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Research Article

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Biologic Comparison of Inhaled Insulin Formulations: Exubera™ and Novel Spray-Dried Engineered Particles of Dextran-10

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Abstract. Inhaled peptides and proteins have promise for respiratory and systemic disease treatment. Engineered spray-dried powder formulations have been shown to stabilize peptides and proteins and optimize aerosol properties for pulmonary delivery. The current study was undertaken to investigate the *in vitro* and *in vivo* inhalation performance of a model spray-dried powder of insulin and dextran 10 in comparison to Exubera™. Dextrans are a class of glucans that are generally recognized as safe with optimum glass transition temperatures well suited for spray drying. A 70% insulin particle loading was prepared by formulating with 30% (*w*/*v*) dextran 10. Physical characterization revealed a "raisin like" particle. Both formulations were generated to produce a similar bimodal particle size distribution of less than 3.5 μm MMAD. Four female Beagle dogs were exposed to each powder in a crossover design. Similar presented and inhaled doses were achieved with each powder. Euglycemia was achieved in each dog prior and subsequent to dosing and blood samples were drawn out to 245 min post-exposure. Pharmacokinetic analyses of post-dose insulin levels were similar for both powders. Respective dextran 10-insulin and Exubera exposures were similar producing near identical area under the curve (AUC), 7,728±1,516 and 6,237±2,621; concentration maximums (C_{max}), 126 and 121 (μU/mL), and concentration–time maximums, 20 and 14 min, respectively. These results suggest that dextran-10 and other dextrans may provide a novel path for formulating peptides and proteins for pulmonary delivery.

KEY WORDS: dextran; inhalation; insulin; pharmacokinetics; spray drying.

INTRODUCTION

The delivery of inhaled peptides and proteins has promise for treatment of local respiratory and systemic indications (1–3). Dornase®, a DNAase, is currently approved for cystic fibrosis and Alpha-1 antitrypsin is in clinical development for pulmonary inhalation treatment of the associated deficiency. Preclinical pharmacology studies are commonly performed with a variety of inhaled biologic molecules for treatment of pulmonary diseases (4,5). The majority of these products and studies have used liquid formulations that are aerosolized with a vibrating mesh or "gentle" jet nebulization.

Recently, dry powder formulation technologies have advanced the field of protein stabilization to a point where small molecules, peptides, and proteins remain active at refrigerated to ambient temperatures for weeks to months at a time (6). These powders may also be formulated to possess exceptional aerosol performance characteristics (7,8).

Dextrans are a class of branched chain glucans of various lengths that represent a class of generally recognized as safe (GRAS) excipients that are used extensively in parenteral formulations (9). Dextrans have a high glass transition temperature (Tg), and good water solubility allowing formulation of a number of dry powders formulations for inhalation delivery (10). Inhalation performance has been shown to be comparable to several standards of care in a pulmonary preclinical setting (10–12).

The current study was undertaken to investigate the *in vitro* and *in vivo* inhalation performance of an example dextran–peptide formulation. Insulin was used as the test peptide and dextran 10 as the excipient. The formulation was compared to Exubera™, a blended, stable dry powder formulation of insulin previously approved and marketed for clinical use. Peptide loading was held similar between the formulations. *In vivo* studies were undertaken in a previously validated beagle dog model of inhaled insulin dosing, clearance, and metabolism (13–15).

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MATERIALS AND METHODS

Materials

Insulin (human recombinant DNA) was generously donated by Pfizer Global Research and Development (Chesterfield, Missouri) and/or purchased from Millipore (Kankakee, Illinois). Technical-grade D10 (marketed as Dextran T10) was purchased from Amersham Biosciences AB (Uppsala, Sweden). Dilute hydrochloric acid (0.01N) from Spectrum Chemicals (Gardena, California) was used to dissolve the solids. Exubera was purchased from a clinical pharmacy.

D10-I, engineered particles containing 70% insulin and 30% D10 (by weight of final solids), was prepared using a custom designed spray drying system that is similar in scale and operation to a Niro PHARMASD™ spray dryer type PSD-1 (GEA Pharma Systems, Wommelgem, Belgium). The spray drying system included custom drying-chamber geometry and a cyclone collector optimized for drying and collecting fine particles relevant to respiratory delivery.

Spray-dried feed solution for dry powder formulation was prepared by completely dissolving D10 and insulin in 0.01N HCL at a total solids content of 2.0 wt%. The pH of the solution was adjusted to 7.4 using dilute sodium hydroxide. The solution was diluted to the optimum spray drying total solids content of 0.2 wt%, using purified water. The spray solution was fed to the spray dryer with a peristaltic pump at 25 g/min and atomized using a two-fluid nozzle (Spraying Systems Co., Wheaton, IL; model no. 1/4J with a part no. 1650 fluid cap and a part no. 70 air cap) with 30-psig nitrogen as the atomizing gas. Nitrogen drying gas was fed to the spray dryer at 1,350 g/min and inlet temperatures of approximately 110°C (as needed to maintain a spray dryer outlet temperature of 55°C). Dry powder yield was 43% at the cyclone collector. The dry powder sample was then dried for 16 h in a vacuum desiccator under 100-mmHg vacuum to ensure sample dryness. The quantification and purity of insulin in the D10-I formulation were assessed with two slightly modified and qualified HPLC assays.

A scanning electron microscope (Hitachi S-3400Ne using S-3400 software at 4,000-fold magnification) was used to capture the morphology of the spray-dried particles, Fig. 1. SEM analysis was carried out with an accelerating voltage of 20 kV.

In Vitro Formulation Characterization

The spray-dried formulation was evaluated by dynamic vapor sorption (DVS), laser diffraction, and modulated differential scanning calorimeter (mDSC). DVS was performed with a TA Instruments, TGA Q5000 Thermal Graphic Analyzer. Briefly, loose spray-dried powder (approximately 15 mg) was added to a metallized quartz basket (TA). The sample was initially dried before measuring the weight percent water uptake from 0 to 90% RH, using nine steps, step size of 10% RH.

Laser diffraction was performed with a Malvern Mastersizer (2000 Scirocco dry cell). The sample tray was filled with spray-dried powder (3–5 g). A dispersive pressure of four bars was used. All samples were run in triplicate, and residuals were <2% with an obscuration of 1–6%.

Modulated DSC was performed with a TA instruments DSC (Q1000 MDSC with auto-sampler). Spray-dried powder (approximately 5 mg) was formed into a compact (compressed pellet of powder to standardize the material contact with the pan surface, ~5 mm in diameter) using a modified press. The compact was loaded into a 30-μL hermetic aluminum pan (Perkin Elmer, Waltham, MA), the pan was then crimped and hermetically sealed before being loaded into the instrument. The sample was heated at a rate of 2.5°C/min with a modulation rate of ±0.7°C/min. The temperature scan was run from −20°C to 200°C for the samples equilibrated at <5% RH and −20°C to 120°C for all other conditions. The materials were tested under ambient conditions (standard pans) and under 50% RH. For the testing at 50% the samples were allowed to equilibrate at 50% RH overnight and then sealed in hermetic pans in a 50% RH environment.

In Vitro Prestudy Device Characterization

Particle size and device delivery efficiency were determined in the Lovelace Bolus Dose Insufflator in preparation for *in vivo* dog experiments below, Fig. 2. Exubera and D10-I were loaded independently in the aerosol generator (Penn Century, Wyndmoor, PA). The device was fired into the expansion chamber and a "puff" of air equivalent was provided by depression of the anesthesia bag. The aerosol was collected at the end of the endotracheal tube for either (1) prestudy "dose" determination onto 47-mm Pallflex filters (PALL Life Sciences; Ann Arbor, MI) or (2) particle sizing with a Mercer-style, seven-stage cascade impactor (IN-TOX; Albuquerque, NM).

Animals and Study Design

All procedures were conducted under protocols approved by the Institutional Animal Care and Use Committee at Lovelace Respiratory Research Institute facilities which are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. The pharmacokinetic studies were performed on pure-bred female beagle dogs from the Lovelace Respiratory Research Institute (LRRI) colony (Albuquerque, NM). Weights (in kilogram) at the time of study were 9.3 (dog 1), 10.2 (dog 2), 12.8 (dog 3), and 9.5 (dog 4). A crossover design was used in which all dogs received doses of both comparator insulin formulations.

In vivo Inhalation Dosing and Experiments

Surgeries and endpoint collections were performed as previously described (13–15). Endogenous glucagon and insulin production were inhibited in these experiments by administration of somatostatin. Exogenous glucose was administered and monitored subsequent to dosing in order to stabilize animals in a euglycemic state. Key endpoint measurements were arterial insulin, glucose, and C-peptide (a measure of endogenous insulin secretion).

Anesthesia was maintained briefly during the inhalation procedure on the first and second days of dosing. For each dose, apnea was induced by ventilating the dog with anesthesia bag 10–15 times. The aerosol generator syringe (described above), previously loaded with ~2 mg D10-I or Exubera, was

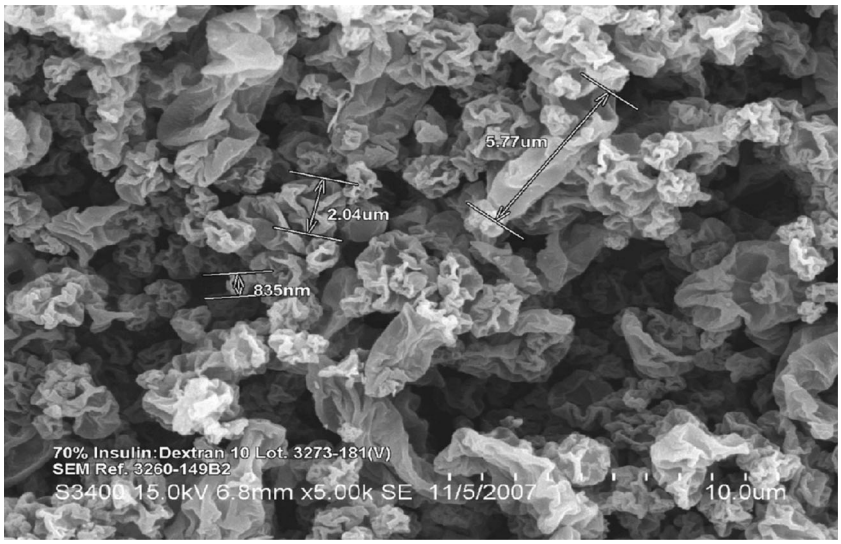

Fig. 1. SEM of the D10-I

depressed and the aerosol generated into the holding chamber of the Lovelace Bolus Dose Delivery System, Fig. 2. A pulse of air from the anesthesia bag was used to move the aerosol from the holding chamber, through the endotracheal tube, to the dogs' lungs. An approximate 3-s breath hold was used subsequent to inhalation dosing to ensure deposition and to mimic clinical delivery. The duration between dosing on the 2 days of study was approximately 48 h.

Approximately 2 mg of D10-I or Exubera containing 1.4 mg of insulin (70% loading) was administered to each dog in a crossover design, Table I. "Presented dose" of the dry powder was determined by differential weight analyses (aerosol generator weight prior to loading plus dry powder weight=total weight; total weight minus aerosol generator weight subsequent to depression/firing=amount of dry powder presented to the dog).

Arterial blood samples were obtained prior to insulin exposure (0 min) and at 5, 10, 15, 20, 35, 50, 65, 80, 95, 125, 155, 185, 215, and 245 min following inhalation of insulin. Somatostatin and glucose infusions were started immediately after the 5-min sample was taken. The exact times were recorded. Additionally, small arterial blood samples were obtained as necessary to monitor and maintain euglycemia (16).

Pharmacokinetic Modeling

Pharmacokinetic modeling was conducted with WinNonlin (Pharsight Corp., Version 5.0.1). Analysis was conducted using non-compartmental methods for each animal to determine the area under the concentration *versus* time curve (AUC), time to maximum concentration (T_{max}), and maximum concentration (C_{max}). The average across the four dogs was then determined. Data presented as means±SE. Time course data were analyzed with repeated measures ANOVA, and univariant *F* tests were used for post hoc comparisons. One-way ANOVA was used for comparisons of mean data and AUC. Statistical significances were accepted at $P<0.05$.

RESULTS

Physical and Chemical Characterization of Spray-Dried Particles

Chemical analysis revealed D10-I contained 70% insulin and showed no major impurities. Figure 1 shows a scanning

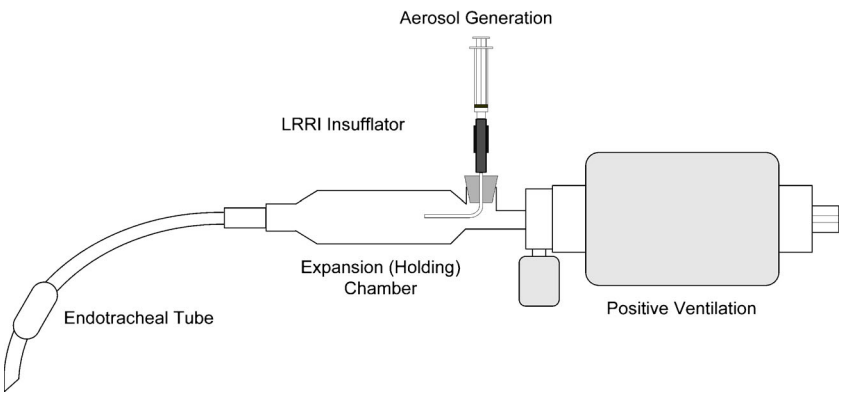

Fig. 2. Lovelace bolus dose delivery system

Table I. Presented Inhaled Dose for Dry Powder Formulations and Insulin

Material	Dog	Total lung presented dose (mg)[a]	Insulin presented dose (×0.7 lung presented dose)[b]	Insulin presented dose (mg/Kg)[c]
Exubera	1	1.71	1.20	0.129
	2	1.80	1.26	0.124
	3	1.81	1.27	0.099
	4	1.90	1.33	0.139
D10-I	1	1.58	1.10	0.086
	2	1.79	1.25	0.132
	3	1.88	1.32	0.141
	4	1.80	1.26	0.124

[a] *Milligrams of powder ejected from the aerosol generator into the inhalation system*
[b] *Insulin approximately 70% of total powder weight for each formulation*
[c] *Dog weights listed in Materials and Methods*

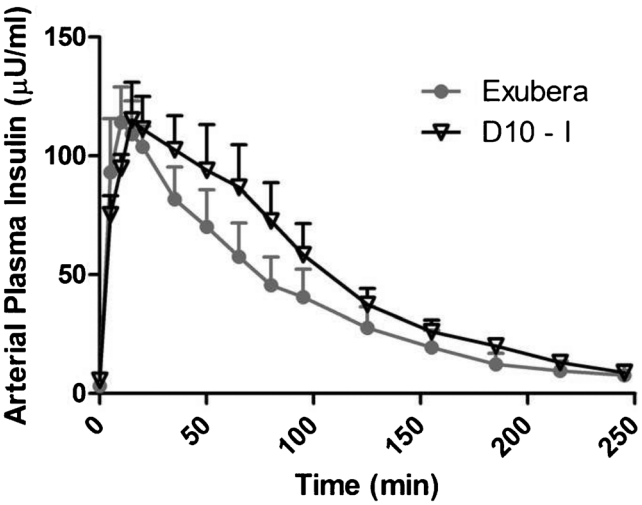

Fig. 4. Average arterial plasma insulin concentrations for both Exubera and the Dextran 10—insulin groups. *Error bars* represent the standard error of the mean

electron micrograph image of the D10-I engineered particles. Particles were "raisin-like" with a ruffled "puffy" appearance.

DVS analysis of the D10-I formulation showed that the formulation absorbed moisture up to 35% at 90% RH. The D10-I formulation absorbed less water at 50% RH (10.8%) when compared to Exubera which absorbs ~15% water when exposed to 50% RH (17).

Analysis of the volume weighted particle size distributions confirmed that the percentage of particles less than 4.6 μm was nearly the same for both formulations, Fig. 3. Interestingly, when the formulations are compared at the lower size ranges (%<1.6 μm), the D10-I formulation has a higher percentage (~13% more D10-I) of particles up until 2.6 μm where the formulations normalize.

DSC analysis of the 50% RH equilibrated formulations (both Exubera and the D10-I) indicated a glass transition temperature of ~10 and ~50°C for Exubera and D10-I, respectively. The samples not equilibrated under 50% RH indicated similar DSC profiles for spray-dried insulin, D10-I and Exubera. Specifically, the thermograms showed a similar exothermic events at ~50°C (relaxation) and at ~110°C (unfolding/denaturation).

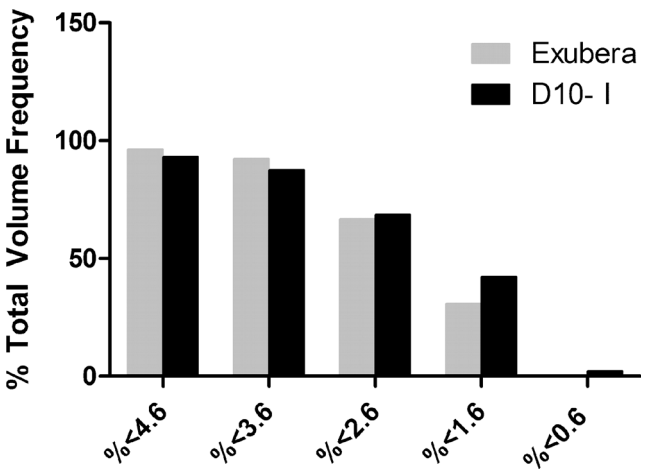

Fig. 3. Laser diffraction particle size distribution of cumulative percent of particles below each size

The measured particle size of both formulations was similar with a slightly bimodal distribution. D10-I retained peaks at 0.28 μm MMAD (1.6 GSD) and 2.8 MMAD (1.7 GSD) while Exubera retained peaks at 0.33 μm MMAD (2.0 GSD) and 3.4 μm MMAD (1.5 GSD). Delivery efficiency (material obtained on the endotracheal tube collection filter/material ejected from the aerosol generator) was generally equivalent for both formulations and ranged from 46% to 75%.

Endpoint and Pharmacokinetic Analyses

Overall, all dogs tolerated both formulations and displayed no adverse clinical signs subsequent to dosing of either Exubera or the D10-I.

C-peptide concentration dropped quickly in response to somatostatin as previously reported (data not shown) indicating that endogenous insulin secretion was suppressed. The arterial plasma insulin levels, followed apparent first-order pharmacokinetics for both formulations and peaked in the Exubera and D10-I groups at 121±21 (at 14 min) and 126±24 μU/ml (at 20 min), respectively, Fig. 4 and Table II. Following the peak in insulin concentration, there was a decline to near basal levels in both groups by the end of the study period. Insulin exposure (AUC, area under the curve) was increased (non-statistically) for the D10-I formulation. There

Table II. Insulin PK Parameters for D10-I and Exubera Following Inhalation Delivery.

	Dextran 10-I	Exubera
T_{max} (min)	20 (10)	14 (8)
C_{max} (μU/mL)	126 (24)	121 (21)
AUC_{0-245} dose normalized	7,728 (1,516)	6,237 (2,621)

Shown as average with standard deviation

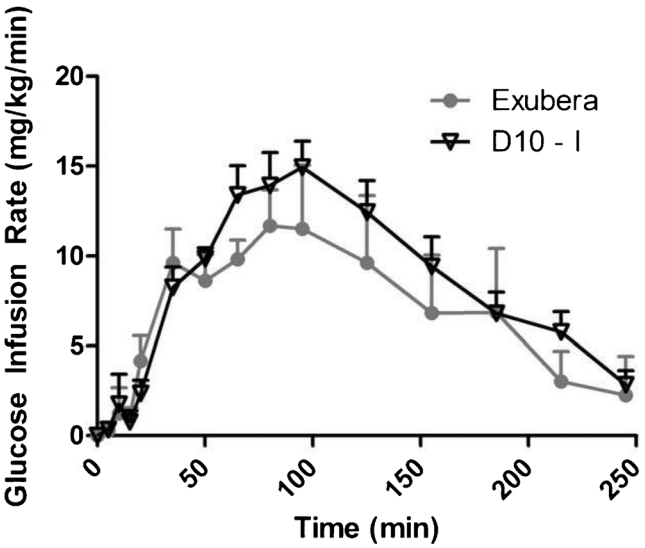

Fig. 5. Average glucose infusion rate for each of the Exubera and Dextran 10—insulin groups. *Error bars* represent the standard error of the mean

were no significant differences in the arterial plasma insulin levels between groups, although between 35 and 95 min there was a tendency for the circulating levels of the insulin to be higher in the Dextran 10-I group.

The arterial blood glucose concentrations were similar for all dogs and between study groups indicating that euglycemia was achieved over the duration of the dosing periods (78±2 and 79±2 mg/dl in the Exubera and D10-I groups, respectively). The glucose infusion rates needed to achieve euglycemia tended to be greater in the Dextran 10-I group, consistent with the notion of greater insulin levels, although no significant difference between formulations was observed, Fig. 5.

DISCUSSION

The objective of this study was to develop a proof of concept formulation of inhaled insulin using the spray drying process and dextran 10 as a novel excipient and to compare aerosol properties and pharmacokinetics for comparison to a known formulation of the same peptide, Exubera.

The novel formulation of D10-I was found to absorb less water compared to Exubera. The dextran polymer is less hygroscopic in the amorphous state relative to the amorphous mannitol and buffer salts. The higher molecular weight and decreased water uptake leads to a higher Tg at conditions of elevated humidity, confirmed by the elevated RH DSC testing. The Tg at elevated humidity is expected to lead to improved physical stability with respect to particle adhesion/fusing and insulin aggregation during storage. Stability studies are required to demonstrate this potential improvement. The DSC thermograms from the spray-dried insulin, D10-I and Exubera showed similar profiles therefore suggesting that the spray drying process did not have any significant impact on the solid state of insulin. The overall DSC profiles for insulin, Exubera and D10-I were similar to those seen by Pikal and Rigsbee (18) with exothermal events for all insulin at ~110°C, suggested to be denaturation.

This particle size of D10-I in our preclinical aerosol characterizations is in line with current literature recommendations for inhaled insulin delivery (19). Additionally, D10-I performed similarly in *in vitro* experiments with a capsule based inhaler, Monodose Inhaler (Plastiape, Osnago, Italy). The D10-I formulation had an increased percentage of particles<1.6 μm. However the number of particle normalized at 2.6 μm between the two formulations. The preclinical device and dog model used for the study were used extensively in the development of Exubera (13–15).

The pharmacokinetic parameters between the D10-I formulation and Exubera were all similar. However, the D10-I formulation resulted in an apparent increase in AUC and a trend of higher circulating insulin levels. Several factors may have contributed to this including that the D10-I formulation was slightly smaller in both MMAD and geometric size the increase which potentially increased deep lung deposition. The potential impact of difference in dissolution is unlikely due to the similar unfolding/denaturation temperatures and water solubility of insulin. These observations combined with D10-I's biological equivalence to Exubera highly suggest translatability of D10-I performance to the clinic.

Dextrans have been shown to be highly versatile for formulating a variety of water soluble molecules, likely due to their optimal glass transition temperatures. Therapeutic, pharmacokinetic, and pharmacodynamics equivalence *vs.* standard of care has been achieved with inhaled dextran 10 formulations of fluticasone, albuterol, and now insulin (10,11). These comparisons have been shown in multiple different preclinical animal models and compared to multiple different standard of care formulations (nebulizer, dry powder). Further, inhaled D10 formulations of camptothecin have been shown to have pharmacokinetic comparability to intravenous formulations in rodent models (20). These data establish the utility of dextrans as a broadly applicable inhalation formulation excipient spanning different drug classes and disease indications.

Dextrans are considered GRAS compounds having been proven safe over many years of parenteral clinical use (9). Additionally, we have briefly reported on the inhaled repeat dose safety of inhaled D10 (neat, spray-dried compound) and other dextrans of varying molecular weights (21,22). Further reporting is needed, but these data suggest that no overt tolerance or pulmonary irritancy is observed and that limited pulmonary inflammation is only present at higher lung burdens. The latter appear to be outside of the drug loading range of most compounds.

CONCLUSION

A novel dextran 10 formulation of insulin compared equivalently to Exubera, an optimized clinical product, in aerosol properties and a dog model of inhaled insulin administration. The thermal and hygroscopic properties of the dextran 10 formulation suggest the potential for increased stability and robustness in environmental handling of the formulation when compared to Exubera. These data, combined with safe clinical use and versatility as a spray drying excipient, suggest dextrans should be considered for future inhalation formulation development of repurposed or novel peptides and proteins.

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