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RESEARCH PAPER

Formulation Development and *In Vivo* Evaluation of a New Dry Powder Formulation of Albuterol Sulphate in Beagle Dogs

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ABSTRACT

Purpose Study objectives were to develop, characterize, and evaluate a novel excipient for dry powder inhalation formulations in a canine model with a model compound.

Methods Dry powder inhalation formulations of albuterol sulphate were developed and compared to a commercially available nebulizer albuterol solution formulation. *In vitro* analysis indicated a high fine-particle fraction (FPF, >70%) and a respirable particle size (~2.5 μm MMAD). Each inhalation formulation, including controls, was delivered targeting a deposited lung dose of 10 μg/kg albuterol. Active formulations were evaluated for pharmacokinetic (PK) profile and bronchodilatory effects in a ragweed-sensitized dog model of allergic airway responses.

Results *In vitro*, the dextran spray-dried formulated materials showed that aerosol performance, including FPF, MMAD, glass transition temperature, and amorphous characteristics, were all largely unaffected by amount of drug loaded. Both the commercial and the dry powder formulations attenuated the ragweed-induced bronchoconstriction by 91.59 ± 3.60 and $81.28 \pm 9.29\%$, respectively. The PK profiles for both albuterol formulations were similar, as were the corresponding T_{max}, C_{max} and $T_{1/2}$.

Conclusions Results indicate that dextran 10 has promise as a novel excipient for dry powder inhalation drug delivery, in a preclinical setting, over a wide range of drug loadings.

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ABBREVIATIONS

ANOVA	analysis of variance
APS	Aerodynamic Particle Sizer
COPD	chronic obstructive pulmonary disorder
DC	dendritic cell
DVS	dynamic vapor sorption
FPF	fine particle fraction
GSD	geometric standard deviation
HPLC-UV	high-pressure liquid chromatography-ultra violet
LC-MS-MS	liquid chromatography tandem mass spectrometry
LRRI	Lovelace Respiratory Research Institute
MDI	metered dose inhaler
mDSC	modulated differential scanning calorimetry
MMAD	mass median aerodynamic diameter
NGI	Next Generation Impactor
PK	pharmacokinetic
PSD	particle size distribution
PXRD	powder X-ray diffraction
RH	relative humidity
RW	ragweed
SEM	scanning electron microscopy, standard error of the mean
SPE	solid phase extraction
Tg	glass transition temperature

INTRODUCTION

Oftentimes, the limiting factor in developing a dry powder inhalation formulation lies in the limited number of excipients that are both safe when delivered to the lungs

and effective in providing the physical properties required for delivery to the lungs (1). Currently, there are a relatively small number of excipients that meet these needs for the development of dry powder inhalation formulations. Therefore, there is a need to both develop and test novel dry powder excipients for inhalation drug delivery.

In an effort to address the shortage of excipients and to potentially circumvent many issues often encountered in formulation (solubility, chemical stability, aerosol performance, etc.), the novel excipient, dextran 10, has been developed for inhalation delivery.

Dextran 10 (molecular weight 10,000 daltons) is a water-soluble polymer that undergoes rapid systemic clearance through the kidneys at molecular weights less than 20,000 daltons (2). Dextan has been shown to clear from the lung in preclinical animals with half lives in the 2–6 h range, depending on charge (3). Various forms of dextran have previously been in human clinical trials for both IV and inhalation delivery (4,5). Inhalation delivery of dextran has been shown to have sufficient human safety to progress into Phase II clinical trials (6). Based on these data, dextran was selected for investigation as a novel excipient.

As a potential novel excipient, dextran 10 can be spray-dried alone or with a single drug, resulting in a powder with exceptional aerosol performance over a wide range of drug loading. In order to test this excipient as a platform for delivery of a dry powder to the lung, the model drug albuterol was selected because its physical properties make it challenging to formulate as a dry powder for inhalation therapy (7). Albuterol sulphate, or albuterol, is a short-acting β_2-adrenergic receptor agonist that is commonly used to alleviate bronchospasm in conditions such as asthma and chronic obstructive pulmonary disorder (COPD) (8). There are several inhalation formulations of albuterol on the market, including both CFC and HFA metered dose inhaler (MDI) formulations and nebulized and IV formulations. However, there is not a dry powder inhalation formulation of albuterol. Thus, selecting albuterol as the model compound to test this delivery platform served as both a technical challenge of the system and the potential for a resulting novel formulation.

In this study, we present a dry powder formulation of albuterol sulphate generated from a spray-drying process with the novel excipient dextran 10. The dry powder formulation was compared to a commercially available albuterol formulation in a ragweed-sensitized beagle dog model for both pharmacokinetic (PK) and pharmacodynamic (bronchoconstriction) end-points. The results of the study indicate that this novel excipient platform is a viable solution for developing dry powder delivery formulations.

MATERIALS AND METHODS

Materials

Albuterol sulphate was purchased from Spectrum (Gardena, CA). Technical grade dextran with a 10,000 Da MW (dextran 10) was purchased from Pharmacosmos (Holbaek, Denmark). Deionized water was used for aqueous spray drying.

Formulation and Manufacture by Spray Drying

Engineered particles containing various concentrations of albuterol sulphate and dextran 10 were manufactured via a spray-drying process. The dry powder test articles were prepared using a novel spray-dryer system similar in scale and operation to a Niro PSD-1. The unique features of the spray-dryer system included a custom drying chamber geometry and cyclone collector optimized for drying and collecting fine particles relevant to respiratory delivery.

Spray-drying feed solutions for all of the dry powder samples were prepared by completely dissolving dextran 10 and albuterol sulphate in deionized water at 2 wt% total solids. The spray solutions were fed to the spray dryer with a peristaltic pump at 25 ml/min. The solutions were atomized using a two-fluid nozzle (Spraying Systems Co. model 1/4J with 1650 fluid cap and 120 air cap; Wheaton, IL) with 42 psig nitrogen as the atomizing gas. Nitrogen drying gas was fed to the spray dryer at 1,350 g/min and at approximately 150°C as needed to maintain a spray-dryer outlet temperature of 50–60°C. Powder yields ranged from 70 to 80% at the cyclone collector. The dry powder samples were secondarily dried for 12–16 h in a vacuum desiccator (100 mmHg vacuum) to ensure sample dryness.

In Vitro Particle Size Distribution Analysis

Aerodynamic particle size distribution (PSD) analysis was conducted with a Next Generation Impactor (NGI, from MSP Corp., Shoreview, MN), operated at 60 L/min. The NGI pans were coated with a light film of silicon oil to eliminate bounce and re-entrapment of particles in the flow. Pans were analyzed with the albuterol chemical assay to determine the mass median aerodynamic diameter (MMAD, in microns) and the geometric standard deviation (GSD). These data were also used to calculate the fine particle fraction (FPF), which is defined as the percentage of particles that enter the NGI that are below 4.6 μm MMAD.

Laser Diffraction

Geometric particle size was studied using laser diffraction (Malvern Mastersizer 2000 Scirocco dry cell). The sample tray was filled with spray-dried powder (3–5 g), and the

mesh basket was completely filled with 3-mm stainless steel balls. 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 Differential Scanning Calorimetry (mDSC)

A modulated differential scanning calorimeter (TA Q1000 MDSC with auto-sampler) was used to study the glass transition temperature (Tg) of the spray-dried powders and how the Tg was plasticized by increasing relative humidities (% RH). 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) and set into an environmental chamber overnight to equilibrate to a given % RH. 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.

Dynamic Vapor Sorption (DVS)

The weight percent water uptake as a function of RH was studied using DVS (TA Instruments, TGA Q5000 Thermal Graphic Analyzer [DVS]). Loose spray-dried powder (approximately 15 mg) was added to a metallized quartz basket (TA). The sample was initially dried (maximum drying time was 1,200 min) before measuring the weight percent water uptake from 0% to 90% RH, using nine steps, with a step size of 10% RH at a rate of 0.001 mg/10 min and a data sampling interval of 10.0 s/point. The balance purge gas flow was set to 10 mL/min, and the humidity gas flow was 200 mL/min.

Powder X-Ray Diffraction (PXRD)

A powder X-ray diffractometer (Bruker AXS D8 Advance with a CuKα source) was used to study the amorphous character of the spray-dried powder. Powder (approximately 50 mg) was dispersed into a plastic cup sample holder (1 mm deep with grooves) and placed in the beam zone. The sample was scanned from a range of 4–40° 2Θ using a step scan mode and a step size of 0.04°/step. The tube angle was 3.0°, and the sample was scanned while being rotated in the φ plane at a rate of 30 rpm.

Scanning Electron Microscopy (SEM)

A scanning electron microscope (Hitachi S-3400Ne using S-3400 software at 4000× magnification) was used to study the morphology of the spray-dried particles. Before observation with SEM, the powder was mounted on aluminum posts (Ted Pella, Inc., Redding, CA; Prod. No. 16111 Specimen Mount, Aluminum, ½″ Slotted Head, 1/8″ Pin) using double-sided tape (Ted Pella, Inc., Prod. No. 16079 Adhesive Tabs) and sputter coated (Hummer 6.2) with AuPd. The SEM analysis was carried out at an accelerating voltage of 20 kV.

Canine Animal Model

All procedures were conducted under protocols approved by the Institutional Animal Care and Use Committee at Lovelace Respiratory Research Institute (LRRI), which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International. Four male beagle dogs, ages 1.5–3 years and between 11.0 and 15.2 kg, from the LRRI allergic beagle colony were used for this study. The dogs were housed in indoor-outdoor kennels where they were provided food (2025 Teklad Global 25% Protein Dog Diet; Harlan Teklad, Madison, WI) once daily and had municipal water *ad libitum*. Prior to exposures for PK analysis the animals were fasted for at least 18 h.

The LRRI allergic beagle dog colony is a selectively bred colony of beagle dogs that were exposed to ragweed (RW) as puppies and have developed an allergic response to RW. These allergic dogs show elevated total and specific serum IgE and increased numbers of eosinophils in their blood and lungs, as well as an increase in airway resistance and a decrease in dynamic compliance after a challenge with RW by inhalation or local instillation in defined lung lobes (9–11).

Canine Dry Powder Exposure System Aerosol Generation

Dry powders were delivered with the LRRI Dry Powder Bolus delivery system, Fig. 1, which facilitates a bolus delivery of dry powder to the lungs of an anesthetized canine via an endotracheal tube. The LRRI Dry powder Bolus delivery system utilizes an aerosol generator coupled to an expansion chamber on positive ventilation to deliver the dry powder to the lungs of a canine via the endotracheal tube. In order to characterize the delivery efficiency of the system for the specific dry powders (95% dextran 10 : 5% albuterol sulphate), the spray-dried material was loaded into the aerosol generator to a target weight of 5 mg of test article. A pre-weighed filter (Pall Life Sciences; Ann Arbor, MI) was fitted to the end of the endotracheal tube, and the aerosol was generated at the same time positive ventilation was applied. The efficiency of the delivery system was determined via differential weight analysis of the device and the filters ($n=3$ for each dry powder). For the purposes of calculating the deposited dose,

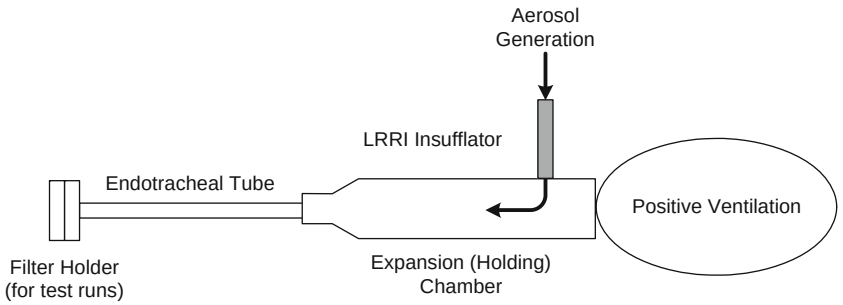

Fig. 1 LRRI dry powder bolus delivery system setup.

it was assumed that 30% of the material presented deposited into the lungs of the canines (12).

Aerosol Collection and Particle Size Distribution

In order to determine the aerosol particle size, expressed as MMAD in microns (μm), aerosols were generated in the same manner except that the filter was replaced with an Aerodynamic Particle Sizer (APS Model 3321; TSI Corp., Shoreview, MN) attached to the end of the endotracheal tube. The APS and the associated Aerosol Instrument Manager were used to determine the MMAD and GSD of the aerosols.

Canine Nebulizer Exposure System Aerosol Generation

The commercially available albuterol (HiTech Pharmaceutical, Amityville, NY) aerosols were generated with a Pari LC Plus compressed air jet nebulizer operated with an inlet pressure of 20 psi. During the administration of this test article (albuterol solution), the canine had its ventilation controlled via a Harvard pump. In order to characterize the aerosols of the 5 mg/mL solution formulation of albuterol, filter samples were collected from the end of the endotracheal tube to determine the aerosol concentration of albuterol via differential weight analysis. Particle size for the albuterol-nebulized aerosols was determined with the APS in the same manner as the dry powder aerosols.

In Vivo Formulation Analysis

In order to determine the efficacy of the newly developed albuterol formulation in the RW-sensitized dogs, one of four formulations—1) dry powder vehicle (dextran 10), 2) nebulized solution vehicle (sterile water), 3) nebulized albuterol aerosol, or 4) dry powder albuterol sulphate (albuterol sulphate with dextran 10)—were given by inhalation to RW-sensitized dogs 30 min prior to challenge with ragweed. The dogs were challenged with a dose of ragweed (5 breaths) that lead to an immediate bronchoconstriction (~500–1,000% increase in airway resistance). Changes in airway resistance were continuously measured for up to 20 min following RW challenge.

Each animal was used as its own control in a crossover study design. A washout period of 1 week was used between all four treatments. All aerosols were conducted to target a deposited dose of 10 μg/kg to the lungs of each dog.

Measurement of Hypersensitivity to Ragweed

Animals were anesthetized (5% induction; 2% maintenance; isoflurane), and an endotracheal tube was placed in the trachea. Each dog was mechanically ventilated (volume = ~210 ml; 18 breaths/min) during each experiment. Airflow and tidal volume were measured using a differential pressure transducer located in front of the endotracheal tube. An esophageal balloon catheter placed in the esophagus was used to determine transpulmonary pressure. Pulmonary resistance and dynamic lung compliance were calculated from the simultaneous measurement of transpulmonary pressure and respiratory flow using Labview software (Version 5.1, National Instuments). Dogs were challenged with RW (RW short, *Ambrosia artemisifolia*, Greer, Lenoir, NC) by inhalation (5 breaths of a 0.3 or 1 mg/mL nebulizer concentration). Immediately following RW challenge, changes in pulmonary resistance and compliance were measured for up to 20 min after challenge.

- Lung compliance = volume change per unit pressure change. Reduced compliance is indicative of increased lung elastic recoil, typical in asthma.
- Airway/pulmonary resistance = pressure difference between alveoli and the mouth divided by the flow rate. Bronchoconstriction increases airway resistance.

Blood Collection

Blood was collected by placing a venous (cephalic or saphenous) catheter in the leg prior to anesthesia or by venipuncture (jugular) using a syringe or needle-vacutainer. Blood (up to 5 mL each draw) was collected into a vacutainer (k3EDTA) at baseline and at 5, 15, 30, 60, 120, 240, and 360 min post-test-article treatment. Blood

was stored as plasma at −80°C until assayed by the liquid chromatography tandem mass spectrometry (LC-MS-MS) method detailed below.

Statistical Analysis

Changes in RW-induced pulmonary resistance (over time) were assessed by repeated measures two-way analysis of variance (ANOVA) with Bonferroni post-test. All other statistical comparisons were made using T-test or ANOVA with the Dunnett's or Tukey's multiple comparison test. A value of $p<0.05$ was considered significant.

Description of LC-MS-MS Bioanalytical Method

Analysis of serum from exposed canines was conducted via the developed LC-MS-MS method. The chromatographic separation was conducted with an Agilent HPLC fitted with a Luna C_{18} (2) column (3 micron, 50×2.0 mm, Phenomenex #00B-4251-B0, Torrance, CA), held at 50°C. The injection volume was 15 μL, and the gradient is described in Table I. Under these conditions, the albuterol retention time was ~1.9 min.

MS-MS analysis was performed using an Applied Biosystems API-365 instrument (Foster City, CA) upgraded to an Ionics EP10+ system with HSID interface (Bolton, ON, Canada). The data station was an Analyst, Version 1.4.2. Analytes were ionized using an electrospray ionization source in positive mode. Multiple reaction monitoring was used with parent/daughter ion pairs of 240/148 m/z for albuterol, settings are shown in Table II.

Serum Extraction Method

Beagle canine serum samples were prepared for analysis using a solid phase extraction (SPE) technique. Throughout the SPE cleanup, the flow rate was never more than 1 to 2 drops per second. The Bond-Elut C_{18} cartridges (100 mg/1 mL, PN: 12102001, Varian Medical Systems, Inc., Palo Alto, CA) were first conditioned with one column volume of methanol followed by one column volume of purified water. Five-hundred microliters of sample were mixed with 500 μL of purified water and 100 μL of 0.1% phosphoric acid solution (linearity spiking solution was prepared in 0.1% phosphoric acid solution) prior to passing it through the SPE cartridge. After passing the sample through the SPE cartridge, the column was washed with one column volume of purified water followed by one column volume of acetonitrile. The tips of the SPE cartridge were dried using a Kim Wipe, and a 5-mL conical shaped polystyrene tube was placed underneath it to collect the sample. Albuterol was eluted off of the SPE C_{18} cartridge using two 750 μL volumes of elution solution (0.5% 1 M ammonium acetate in methanol). The solvent was evaporated off under a gentle stream of nitrogen in a 50°C water bath. The sample was reconstituted with 100 μL of 0.1% phosphoric acid solution. The sample was vortexed for 3 min, and then the final extract was analyzed by LC-MS-MS.

Determination of Deposited Dose

The amount of material deposited in the lungs of the canine was determined as follows. For the nebulized solution, the aerosol concentration of the 5 mg/mL albuterol was determined to be 0.13 mg/L. The aerosol concentration can be used to calculate a deposited dose based on Guyton's formula (see Eq. 1), assuming a 10-kg canine, 0.75-min exposure, and 30% deposition fraction.

$$\mathrm{DD}(\mu\mathrm{g}) = \frac{\mathrm{Ce}\,(\mu\mathrm{g/L}) \times RMV\,(\mathrm{L/min}) \times \mathrm{T}\,(\mathrm{min})}{DF} \quad (1)$$

where: DD = deposited dose, respiratory minute volume (RMV) = estimated (13), aerosol exposure concentration (Ce) = measured from exposure chamber, T = time, and DF = deposition fraction

Based on these data, the exposure time of 45 s was selected to deposit ~10 μg/kg.

The deposited dose for dry powder animals was based on the efficiency of the device to deliver material to the end of the endotracheal tube. Of the material presented at the terminus of the endotracheal tube, it was assumed that 30% deposited in the lungs. A targeted total deposited dose of 200 μg/kg, (dextran 10 and 5% albuterol sulphate : 95% dextran 10) was determined because this would facilitate the same 10-μg/kg deposited dose of albuterol as the nebulizer.

Table I Liquid Chromatographic Conditions

Time (min)	Flow (μl/min)	0.1% Trifluoroacetic Acid in Purified Water	100% Methanol
0.0	700	95	5
0.5	700	95	5
3.5	700	60	40
3.7	700	95	5
5.0	700	95	5

Table II Source and MS/MS Operational Settings

Ionization Source Parameters		MS Parameters	
Electrospray ionization source	ESI (positive)	Decluster potential (V)	16
Curtain gas	12	Focus potential (V)	50
Nebulizer gas	7	Collision energy (V)	23
Collision gas setting	2	Collision cell exit potential (V)	22
Ionization voltage (V)	5,500	Entrance potential (V)	4.5
Temperature (°C)	400	Q1 resolution	Unit
		Q3 resolution	Unit

Pharmacokinetic Analysis

Relevant PK parameters (maximum blood concentration, time to maximum blood concentration, and half-life) were conducted with WinNonLin software (Pharsight Corp., Version 5.0.1). Analysis was conducted using non-compartmental methods with the average concentration ($n=4$) at each time-point for each of two test groups (albuterol nebulized and albuterol sulphate dry powder [DPI]). No blood was collected from either control exposure groups for analysis.

RESULTS

Physical Characterization of Spray-Dried Particles

Dextran 10 and albuterol sulphate were completely dissolved in deionized water and then spray dried to produce solid particles comprising a homogeneous amorphous dispersion. The spray-dry process and equipment were specifically designed to manufacture and collect respirable size particles (1–5 μm) with a tight distribution span. Table III shows geometric particle size distributions measured by laser diffraction for five different formulations of spray-dried albuterol sulphate : dextran 10 formulations. Because the atomization and drying conditions leading to the preferred size and distribution can be largely independent of solution composition, this process enables formulation of a wide range of albuterol sulphate loadings while maintaining a similar geometric size and distribution.

The spray-dried powders were characterized by PXRD and mDSC to show that they are homogenous amorphous dispersions. Fig. 2 shows the results of the PXRD analysis, illustrating that the powders are indeed amorphous. Similarly, the mDSC analysis revealed a single Tg, which is indicative of a single phase, homogeneous amorphous dispersion. The homogeneous dispersion is advantageous because it takes on the composite properties of the components. In this case, the particles largely take on the properties of the dextran excipient. The aerosol and powder handling properties are therefore largely independent of the albuterol sulphate loading in the formulation.

The dextran excipient is important for the physical stability of these powders, for both stabilizing the amorphous dispersion and maintaining a discrete particle of a respirable size upon storage. Dextran is a high Tg material, which aids in kinetically stabilizing the amorphous state of

Table III Comparison of Geometric Particle Size and Distribution for Albuterol Sulphate : Dextran 10 Formulations

Formulation	D_{90} (μm)[a]	D_{50} (μm)[b]	D_{10} (μm)[c]	Span[d]	$D_{[4.3]}$ (μm)[e]	$D_{[3.2]}$ (μm)[f]
5/95	4.3	2.3	1.2	1.3	2.6	2.1
10/90	4.9	2.4	1.2	1.5	2.9	2.4
25/75	4.9	2.3	1.1	1.6	2.8	2.0
50/50	4.2	2.1	1.0	1.5	2.4	1.8
75/25	4.2	2.1	1.0	1.5	2.4	1.8

[a] D_{90}: Size encompassing 90% of particle

[b] D_{50}: Size encompassing 50% of particle

[c] D_{10}: Size encompassing 10% of particle

[d] Span: Distribution of particle

[e] $D_{[4.3]}$: Volume moment mean of particles

[f] $D_{[3.2]}$: Surface moment mean of particles

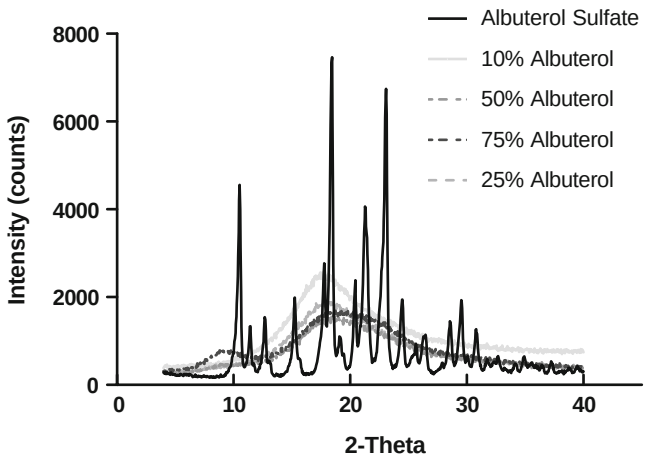

Fig. 2 Powder X-ray for albuterol sulphate:dextran 10 formulations.

the material. For all formulations manufactured, the dry Tg of the amorphous dispersions were >100°C. Additionally, for a very water-soluble material, dextran has relatively low water uptake properties at relevant relative humidities. Specifically, weight percent water was <25% up to 80% relative humidity.

The robust spray-dry process leads to manufacture and collection of respirable-sized particles. Dextran acts as a film former in these formulations and leads to shriveled raisin morphology that can be observed in the SEM images shown in Fig. 3.

Four formulations were analyzed for aerosol performance using impaction. An NGI was used to determine the MMAD and FPF. Fig. 4 shows the results of this test. All formulations (10% active loading to 75%) had excellent aerosol performance with MMADs near 2.5 μm and FPFs near 80%. This result demonstrates that the aerosol performance of the formulation can be independent of active loading up to at least 75% active. This type of formulation could be extremely useful as a preclinical tool to assess toxicity of a compound by changing the dose without changing aerosol performance.

Canine Dry Powder Aerosol Method Development

Testing was conducted with each of the two test articles to be aerosolized with the device, dextran 10 and 5% albuterol sulphate: 95% dextran 10, in order to characterize the efficiency of the LRRI Dry Powder Bolus delivery system. Initial aerosol efficiency testing indicated that the delivery for each of the dextran 10 and the 5% albuterol sulphate: 95% dextran 10 was highly variable with relative

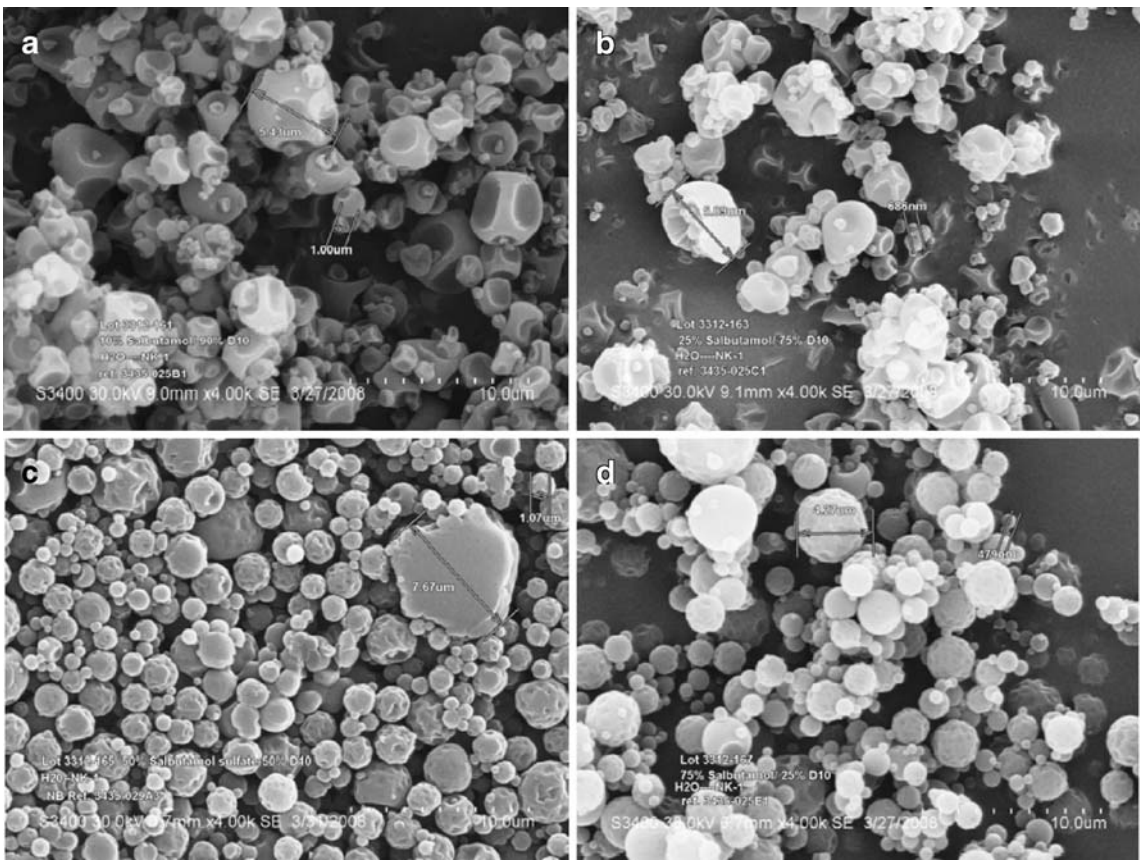

Fig. 3 SEM images for **a** 10%, **b** 25%, **c** 50%, **d** 75% albuterol sulphate-loaded inhalable particles.

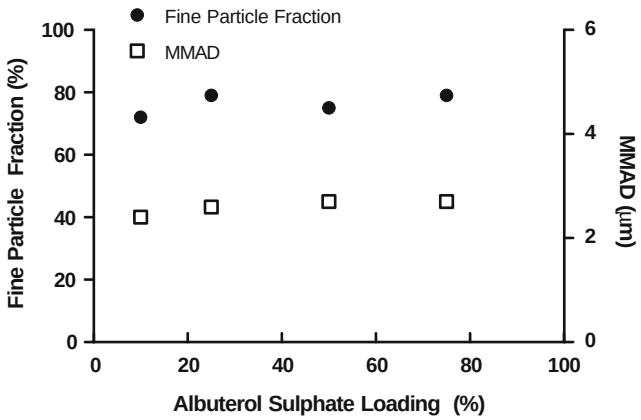

Fig. 4 *In vitro* aerosol performance of albuterol sulphate:dextran 10 formulations.

standard deviation values >30%. Based on this testing, the materials were placed in a 40°C oven for 4 days. Repeat testing indicated that the ratio of material ejected to material collected at the end of the endotracheal tube (delivery efficiency) was >10% for both materials; data are shown in Table IV.

These data indicate that both of the materials, after a period of drying, resulted in more consistent delivery efficiency. In order to ensure that the heating of the material in the oven did not decompose or degrade the albuterol, a high-pressure liquid chromatography-ultra violet (HPLC-UV) analysis was conducted with albuterol API (5% albuterol sulphate: 95% dextran 10) that was not heated and 5% albuterol sulphate: 95% dextran 10 that was dried in the oven. This analysis was conducted on a previously established HPLC-UV for albuterol. These data indicated that there was a <1% difference between the dried and the undried material, as reflected in detector response and retention time. Therefore, this process of heating the material prior to aerosol generation was utilized for animal exposures. The determined efficiency of 75% was used to calculate the amount of material required to achieve the desired deposited doses in the *in vivo* studies.

Canine Dry Powder Delivery Particle Size Analysis

Particle size analysis was conducted for both the dried and undried materials. The data are shown in Table V. All particle sizes, regardless of whether or not the material was dried, were shown to be viable for canine aerosol exposures.

Table IV Aerosol Delivery Efficiency, Albuterol Sulphate : Dextran 10

Material	% Ejected	% RSD	Delivery Efficiency
Undried	58.1	38.9	73.1%
Dried	66.6	22.3	82.7%

Table V Particle Size Distribution for Each of the Dextran 10 and 5% Albuterol Sulphate Test Articles

Formulation	Dried or Undried	MMAD[a] (μm)	GSD[b]
Dextran 10	Dried	2.36	1.60
Dextran 10	Undried	3.02	1.71
5% Albuterol Sulphate	Dried	2.19	1.61
5% Albuterol Sulphate	Undried	2.47	1.66

[a] Mean median aerodynamic diameter

[b] Geometric standard deviation

Canine Albuterol Nebulizer Aerosol Method Development

The 5-mg/mL albuterol solution was shown to result in an aerosol concentration of 0.13 mg/L as used on this aerosol system with an MMAD of ~1.0 μm. Based on these data, the solution was nebulized for 0.75 min to achieve a deposited dose of 9.4 μg/kg to each animal, as determined with Eq. 1.

In Vivo Formulation Analysis

A total of four RW-sensitized dogs were used in a crossover-style study to compare the efficacy of a nebulized *versus* dry powder formulation of albuterol suphate. Initially, the dogs were each treated with vehicle (water) and challenged with RW to induce an immediate bronchoconstriction (e.g., increase in airway resistance). Each subsequent week, the

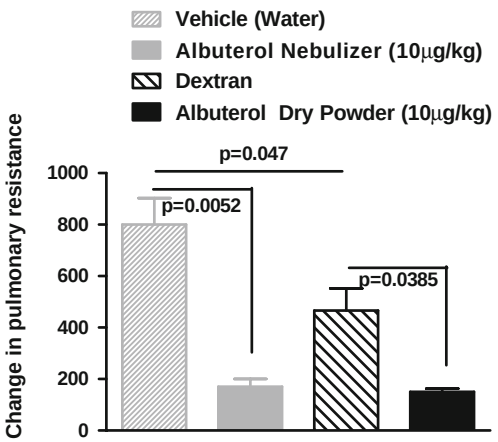

Fig. 5 Percent change in pulmonary resistance from baseline after ragweed challenge by inhalation expressed as mean ± SEM. Ragweed was given 30 min after the end of treatment with vehicle (water), albuterol, dextran or dry powder albuterol by inhalation. P values indicate statistical significance to appropriate control determined by paired t-test.

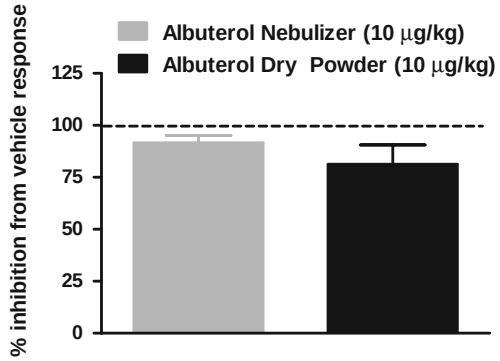

Fig. 6 Percent inhibition from vehicle response after ragweed challenge by inhalation expressed as mean ± SEM. Ragweed was given 30 min after the end of treatment with vehicle (water), nebulized albuterol, dextran or albuterol treatment by inhalation.

same dogs were treated with nebulized albuterol (~10 μg/kg), dextran 10 (170 μg/kg), or dry powder albuterol sulphate (~10 μg/kg; 5% albuterol sulphate: 95% dextran 10 formulation) and challenged with the same dose of RW.

The albuterol 5-mg/mL solution was nebulized for 0.75 min, as determined above, to achieve a deposited dose of 9.4 μg/kg to each animal. The inhalation exposures to the dextran 10 (dry powder vehicle control) resulted in an average deposited dose of 173 μg/kg. The 170-μg/kg dose was selected, as it is the equivalent dextran dose to that used for the dry powder albuterol sulphate. The inhalation exposures to the 5% albuterol sulphate: 95% dextran 10 resulted in an average total deposited dose of 169.3 μg/kg and an albuterol deposited dose of 8.46 μg/kg.

Following exposure to each control or test article, the RW challenge led to an ~800% (range = 560–1,000%) increase in the peak airway resistance in the vehicle control group (Fig. 5). Treatment with the nebulized or dry powder

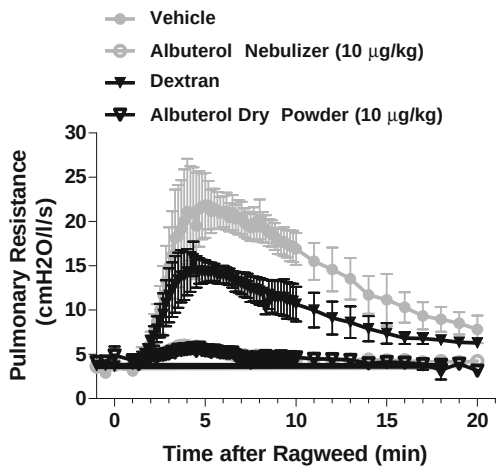

Fig. 7 Pulmonary resistance measured before (time 0) and for up to 20 min after ragweed challenge (mean ± SEM) for each treatment group. Ragweed was given 30 min after the end of inhalation treatment with vehicle (water), nebulized albuterol, dextran or dry powder albuterol.

Table VI Relevant PK Parameters for Albuterol Nebulizer Formulation and 5% Albuterol Sulphate: 95% Dextran 10 Formulation, Both at 10 μg/kg

Parameter	Albuterol Nebulizer	5% Albuterol Sulphate: 95% Dextran 10
T_{max}[a] (min)	15	5
C_{max}[b] (ng/mL)	23.8	38.3
$T_{1/2}$[c] (min)	58.3	58.3

[a] Time to maximum blood concentration

[b] Maximum blood concentration

[c] Half-life

formulations of albuterol led to an equivalent percent reduction in airway resistance, 91.59±3.60 and 81.28±9.29%, respectively (Fig. 6). Interestingly, treatment with dextran 10 alone attenuated the RW-induced airway resistance (Fig. 5). Both nebulized and dry powder formulations had a similar effect on attenuating the RW-induced increase in airway resistance (Fig. 6). Individual dog (not shown) and summary data (Fig. 7) for the time course of the response after RW challenge are also shown.

PK Analysis

LC-MS-MS analysis of all samples was conducted to determine the concentration of albuterol in the blood at time-points 0, 5, 15, 30, 60, 120, 240, and 360 min post-exposure. The concentration for each of the four canines at each time-point was averaged and used for PK analysis. Analysis of the data indicated that the elimination appeared to follow apparent first-order elimination; therefore, first-order kinetic principles were used in analysis. The relevant PK parameters are listed for each of the two formulations in Table VI. These data indicated similar behavior for both the albuterol nebulized formulation and the 5% albuterol sulphate: 95% dextran 10 formulation.

DISCUSSION

The goals of this study were twofold: first, to develop and characterize the spray drying of a novel excipient, dextran 10, for use in dry powder inhalation formulations. The second goal of the study was to conduct *in vivo* analysis of a novel dry powder formulation that utilized the novel excipeint, dextran 10, with a model compound, albuterol, in a RW-sensitized dog model of allergic airway responses.

The *in vitro* analysis indicated that due to the refinement of the atomization and drying conditions, preferred size and distribution can be largely independent of solution composition, which enables formulation of a wide range of drug

loadings while maintaining a similar geometric size and distribution. Analysis of the spray-dried powders via PXRD and mDSC indicated that they are homogenous amorphous dispersions with a single Tg regardless of drug loading. As all four formulations (10% active loading to 75%) had excellent aerosol performance with MMADs near 2.5 μm and FPFs near 80%, the aerosol performance of the formulation was independent of active loading up to at least 75% active. This makes the formulation extremely useful as a preclinical tool to assess efficacy and/or toxicity of a compound by changing the dose without changing aerosol performance.

The efficacy of the novel formulation and the PK of the same deposited dose were determined in a four-canine crossover study that was designed to determine the bronchodilatory effect of pretreating RW-sensitized dogs with a novel formulation of albuterol sulphate via inhaled administration. This novel formulation was compared to an existing on-the-market formulation of albuterol. The dry powder formulation of albuterol sulphate consisted of 95% dextran 10 and 5% albuterol sulphate. Both the current "off-the-shelf" nebulized albuterol formulation and the dry powder albuterol sulphate formulation provided the same degree of protection against RW-induced bronchoconstriction in RW-sensitized beagle dogs.

Interestingly, treatment with dextran 10 alone also appeared to attenuate the RW-induced increase in airway resistance. There is limited evidence in the literature about the effects of inhaled dextran on allergen-induced bronchoconstriction. A 6-h pretreatment with dextran in ovalbumin-sensitized guinea pigs did not have any effect on methacholine-induced changes in airway resistance (8). Low molecular weight dextran sulfate (m. w. 5000) is known to inhibit alternative, classical, and lectin complement pathways (7) as well as the coagulation cascade (14). It acts as an endothelial cell protectant inhibiting complement-mediated endothelial cell damage (15,16) as well as preventing phenotypic maturation of monocyte-derived dendritic cells (DCs) and peripheral myeloid DCs (17). Whether the present formulation of dextran shares these same properties is unclear.

There is an increasing appreciation for the role of complement in the development and pathogenesis of asthma, and thus complement inhibition by dextran could potentially attenuate asthma symptoms (18). Complement activation products C3a and C5a (anaphylatoxins) are known to induce smooth muscle contraction and mucus hypersecretion, recruitment of inflammatory cells, and vascular permeability (19–22). In rodent models, complement inhibition has been more closely tied to the attenuation of inflammation and airway hyperresponsiveness rather than immediate bronchoconstriction (23,24). Elevated levels of C3a in plasma of patients with severe acute asthma have been related to airway inflammation and bronchoconstriction in acute asthma exacerbation (25). Thus, although there is no specific causative link, there is the mechanistic potential for dextran alone to inhibit allergen-induced bronchoconstriction. In general, the 95% dextran 10 and 5% albuterol sulphate dry powder formulation showed an equivalent attenuation of allergen-induced bronchoconstriction in ragweed-sensitized dogs and a similar PK profile when compared to an "off-the-shelf" albuterol formulation.

These data indicate that the novel excipient, dextran 10, may prove to have value as a generic carrier particle for inhalation drug delivery over a wide range of drug loadings in a preclinical setting. However, despite the previous toxicity studies, future studies will include a more complete characterization of the excipient with regards to its safety.

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