In Vivo Lung Deposition of Hollow Porous Particles from a Pressurized Metered Dose Inhaler

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Received November 20, 2001; accepted November 30, 2001.

Purpose: PulmoSphere™ particles are specifically engineered for delivery by the pulmonary route with a hollow and porous morphology, physical diameters < 5 μm, and low tap densities (circa 0.1 g.cm^{-3}). Deposition of PulmoSphere particles in the human respiratory tract delivered by pressurized metered dose inhaler (pMDI) was compared with deposition of a conventional micronized drug pMDI formulation.

Methods: Nine healthy nonsmoking subjects (5 male, 4 female) completed a two-way crossover gamma scintigraphic study, assessing the lung and oropharyngeal depositions of albuterol sulfate, formulated as ^{99m}Tc-radiolabeled PulmoSphere particles or micronized particles (Ventolin Evohaler™, GlaxoSmithKline, Ltd.) suspended in HFA-134a propellant.

Results: Mean (standard deviation) lung deposition, (% ex-valve dose) was doubled for the PulmoSphere formulation compared with Evohaler pMDI (28.5 (11.3) % vs. 14.5 (8.1) %, $P < 0.01$), whereas oropharyngeal deposition was reduced (42.6 (9.0) % vs. 72.0 (8.0) %, $P < 0.01$). Both PulmoSphere and Evohaler pMDIs gave uniform deposition patterns within the lungs.

Conclusions: These data provided "proof of concept" *in vivo* for the PulmoSphere technology as a method of improving targeting of drugs to the lower respiratory tract from pMDIs, and suggested that the PulmoSphere technology may also be suitable for the delivery of systemically acting molecules absorbed via the lung.

KEY WORDS: metered dose inhaler; particle engineering; spray-drying; lung deposition; PulmoSphere; homodispersion.

INTRODUCTION

Inhaled aerosol particles have been used for many years as a means of delivering drugs to the lungs in the treatment of local respiratory diseases, particularly asthma (1). More recently, there has been considerable interest in developing novel methods for the delivery of peptides and proteins that exhibit poor oral absorption, and which currently have to be administered by injection (2). Pulmonary delivery offers an acceptable noninvasive alternative to the needle for systemic administration.

Administration of drugs by the pulmonary route is technically challenging as oropharyngeal deposition may be high, and variations in inhalation technique may affect the quantity of drug delivered to the lungs (3–5). The pressurized metered dose inhaler (pMDI) was first introduced in 1956. When used to deliver conventional formulations consisting of micronized suspensions it is inefficient, with most formulations depositing no more than 10% to 15% of the dose in the lungs, and depositing the majority of the dose in the oropharynx (6,7). Hence, there have been considerable efforts to produce more efficient and reproducible aerosol systems, either through improved drug delivery devices (8,9) or through better formulations that disperse more readily during inhalation (10).

The traditional approach to formulations for pMDIs has involved formulating micronized particles with densities in the range of 1.0 to 1.5 g cm^{-3}. Micronization results in a broad particle size distribution, with little control over other particle attributes such as morphology, density, and surface energy. It is these same attributes that are critical, however, to achieving improved powder dispersion and dispersibility from propellants in pMDIs (11). An alternative approach to the traditional micronized particles is to engineer particles specifically with dispersibility in mind (e.g., the PulmoSphere™ technology).

PulmoSphere particles are prepared by a proprietary spray-drying method and are designed to be both hollow and porous with geometric diameters between 3 and 5 μm, and tap densities of about 0.1 g cm^{-3} (12,13). When the hollow porous PulmoSphere particles are dispersed in propellant, the propellant permeates within the particle, forming a remarkably stable suspension termed a homodispersion™. In essence, the continuous and dispersed phases are equivalent in a homodispersion, separated by a thin-walled interfacial region comprised of drug and excipients. The propellant-filled particles exhibit little difference in density from the neat propellant, and decreased interparticle attractive forces, thereby decreasing the tendency for the particles to cream and flocculate. The improvements in suspension stability could improve uniformity of both emitted dose and delivery to the lung.

In this study we assessed the *in vivo* respiratory tract deposition of a PulmoSphere particle formulation, containing the beta$_2$-agonist albuterol sulfate, suspended in hydrofluoroalkane (HFA-134a; 1,1,1,2-tetrafluoroethane) propellant. A comparison was made against a conventional micronized formulation involving the same propellant. The study also assessed the safety and tolerability of the PulmoSphere pMDI formulation in healthy volunteers.

MATERIALS AND METHODS

Metered Dose Inhalers

The hollow porous albuterol sulfate particles were manufactured by a two-step process involving emulsification followed by spray drying, which has been described previously (12). Spray-dried powders were then hand-filled into aluminum canisters (Presspart Inc., Cary, North Carolina), and the can was sealed with a DF30/50act 50 μl-metering valve (Valois Pharmaceuticals, Marly-le-Roi, France). HFA-134a propellant (DuPont Fluoroproducts, Wilmington, DE) was then loaded into the canister by overpressure through the valve stem. The powder was dispersed by first sonicating the canisters for 10-15 s, and then placing them on a wrist-action

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shaker for ca. 30 min. The albuterol PulmoSphere formulation was administered using the standard actuator for a Proventil® HFA pMDI (Key Pharmaceuticals, Kenilworth, New Jersey). The PulmoSphere particles contained 41% w/w albuterol sulfate (FDC Limited, Mumbai, India). Excipients included distearoylphosphatidylcholine, (DSPC; Genzyme Pharmaceuticals, Cambridge, Massachusetts), calcium chloride dihydrate (Fisher Scientific, Pittsburgh, Pennsylvania), and residual levels of perfluorooctyl bromide (LiquiVent®, Alliance Pharmaceutical Corp., San Diego, California) and water. The pMDI metered about 85-μg albuterol sulfate from the valve per actuation. An already marketed albuterol HFA pMDI comprised of micronized drug (Ventolin Evohaler, Glaxo-SmithKline Ltd, London, United Kingdom) was used as a comparator product. This formulation metered 100 μg of albuterol sulfate per actuation.

Physicochemical Testing

A Sympatec laser diffraction analyzer (HELOS H1006, Clausthal-Zellerfeld, Germany) equipped with a RODOS type T4.1 vibrating trough was used to characterize the volume-weighted mean geometric diameter of the spray-dried powder. Approximately 1–3 mg of powder was placed in the powder feeder, which was subsequently atomized through a laser beam using 1 bar of air pressure, 60 mbar of vacuum, 70% feed rate, and a 1.30 mm funnel gap. Data were collected over an interval of 0.4 s, with a 175-μm focal length lens, triggered with 1% obscuration. Particle size distributions were determined using a Fraunhofer model. Particle densities were estimated from tap density measurements as determined with a Van Kel Industries (Edison, New Jersey) unit. Dose uniformity measurements were collected in the standard United States Pharmacopeia (USP) unit spray apparatus <601> and quantitated for albuterol sulfate content by high-performance liquid chromatography (HPLC). These measurements were conducted independently by scientists at Magellan Laboratories (Research Triangle Park, North Carolina). Aerodynamic particle size measurements were made using an Andersen Cascade Impactor (ACI; Copley Instruments, United Kingdom) operated at 28.3 l/min and fitted with a USP induction port (14). The particle size distributions were fractionated into mass of drug or amount of radiolabel deposited on the pMDI actuator, ACI induction port, eight stages, and terminal filter. The percentage of the total dose deposited from stage 4 to the terminal filter (corresponding to particles less than 3.3 μm) was considered to be the fine particle fraction ($FPF_{3.3}$).

Radiolabeling Methods

The radiolabeling method used for the pMDI formulations was based on previously described methods (15,16). ^{99m}Tc-pertechnetate (Nycomed Amersham, Amersham, United Kingdom) was extracted from a saline solution into methyl ethyl ketone (MEK) and subsequently transferred into an empty pMDI canister. The MEK was then evaporated by gentle heating in a stream of air. A filled pMDI containing either PulmoSphere or Evohaler formulations was cooled in liquid nitrogen, the metering valve removed, and the liquefied contents poured quickly into the canister containing the ^{99m}Tc-pertechnetate. A new metering valve was crimped in place and the canister was immersed in water to ensure that the seal was intact. The new cans and valves were identical to those used for the "unlabeled" product. The radiolabeled pMDI was sonicated for 10 min and primed before use by firing 10 doses to waste. The amount of radiolabel was adjusted to ensure that each actuation delivered at least 3 MBq, but no more than 5 MBq, of ^{99m}Tc.

Validation of Radiolabeling Methods

Before starting the clinical phase of the study, a series of experiments was performed to assess whether the radiolabeling process had any effect on the particle size distribution of albuterol sulfate from the two pMDIs and to determine whether the radiolabel would accurately reflect the distribution of the drug substance. The particle size distribution of albuterol sulfate before labeling was compared with that after labeling, and also with the particle size distribution of the ^{99m}Tc radiolabel, using a series of replicate inhalers prepared on different days. Stages of the ACI were washed quantitatively into volumetric flasks, and the drug and radiolabel content of the washes were determined by HPLC (Agilent HP1100 with Chemstation software detecting ultraviolet radiation at 276 nm) and by gamma counting, respectively. The ACI was fitted with a USP induction port, and operated at 28.3 l/min as described above. Ten doses were used in each of the ACI tests.

Study Population

The clinical study was a two-way randomized crossover investigation involving a comparison of the two pMDI formulations on 2 study days between 5 and 14 days apart. Nine healthy volunteers (five male, four female, age range 24 to 62 y) were included in the study, all of who had lung function values within the normal limits (forced expiratory volume in one second, FEV_1 > 80% of predicted, 17). All had not smoked for at least 1 y and were declared healthy by a physician as being free from clinically significant pathology, and passing a physical examination. Prior to recruitment, the nature of the study was explained both verbally and in writing to each volunteer, and each volunteer provided written consent. The objectives and methods used in the study were approved by the Quorn Research Review Committee (Leicestershire, UK). The administration of radioactivity to the subjects was approved by the Department of Health, UK. The study was performed in accordance with the Declaration of Helsinki.

Administration of Radiolabeled Aerosols

Prior to administration of the radiolabeled aerosol, subjects practiced the inhalation maneuver with the aid of a placebo pMDI. Subjects were instructed to empty their lungs before taking a slow deep inhalation with a mean inhaled flow rate of 30 l/min. The investigator fired the pMDI approximately 1 s after the beginning of inhalation. At the end of the inhalation, a 10 s breath-holding pause was observed before the subject breathed out through a filter to trap any radioactive aerosol in the exhaled air. When the subject had demonstrated competence in the inhalation maneuver, the placebo pMDI was replaced with the radiolabeled inhaler. Each subject received two doses of the radiolabeled aerosol, and the pMDI was shaken between doses. During the aerosol inhalations the inhaled volume and inhaled flow rate were recorded by a Vitalograph pMDI Compact-Spirometer (Vitalograph Ltd., Buckingham, UK) connected in series with the pMDI.

Scintigraphic Measurements and Data Analysis

Immediately following administration of the radiolabeled aerosol, scintigraphic images of the chest (posterior and anterior, duration 100 s), lateral oropharynx (duration 30 s), actuator, and exhalation filter were recorded using a gamma camera (General Electric Maxicamera, Milwaukee, WI). During 1 study day, a posterior ventilation scan was performed using the radioactive inert gas ^{81m}Kr. This scan was omitted for subjects who had a ventilation scan available from a previous study conducted within the previous five years. All images were recorded on a Park Medical Micas X plus computer system (Park Medical, Farnborough, Hampshire, United Kingdom) and were stored on digital audiotape (DAT, Seagate, Amsterdam, Netherlands) for subsequent analysis.

Regions of interest were drawn around the oropharynx, esophagus, and stomach. The counts obtained within these regions were corrected for background radioactivity, radioactive decay, and tissue attenuation of gamma rays (18). In regions where both anterior and posterior images were recorded, the geometric mean of counts in both images was calculated. Determination of the percentage of the dose deposited in the oropharynx included activity adhering to the mouth and pharynx together with any swallowed activity detected in the esophagus, stomach, and intestine. The counts for each area were expressed as a percentage of the metered dose, which was determined from the sum of the total body counts in addition to those deposited on the actuator and the exhalation filter.

To assess regional lung deposition, the lung outlines from the ^{81m}Kr ventilation scan were used to define the borders of the lung fields on the aerosol views. The lungs were divided into central, intermediate, and peripheral regions of interest (15). The peripheral lung zone to central lung zone deposition ratio (P/C ratio) was calculated as an index of regional lung deposition.

Safety Assessments

Lung function tests (FEV_1, peak expiratory flow rate, and forced vital capacity) were performed prior to dosing, 5 min postdose, and 1 h and 8 h postdose, using a Microloop Spirometer (Micro Medical Ltd, Rochester, United Kingdom). Blood samples for hematology and serum chemistry, and urine samples for urinalysis, were obtained prior to dosing and again at 180 min postdose. Blood pressure and pulse rate were measured prior to dosing, and at intervals up to 8 h postdose. An electrocardiogram (ECG) was performed prior to dosing and again at 30 min postdose. All of the tests were repeated at the poststudy medical examination, 5 to 7 d postdose.

Statistical Methods

The Wilcoxon matched-pairs signed-ranks test was used to compare the lung deposition data for the PulmoSphere and Evohaler formulations.

RESULTS

Physicochemical Properties of Albuterol PulmoSphere Powder

The albuterol PulmoSphere spray-dried powder exhibited a volume-weighted mean geometric diameter by laser diffraction of 3.8 μm with a geometric standard deviation (GSD) of 1.8. The ultralight nature of the particles was confirmed by a measured tap density of 0.12 g cm^{-3}. Albuterol PulmoSphere suspensions in HFA-134a exhibited little particle aggregation and creaming times in the order of several hours.

The aerodynamic particle size distribution of the albuterol PulmoSphere particles was assessed by ACI (Table I). The mass median aerodynamic diameter and GSD remained constant following storage for 7 months at ambient room temperature (n = 3 canisters) with values of 3.6 μm and 1.8, respectively. Within the error of the measurement, no variation was noted in the $FPF_{3.3}$. Dose content uniformity measurements were conducted according to the proposed Food and Drug Administration (FDA) specifications (Fig. 1). Mean emitted doses were 85.8 μg and 82.0 μg before and after storage, respectively. The relative standard deviations were 5.8% at the initial time point, and 4.2% after 8 months. Chemical stability of the drug substance was assessed according to the current USP test method. The USP specification for degradation products in albuterol sulfate allow for no more than 2% w/w impurities as measured via thin layer chromatography relative to a USP albuterol sulfate reference standard (USP XXIV method <621>). In this regard, the level of degradation products following spray-dry manufacture or after storage of the albuterol PulmoSphere formulation for 7 months at ambient room temperature was less than the 2% limit and acceptable.

Radiolabeling Validation

The results of the radiolabeling validation tests for the PulmoSphere and Evohaler formulations are summarized in Fig. 2 and 3. For the PulmoSphere formulation, drug and radiolabel matched closely throughout the stages of the ACI. The mean $FPF_{3.3}$s and standard deviations (in parentheses) of drug before labeling, drug after labeling, and radiolabel were 33.3 (3.3) %, 33.6 (1.3) % and 36.6 (1.9) %, respectively. For the Evohaler pMDI, there was a slight mismatch between drug and label on the inlet "throat" and impactor stage 0, but as drug deposited on both these stages would be deposited in the oropharynx *in vivo*, this mismatch was not considered

Table I. Particle Size Distribution of Albuterol PulmoSphere Clinical Formulation over Time

Particle size attribute	Can no.	Initial	7 Months (ART)
$FPF_{3.3}$ (% < 3.3 μm)	1	27	28
	2	27	27
	3	26	28
	Mean	27	28
MMAD (μm)	1	3.56	3.66
	2	3.65	3.63
	3	3.62	3.46
	Mean	3.61	3.55
GSD	1	1.76	1.79
	2	1.79	1.83
	3	1.82	1.73
	Mean	1.79	1.78

Abbreviations: $FPF_{3.3}$: fine particle fraction; MMAD: mass median aerodynamic diameter; GSD: geometric standard deviation; ART: ambient room temperature.

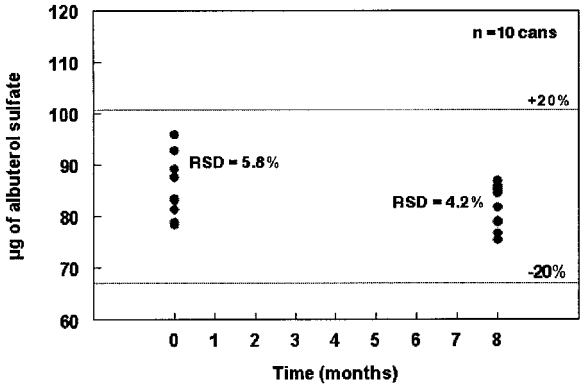

Fig. 1. Plot of the dose content uniformity for the PulmoSphere formulation immediately after manufacture and following storage for 8 months at ambient room temperature. (RSD = relative standard deviation.)

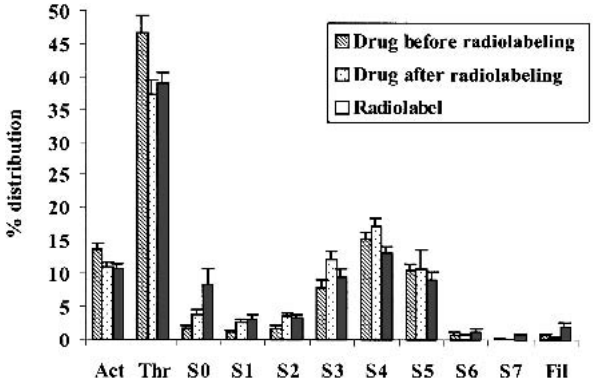

Fig. 3. Radiolabeling validation data for Evohaler formulation, showing distributions in an ACI of drug before labeling, drug after labeling, and radiolabel. Act: actuator; Thr: inlet throat; S0 to S7: stages 0 to 7; Fil: final filter (n = 5 unlabeled, n = 11 radiolabeled).

important. There was a close agreement between drug and label within the "respirable" part of the size distribution (i.e., stage 4 to filter inclusive). The mean $FPF_{3.3}$s of drug before labeling, drug after labeling, and radiolabel for the Evohaler pMDI were 27.3 (1.9) %, 29.0 (3.9) % and 26.0 (2.8) %, respectively. The ratios of radiolabel $FPF_{3.3}$ to $FPF_{3.3}$ of drug before labeling were 1.10 and 0.95 for the PulmoSphere and Evohaler pMDIs, respectively. These data demonstrated that the radiolabel deposition patterns would be representative of drug deposition. Mean fine particle masses (masses of drug recovered from stages 2 to filter in the ACI, inclusive) before and after labeling for the PulmoSphere formulation were 44.3 and 43.3 µg, respectively, compared with 44.2 and 56.5 µg, respectively, for the Evohaler ($P < 0.05$).

Before administering radiolabeled formulations to volunteers in the clinical study, each pMDI was tested to ensure that the $FPF_{3.3}$ of the radiolabel was comparable to those obtained in the radiolabeling validation experiments. The mean radiolabel $FPF_{3.3}$s for study-day PulmoSphere and Evohaler pMDIs were 30.4% and 23.1%, respectively.

Deposition Data

The *in vivo* fractionation of the dose, expressed as a percentage of the ex-valve (metered) dose, is summarized in Fig. 4, and individual lung deposition data are listed in Table II. Mean (SD) lung deposition was 28.5 (11.3) % and 14.5 (8.1) % for the PulmoSphere and Evohaler pMDIs, respectively, and this difference was statistically significant ($p < 0.05$). These values correspond to mean lung doses of 48 and 29 µg of albuterol sulfate deposited in the lungs for the two products, respectively. With regard to regional lung deposition, deposition in each of the peripheral, intermediate, and central lung regions followed the same order as whole lung deposition, i.e., greater deposition for the PulmoSphere pMDI (Table III). For both pMDIs, mean peripheral lung deposition averaged greater than intermediate lung deposition, which in turn averaged greater than central lung deposition. These data were supported by the P/C ratios with mean (standard deviation [SD]) values of 1.8 (0.9) and 1.7 (0.4) recorded for the PulmoSphere and Evohaler pMDIs, respectively.

Mean (SD) deposition in the oropharynx was significantly lower ($p < 0.01$) for the PulmoSphere pMDI (42.6 (9.0) %) than the Ventolin Evohaler (Fig. 4) (72.0 (8.0) %). Mean (SD) actuator depositions were 28.6 (7.6) % and 12.6 (2.5) % ($P < 0.01$) and the percentage of the dose deposited on the exhalation filter was 0.3 (0.3) % and 0.7 (0.6) % for the PulmoSphere and Evohaler pMDIs, respectively.

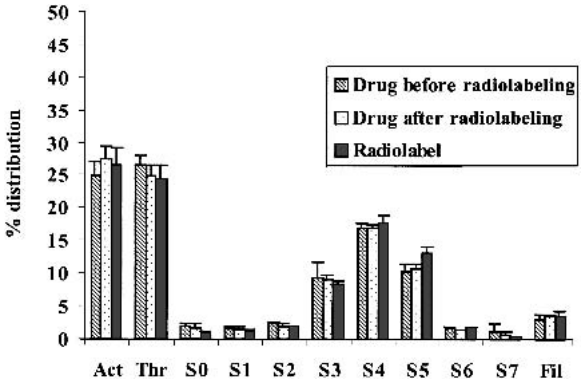

Fig. 2. Radiolabeling validation data for PulmoSphere formulation, showing distributions in an ACI of drug before labeling, drug after labeling, and radiolabel. Act: actuator; Thr: inlet throat; S0 to S7: stages 0 to 7; Fil: final filter (n = 5).

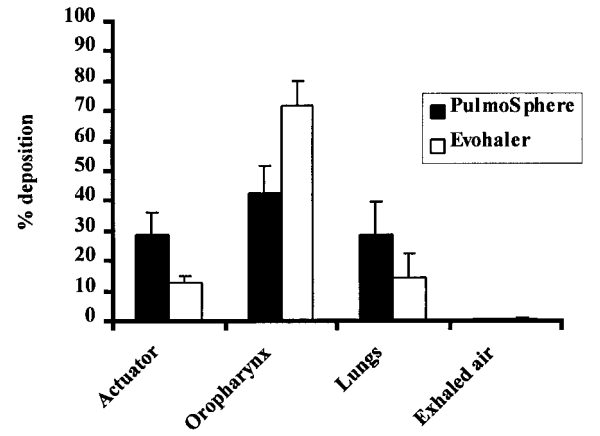

Fig. 4. Fractionation of the dose between the actuator, oropharnyx, whole lungs, and exhaled air filter for PulmoSphere and Evohaler formulation (n = 9).

Table II. Individual Lung and Oropharyngeal Deposition Data, Expressed as Percentage Ex-Valve (Metered) Dose ($n = 9$)

	PulmoSphere formulation		Evohaler formulation	
Subject	Oropharynx	Lungs	Oropharynx	Lungs
1	41.4	18.7	78.2	10.3
2	32.1	36.2	63.0	23.7
3	42.6	39.5	70.1	18.1
4	43.8	21.2	74.5	10.1
5	33.7	33.0	77.2	10.3
6	54.6	20.3	78.6	3.3
7	31.4	47.2	56.3	29.9
8	50.5	28.4	79.6	11.0
9	53.5	12.2	70.5	13.6
Mean	42.6	28.5	72.0	14.5
SD	9.0	11.3	8.0	8.1

Inhalation Details

The inhalation details are summarized in Table IV. The peak inhaled flow rate and the mean inhaled flow rate were recorded for both pMDIs. The mean inhaled flow rates were close to the targeted rates of 30 l/min. Inhaled volume averaged 2.9 l for PulmoSphere formulation, and 1.5 l for Evohaler formulation. The lower inhaled volume for the Evohaler formulation may have been an artifact caused by some of the volunteers pausing during inhalation as a reaction to the propellant spray hitting the back of the throat, which results in the Spirometer stopping recording.

Safety Assessments

Lung function remained constant throughout the study (Table V), being similar before and after dosing, and at the poststudy medical examination. The PulmoSphere formulation was well tolerated: six subjects reported no adverse events, and in only one subject was an adverse event reported that could have been product related (mild dizziness and headache). No significant abnormalities were detected in the blood samples taken for hematology and clinical chemistry or the urine samples taken for urinalysis. Similarly, the pre- and postdose vital signs and ECG measurements did not detect any significant abnormalities.

DISCUSSION

This was the first human study to investigate the deposition of the PulmoSphere pMDI technology. PulmoSphere particles are produced using a two-step process (12,13). First, a submicron fluorocarbon-in-water emulsion is produced by high-pressure homogenization. The emulsion is stabilized by a monolayer of phospholipid (e.g., DSPC) at the fluorocarbon/water interface. The emulsion is then combined with a second aqueous phase containing the drug. The resulting aqueous dispersion is then used as the feedstock in the subsequent spray-drying step. The fluorocarbon in the emulsion droplets serves as a "blowing agent or "inflation agent" and creates the hollow porous morphology as the droplets dry. Varying the ratio of fluorocarbon to phospholipid in the emulsion controls the porosity and bulk density of the particles.

The resulting particles form very stable homodispersions in HFA propellants, leading to highly reproducible dosing *in-vitro* (relative standard deviation < 6%), independent of canister storage orientation. In the present study the albuterol PulmoSphere formulation easily met the proposed FDA specifications for content uniformity, both after manufacture and following 8 months of storage at ambient room temperature. According to the guidance, ten canisters are tested and the amount of active per determination should not be outside of 80–120% of the label claim for more than one canister, and none of the determinations should be outside of 75–125%. Overall, the mean should not be outside of 85–115% of label claim. The albuterol PulmoSphere sample utilized in the clinical study met the proposed FDA specifications both after manufacture and after storage for 8 months at ambient room temperature. None of the determinations were outside of 85–115%. These errors are comparable to the errors associated with hand-filling of powder into the canisters during manufacturing, and may be reduced further if homogeneous suspensions are utilized in the filling process as would be the case in large-scale manufacturing operations. In addition, no changes in the aerodynamic particle size distribution or chemical stability of the drug substance were noted.

The principal forces leading to particle flocculation in nonaqueous media are believed to van der Waals' attractive forces acting over extremely short ranges. It was predicted on theoretical grounds that particles that are both hollow and porous would be subject to reduced van der Waals' forces,

Table III. Mean (SD) Regional Lung Deposition Data

	PulmoSphere formulation	Evohaler formulation
Peripheral region (%)	11.7 (4.0)	5.9 (2.9)
Intermediate region (%)	9.3 (4.1)	4.5 (2.6)
Central region (%)	7.5 (4.5)	4.3 (2.9)
P/C ratio	1.8 (0.9)	1.7 (0.4)

Deposition in peripheral, intermediate, and central regions are expressed as a percentage of metered dose. The peripheral lung zone/central lung zone deposition ratio (P/C ratio) is also shown ($n = 9$).

Table IV. Inhalation Parameters

	PulmoSphere formulation	Evohaler formulation
Mean inhaled flow (l/min)	29.8 (27.8)	25.4 (7.5)
Peak inhaled flow rate (l/min)	40.4 (42.0)	34.1 (5.6)
Inhaled volume (l)	2.9 (1.5)	1.5 (0.7)

$n = 9$.

Table V. Forced Expiratory Volume in 1 s (FEV_1, l) before the Study, before Dosing, at Timed Intervals after Dosing and at the Poststudy Medical ($n = 9$)

	PulmoSphere particles	Evohaler formulation
Prestudy	3.74 (0.89)	3.74 (0.88)
Predose	3.72 (0.99)	3.70 (0.87)
5 min postdose	3.70 (0.92)	3.73 (0.86)
1 h postdose	3.65 (0.97)	3.75 (0.87)
8 h postdose	3.57 (0.94)	3.64 (0.88)
Poststudy	3.69 (0.88)	3.69 (0.87)

together with reduced interfacial tensions between the particle surface and the liquid medium (12,13). Visible inspection of PulmoSphere particles has revealed a very low incidence of aggregates (12). The scintigraphic data confirmed this prediction, and lung deposition of albuterol sulfate was approximately doubled for the PulmoSphere formulation compared with the micronized Evohaler formulation, whereas oropharyngeal deposition was correspondingly reduced. Differences between the products in actuator orifice diameter could also contribute to the observed differences in fine particle fraction and lung dose (19).

Albuterol sulfate was used as test drug in this study, although in clinical practice there is little real advantage in improving targeting to the lungs for inhaled $beta_2$-agonists. However, other drugs including inhaled corticosteroids, and drugs intended for systemic delivery, would benefit from being delivered more efficiently to the lungs. This could result in lower treatment doses of inhaled corticosteroids, so that the therapeutic ratio is increased, and more cost-effective delivery of expensive systemically acting drugs, leading to products that are more commercially viable to develop. Other types of pMDI formulations can also improve targeting drugs to the lungs, including some solution formulations containing ethanol as a cosolvent (20). However, it has been suggested (12) that the presence of cosolvents may lead to chemical instability of some drug substances, extraction of elastomers from the metering valve, enhanced Ostwald ripening, and unacceptable taste. As well, the maximum dose for solution-based formulations may be limited for some drugs with limited solubility.

Lung deposition was doubled for PulmoSphere particles compared with the micronized formulation (28.5 vs. 14.5% of the ex-valve dose $p < 0.05$), although there was less difference between the fine particle fractions (33.3 vs. 27.3%). The failure of *in vitro* tests to accurately predict the relationship between drug deliveries *in vivo* for two products has been observed previously, especially for pMDI formulations (21). In one study (22), sprays delivered from an AERx® (Aradigm Corporation, Hayward, California) multidose liquid spray device and from a pMDI had $FPF_{5.8}$s (particles less than 5.8 μm) that were quite similar (55 vs. 44%), whereas lung depositions were markedly different (53 vs. 21% $p < 0.0001$). Values of $FPF_{5.8}$ obtained *in vitro* systematically overestimate drug delivery to the lungs *in vivo* (21). These considerations underline that the major roles of *in vitro* particle size data lie in obtaining rapid data in product development, and also in quality control. *In vivo* tests to assess drug delivery are desirable when products are compared, or when some prediction of performance in man is needed. The radiolabeling validation data showed that the ^{99m}Tc radiolabel was an accurate marker for drug in both formulations. The fine particle mass for the Evohaler formulation increased significantly after labeling, but this had no bearing on the results of the study, as we were not seeking to assess either pharmacokinetic or pharmacodynamic endpoints.

This study has shown *in vivo* "proof of concept" for an engineered formulation of PulmoSphere particles, and has demonstrated that they can be delivered more efficiently to the lungs from pMDIs than from a conventional micronized formulation. The data suggest that PulmoSphere formulations could provide a useful delivery system for both locally acting and systemically acting drugs given by the pulmonary route.

ACKNOWLEDGMENT

This study was cosponsored by Alliance Pharmaceutical Corp., San Diego, California, and Inhale Therapeutic Systems, Inc., San Carlos, California.

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