Pulmonary Delivery of Deslorelin: Large-Porous PLGA Particles and HPβCD Complexes

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Purpose. To compare the systemic delivery of deslorelin following intratracheal administration of different deslorelin formulations. The formulations included dry powders of deslorelin, large-porous deslorelin-poly(lactide-co-glycolide) (PLGA) particles, and small conventional deslorelin-PLGA particles. Also, solution formulations of deslorelin and deslorelin-hydroxy-propyl-beta-cyclodextrin (HPβCD) complexes were tested.

Methods. Dry powders of deslorelin, large-porous (mean diameter, 13.8 μm; density, 0.082 g/cc), and small conventional (mean diameter, 2.2 μm; density, 0.7 g/cc) deslorelin-PLGA particles and solutions of deslorelin with or without HPβCD were administered intratracheally to Sprague-Dawley rats. Blood samples were collected at 3 h, 1, 3, and 7 days postdosing, and plasma deslorelin concentrations were determined using enzyme immunoassay. At the end of 7 days, lungs were isolated, and bronchoalveolar lavage fluid was collected and analyzed for deslorelin.

Results. At the end of 7 days, deslorelin plasma concentrations in the large-porous deslorelin-PLGA particle group were 120-fold and 2.5-fold higher compared to deslorelin powder and small conventional deslorelin-PLGA particles, respectively. Co-administration of HPβCD resulted in 2-, 3-, and 3-fold higher plasma deslorelin concentrations at 3 h, 1 and 3 days, respectively, compared to deslorelin solution. On day 7, deslorelin concentrations in bronchoalveolar lavage fluid as well as plasma were in the order: large porous particles > small conventional particles > deslorelin-HPβCD solution > deslorelin powder > deslorelin solution.

Conclusions. Large-porous deslorelin PLGA particles can sustain deslorelin delivery via the deep lungs. Co-administration of HPβCD enhances the systemic delivery of deslorelin. The pulmonary route is useful as a noninvasive alternative for the systemic delivery of deslorelin.

KEY WORDS: deslorelin; HPβCD; intratracheal; large-porous particles; PLGA.

INTRODUCTION

The pulmonary route is an attractive noninvasive alternative for the systemic delivery of macromolecules (1). Indeed, the clinical development of inhaled insulin has propelled commercial and scientific interest in the use of inhalation systems for the treatment of nonpulmonary diseases (2). Currently, a number of peptides and proteins including insulin, growth hormone, calcitonin, and leuprolide are being investigated for delivery via the deep lungs (3). In our studies, we are investigating the pulmonary route for the systemic delivery of deslorelin. Deslorelin, a nonapeptide of molecular weight 1.3 kDa, is a potent synthetic leutinizing hormone-releasing hormone (LHRH) agonist. Deslorelin has potential therapeutic application in the treatment of endometriosis, uterine fibroids, and cancers of the breast and prostate (4, 5). The pulmonary route offers a number of advantages including large surface area (~100 m^2), ample blood supply, and avoidance of hepatic first-pass metabolism (6). However, proteolytic degradation limits the bioavailability of macromolecules administered by this route (7).

We have previously demonstrated that deslorelin is degraded in the respiratory epithelium (8). Also, we identified the degradation-susceptible bonds in deslorelin and determined the kinetics of metabolite formation. Our results indicated that deslorelin is susceptible for degradation at the Trp^3-Ser^4 and the Ser^4-Tyr^5 bonds (8). One way to decrease deslorelin degradation and improve bioavailability is to use an adjuvant, which would protect the enzymatically labile sites from degradation. Cyclodextrins, cyclic oligomers of glucose, have a cone-like structure, the interior of which is hydrophobic (9). In our previous studies, using spectroscopic and calorimetric techniques, we observed that the hydrophobic cavity of hydroxy-propyl-beta-cyclodextrin (HPβCD) interacts with the aromatic amino acids (tryptophan and tyrosine) of deslorelin and reduces its proteolytic degradation (10). Thus, cyclodextrin-deslorelin complexes offer an opportunity to protect deslorelin against degradation. Therefore, one goal of this study was to test whether HPβCD enhances deslorelin delivery following intratracheal administration as a solution in a rat model.

Another goal of this study was to sustain deslorelin delivery via the lungs. Using drug-polymeric particles, the drug release and the duration of action can be sustained (11). However, sustained drug delivery via the lungs is a challenge because alveolar macrophages within the deep lungs rapidly clear insoluble particles (12). This limitation can be overcome using large porous particles with high geometric diameters (~10–20 μm) and low bulk density (<0.4 g/cc). These particles, by virtue of their large size, will likely escape clearance by the alveolar macrophages and sustain the delivery of macromolecules *in vivo* (13). The porosity of these particles lends them an aerodynamic diameter much less than their geometric diameter, facilitating their deep lung deposition.

We have developed a supercritical fluid (SCF) process for the preparation of large porous polymeric (polylactide-co-glycolide) (PLGA) particles of deslorelin (14). SCF technology for porous particle preparation offers several advantages: a) an environmentally benign process, b) mild processing conditions suitable for macromolecules, and c) processes that can easily be tailored to produce particles with desired morphologies, densities, and low residual solvents (15, 16). Using an SCF process, we could modify conventional deslorelin-PLGA particles to large porous particles (14). Our results indicated that upon supercritical (SC) CO_2 treatment (1200 psi, 33°C for 30 min), the mean particle size of the deslorelin-PLGA microparticles increased from 2.2 to 13.8 μm, and the mean bulk density reduced from 0.7 to 0.082 g/cc. Also, mass spectrometry indicated structural integrity of released deslorelin, circular dichroism spectra indicated stabilization of β-turn conformation, and scanning electron microscopy confirmed increased particle size and pore formation. The des-

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lorelin release was sustained during the 7-day *in vitro* study period. Also, the residual solvent content was reduced from 4500 ppm to below detection limit (<25 ppm). Compared to the conventional particles, these porous particles exhibited reduced cellular uptake by alveolar macrophages, airway epithelial cells, and alveolar epithelial cells. This suggests that these particles can remain for longer periods in the lungs to sustain drug delivery (14). In the current study, we evaluated the ability of large porous deslorelin-PLGA particles to sustain systemic delivery of deslorelin following intratracheal administration in rats.

MATERIALS AND METHODS

Drugs and Chemicals

Deslorelin was a gift from Balance Pharmaceuticals, Inc. (Santa Monica, CA, USA). PLGA 50:50 with acid end groups and molecular weight of 23.2 (i.v. 0.26 dl/g) kDa was obtained from Birmingham Polymers, Inc. (Birmingham, AL, USA). HPβCD was obtained from Cerestar US (Hammond, IN, USA). Poly(vinyl alcohol) (average molecular weight 30,000–70,000) and the anesthetic, a mixture of ketamine HCl and xylazine HCl, were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Enzyme immunoassay kits for plasma deslorelin analysis and C18 Sep Pak columns for extraction of deslorelin from plasma were obtained from Peninsula Laboratories (San Carlos, CA, USA).

Preparation of Deslorelin Formulations

Prior to *in vivo* use, dry powders of conventional and large porous deslorelin-PLGA (50:50) microparticles and solutions of deslorelin with and without HPβCD were formulated.

Conventional Deslorelin PLGA (50:50) Microparticles

Conventional deslorelin-PLGA microparticles were prepared using an emulsion-solvent evaporation method. Weighed (900–1100 mg) quantities of the polymer were placed in methylene chloride (7 ml) and allowed to dissolve under intermittent vortexing at room temperature. Deslorelin (100–200 mg) was dissolved in methanol (3 ml), and this solution was then added to the polymer solution to form the dispersed phase. This dispersed phase was then added over 1 min to 50 ml of aqueous continuous phase containing 2% w/v poly(vinyl alcohol) on ice under sonication (Misonix Inc. Farmingdale, NY, USA) at 50 W to form an O/W emulsion. This emulsion was then added dropwise to 500 ml of 2% w/v aqueous poly(vinyl alcohol) solution under rapid stirring and allowed for solvent evaporation at 25°C for 12 h. The microparticles were then separated by ultracentrifugation at 100,000 × g for 30 min at 4°C and washed with 200–500 ml of double-distilled water. The microparticle pellet was suspended in distilled water and lyophilized for 24–48 h to obtain dry particles.

SC CO_2 Pressure Quench Technique for the Preparation of Large Porous Polymeric Microparticles

Large, porous deslorelin-PLGA particles were prepared using a previously described SC CO_2 pressure quench technique (14). Briefly, small conventional deslorelin-PLGA particles prepared using an emulsion-solvent evaporation process, as described above, were placed in a high-pressure vessel (High Pressure Equipment Company, Erie, PA, USA), tightly sealed, and equilibrated to 33°C over 15 min in a temperature-controlled water bath. Subsequently, the vessel was filled with CO_2 using a pump (Model 37-6-30, High Pressure Equipment Company, Erie, PA) to attain a pressure of 1200 psi. These pressure and temperature conditions are above the critical values for CO_2. After exposing the particles to SC CO_2 for 30 min, the pressure within the high-pressure vessel was quenched over 1 min. The particles were recovered and characterized for their morphology, mean size, density, and porosity.

Deslorelin-HPβCD Solution

Deslorelin-HPβCD complex in solution was formed as described previously (10) by mixing deslorelin (1 mg/ml) with 50 mM HPβCD in phosphate-buffered saline (PBS).

In Vivo Studies

In all studies, male Sprague-Dawley rats weighing 150–200 g, purchased from SASCO (Wilmington, MA, USA), were used. The animals were fed a normal diet (Purina, Inc., Richmond, IN, USA) and water *ad libitum* and housed in a 12-h light/12-h dark cycle (lights on at 6 a.m. and off at 6 p.m.) in a constant temperature environment of 22°C. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center, an American Association for Accreditation of Laboratory Animal Care (AAALAC) approved facility.

The animals were randomly divided into 5 groups of 4 animals each to receive different treatments. Group 1: rats administered a single intratracheal dose of deslorelin solution; group 2: rats administered a single intratracheal dose of deslorelin-HPβCD solution; group 3: rats administered a single intratracheal dose of deslorelin powder; group 4: rats administered a single intratracheal dose of large-porous deslorelin-PLGA particles; group 5: rats administered a single intratracheal dose of conventional deslorelin-PLGA particles. Blood was collected at 3 h, 1, 3, and 7 days following dose administration in all groups, and all animals were sacrificed 7 days post-dosing. Following sacrifice, lungs were isolated and bronchoalveolar lavage fluid was collected.

For deslorelin and deslorelin-HPβCD solutions, 150 μl of preparation containing 1 mg/ml deslorelin with or without 50 mM HPβCD was used. For large porous and small conventional microparticles, about 2.2 mg of powder containing 150 μg of deslorelin was administered. For plain deslorelin powder formulation devoid of any excipients, about 1.1 mg of deslorelin was administered.

Intratracheal Instillation

All intratracheal instillations were performed after anesthetizing animals with an intraperitoneal injection of ketamine/xylazine (90/20 mg/kg body weight). Dry powders of deslorelin, large-porous deslorelin-PLGA particles, and conventional deslorelin-PLGA particles were administered to the rat lungs as previously described (17) using a PennCentury Dry Powder Delivery device equipped with an air pump (AP-1, Penn-Century, Inc. Philadelphia, PA, USA). The adminis-

tration of the powder was made by insufflation of 3 ml of air contained in the air pump connected to the device. The DP-4 Dry Powder Insufflator (Penn-Century, Inc., Philadelphia, PA) was selected as a simple, easy to use, commercially available device that could aerosolize powders to be delivered to the rat lung. The insufflator was weighed before and after powder filling and after administration to determine the actual amount of sample emitted and aerosolized into the lungs. Intratracheal instillation of solutions was performed using spray instillation. For spray instillation, a Microsprayer (model IA-1C, Penn-Century, Inc. Philadelphia, PA), which consists of a thin, flexible, stainless steel tube (0.64-mm diameter; 23 gauge) equipped with a high-pressure syringe (FMJ-250, Penn-Century Inc. Philadelphia, PA) was used to aerosolize 150 μl of liquid into the lungs.

Lung Isolation and Bronchoalveolar Lavage

Isolation of lungs and lung lavage was done as described previously (18). Briefly, after animals were euthanized, an incision was made at the level of the esophagus and the lung lobes were isolated along with the trachea. Following isolation, 1 ml of phosphate-buffered saline was perfused through the trachea using a 27-gauge needle, and the lavage fluids were collected by making an incision on the lung lobes. The lungs were then reperfused two more times with the recovered fluid. The fluid thus obtained was centrifuged (10,000 × *g* for 10 min at 4°C) to remove suspended cells. The supernatants were stored at −20°C until analysis.

Enzyme Immunoassay for Deslorelin in Plasma

Blood Processing To Obtain Plasma

Blood from all animals was collected under ether anesthesia from orbital plexus into polypropylene tubes containing EDTA (1 mg/ml) and aprotinin (500 KIU/ml of blood). The tubes were then centrifuged at 8000 × *g* for 15 min to separate plasma. Plasma was transferred to a fresh polypropylene tube and stored at −20°C until analysis.

Deslorelin Extraction from Plasma

Prior to analysis by enzyme immunoassay, deslorelin was extracted from plasma using C-18 Waters Sep-Pak columns (Peninsula Laboratories). Figure 1 depicts the preparation of column and extraction procedure. Briefly, the Sep-Pak columns were equilibrated by first washing with 1 ml of acetonitrile followed by 3 washes with 3 ml of distilled water containing 1% trifluoroacetic acid. Plasma was diluted with an equal volume of 1% trifluoroacetic acid solution and centrifuged at 10,000 × *g* for 15 min to remove any particulate matter. This acidified, clarified, plasma was loaded onto the prepared C-18 Sep-Pak column. This was then eluted slowly with 1 ml of a 60:40 mixture of acetonitrile and 1% trifluoroacetic acid solution. This eluant was then dried under nitrogen and stored at −20°C until analysis. This dried extract was reconstituted in EIA buffer just prior to analysis.

Enzyme Immunoassay

Deslorelin plasma concentrations were determined using a commercially available enzyme immunoassay kit from Peninsula Laboratories (San Carlos, CA). The kit is a competitive

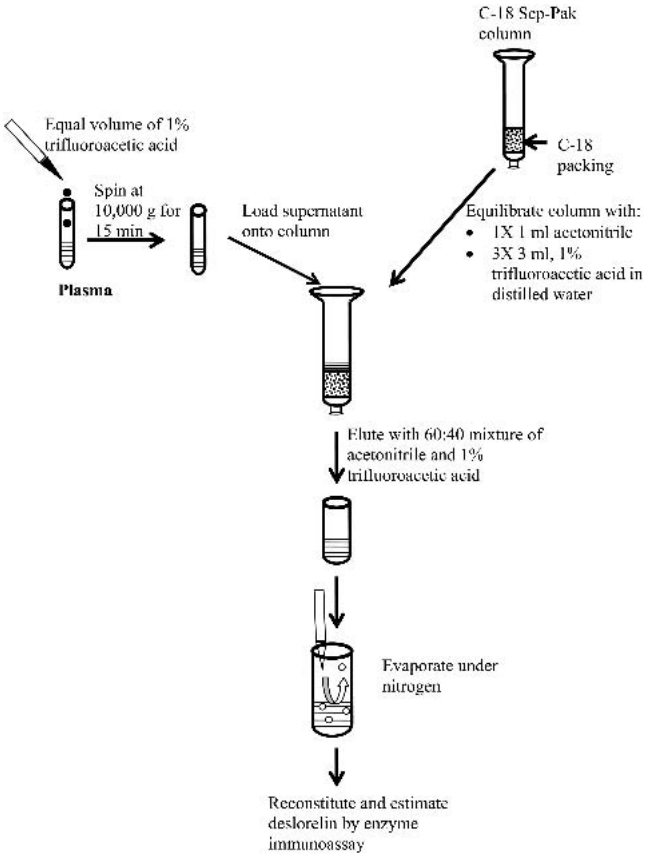

Fig. 1. Extraction of deslorelin from rat plasma.

enzyme immunoassay (EIA) kit composed of biotinylated peptide and peptide antibody. The unknown or standard deslorelin competes with the biotinylated peptide for the peptide antibody. The reaction of TMB (3,3′, 5,5′-tetramethyl benzidine dihydrochloride) with streptavidin conjugated horseradish peroxidase (SA-HRP) was used for the detection and quantification of the peptide. Deslorelin standard curve was constructed in plasma. The extraction recovery of deslorelin from plasma was estimated over a concentration range of 16 pg/ml to 2 ng/ml. The extraction recoveries were >75% for all concentrations except for 16 pg/ml, which was low, ~50%. The log concentration vs. absorbance was plotted and was linear over the concentration range of 31 pg/ml to 2 ng/ml. Whenever appropriate, the samples were diluted prior to the final analysis.

Data Analysis

Each treatment group consisted of four animals. Data were analyzed and mean values between different treatments were compared using two-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis using SPSS (version 8.0) software. A probability level of $p < 0.05$ was considered to be statistically significant.

RESULTS

Large Porous Deslorelin-PLGA Particles Sustain Pulmonary Drug Delivery

Following intratracheal administration of dry powder formulations of large porous and conventional (small-

nonporous) deslorelin-PLGA particles, plasma deslorelin was estimated and compared at 3 h, 1, 3, and 7 days. Also, plain deslorelin powder was delivered as a control and plasma deslorelin was estimated at the same time points. At the end of 3 h, the dose-normalized plasma deslorelin levels were the highest for large porous particles (Fig. 2). Interestingly, for the small conventional deslorelin-PLGA particles, the peak plasma deslorelin levels were observed at 1 day, following which there was a continuous decrease in plasma concentrations. In contrast, the large porous particles maintained an elevated level even on day 7 (Fig. 2). For large porous particles, no significant difference was observed between plasma levels on days 1 and 7. At the end of 7 days, the deslorelin levels following large porous particle administration were 2.4-fold and 120-fold higher compared to small conventional particles and deslorelin powder, respectively, indicating that the porous particles are cleared less rapidly compared to conventional particles and deslorelin powder.

Deslorelin-HPβCD Complexes Enhance Deslorelin Delivery

Dose-normalized plasma levels of deslorelin were compared at 3 h, 1, 3, and 7 days following a single intratracheal spray-instillation of deslorelin solution and deslorelin-HPβCD complexes (Fig. 3). The plasma deslorelin concentration following deslorelin-HPβCD solution administration was 2-, 3-, and 3- fold higher compared to deslorelin solution at 3 h, 1, and 3 days, respectively. On day 7, the drug levels in the deslorelin-HPβCD group were 33.8 ng/ml per mg dose. On the other hand, in the deslorelin solution group they were below detection limit.

Dry Powder vs. Solution Formulations for Deslorelin Delivery

Dose-normalized plasma deslorelin concentrations were compared following solution and dry-powder administrations (Fig. 4). The results indicated that at 3 h, the plasma deslorelin following powder administration (317 ng/ml per mg dose) was 1.9-fold greater compared to that following solution administration. On days 1 and 3, there was no significant difference between the two formulations. On day 7, drug was detectable with the powder but not solution formulation. The dose-normalized plasma deslorelin levels at the end of 7 days following intratracheal administration of different formulations was in the order large porous deslorelin-PLGA particles > small conventional deslorelin-PLGA particles > deslorelin-HPβCD complex > deslorelin powder > deslorelin solution (Fig. 5). Significantly ($p < 0.05$) higher levels were observed with both types of deslorelin-PLGA microparticles on days 3 and 7 compared to deslorelin-HPβCD complexes (Figs. 2 and 3). The bronchoalveolar lavage (BAL) concentration on day 7 was also significantly higher with large-porous microparticle formulation compared to deslorelin-HPβCD complexes.

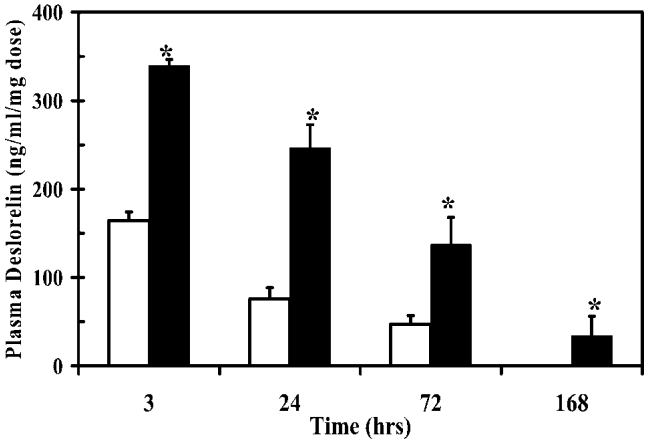

Fig. 3. Dose-normalized deslorelin plasma concentrations following single intratracheal instillation of deslorelin solution (open bars) and deslorelin-HPβCD complexes in solution (closed bars). Samples were collected at 3 h, 1, 3, and 7 days and the data is expressed as mean ± SD for n = 4. *Indicates significant difference at $p < 0.05$ compared to the intratracheal deslorelin solution formulation.

Bronchoalveolar Lavage Levels

Bronchoalveolar lavage deslorelin concentrations were compared on day 7 following intratracheal instillation of so-

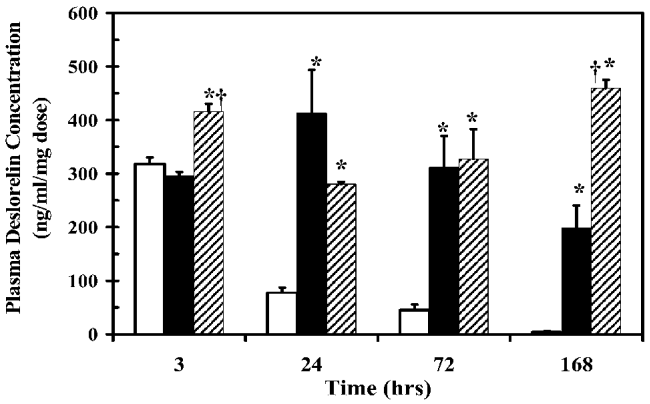

Fig. 2. Dose-normalized deslorelin plasma levels at 3 h, 1, 3, and 7 days following intratracheal instillation of deslorelin powder (open bars), small conventional deslorelin-PLGA particles (closed bars), and large porous (hatched bars) deslorelin-PLGA particles. *Indicates significant difference compared to deslorelin powder; †indicates significant difference compared to conventional particles at $p < 0.05$. Data is represented as mean ± SD for n = 4.

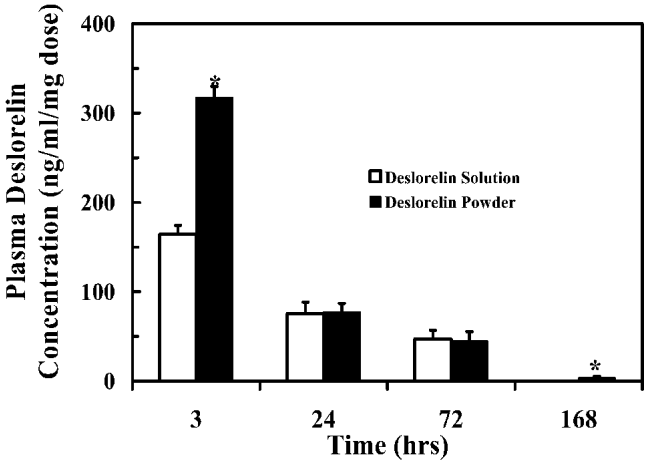

Fig. 4. Dose-normalized deslorelin levels in plasma at 3 h, 1, 3, and 7 days following single intratracheal administration of deslorelin solution and deslorelin powder. *Indicates significant difference compared to the intratracheal deslorelin solution formulation at $p < 0.05$. Data is represented as mean ± SD for n = 4.

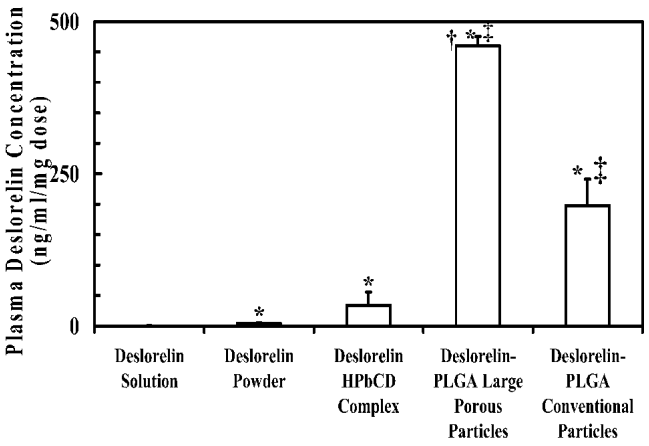

Fig. 5. Dose normalized (per mg dose) plasma deslorelin concentrations at the end of 7 days following single intratracheal administration of different deslorelin formulations. Data is expressed as mean ± SD for n = 4. *Indicates significant difference compared to solution; †indicates significant difference compared to conventional particles; ‡indicates significant difference compared to deslorelin-HPβCD complexes. In all cases, values were considered significant at $p < 0.05$.

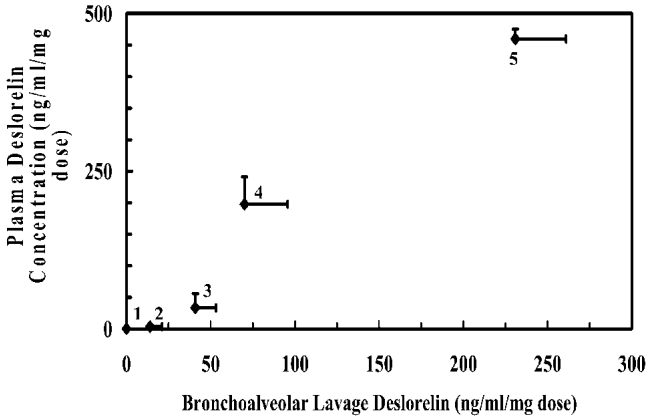

Fig. 6. Correlation of deslorelin levels in the plasma and bronchoalveolar lavage of rats on day 7 following intratracheal instillation of 1) deslorelin solution, 2) deslorelin powder, 3) deslorelin-HPβCD complex, 4) small conventional deslorelin PLGA particles, and 5) large porous deslorelin-PLGA particles.

lutions of deslorelin and deslorelin HPβCD and powders of deslorelin, large porous deslorelin-PLGA particles, and small conventional deslorelin-PLGA microparticles (Table I). The BAL deslorelin concentration was below the detection limit following the administration of deslorelin solution. Also, at the end of 7 days after large-porous particle administration, the dose-normalized BAL deslorelin was 3.3-fold, 5.75-fold, and 16.5-fold higher compared to small conventional deslorelin PLGA particles, deslorelin-HPβCD complexes, and deslorelin powder, respectively. This trend was similar to that observed for plasma deslorelin concentrations at 7 days. A comparison of plasma and BAL levels indicated a correlation (Fig. 6).

DISCUSSION

The goals of this study were 2-fold: 1) to improve systemic delivery of deslorelin via the deep lungs using a stabilizing formulation adjuvant HPβCD and 2) to sustain systemic deslorelin delivery using a dry powder formulation consisting of large-porous deslorelin-PLGA particles. We have shown that cyclodextrin complexes and large-porous polymeric particles provide potential platforms for the improved systemic delivery of deslorelin via the respiratory tract. The following findings support our conclusions: a) after intratracheal spray instillation, deslorelin-HPβCD solution provided higher systemic deslorelin levels compared to deslorelin solution, and b) on day 7 following intratracheal administration, large-porous deslorelin-PLGA particles provided 120-fold and 2.4-fold higher plasma levels compared to deslorelin powder and conventional deslorelin-PLGA particles, respectively.

Table I. Dose-Normalized Deslorelin Concentrations in Bronchoalveolar Lavage at the End of 7 Days Following Intratracheal Instillation of Different Deslorelin Formulations

Formulation	BAL deslorelin concentration (ng/ml per mg dose)
Deslorelin solution	n.d.
Deslorelin powder	13.95 ± 7.52*
Deslorelin-HPβCD complexes (solution)	40.84 ± 12.22*†
Deslorelin large-porous particles	230.8 ± 30.5*†‡
Deslorelin small conventional particles	70.56 ± 25.54*†

BAL, bronchoalveolar lavage; n.d., not detected. Data is presented as mean ± SD for n = 4.
* Indicates significant difference compared to solution.
† Indicates significant difference compared deslorelin powder.
‡ Indicates significant difference compared to small conventional particles.

It has long been recognized that efficient absorption of inhaled peptide drugs is limited by the barrier property of the alveolar epithelium and local proteases (19). Therefore, one approach to improve bioavailability of inhaled peptides is to use formulation adjuvants such as HPβCD that would decrease proteolytic degradation of peptides. In our studies, co-administration of HPβCD provided higher plasma deslorelin compared to the same dose of deslorelin without HPβCD (Fig. 3). Consistent with our observations, Matsubara *et al.* reported an improved nasal biovailability of buserelin, another LHRH agonist, when co-administered with dimethyl-β-cyclodextrin (DMβCD) (20). Also, β cyclodextrin improved pulmonary absorption of FK224, a cyclopeptide, in a β-cyclodextrin dose-dependent manner (21). Furthermore, Kobayashi *et al.* demonstrated that dimethyl-β-cyclodextrin (250 μg/dose) significantly enhanced the pulmonary absorption of calcitonin in rats (22).

Elevated plasma concentrations of deslorelin could in part be attributed to the ability of HPβCD to interact with the aromatic amino acids in deslorelin, thereby protecting it from enzymatic degradation. Indeed, our previous studies indicated an interaction of HPβCD with Trp3 and Tyr5 of deslorelin (10). Interestingly, *in vitro* degradation studies of deslorelin in respiratory epithelial (Calu-1) cells indicated that the primary sites in deslorelin susceptible to degradation are the Trp3-Ser4 and the Ser4-Tyr5 linkages (8). The interaction of HPβCD at the Trp3 and Tyr5 likely provides steric hindrance to enzymatic degradation, thereby stabilizing deslore-

lin within the lung. However, other mechanisms including disruption of the alveolar epithelium, extraction of membrane lipids and proteins, and complexation of proteolytic enzymes by HPβCD cannot be ruled out (8, 23).

The toxicity of cyclodextrins has been assessed in mucosal tissues of the trachea and the nose. The ciliostatic effects of cyclodextrins (5% w/v) on chicken embryo trachea were in the order DMβCD > α-cyclodextrin > γ-cyclodextrin = HPβCD. The toxicity of different cyclodextrins (5% w/v) in nasal mucosa was assessed based on the release of membrane proteins or phospholipids, membrane bound 5′-nucleotidase, and intracellular lactate dehydrogenase (24). Among the cyclodextrins tested, HPβCD was the least toxic as evidenced by a slight increase in 5′ nucleotidase release and lack of effect on lactose dehydrogenase release and phospholipid release. The observed toxicity was in the order DMβCD > α-cyclodextrin > β-cyclodextrin > γ-cyclodextrin > hydroxypropyl-β-cyclodextrin (23, 25). Furthermore, intravenous HPβCD at a dose of 500 mg/kg caused low hemolysis (<5%) compared to other cyclodextrins including β-cyclodextrin, DMβCD, and α-cyclodextrin, which caused 25% or greater hemolysis (26). The above toxicity studies were done in isolated tissue systems or following intravenous administration. Prior to clinical development, future studies should establish the safety of any new excipients such as HPβCD or PLGA in the whole animal models following lung administration.

Apart from improving the systemic availability of deslorelin administered via the lungs, another goal of our study has been to sustain deslorelin delivery. Using a mild supercritical carbon dioxide process, we prepared large porous deslorelin PLGA particles that maintained deslorelin integrity, sustained *in vitro* release up to a period of 7 days, and exhibited decreased alveolar macrophage and epithelial cell uptake (14). Following *in vivo* delivery of dry powder formulations of large porous deslorelin PLGA particles, plasma deslorelin was significantly higher compared to small conventional deslorelin PLGA particles on day 7 (Fig. 2). An observation of the time course of plasma deslorelin following intratracheal instillation of large porous and small conventional particles clearly indicated an elimination-like phase for the small conventional particles after a peak at 24 h (Fig. 2). Interestingly, with large porous particles, day 7 plasma deslorelin was not significantly different from that at 3 h, suggesting maintenance of drug levels.

The PLGA 50:50 used in this study has a molecular weight of 23 kDa with free carboxyl end groups. Previous *in vitro* degradation studies with microspheres prepared using this polymer indicated 95% degradation in 35 days, as evidenced by mass loss (27). *In vivo* degradation studies with PLGA 50:50 of MW 15,000–25,000 following subcutaneous injection indicated an average degradation time (defined as >80% decrease in weight average molecular weight) of 1 month (28). Apart from polymer molecular weight and copolymer ratio, other factors such as the site of administration, pH of the surrounding medium, and the nature of the drug also influence the hydrolytic degradation rate of PLGA polymers (29).

Polymer degradation *in vivo* as well as several other factors determine the residence time of particulate systems *in vivo* (30). Though the mucociliary escalator efficiently clears particles in the upper airways, macrophages in the lower airways scavenge particles and shorten drug action. Alveolar macrophages can clear particles from the alveolar region by one or more of the following mechanisms: 1) transport along the alveolar surface to the mucociliary escalator and subsequent removal from the lung within 1 day. Particles cleared by this pathway are swallowed and excreted via feces. 2) Degradation of susceptible particles by enzymatic hydrolysis, and 3) translocation into the interstitium across the epithelial layer and subsequent entry into the tracheobronchial lymphatic system and lymph nodes. Removal from the lymph nodes is possible following particle dissolution in the phagolysosomes of macrophages. Dissolved material enters the circulation and is excreted via urine if it does not undergo metabolism or resorption by other organs (31).

PLGA is an FDA-approved biodegradable and biocompatible polymer (29). Though it is widely used for parenteral administration, its use in lung is not established. There are no literature reports assessing the toxicity of PLGA in the lungs. Future investigations should address this prior to clinical development of PLGA based systems for lung delivery.

The higher systemic drug levels provided by large porous particles compared to the small conventional particles can generally be due to 1) reduced oropharyngeal deposition due to lower density and reduced aggregation, 2) higher fraction of large porous particles deposited in the deep lungs leading to increased systemic access, and 3) lower clearance of large porous particles by alveolar macrophages (32–34). In our study, the first reason cannot be considered because we have delivered particles at the level of the carina using the PennCentury device, thereby circumventing the mouth and oropharynx (17). However, even within the lower airways, large-porous particles may have penetrated better as small conventional particles have a tendency to aggregate, which decreases their flow and diffusion (35). Indeed, it was observed that with an engineered pulmosphere powder formulation, the percent dose emitted and the fraction deposited in the deep lungs were greater than conventional particles of comparable aerodynamic diameter (36). However, a major contribution toward the increased plasma levels observed with large porous particles on day 7 would be the lower alveolar macrophage clearance and consequently the increased retention of large porous particles in the lungs. Previous studies suggested that uptake by alveolar macrophages represents a degradation pathway for inhaled macromolecules that competes with the absorption of these macromolecules across pulmonary epithelia, thereby lowering drug absorption (2). Indeed, following intratracheal instillation of large porous particles, it was reported that the number of particles remaining in the lungs was approximately an order of magnitude greater than that of small conventional particles of comparable aerodynamic diameter (13). An additional reason for the differences in the drug levels of the two microparticle formulations could be a difference in the drug release properties of these systems. However, this reason cannot explain the observed differences because under the conditions used for the preparation of microparticles, the *in vitro* drug release was slightly lower for large porous particles compared to conventional deslorelin-PLGA particles (14).

Also, in our studies with the BAL (Table I), we observed that the deslorelin concentrations with large porous particles was 3.2-fold higher compared to the small conventional particles, indicating an increased retention of large porous particles in the lungs. The BAL was separated from the cells in

this study and hence, it is reflective of drug present in the lung fluids. We believe that this drug fraction, escaping the circulating macrophages and degradation, represents the amount of drug that is available for systemic absorption. Indeed we observed a correlation ($r^2 = 0.9615$) between plasma and BAL deslorelin concentrations on 7 day (Fig. 6). Our BAL isolation procedure did not include a perfusion of lung blood vessels. Therefore, the possibility of contamination from systemic circulation cannot be ruled out. However, we expect this to be minimal because the lungs were completely isolated from the animal before lavage and the lavage was performed rapidly within 1–2 min, reducing the chances of contamination due to solute/fluid influx from systemic circulation. Furthermore, contamination from systemic circulation is a bigger concern for small molecules and possibly secretory proteins such as IgG and IgM (37). Previously, we have demonstrated that deslorelin is preferentially transported across the respiratory epithelium from the mucosal to serosal side and the serosal to mucosal transport is very low (38).

Many studies have focused on sustaining the delivery or effect of small molecules such as testosterone, estrogen, and carbachol (39,40). Suarez *et al.* have demonstrated a 28-day sustained effect with rifampicin particles for the treatment of lung infections. It should be noted that the drug effect in many cases may exceed the drug residence time (41). Although some previous studies with large-porous particles have investigated the possibility of sustaining lung delivery of macromolecules, the maximum duration of sustained delivery reported was 96 h (13). On the other hand, the current study measured plasma and BAL drug levels and demonstrated the utility of large-porous deslorelin particles in sustaining systemic levels of a macromolecule for 168 h.

This study answers important questions regarding strategies to improve pulmonary delivery of molecules. For instance, it is interesting to note that deslorelin-HPβCD complexes, while enhancing and sustaining the initial delivery of deslorelin compared to plain deslorelin, are unable to sustain drug levels beyond day 3 (Figs. 3 and 4). In contrast, large porous particles can provide sustained drug levels even on day 7 (Fig. 2).

Currently, some LHRH agonists are administered as nasal sprays to children for the treatment of precocious puberty (42). Inhaled deslorelin-HPβCD complexes can potentially be used for this purpose. Pulmonary route offers greater peptide bioavailability compared to the nasal route. Use of HPβCD complexes as opposed to plain deslorelin will likely result in further enhancement of deslorelin delivery. On the other hand, large-porous particles will likely be useful for short-term sustained release in the treatment of disorders such as premenstrual syndrome in humans and controlled ovulation and *in vitro* fertilization programs in animals (5). A commercial formulation of itraconazole containing HPβCD (Sporanox, Janssen, Titusville, NJ, USA) is approved in the United States for oral and intravenous use. Also, several LHRH agonist formulations with PLGA including Trelstar, De-Capeptyl, Lupron Depot, and Zoladex are approved for subcutaneous and intramuscular use. Therefore, HPβCD complexes and PLGA particles will likely find a clinical application in pulmonary delivery. However, prior to clinical application, lack of toxicity of these formulations in the lung needs to be ascertained.

CONCLUSIONS

In conclusion, the lung is a suitable route for the systemic delivery of deslorelin, and HPβCD is an effective additive for improving the pulmonary absorption of deslorelin. Deslorelin-PLGA particles are useful for the systemic delivery of deslorelin via the respiratory tract. Dry powder formulations of large-porous deslorelin PLGA particles can sustain systemic deslorelin levels better than small conventional particles.

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