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

Francesca Buttini1, Veronica Chierici1, Eride Quarta1, Lisa Flammini1, Adriana Rocha Clementino1, Andrea Grandi1

Susanna Ecenarro2, Massimiliano Tognolini2, Elisabetta Barocelli2, Paolo Colombi1 & Ruggero Bettini1

1 Food and Drug Department, University of Parma, Parco area delle Scienze 27a, 43124, Parma, Italy
2 Qualicaps Europe, S.A.U., Alcobendas, Madrid, Spain

*Email: francesca.buttini@unipr.it*

**INTRODUCTION**

Afrezza® (McNeilking, US) is a powder for inhalation containing a 15% of recombinant human insulin and it is available in different IU strengths. Afrezza® has to be stored at 2-8°C and it must be used within 72 hours from blister opening. A pure multi-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 paper, the percentage of decomposition products (A2I, 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® capsules, sealed in Inflex 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 glycaemic plasma profile in rats was measured after pulmonary insufflation of Ins_SD and Afrezza® at a dose of 10 IU/Kg.

**EXPERIMENTAL METHODS**

Ins_SZ was prepared by spray drying using a mini Spray Dryer Buchi B-290 (Buchi®, 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 (Plastipak, Italy) to aerosolize the formulation. In vivo study was conducted in male Wistar rats (Charles River, LC, Italy) and the glycaemic plasma profile (Glucose Codiesa Werfen) was determined after pulmonary insufflation of Ins_SD and Afrezza® powders. Ins_SD and Afrezza® were blended with mannitol spray-dried (Ins 4% w/v) to achieve a mass of powder sufficient to be loaded in the device. The insulin powders were immediately loaded into a (IT) administered (n=9) using a powder device DP-4 insufflator® (T Mor-Ventury, Inc, US). For SC administration insulin powders were intratracheally (IT) administered (n=9) using a powder device DP-4 insufflator® (T Mor-Ventury, Inc, US). HPMC capsules size 3 (Quali-V®-I, Qualicaps Europe, ES) were semi-automatically filled with 2 mg of Ins_SD powder. The aerodynamic parameters of Ins_SD and Afrezza® formulations were measured using the Next Generation Impactor (NGI) (Copley Scientific, UK). The 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**

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

**Table 1.** Aerodynamic parameters of Ins_SD and Afrezza®-powder (IU/Kg/mL solution) (n=3).

<table>
<thead>
<tr>
<th>Ins_SD</th>
<th>Dose (mg)</th>
<th>MMAD (µm)</th>
<th>Standard Deviation</th>
<th>FPF %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ins_SZ</td>
<td>600</td>
<td>3.19 ± 0.03</td>
<td>0.41 ± 0.01</td>
<td>3.39 ± 0.96</td>
</tr>
<tr>
<td>Afrezza®</td>
<td>300</td>
<td>3.64 ± 0.05</td>
<td>0.46 ± 0.02</td>
<td>3.32 ± 0.96</td>
</tr>
</tbody>
</table>

**Figure 3** Insulin (mU/mL) and glucose level (mg/dl) 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 ± standard deviation (n=9). **A:** Ins_SD vs Afrezza®; **B:** Insulin SC - Ins_SD;  **C:** Ins_SD vs mannitol. The histological analysis showed that there was no inflammation in the lung tissue after insulin administration.

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

**Figure 5** Leukocytes profile (x10⁶/ml) in the BALF (n=9) after intratracheal insulin SC administration. There is significant difference SC and intratracheal between groups (P<0.05). 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 Cmax (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.

**REFERENCES**


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