Pharmacokinetic Profile Investigation of Pulmonary Semaglutide Engineered Powder

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Summary

The study highlights that an inhalable formulation of the glucagon-like peptide-1 (GLP-1), semaglutide, could represent a promising alternative to the subcutaneous route. The inhalation route provides rapid systemic absorption while mitigating gastrointestinal degradation typically seen with peptide hormone delivery. This method could enhance patient compliance and outcomes by allowing for more convenient dosing regimens.

In the spray drying production our lead formulation candidate was prepared using trehalose and leucine as excipients, as previously described. Production yield was high (> 75%) and the FPF was about 43%.

The MTT analysis demonstrated that the semaglutide peptide is non-toxic to A549 cell lines at concentrations up to 64 µg/mL. Pharmacokinetic (PK) studies in rats revealed distinct absorption profiles, with intratracheal (IT) administration yielding a maximum concentration (Cmax) of 0.341 µg/mL at 3 hours, whereas subcutaneous (SC) administration resulted in a Cmax of 0.406 µg/mL at 4 hours. Even though, the AUC of the IT administered lead spray dried candidate was about 20% of what observed for the peptide SC administration, SMG was promptly absorbed through the lungs and the data set collected was in line with what reported in other recent works.

Key Message

This work demonstrates that an engineered powder containing Semaglutide could be successfully prepared by spray drying and that when administered intratracheally (400 µg/kg) to rats it was promptly absorbed with an AUC of 2.44 µg/mL*h.

Introduction

Glucagon-like peptide-1 (GLP-1) is an incretin hormone that plays a critical role in glucose metabolism and has garnered significant interest for its potential in inhalable formulations [1]. Inhalation as a route of administration may provide rapid systemic absorption while bypassing some of the gastrointestinal degradation that typically affects peptide hormones, offering a novel approach for delivering GLP-1 agonists to patients with type 2 diabetes and other metabolic conditions. To formulate the GLP-1 agonist as a dry powder for inhalation, spray drying could be useful to allow particle engineering but may degrade the peptide and have negative effects on its stability.

Among GLP-1 receptor agonists, semaglutide (SMG) is less sensitive to enzymatic degradation, with a half-life of 165h, marketed for the treatment of type 2 diabetes and weight loss both as subcutaneous or oral administration.

It was previously demonstrated that SMG was chemically stable when solubilised both in TRIS and phosphate buffer [2]. However, the powder produced showed significant differences in the aerodynamic performance: TRIS buffer was worsening in all cases it was used. Moreover, the investigation of the type of bulk excipient highlighted that trehalose was the best material to maximize the respirability of the products [3].

The aim of this project was to evaluate the lead SMG-engineered powder formulated with trehalose and phosphate buffer, both *in vitro* and *in vivo*, to assess its toxicity on the A549 cell line and to determine its pharmacokinetic (PK) profile in rats, in comparison with subcutaneous (SC) administration of SMG.

Methods

SMG (Hangzhou Go Top Peptide Biotech Co., China) and excipients were dissolved in phosphate buffer (pH= 7.4) and inhalation powders were produced employing a Mini Spray Dryer (B-290, Buchi, CH). The process parameters set up were feed rate of 3.5 mL/min, air flow rate of 600 L/h, aspiration of 35 m^3/h and inlet temperature of 135°C. It is not currently possible to disclose the qualitative and quantitative composition of the powder. BioHale®trehalose dihydrate (DFE Pharma, DE) was chosen as the bulk excipient. Reverse phase high pressure liquid chromatography (RP-HPLC), Gel-Filtration Chromatography (GF-HPLC) and mass spectroscopy (MS) were used to investigate the SMG concentration, formation of aggregates and eventual chemical degradation of the peptide sequence. Residual water content and solid state were characterized by Thermogravimetric Analysis (TGA, Mettler Toledo, USA) and Dynamic Vapour Sorption (DVS, ProUmid, DE), respectively. The aerodynamic parameters of emitted fraction (EF), mass median aerodynamic diameter (MMAD), fine particle mass (FPM< 5 µm), fine particle fraction (FPF) and extra-fine particle fraction (EFPF) were investigated employing a Next Generation Impactor (NGI, Copley Scientific Ltd, UK). For the analysis, a capsule of hydroxypropyl methylcellulose (HPMC) size 3 (Roquette, FR) filled with 20 mg of powder was aerosolized in the NGI using a RS01 (Plastiape, IT) mid resistance device activated at 65 L/min to reach a 4 kPa pressure drop. SMG deposited in each stage of the impactor was quantified by RP-HPLC. Particles morphology was assessed by Scanning Electron Microscopy (SEM, Carl Zeiss, DE). An MTT assay was carried out on A549 cell line to assess the cells viability when treated with SMG. The powder was dissolved in cell medium to obtain different SMG concentrations in the range 2-64 µg/mL and the cells were treated for 24h. Viability above 80% was considered acceptable (ISO 10993–5).
For PK studies SMG were dosed via SC or intratracheal (IT) administration to Sprague-Dawley rats. For the SC study, SMG was solubilized in PBS ad injected at the dose of 100 nmol/kg (= 400 µg/kg). The SMG formulated as a dry powder was administered IT at the same dose. For the IT administration, the animals were lightly anesthetized with 5% isoflurane, lane back at an angle of 45/50° and, using a small laryngoscope, a catheter (BD Insyte 22G, Becton, Dickinson & Co., USA) was introduced in the trachea through which the device Penn-Century Dry Powder Insufflator TM (Model DP-4 M, Penn-Century, USA) was inserted. The powder loaded in the insufflator was delivered into the lungs by the discharge of 4 mL of air.
Blood samples (200 µL each) were collected in heparinized test tubes at the following time-points: 1) for SC at 0, 0.5, 1, 2, 4, 7, 11, 24, 36, 48, 72h and 2) for the IT at 0, 0.25, 0.5, 0.75, 1, 2, 4, 7, 11, 24, 36h post-administration. Blood samples were centrifuged to collect the serum that was analysed to determine the concentration of SMG by ELISA kit (KRIBIOLISA™ semaglutide (Ozempic™) ELISA by KRISHGEN BioSystem) according to manufacturer instruction.

Results

A SMG spray-dried powder was produced. Trehalose was employed as bulking agent excipient and L-leucine was added to increase the powder respirability.

Table 1. Process yield and aerodynamic parameters obtained by NGI for the SMG lead powder produced (n=3, mean ± standard deviation).

Product	Yield (%)	Aerodynamic Performance			
		EF (%)	MMAD (µm)	FPF (%)	EFPF (%)
SMG lead powder	79.1	94.5±5.9	4.48±0.04	43.6±2.6	20.6±2.6

The production yield was sufficiently high (> 75%) (**Table 1**) to consider the process efficient and scalable. The SMG content assessed by RP-HPLC was in the range between 95 and 125% in respect to the theoretical value indicating that no peptide was lost during the spray drying process. Moreover, as determined by GF-HPLC and MS SMG did not report any aggregation or degradation in the powder prepared.

From the DVS analysis it was highlighted that the mass of the powder did not return to their original dry weight after the humidity was cycled back down. The retention of water is well documented for typically amorphous structures where the water is entrapped into the particle matrix. The moisture content of the

powder was about 3.5% w/w, as assessed by TGA. The NGI analysis indicated that the mass of powder loaded in the device (20 mg) was efficiently delivered (EF > 90%) and aerosolised (FPF ~ 43%).

The lead spray dried powder reached the stage 8 of the instrument, leading to an extra fine particle fraction (< 2 µm) of about 20%. This value is very favourable considering that the target delivery area of the formulation is the alveoli where the peptide may be absorbed.

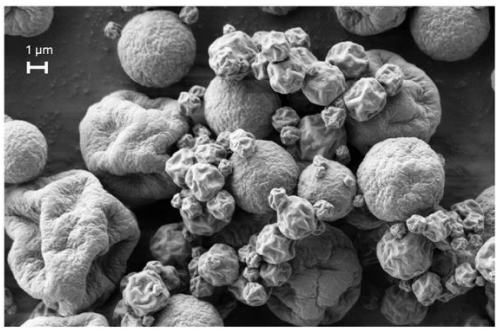

Figure 1. SEM pictures at 10.000X of SMG lead powder.

The morphological analysis showed (**Figure 1**) particles of 3-4 µm in size, with a rounded shape and a crumpled surface.

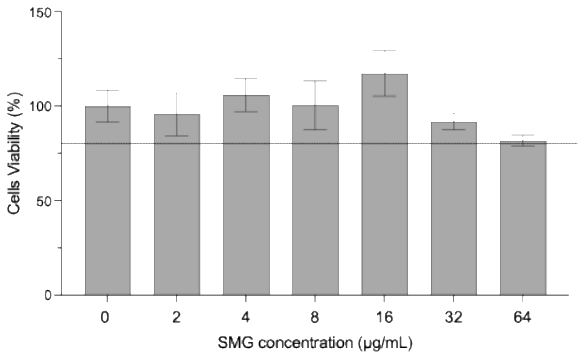

Figure 2. A549 cells viability (%) after treatment with resuspended spray-dried powder to obtain different SMG concentrations (n=3, mean ± std deviation).

The MTT analysis carried out on a wide range of SMG concentrations, showed that the peptide is well tolerated and not toxic on A549 cell line up to a 64 µg/mL concentration investigated (**Figure 2**). The variability observed in the experiment is in line to that commonly observed for cell culture MTT test.

From the PK study, the plasma concentration profile of SMG indicates that formulation administered via IT route was rapidly absorbed, reaching a Cmax of 0.341 µg/mL at a Tmax of 3 hours, (**Table 2**). When administered SC, SMG reached a Cmax of 0.406 µg/mL at a Tmax of 4 hours.

As expected, the depot effect of SC injection resulted in a prolonged and delayed exposure to SMG, as reflected in the PK profile. This translated into an AUC statistically significantly higher than that observed after IT administration (**Table 2**).

It is well known that pulmonary delivery may result in partial dose loss within the device, whereas SC administration delivers the full dose directly into the tissue, contributing to the observed differences in systemic exposure. The data obtained were comparable with other two recent works [2,3], as the SMG was promptly absorbed but the bioavailability was lower with respect to the SC administration, as expected.

Table 2. Main SMG pharmacokinetic parameters in rats after administration via SC injection (n = 3) or IT insufflation (n = 4) of a dose of 100 nmol/kg of SMG (corresponding to 410 µg/kg) (mean ± std deviation).

	SC	IT
AUC (µg/mL*h)	12.66 ± 5.18	2.44 ± 1.57
Cmax (µg/mL)	0.406 ± 0.066	0.341 ± 0.105
Tmax (h) Median (range)	4 (2-4)	3 (0.75-4)

Conclusions

In summary, inhaled GLP-1 represents a promising advancement in weigh management, aligning with the growing trend of non-invasive delivery systems for peptide-based therapies. Future studies will be dedicated to investigating the drug's effectiveness on weight loss in animals for an observation period of at least one week.

References

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