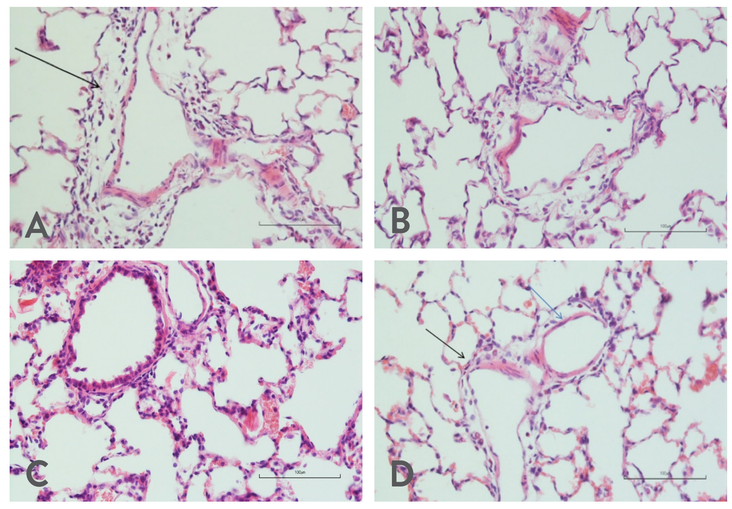

Figure 4. Lung tissue 3h post administration of the different formulations (magnification: 20x hematoxylin-eosin). **A**: mannitol; **B**: insuline S.C. ; **C**: Ins_SD ; **D**: Afrezza®. The histologycal analysis showed that there was no inflammation in the lung tissue after insulin administration.

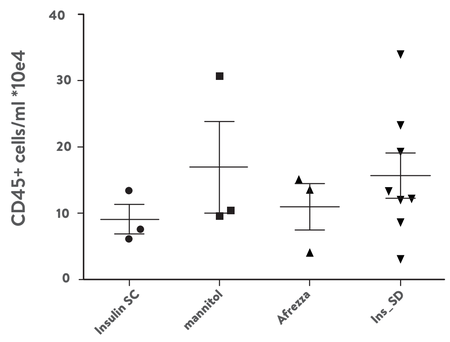

Figure 5. Leukocytes profile (cell/ml x 10^4) in the BALF (n=9) after intratracheal vs SC insulin administration. There is significant difference SC and intratracheal between groups ($p < 0.01$). The local inflammation was attributed to the mechanical insertion of the device needle.

CONCLUSION

Ins_SD presented a very favourable respirability (FPF 91%). *In vivo* data showed that Ins_SD provided a rapid glucose reduction, similar to Afrezza®. Compared to Afrezza®, Ins_SD showed a more rapid absorption, with a significantly higher C_{max} ($P<0.01$). As expected, both inhaled insulin powders were more rapidly bioavailable than SC Ins, and more rapidly eliminated. Moreover, SC Ins showed a higher AUC with respect to IT administered powders, resulting in a longer lasting hypoglycaemic effect due to the prolonged insulin concentration in the plasma.

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