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Evaluation of Novel Particles as Pulmonary Delivery Systems for Insulin in Rats

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ABSTRACT

The purpose of the study was to evaluate the influence of calcium phosphate (CAP) and polyethylene glycol (PEG) particles on the systemic delivery of insulin administered by the pulmonary route. Two methods of pulmonary delivery were employed: intratracheal instillation and spray instillation. Insulin-CAP-PEG particles in suspension (1.2 U/kg, 110-140 μL) were administered to the lungs of fasted rats by intratracheal instillation (INCAPEG) or spray instillation (SINCAPEG). Control treatments consisted of insulin solution (1.2 U/kg) by intratracheal instillation, spray instillation, and subcutaneous administration (SC). Plasma concentrations of insulin and glucose were determined by chemiluminescence and colorimetric methods, respectively. Data were analyzed by compartmental and non-compartmental methods, and pharmacokinetic (PK) and pharmacodynamic (PD) parameters of insulin disposition were determined. PK analysis suggested that insulin administered in particles had a longer half-life, a longer mean residence time, and a smaller rate of elimination than insulin in solution. In addition, insulin bioavailability after SINCAPEG was 1.8-fold that of insulin solution administered SC. PD analysis showed that smaller areas under the effect curve and, conversely, larger areas above the effect curve were obtained after INCAPEG in comparison to insulin solution. The magnitude of this effect was increased after SINCAPEG. The presence of CAP-PEG particles appears to positively influence the disposition of insulin administered to the lungs of Sprague-Dawley rats. Spray instillation appears to be a more efficient method of delivering insulin to the lungs of rats than intratracheal instillation.

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INTRODUCTION

The reduced bioavailability observed after the administration of inhaled insulin has been a major concern for the pulmonary delivery of insulin. Several approaches, including the use of co-administration of permeation enhancers or delivery agents and encapsulation of insulin in proprietary particles, have been used to increase the bioavailability of inhaled insulin. We have previously reported that co-administration of insulin with the delivery agent hydroxymethyl amino propionic acid (H-MAP) significantly improved the bioavailability of insulin in the rat lung in a dose-dependent fashion and without adverse effects in lung histology and function.[1,2] Other insulin particle technologies, including Technospheres™ (Pharmaceutical Discovery Corporation, Elmsford, NY),[3] ProMaxx® (Epic Therapeutics, Inc, Norwood, MA),[4] SoliDose® (Elan Drug Delivery, Nottingham, England),[5] and AIR™ technology (Alkermes, Cambridge, MA)[6-8] have been developed as aids to pulmonary drug delivery. These vary in composition and purpose. In general, they employ components generally regarded as safe (GRAS) from a regulatory standpoint and are intended to enhance targeted and/or controlled delivery of drug.

The particles used in the present study also employ GRAS excipients: calcium phosphate (CAP) and polyethylene glycol (PEG). They are prepared by a controlled precipitation technique[9] that yields solid particles in a uniform micron size range. Their influence on drug disposition from the lungs of rats has not previously been described. CAP particles have shown high loading capacities and a potential for controlled drug release. The inclusion of PEG in the formulation improved insulin loading capacity, possibly by masking surface negative charges in the CAP particles due to the presence of PO_4 ions.[9] Other reports also

indicate enhanced protein absorption for polymeric microparticles[10] and hydrogels[11] in the presence of PEG.

Historically, accurate dose delivery to laboratory animals has been difficult to achieve. Previous studies have employed intratracheal instillation or spray instillation. The goal of the present study was to evaluate the influence of CAP-PEG particles on the systemic delivery of insulin administered by the pulmonary route. In addition, 2 methods of delivery, intratracheal liquid instillation and spray instillation, were compared to assess their effect on delivery. A study of the effects of these particles on insulin disposition in the rat model will assist in evaluating the particles' prospects for enhancing delivery of this drug, using the respiratory tract as a route of administration.

MATERIALS AND METHODS

Materials

Recombinant human insulin expressed in *Escherichia coli* (28.6 USP units/mg) was obtained from Sigma Chemical Co (St Louis, MO). Insulin-CAP-PEG particles were manufactured by BioSante Pharmaceuticals, Inc (Smyrna, GA) as a 0.58 mg/mL insulin suspension (1 mg of solids per mL of suspension). The mean volume diameter was 0.315 μm and was determined by photon correlation spectroscopy using a Beckman N4 Plus submicron particle sizer (Brea, CA). Sterile saline solution (Abbott Laboratories, North Chicago, IL) was used to replace the blood volume taken during sampling. Sodium heparin injection (1000 USP U/mL, Elkins-Sinn, Inc, Cherry Hill, NJ) was used after dilution with sterile saline (15 U/mL); a fresh supply was prepared each day of the study. Ketamine, acepromazine (Fort Dodge, Fort Dodge, IA), and xylazine (Phoenix Scientific Inc, St Joseph, MO) were used in the appropriate doses for anesthesia.

Preparation of Insulin Solution or Suspension

Insulin solution was prepared by dissolving recombinant human insulin powder in 2 mL of water for injection (WFI) pH 3. Hydrochloric acid (0.1N) was added in 30 μL increments until solution was achieved. Subsequently, 3 mL of WFI pH 7.4 was added, and the pH was adjusted to physiological pH (7.4) with 0.1N sodium hydroxide solution. The final volume for the desired concentration was achieved with additional WFI. Insulin-CAP-PEG particles were prepared by BioSante according to the standard operating procedures. Briefly, 1 volume of insulin from a stock solution of 20 mg/mL in 0.01N HCl was diluted to 1 mg/mL in 1% (wt/vol) PEG3350. One volume of calcium chloride (125 mM) and 0.2 volume of sodium citrate (156 mM) were injected into PEG-hIns solution, simultaneously, while stirring. One volume of 125 mM sodium dibasic phosphate was added to initiate the formation of calcium phosphate. Stirring was continued for 48 hours at room temperature for maximum insulin incorporation. The resulting particle suspension was sonicated at 5 to 10°C to obtain stable particles in the size range of 2 to 4 μm. Working CAP-PEG incorporated insulin doses were prepared by diluting the supplied suspension with WFI until the desired insulin concentration was achieved. All solutions and suspensions were prepared immediately prior to administration into animals.

Droplet Size Determination

The droplet size of the spray emerging from the spray instillator (Penn Century, Philadelphia, PA) was measured using a laser diffraction instrument (Malvern 2600c, Southborough, MA) fitted with a 63-mm lens (0.5- to 118-μm size range) and an external timing trigger. The measurement was synchronized with spray emission from the Penn Century device. Insulin solutions and suspensions were measured in a Hamilton syringe (Hamilton Company, Reno, NV) and attached to the spray instillator. The device was positioned at 4 cm from the laser beam (the aerosol plume center was projected across the laser). Particle size data were collected throughout the passage of the spray through the laser region. The positioning of the device in this study was such that the orifice of the microsprayer was within the lens cut-off distance, the device did not deposit aerosol droplets on the detector lens surface, and the actuator orifice was aligned with the height of the laser path. Particle size estimates were based on Fraunhofer diffraction theory.

Animals

Female Sprague-Dawley rats (Hilltop Laboratory Animals Inc, Scottdale, PA) weighing 227-284 g were housed in a 12-hour light/12-hour dark cycle and constant temperature environment of 22°C. A standard diet (Prolab RMH 3000, PMI Nutrition International, Inc, Brentwood, MO) and water were supplied ad libitum during a period of acclimatization. However, animals were fasted 10.5 to 12 hours before dosing. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina, an American Association for Accreditation of Laboratory Animal Care (AALAC) approved facility. The day of the study, each rat underwent cannulation of the right external jugular vein with silicone polymer tubing

Table 1. Summary of Treatments*

Group	Route	Formulation	Delivery Device	Insulin Dose	Group Code	Number of Animals
A	Subcutaneous	Insulin solution	Injection	1.2 U/kg	SC	6
B	Intratracheal	Insulin solution	Liquid instillation	1.2 U/kg	ITI	6
C	Intratracheal	Insulin-CAP-PEG suspension	Liquid instillation	1.2 U/kg	INCAPEG	7
D	Intratracheal	Insulin solution	Spray instillation	1.2 U/kg	SI	6
E	Intratracheal	Insulin-CAP-PEG suspension	Spray instillation	1.2 U/kg	SINCAPEG	8
F	Intratracheal	Empty CAP-PEG suspension	Liquid instillation	None	—	5
G	Subcutaneous	Empty CAP-PEG suspension	Injection	None	—	2
H	Untreated controls	None	None	None	—	2

*CAP-PEG indicates calcium phosphate–polyethylene glycol particles; SC, subcutaneously; ITI, insulin solution by intratracheal instillation; SI, insulin solution by spray instillation; INCAPEG, insulin CAP-PEG particles by intratracheal instillation; SINCAPEG, insulin CAP-PEG particles by spray instillation.

connected to polyethylene PE-50 tubing. The surgery was performed after anesthetizing animals with an intraperitoneal injection of ketamine:xylazine:acepromazine cocktail (50:3.4:3.3 mg/kg, respectively). The cannula was routed subcutaneously, externalized at the neck, and secured to musculature.

Treatments

Animals were randomly divided into 8 groups to receive different treatments (**Table 1**). One group received insulin solutions administered subcutaneously to the right thigh of each rat. Some other groups received insulin solution or suspension by intratracheal liquid instillation or spray-instillation. The procedure involved intubating animals approximately 1 hour after surgery, using a fiber optic laryngoscope (Dolan-Jenner Industries Inc, Lawrence, MA). The tip of the delivery device (oral gavage needle or spray instillator) was placed at the level of the tracheal bifurcation to the main bronchi. Suspensions or insulin solution (110-140 µL) were instilled/sprayed into the lower airways of the rats using a Hamilton syringe attached to the delivery device. After administration, the delivery device was removed and the animal was held in an upright position for 1 minute to ensure deposition of the dose. After this period, the animal was placed on its side until recovery from anesthesia, approximately 1.5 hours after dosing. From that point on, animals were free to move in their cages. Each animal's respiration was monitored visually until the animal recovered from anesthesia. In addition to the experimental groups receiving insulin solutions or suspensions, 3 groups were studied as negative controls. These animals received empty CAP-PEG suspensions SC, by intratracheal instillation or spray instillation or were untreated controls (**Table 1**). The stability of the glucose baseline was verified by measuring glucose levels at 3 time points before insulin administration: immediately on completion of surgery, 15 minutes before dosing, and immediately before dosing.

Blood samples (450 µL) were collected from each animal into clean Eppendorf tubes previously coated with Sigmacote™ (Sigma Chemical Co, St. Louis, MO) (to avoid insulin adsorption onto the tube walls) at 0, 0.17, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, and 12 hours. Sterile saline solution was used to replace the blood volume lost through sample collection. Blood samples were immediately centrifuged and serum was collected into clean Eppendorf tubes previously coated with Sigmacote™. Samples were stored at -80°C until analyzed. The body temperature of each animal was maintained at 37°C using heated surgical pads and incandescent lamps to prevent hypothermia during anesthesia.

After recovery from anesthesia, animals were placed in individual cages until the end of the study.

Sample Analysis

Glucose Determination

The glucose concentration in serum samples was determined using the VT 250 automatic chemical analyzer (Johnson & Johnson Clinical Diagnostics Inc, Rochester, NY). Analysis is based on the enzyme-catalyzed reaction of glucose with molecular oxygen, followed by a second reaction that produces a highly colored red dye. The intensity of the color is proportional to the quantity of glucose in the sample. This analytical method determines plasma glucose concentrations in the range of 20 to 450 mg/dL with a ±2% precision.

Insulin Determination

Insulin serum concentrations were determined by the Beckman Access Ultrasensitive Insulin Assay, which is a simultaneous one-step immunoenzymatic ("sandwich") assay performed by the automated Access Immunoassay System (Beckman Coulter, Brea, CA). A sample (20 µL) was added to a reaction vessel with mouse monoclonal anti-insulin alkaline phosphatase conjugate and paramagnetic particles coated with mouse monoclonal anti-insulin antibody. A chemiluminescent substrate, Lumi-Phos 530, was added to the reaction vessel, and light generated by the reaction was measured with a luminometer. The photon production is proportional to the amount of conjugate bound to the solid support. The amount of analyte in the sample was determined by means of a stored, multipoint calibration curve. This assay is linear in the interval of 1 to 300 µIU/mL with a ±5% precision.

Data Analysis

Pharmacokinetic Analysis

Insulin plasma concentration-time data was initially analyzed by fitting the data to a one-compartment body model, first order absorption, first order elimination, no lag time for all treatments using the WinNonlin computer analysis program (Pharsight Corp., Mountain View, CA):

$$C = D\,K_a / V / (K_a - K) * (EXP(-KT) - EXP(K_aT)) \quad (1)$$

The following pharmacokinetic (PK) parameters were obtained: area under the plasma concentration-time curve (AUC), maximum insulin concentration (C_{max}), time to obtain C_{max} (T_{max}), first order absorption constant (K_a), and first order elimination constant (K). The criteria to determine the best fitting curve were the Akaike criteria, the model selection criteria, the coefficient of variation, and the width of the confidence interval for each parameter estimate.

Insulin data were subsequently analyzed by non-compartmental methods (LaGran computer analysis program[12]) to obtain AUC, area under the first moment curve, apparent total body clearance (CL), mean residence time (MRT), and half-life ($T_{1/2}$). Estimates of K were obtained by supplying the program with the number of points in the terminal phase of the concentration versus time plots. C_{max} and T_{max} were determined from the non-fitted plasma versus time profiles for each animal. The relative bioavailability (F′) was calculated using the following equation:

$$F' = \frac{AUC_{0 \to \infty\ (lung)}}{AUC_{0 \to \infty\ (SC)}} * \frac{Dose_{(SC)}}{Dose_{(lung)}} * 100 \quad (2)$$

Pharmacodynamic Analysis

The percentage minimum plasma glucose concentration (%MPGC) and the time to obtain each %MPGC ($T_{\%MPGC}$) were determined from the mean plasma glucose level versus time profile for the treatments. The area above the effect curve (AACE) was calculated by:

$$AAC_E = Total\ Area - AUC_E \quad (3)$$

The area under the effect curve (AUC_E) was calculated by the trapezoidal rule.

The percentage total reduction in plasma glucose (%TRPG) from 0 to 8 hours was calculated using the following equation:

$$\%TRPG_{0-8} = 100 * \frac{AAC_{E0-12}}{AUC_{E0-12}} \quad (4)$$

Statistical Analysis

Data were analyzed with analysis of variance and the least-squares significant-differences multiple comparison method. A probability level of <0.05 was considered to be statistically significant.

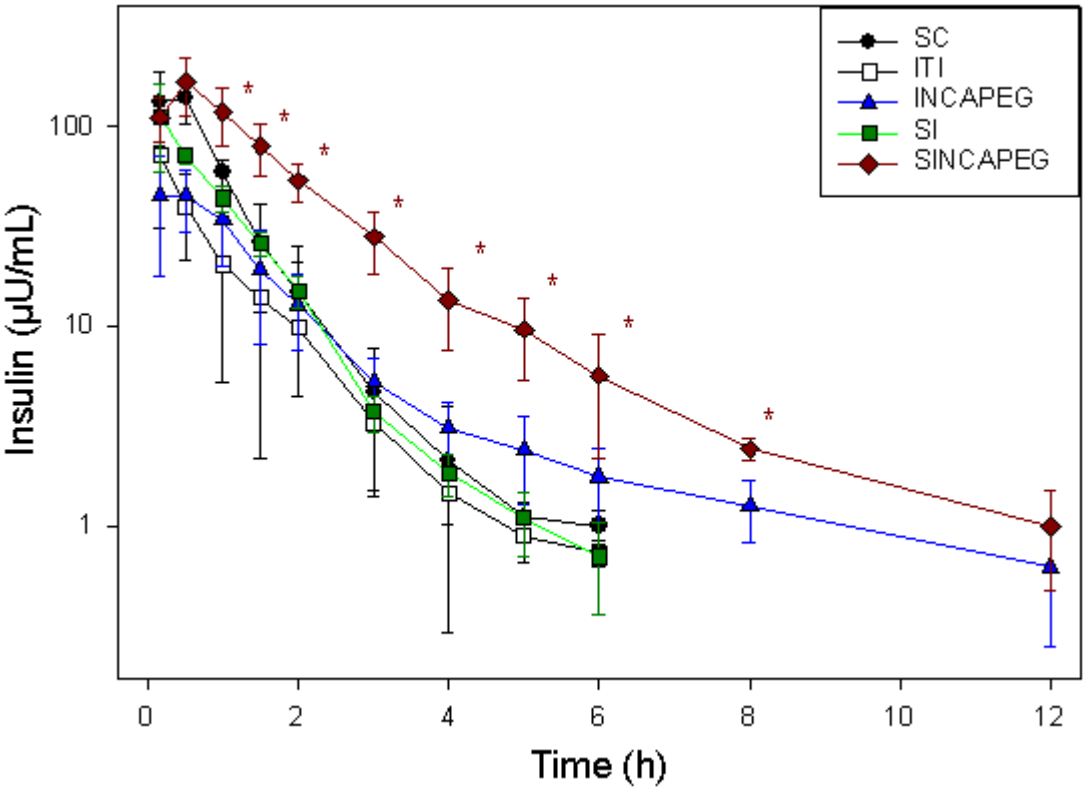

Figure 1. Serum concentration versus time curves after administration of insulin solution (1.2 U/kg) by the subcutaneous (SC) or intratracheal routes: liquid instillation (ITI) or spray-instillation (SI) and insulin-CAP-PEG particles by intratracheal route: liquid instillation (INCAPEG) or spray-instillation (SINCAPEG) (mean ± SD, n = 4-8). Asterisk indicates significantly different from SC and SI insulin solution ($P < 0.05$).

RESULTS

Droplet Size Determination

The droplet size of the spray emerging from the Penn Century was 45.5 ± 3.7 µm (n = 7) as estimated by laser diffraction. There was no significant difference in the droplet size as a consequence of sprayed volume.

Pharmacokinetic Data Analysis

Figure 1 shows the plasma concentration versus time curves after administration of insulin solution (1.2 U/kg) or suspensions by the different routes. Significantly, the highest insulin plasma concentrations were obtained at all time points after spray instillation of insulin-CAP-PEG particles (SINCAPEG). Although higher insulin concentrations were observed in animals treated SC during the first hour, they were comparable until 6 hours for all insulin treatments. After 6 hours, insulin concentrations were under the analytical detection limits for all groups receiving solutions but remained detectable in groups receiving insulin particles. Insulin concentrations after SI were notably higher than those after ITI for the first 2 hours, but they were comparable for the remainder of the study. Likewise, insulin concentrations after particle administration were significantly higher following spray instillation (SINCAPEG) than they were following liquid instillation (INCAPEG).

Tables 2 and **3** show a summary of the PK parameters obtained by compartmental and non-compartmental analysis, respectively. Smaller standard deviations associated with parameter estimation were observed when calculated by non-compartmental methods. The K_a of insulin solution ITI was significantly faster than that by SC or that after administration of particles (INCAPEG, SINCAPEG). Insulin solution administered SC was eliminated (K) significantly faster than by intratracheal delivery (ITI or SI). Furthermore, insulin was eliminated significantly faster when administered in solution (SC, ITI, or SI) than in particles (INCAPEG, SINCAPEG). The clearance rate was significantly higher for insulin solution ITI, and it was comparable for the rest of the experimental groups. This probably reflects its dependency on the absorption process. Com

Table 2. Summary of the Pharmacokinetic Parameters Obtained by Compartmental Analysis After Administration (SC, intratracheal instillation or spray instillation) of Insulin Solution or Particles (mean ± SD, n = 4-8)*

Parameter	SC Insulin Solution 1.2 U/kg (SC)	ITI Insulin Solution 1.2 U/kg (ITI)	SI Insulin Solution 1.2 U/kg (SI)	ITI Insulin-CAP-PEG Particles 1.2 U/kg (INCAPEG)	SI Insulin-CAP-PEG Particles 1.2 U/kg (SINCAPEG)
AUC (μU·h/mL)	149.73 ± 14.62[2]	54.05 ± 12.54[4]	109.84 ± 11.23[3]	76.54 ± 33.43[3,4]	260.89 ± 35.19[1]
K_a (h^{-1})	3.96 ± 2.40[2]	48.16 ± 28.57[1]	13.79 ± 4.76[2]	8.31 ± 8.36[2]	4.66 ± 1.91[2]
K (h^{-1})	2.32 ± 0.72[1]	1.52 ± 0.56[2]	1.05 ± 0.11[2,3]	0.92 ± 0.18[2, 3]	0.88 ± 0.16[3]
$T_{½}$ (h)	0.33 ± 0.12[3]	0.52 ± 0.22[2, 3]	0.67 ± 0.07[2]	0.78 ± 0.13[1]	0.86 ± 0.18[1]
C_{max} (μU/mL)	168.10 ± 38.01[1]	76.83 ± 38.09[2]	88.28 ± 9.80[2]	54.54 ± 24.96[2]	150.92 ± 34.94[1]
T_{max} (h)	0.35 ± 0.10[1,2]	0.10 ± 0.05[3]	0.21 ± 0.06[2,3]	0.39 ± 0.19[1]	0.43 ± 0.08[1]
F′	—	0.29 ± 0.18[3]	0.70 ± 0.05[2]	0.57 ± 0.21[2,3]	1.74 ± 0.24[1]

*Numeric superscripts show the relative rank (starting from the highest values). When the means are not significantly different, the same superscript is used.

Table 3. Summary of the PK Parameters Obtained by Non-compartmental Analysis After Administration (SC, intratracheal instillation or spray instillation) of Insulin Solution or Particles (mean ± SD, n = 4-8)*

Parameter	SC Insulin Solution 1.2 U/kg (SC)	ITI Insulin Solution 1.2 U/kg (ITI)	SI Insulin Solution 1.2 U/kg (SI)	ITI Insulin-CAP-PEG Particles 1.2 U/kg (INCAPEG)	SI Insulin-CAP-PEG Particles 1.2 U/kg (SINCAPEG)
AUC (μU·h/mL)	158.00 ± 15.48[2]	49.16 ± 17.33[4]	115.04 ± 21.29[3]	99.04 ± 23.95[3]	297.10 ± 37.01[1]
CL x 10^{-3} (mL/hkg)	0.01 ± 0.00[2]	0.06 ± 0.06[1]	0.011 ± 0.002[2]	0.01 ± 0.00[2]	0.004 ± 0.001[2]
K (h^{-1})	0.80 ± 0.21[1,2]	0.66 ± 0.11[2]	0.94 ± 0.34[1]	0.22 ± 0.05[3]	0.37 ± 0.07[3]
$T_{½}$ (h)	0.93 ± 0.25[3]	1.08 ± 0.22[3]	0.82 ± 0.32[3]	3.25 ± 0.87[1]	1.93 ± 0.37[2]
MRT (h)	0.89 ± 0.17[3]	1.08 ± 0.17[3]	1.06 ± 0.05[3]	2.64 ± 0.90[1]	1.97 ± 0.21[2]
C_{max} (μU/mL)	166.15 ± 20.75[1]	54.60 ± 23.49[2]	85.87 ± 11.26[2]	58.53 ± 23.62[2]	164.6 ± 39.00[1]
T_{max} (h)	0.39 ± 0.17[1,2]	0.22 ± 0.14[2,3]	0.17 ± 0.00[3]	0.37 ± 0.18[1,2]	0.50 ± 0.00[1]
F′	—	0.31 ± 0.11[3]	0.66 ± 0.05[2]	0.63 ± 0.15[2]	1.88 ± 0.23[1]

*Numeric superscripts show the relative rank (starting from the highest values). When the means are not significantly different, the same superscript is used.

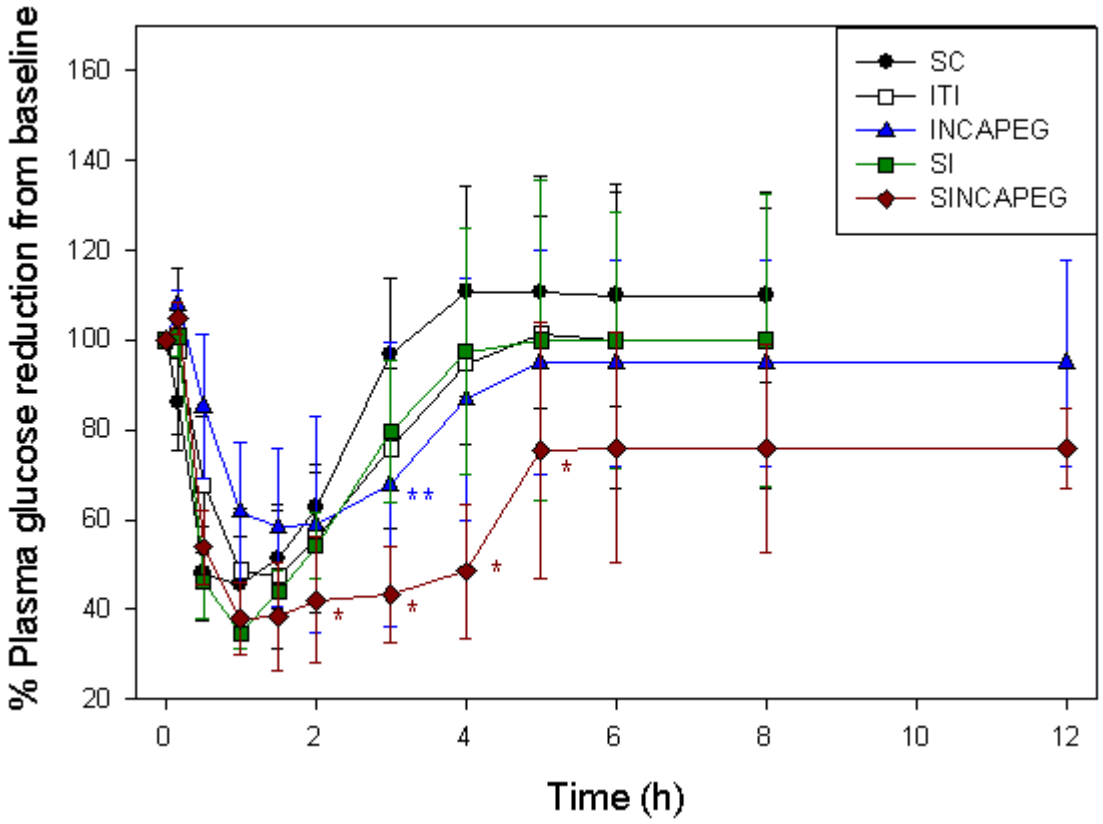

Figure 2. Percentage of plasma glucose reduction from baseline versus time curves after administration of insulin solution (1.2 U/kg) (mean ± SD, n = 4-8) by the subcutaneous (SC) or intratracheal routes: liquid instillation (ITI) or spray-instillation (SI) and insulin-CAP-PEG particles by intratracheal route: liquid instillation (INCAPEG) or spray-instillation (SINCAPEG). Asterisk indicates significantly different from SC and SI insulin solution ($P < 0.05$); double asterisk, significantly different from SC solution ($P < 0.05$).

partmental analysis indicated that the insulin half-life in animals receiving particles intratracheally using either device was significantly longer than solution administration using either device or route. In addition, non-compartmental analysis revealed that the half-life and MRT of INCAPEG were statistically longer than those of SINCAPEG. Insulin C_{max} was comparable in animals receiving solution SC or SINCAPEG and significantly higher than those of groups receiving SI or ITI solution or INCAPEG. T_{max} was reached faster after SI of insulin solution. T_{max} values were similar after administration SC or ITI of insulin solution and significantly longer after SINCAPEG. AUC was significantly largest for animals that received SINCAPEG, followed by those that received solution SC. Relative bioavailability (F′) after SINCAPEG was 1.74-fold (compartmental analysis) and 1.88-fold (non-compartmental analysis) that after administration SC of insulin solution.

Pharmacodynamic Data Analysis

Figure 2 shows the percentage plasma glucose reduction from baseline versus time curves after administration of insulin (1.2 U/kg) solutions or suspensions by the different routes. A rapid and large initial decrease in glucose levels was observed after administration of insulin SC, SI, and SINCAPEG. However, the %MPGC achieved was smaller after SI and INCAPEG than for other groups. Glucose levels remained significantly lower after SINCAPEG than for other groups for the rest of the study (**Figure 2**). No significant difference was observed in the levels of glucose from baseline levels among the 3 negative control treatments.

Table 4 shows the mean pharmacodynamic (PD) parameters following administration of the different treatments. The calculated %MPGC was significantly smaller in rats receiving SI and comparable for other treatments. The $T_{\%MPGC}$ was shorter for animals receiving insulin SC and

Table 4. Mean PD Parameters Obtained After Administration (SC, intratracheal instillation or spray instillation) of Insulin Solution or Particles (mean ± SD, n = 4-8)*

PD parameter	SC Insulin-solution 1.2 U/kg (SC)	ITI Insulin–solution 1.2 U/kg (ITI)	SI Insulin –solution 1.2 U/kg (SI)	ITI Insulin-CAP-PEG particles 1.2U/kg (INCAPEG)	SI Insulin-CAP-PEG particles 1.2U/kg (SINCAPEG)
%MPGC	$42.38 \pm 7.16^{1,2}$	$39.54 \pm 5.43^{1,2}$	34.85 ± 3.73^{2}	44.24 ± 11.83^{1}	$38.24 \pm 8.01^{1,2}$
$T_{\%MPGC}$	0.83 ± 0.26^{2}	1.25 ± 0.27^{1}	$1.00 \pm 0.00^{1,2}$	1.4 ± 0.54^{1}	1.38 ± 0.44^{1}
AUC_E	692.32 ± 11.27^{1}	$678.68 \pm 51.75^{1,2}$	$667.50 \pm 28.50^{1,2}$	611.94 ± 59.69^{2}	486.50 ± 73.70^{3}
AAC_E	107.68 ± 11.29^{3}	$121.38 \pm 51.62^{2,3}$	$132.50 \pm 28.50^{2,3}$	188.05 ± 59.67^{2}	313.50 ± 73.70^{1}
%TRPG	15.58 ± 1.87^{2}	21.8 ± 4.35^{2}	20.0 ± 5.20^{2}	27.79 ± 14.68^{2}	67.40 ± 23.30^{1}

*Numeric superscripts show the relative rank (starting from the highest values). When the means are not significantly different, the same superscript is used.

comparable for animals receiving other treatments. The AUC_E was significantly smaller after administration of SINCAPEG and significantly larger for animals receiving insulin SC. Likewise, AAC_E was significantly larger after administration of SINCAPEG and significantly smaller for animals receiving insulin SC. In addition, %TRPG was significantly larger for animals treated with SINCAPEG.

DISCUSSION

We have previously reported the dose-effect relationship of insulin contained in CAP-PEG particles over a range of doses (0.24-2.4 U/kg), supporting the potential use of CAP-PEG particles to increase the effectiveness of delivery through the lungs.[13] The present study evaluated the influence of CAP-PEG particles on delivery and transport of insulin in the lungs of Sprague-Dawley rats compared to the traditional subcutaneous method of delivery and measured as enhancement of the PK and PD parameters. The effectiveness of 2 intratracheal delivery devices was also evaluated.

Clinical data reported by Cefalu et al[14] suggest that inhaled insulin is safe over 2 years of use; however, the possibility of immunologic reactions to inhaled proteins and peptides is still a major concern. Preclinical studies conducted by BioSante[15] reported minimum IgE responses to vaccine antigens formulated with CAP as an adjuvant and very little inflammation at the site of injection. Therefore, it is anticipated that therapeutic proteins formulated in CAP particles will invoke little or no IgE response relative to the responses that may be induced by other drug carriers. Also, earlier preclinical acute toxicity and inflammatory response studies of CAP (IIT Research Institute, Chicago, IL) using various routes of administration, including inhalation, reported no significant adverse effect at the administration sites as a result of 2 weeks-exposure to CAP. In the same studies, bronchoalveolar lavage (BAL) assessment indicated no significant biological effects on any protein or enzyme parameter in the BAL fluid of interim or terminal inhalation groups. Gonda[16] developed a mathematical model that describes the release of drug from a carrier deposited in the respiratory tract and the clearance of the drug by mucociliary and nonmucociliary mechanisms. This model also accounts for the possibility of accumulation of carrier materials during chronic administration as a function of release rate of the drug. According to the in vitro release profile of insulin from CAP-PEG particles (100% of insulin released in 18 hours and cumulative mass loss <40% during that period), it is unlikely that these particles would be resident for sufficient time to pose a risk of toxicity. Nevertheless, all of these observations require experimental evaluation before definitive conclusions can be drawn regarding safety.

Although several studies have demonstrated that inhaled insulin is absorbed faster in the presence of specific delivery aids than insulin delivered SC,[17,18] the reduced bioavailability observed in all systems used for administration has been called insurmountable.[18] The present study indicates that CAP-PEG particles significantly reduce the elimination (K) of insulin, increasing its systemic residence time (MRT and half-life). Thus, insulin bioavailabil-

ity and duration of action are enhanced when administered to the lungs compared to the SC route.

The AUC of animals receiving SINCAPEG was almost twice that of the SC group. The insulin elimination rate (K) was significantly smaller when it was included in the CAP-PEG particles, which correlates with the longer MRTs and half-lives observed after administration of particles. Insulin half-life and MRT after SINCAPEG were respectively, 2- and 3-fold longer than after insulin SC (**Table 3**). This is also reflected in the significantly higher insulin serum concentrations observed from 1 to 8 hours (**Figure 1**). The rate of appearance of insulin in serum correlated well with the in vitro release profiles of insulin from CAP-PEG particles.[9]

The relative bioavailability of insulin in SINCAPEG was 1.74-fold (compartmental) and 1.88-fold (non-compartmental) that of insulin SC. It should be noted that this relative bioavailability is almost 3-fold that observed after INCAPEG, which may reflect a more efficient dose distribution. This may be seen in **Figures 1** (better and faster absorption) and **2** (longer duration of action). Spray instillation may be more efficient than intratracheal instillation in delivering insulin particles because distribution of the dose after intratracheal instillation is frequently localized (<5% of lung surface).[17] Insulin absorption after intratracheal instillation might be expected to proceed from coarse droplets or liquid deposited on a small fraction of the total surface area of the lung, whereas droplets produced by SI present a spray to many regions of the lung.

The pharmacodynamics of insulin are complex and depend on various factors such as route of administration, liver function, or glucose concentration. The hypoglycemic effect is based on the sum of several biochemical and physiological processes that occur at different sites. The practical solution to overcome this complexity is to monitor glucose levels and titrate patients according to the overall response to the administered insulin.[19] Studies in humans have evaluated the effects of different insulin formulations using the glucose clamp technique to maintain baseline levels.[18, 20-22] However, in the present study the glucose baseline levels were maintained by fasting the animals 10.5 to 12 hours before and during the experiment.

AUC_E and AAC_E after administration of SINCAPEG or INCAPEG were both significantly different than those after insulin SC. In addition, %TRPG was significantly larger in animals receiving SINCAPEG than for any other treatment. Plasma glucose levels in animals receiving SINCAPEG remained lower for longer time periods than those in animals receiving any other treatment (**Figure 2**). The extended hypoglycemic effect of insulin-CAP-PEG was comparable to that observed in a diabetic mouse model and could be explained by a sustained release of insulin from particles as observed in the in vitro release profile.[9]

Patton et al[17] defined the efficiency of aerosol insulin as a sum of the efficiency of the device, the efficiency of deposition, and the efficiency of absorption. We acknowledge the fact that the 2 first terms were circumvented in the present studies by directly spraying/instilling the dose into the rat's trachea. However, the efficiency of absorption should be optimized before deposition and device efficiency can be optimized. The high bioavailability observed with CAP-PEG particles (>170%) at the low dose of insulin evaluated in the present studies, is a promising observation with regard to the efficiency that might be achieved upon optimizing device and deposition characteristics.

A number of studies have evaluated the efficacy of inhaled insulin. Comparisons among these studies become subjective, as there are many variables involved that can influence the evaluation of a particular approach. Among these variables are the type of insulin, formulation, dose, means of administration, and species employed. Human insulin tends to have faster rates of absorption from the site of subcutaneous administration and shorter duration of action compared with animal insulin.[19] Likewise, it has been reported that pulmonary bioavailability of insulin is underestimated in rats and overestimated in rabbits and monkeys when compared to that of humans.[17] **Table 5** summarizes the efficacy studies of insulin delivered through the lungs that are relevant for comparison to the present study.[3-5, 7, 18, 21-27] Among the studies that evaluate insulin formulations in rats using direct means of delivery, CAP-PEG particles yield the highest bioavailability using the smallest dose.

In summary, insulin contained in CAP-PEG particles delivered intratracheally to Sprague-Dawley rats by intratracheal instillation or spray instillation appeared to have a longer half-life and MRT and to be eliminated more slowly than insulin solution. Moreover, the selection of an appropriate delivery device significantly increased the efficiency of this approach. Results obtained in the present study may support the potential use of insulin-CAP-PEG particles to increase the effectiveness of drug delivery through the lung.

Table 5. Efficacy Studies of Insulin Delivered Through the Lungs*

Formulation	Type of Insulin	Dose	Device	Subject	F′ (%)	T_{max} (min)	T_{glumin} (min)	Duration of Effect (h)	Reference
Liquid insulin + protease inhibitors or surfactants	Recombinant human	3-7.5 U/kg	Instillation or MDI	3 Wistar rats	27-98†	—	—	—	(23)
Dry powder alone or combined with citrates	Recombinant human	3 U/kg	Insufflation	5 Wistar rats	6.5 or 17.5	—	—	—	(24)
Aqueous suspension nanospheres	Bovine from pancreas	3.9 U/kg	Ultrasonic nebulizer NE-U03	5 guinea pigs	—	—	240	48	(25)
Sodium hyaluronate solutions	Recombinant human	5 U/kg	Instillation	4 Wistar rats	20.24‡	10	120	4	(26)
AIR particles (powder)	NS	1.8 mg	Insufflation	3 rats	87.5	—	—	96	(7)
Solidose particles (powder)	NS	56 U/kg	Insufflation	Rats	50-100§	—	—	—	(5)
ProMaxx particles (powder)	Human	—	—	Rats	—	—	—	6	(27)
			DPI	Dog	—	—	100	3‖	(4)
BioSante CAP-PEG particles	Recombinant human	1.2 U/kg	Intratracheal instillation and spray instillation	6-8 Sprague-Dawley rats	63-188¶	22-30¶	83	12	—
Technosphere particles (powder)	NS	1.2 and 2.4 U/kg	Inhalator M	6 beagle dogs	—	11 and 7.5	30	—	(3)
	NS	100 U	—	5 patients	25.8	—	—	—	(21)
	Regular human	100 U	Inhalator M	5 patients	14.6#	13	39	5	(22)
Liquid insulin	Human soluble	1.2 U/kg	AERx	18 diabetic patients	13**	62	157	8	(18)

*T_{glumin} indicates time to reach minimum glucose concentration; MDI, metered dose inhaler; NS, not specified; DPI, dry powder inhaler;
† Depending on aerosol or instillation delivery and depth of intratracheal administration.
‡ Calculated from intravenous instead of subcutaneous dose.
§ Depending on the formulation tested.
‖ Dog was fed at 200 minutes.
¶ Lower value corresponds to intratracheal instillation and upper value to spray-instillation.
# Calculated from area under the curve and subcutaneous and inhaled doses.
** Reported as system efficiency—that is, relative bioavailability calculated from the amount of insulin placed in the inhalation device versus that placed in the injection system.

REFERENCES

1. Garcia-Contreras L, Sarubbi D, Flanders E, et al. Immediate and short-term cellular and biochemical responses to pulmonary single-dose studies of insulin and H-MAP. Pharm Res. 2001;18(12):1685-1693.

2. Suarez S, García-Contreras L, Sarubbi D, et al. Facilitation of pulmonary insulin absorption by H-MAP: pharmacokinetics and pharmacodynamics in rats. Pharm Res. 2001;18(12):1677-1684.

3. Pohl R, Muggenburg BA, Wilson B, Woods RJ, Burrell BE, Steiner SS. A dog model as predictor of the temporal properties of pulmonary technosphere/insulin in humans. In: Dalby RN, Byron PR, Peart J, Farr SJ, eds. Respiratory Drug Delivery VII. Raleigh, NC: Davis Horwood International Publishing; 2000; 463-465.

4. Brown LR, Rashba-Step J, Scott T, et al. Pulmonary delivery of novel insulin microspheres. In: Dalby RN, Byron PR, Peart J, Farr SJ, eds. Respiratory Drug Delivery VIII. Raleigh, NC: Davis Horwood International Publishing; 2002; 431-433.

5. Blair J, Coghlan D, Langner E, Jansen M, Askey-Sarvar A. Sustained delivery of insulin via the lung using Solidose technology. In: Dalby RN, Byron PR, Peart J, Farr SJ, eds. Respiratory Drug Delivery VIII. Raleigh, NC: Davis Horwood International Publishing; 2002; 411-413.

6. Edwards DE, Ben-Jebria A, Langer R. Recent advances in pulmonary drug delivery using large, porous inhaled particles. J Appl Physiol. 1997;84(2):379-385.

7. Edwards DE, Hanes J, Caponetti G, et al. Large porous particles for pulmonary drug delivery. Science 1997;276:1868-1871.

8. Vanbever R, Mintzes JD, Wang J, et al. Formulation and physical characterization of large porous particles for inhalation. Pharm Res. 1999;16(11):1735-1742.

9. Morcol T, Nagappan P, He Q, Bell SJD Utilizing calcium phosphate particles as drug delivery vehichles for therapeutic proteins, IIR-USA, ed. IIR-USA 1st Annual Protein and Peptide Formulation Strategies, August 19-21, 2002;9-15.

10. Yeh MK, Jenkins PG, Davis SS, Coombes AGA. Improving the delivery capacity of microparticle system using blends of poly(DL-lactide co-glycolide) and poly(ethylene glycol). J Control Release 1995;37:1-9.

11. Gehrke SH, Uhden LH, McBride JF. Enhanced loading and activity retention of bioactive proteins in hyrdogel delivery systems. J Control Release 1998;55:21-33.

12. Rocci ML, Jusko WL. LAGRAN program for area and moments in pharmacokinetics. Comp Prog Biomed. 1983;16:203-211.

13. Garcia-Contreras L, Morcol T, Bell SJD, Hickey AJ. Evaluation of novel calcium phosphate particles as pulmonary delivery systems for insulin in rats. AAPS PharmSci (Suppl) 2001;3(3).

14. Cefalu WT, Balagtas CC, Landschultz WH, Gelfand RA. Sustained efficacy and pulmonary safety of inhaled insulin during 2 years of outpatient therapy. Diabetes 2000;49(Suppl 1):A406.

15. He Q, Mitchell AR, Johnson SL, Wagner-Bartak C, Morcol T, Bell SJD. Calcium phosphate nanoparticle adjuvant. Clin Diagnos Lab Immunol. 2000;7:899-903.

16. Gonda I. Drugs administered directly into the respiratory tract: modeling of the duration of effective drug levels. J Pharm Sci 1988;77(4):340-346.

17. Patton JS, Bukar J, Nagarajan S. Inhaled insulin. Adv Drug Deliv Rev 1999;35:235-247.

18. Brunner GA, Balent B, Ellmerer M, et al. Dose-response relation of liquid aerosol inhaled insulin in type I diabetic patients. Diabetologia 2001;44:305-308.

19. Hoffman A, Ziv E. Pharmacokinetic considerations of new insulin formulations and routes of administration. Clin Pharmacokinet. 1997;33(4):285-301.

20. Rave K, Heise T, Pfutzner A, Steiner SS, Heinemann L. Assesment of dose-reponse characteristics for a new pulmonary insulin formulation and an inhaler. Exp Clin Endocrinol Diabetes 2000;108(Suppl. 1):S161.

21. Steiner SS, Rave K, Heise T, et al. Pharmacokinetic properties and bioavailability of inhaled dry powder TechnosphereTM/Insulin. Exp Clin Endocrinol Diabetes 2000;108(Suppl. 1):S161.

22. Steiner SS, Pfutzner A, Wilson B, Harzer O, Heinemann L, Rave K. TechnosphereTM / Insulin: proof of concept study with a new insulin formulation for pulmonary delivery. Exp Clin Endocrinol Diabetes 2002;110:17-21.

23. Okumura K, Iwakawa S, Yoshida T, Seki T, Komada F. Intratracheal delivery of insulin absorption from solution and aerosol by rat lung. Int J Pharm. 1992;88:63-73.

24. Komada F, Iwakawa S, Yamamoto N, Sakakibara H, Okumura K. Intratracheal delivery of peptide and protein agents: absorption from solution and dry powder by rat lungs. J Pharm Sci 1994;83:863-867.

25. Kawashima Y, Yamamoto H, Takeguchi H, Fujioka S, Hino T. Pulmonary delivery of insulin with nebulized DL-lactide/glycolide copolymer (PLGA) nanospheres to prolong hypoglycemic effect. J Control Release. 1999;62:279-287.

26. Morimoto K, Metsugi K, Katsumata H, Iwanaga K, Kakemi M. Effects of low viscosity sodium hyaluronate preparation on the pulmonary absorption of rh-insulin in rats. Drug Dev Ind Pharm. 2001;27(4):365-371.

27. Scott T, Sullivan A, Proos R, et al. Novel technology for fabrication of therapeutic microspheres for pulmonary delivery. In: Dalby RN, Byron PR, Peart J, Farr SJ, eds. Respiratory Drug Delivery VIII. Raleigh, NC: Davis Horwood International Publishing; 2002; 435-437.