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RESEARCH ARTICLE

Inhaled PYY(3–36) dry-powder formulation for appetite suppression

Philip J. Kuehl[1], Tracey Boyden[2], Dan E. Dobry[3], Melanie Doyle-Eisele[1], Dwayne T. Friesen[3], Jacob D. McDonald[1], Brice G. Murri[3], David T. Vodak[3], and David K. Lyon[3]

[1]*Lovelace Respiratory Research Institute, Albuquerque, NM, USA,* [2]*Pfizer Inc., Groton, CT, USA, and* [3]*Bend Research Inc., Bend, OR, USA*

Abstract

Objective: Peptide YY3–36 [PYY(3–36)] has shown efficacy in appetite suppression when dosed by injection modalities (intraperitoneal (IP)/subcutaneous). Transitioning to needle-free delivery, towards inhalation, often utilizes systemic pharmacokinetics as a key endpoint to compare different delivery methods and doses. Systemic pharmacokinetics were evaluated for PYY3–36 when delivered by IP, subcutaneous, and inhalation, the systemic pharmacokinetics were then used to select doses in an appetite suppression pharmacodynamic study.

Methods: Dry-powder formulations were manufactured by spray drying and delivered to mice via nose only inhalation. The systemic plasma, lung tissue, and bronchoalveolar lavage fluid pharmacokinetics of different inhalation doses of PYY(3–36) were compared to IP and subcutaneous efficacious doses. Based on these pharmacokinetic data, inhalation doses of 70:30 PYY(3–36):Dextran T10 were evaluated in a mouse model of appetite suppression and compared to IP and subcutaneous data.

Results: Inhalation pharmacokinetic studies showed that plasma exposure was similar for a 2 × higher inhalation dose when compared to subcutaneous and IP delivery. Inhalation doses of 0.22 and 0.65 mg/kg were for efficacy studies. The results showed a dose-dependent (not dose proportional) decrease in food consumption over 4 h, which is similar to IP and subcutaneous delivery routes.

Conclusions: The pharmacokinetic and pharmacodynamics results substantiate the ability of pharmacokinetic data to inform pharmacodynamics dose selection for inhalation delivery of the peptide PYY(3–36). Additionally, engineered PYY(3–36):Dextran T10 particles delivered to the respiratory tract show promise as a non-invasive therapeutic for appetite suppression.

Keywords

Inhalation excipients, pharmacodynamics, pharmacokinetics, protein/peptide inhalation drug delivery, PYY

History

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Introduction

Peptide tyrosine-tryosine (PYY) is an endogenous peptide secreted from the endocrine L cells found in the distal intestine[1]. Within the L cells, proPYY is processed to PYY(1–36)[1]. During and after a meal, PYY(1–36) is secreted into systemic circulation, where it is rapidly converted at its n-terminus to PYY(3–36)[2].

While PYY(1–36) has broad activity across multiple receptors (i.e. Y1, Y2, Y4, and Y5), PYY(3–36) has a specific affinity for the Y2 receptor[3]. It is the interaction with this receptor that is thought to suppress appetite and thereby inhibit food consumption[3]. PYY(3–36) food-consumption inhibition and appetite suppression is thought to be mostly hormonal in nature, with the hypothalamic arcuate nucleus the most likely site of action[3]. PYY(3–36) inhibits food intake by reducing the amount of food consumed per meal, without significant changes in meal frequency.

PYY(3–36) appears to be anorexogenic when dosed exogenously[3,4]. For instance, a study demonstrated that PYY(3–36) inhibits food intake by approximately 20–45% in a dose-dependent manner for 3 to 4 h after intraperitoneal (IP) administration to mice and with no effect on food intake after 12 h[5]. In fact, many studies have now shown that exogenous administration of PYY(3–36) using intravenous (IV) or subcutaneous (SC) delivery routes can significantly suppress food consumption in animals and humans[6–11]. Delivery of PYY(3–36) to the lung has been reported to provide a better pharmacodynamic response than IV infusion by limiting C_{max} and systemic exposure, which may lead to side effects such as nausea[12]. In a recent study, a solution of PYY(3–36) was delivered to the lungs of rats via intra-tracheal administration and was shown to decrease food intake[13]. These data provide proof of concept data for the efficacy of PYY(3–36), when delivered to the lungs. However, because of the known issues with intra-tracheal administration to the lungs of rodents and poor representation of inhalation drug delivery the utility and ability to interpret these data are minimal.

The goal of this study was to demonstrate that a dry-powder aerosol formulation of PYY(3–36) would suppress appetite in a mouse model after inhaled exposure. We hypothesized that a similar pharmacodynamic endpoint could be achieved for spray-dried engineered particles delivered to the mouse lung using a nominally similar dose. However, the selection of a similar inhalation dose when converting from an injection delivery of a

Address for correspondence: Philip J. Kuehl, PhD, Lovelace Respiratory Research Institute, 2425 Ridgecrest Drive SE, Albuquerque, NM 87108, USA. Tel: 1-505-348-9745. Fax: 1-505-348-4980. E-mail: pkuehl@lrri.org

peptide is not straightforward. Nominally similar doses were determined based on pharmacokinetic studies conducted with IP, SC and inhalation dosing. This was enabled by the fact that PYY(3–36) is a peptide and likely has no oral bioavailability and therefore the systemic exposure following inhalation delivery will be the result of the absorption of the pulmonary dose.

In order to test our hypothesis, the pharmacokinetics and pharmacodynamics of PYY(3–36) was determined for more standard peptide delivery methods (IP and SC injections). Pharmacokinetic evaluations of the novel spray-dried formulations were also performed. These data were used to select inhalation doses for pharmacodynamic assessment of inhalation PYY(3–36) in a mouse appetite suppression model.

Materials and methods

Materials

PYY(3–36), amidated at the C-terminus, was purchased from GenScript USA Inc. (Piscataway, NJ). Technical-grade D10 (marketed as Dextran T10) was purchased from Pharmacosmos A/S (Holbæk, Denmark). Deionized water was used for aqueous spray drying.

Formulation and manufacture by spray drying

Engineered particles containing PYY(3–36) and D10 were prepared using a custom designed spray drying system that is similar in scale and operation to a Niro PHARMASD™ spray dryer type PSD-1 (GEA Pharma Systems, Wommelgem, Belgium). The spray drying system includes customized drying-chamber geometry and a cyclone collector optimized for drying and collecting fine particles relevant to respiratory delivery.

Spray-dried feed solutions for all dry-powder formulations were prepared by completely dissolving D10 and PYY(3–36) in deionized water at a total solids content of 1.5 wt%. The pH of the spray solution was adjusted to 6.5 using dilute sodium hydroxide. The spray solutions were fed to the spray dryer with a peristaltic pump at 25 g/min. The solutions were atomized using a two-fluid nozzle (Spraying Systems Co., Wheaton, IL; Model No. 1/4J with a Part No. 1650 fluid cap and a Part No. 120 air cap) with 45-psig nitrogen as the atomizing gas. Nitrogen drying gas was fed to the spray dryer at 1350 g/min and inlet temperatures of approximately 143 °C for first batch and 117 °C for second a batch (as needed to maintain a spray dryer outlet temperature of 55 °C). Dry-powder yields ranged from 66% to 67% at the cyclone collector. The dry-powder samples were then dried for 12–16 h in a vacuum desiccator under 100-mmHg vacuum to ensure sample dryness.

In vitro characterization

Formulations were characterized *in vitro* for (a) content and chemical purity using high-performance liquid chromatography (HPLC); (b) particle-size distribution using a M170 Next Generation Impactor™ (NGI™) (MSP Corp., Shoreview, MN); and (c) morphology by imaging the powders using a scanning electron microscopy (SEM).

Content and chemical purity

Content and chemical purity were determined using an HPLC gradient method with 0.1% trifluoroacetic acid (TFA) (Sigma Aldrich®, St. Louis, MO) in water, starting at 90% and increasing the amount of 0.1% TFA in acetonitrile from 10% to 65% using a Jupiter® column (300Å C18, 250 mm by 4.6 mm, 5 µm; Phenomenex®, Torrance, CA) with a column temperature of 25 °C and absorbance of 214 nm. The run time was 16 min.

Particle-Size determination

Aerodynamic analysis to determine the particle-size distribution was conducted with an NGI. The NGI pans were analyzed with the PYY(3–36) HPLC assay to determine the mass median aerodynamic diameter (MMAD) in microns and the geometric standard deviation (GSD). These data were also used to calculate the fine particle fraction (FPF), which is defined as the percentage of particles that enter the NGI that have MMADs of less than 4.6 µm. Experiments were performed using a monodose inhaler (Plastiape® SPA, Osnago, Italy) with powder in Size 3 hydroxypropyl methylcellulose (HPMC) capsules using an inhaled flow rate of 60 L/min for 4 s.

Morphology

A scanning electron microscope (Hitachi S-3400Ne using S-3400 software at 4000-fold magnification) was used to study the morphology of the spray-dried particles. The dry-powder particles were mounted on aluminum posts (Product No. 16111 specimen mount, aluminum, ½-inch slotted head, ⅛-inch pin, Ted Pella Inc., Redding, CA) using double-sided tape (Product No. 16079 adhesive tabs, Ted Pella Inc.,) and sputter-coated (Ladd/Hummer™ 6.2, Ladd Research, Williston, VT) with gold palladium (AuPd). SEM analysis was carried out with an accelerating voltage of 20 kV.

Mouse animal model

In vivo procedures were conducted under protocols approved by the Institutional Animal Care and Use Committee. Facilities were accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Animals were examined twice per day (morning and afternoon) on each day of the study. No adverse clinical signs were noted during the course of these studies. Animals were fed a certified diet twice daily and allowed water *ad libitum*.

IP and SC administration

IP and SC injections ($n = 3$ at each timepoint) were performed from aqueous solutions of PYY(3–36) by trained animal technicians. Sterile solutions were prepared in 20 mM sodium acetate (pH 4.5). Doses were modulated by injection volume.

Dry-powder exposure system: aerosol generation

Aerosols of the developed 70:30 PYY(3–36):D10 formulation were generated with a Palas rotating brush generator (RBG) (Palas® GmbH, Karlsruhe, Germany) connected to the nose-only flow past inhalation exposure system. All exposures were conducted for 30 min; dose was modulated by exposure to different aerosol concentrations. The aerosol concentration (mg/L) of PYY(3–36) at the breathing zone of the nose only inhalation exposure system was determined by gravimetric analysis of filter samples from Pallflex® 47-mm membrane filters (Pall Life Sciences; Ann Arbor, MI). Aerosol concentration was modulated through control of the RBG piston feed speed.

Particle-size distributions were determined at the breathing zone for the exposure chamber using an Aerodynamic Particle Sizer® (APS) (Model 3321, TSI Inc., Shoreview, MN). Particle-size characteristics – i.e. MMAD and GSD – were determined using Aerosol Instrument Manager® (AIM) software (Version 8.1, TSI Inc.).

Prior to *in vivo* studies, each aerosol concentration was characterized at the breathing zone for aerosol concentration homogeneity, aerosol concentration (total and PYY(3–36)), and aerodynamic particle-size distribution.

Table 1. Calculated deposited dose for each group for the PK and PD studies.

Study	Group	Total average aerosol conc. (mg/L)	PYY(3–36) average aerosol conc. (mg/L)	Deposited dose of PYY (3–36) (mg/kg)
PK	Low	0.012	0.008	0.006
PK	Mid	0.038	0.027	0.02
PK	High	0.388	0.272	0.21
PD	Low	0.41	0.287	0.22
PD	High	1.22	0.854	0.65

The pharmacokinetic study exposures actual total aerosol concentrations were 0.012, 0.038, and 0.388 mg/L or 0.008, 0.027 and 0.272 mg/L PYY(3–36). These aerosol concentrations correlate to pulmonary deposited doses of 0.006, 0.02 and 0.21 mg/kg (Determination of Deposited Dose, Table 1).

Inhalation pharmacodynamic dose selection

Based on the results from the inhalation, IP and SC pharmacokinetic studies, two inhalation doses were selected to achieve elevated PYY(3–36) plasma concentrations and were dosed using the same nose-only exposure system to animals ($n = 20$) in the PK study. The target total aerosol concentrations were 0.0 (for the control group, which breathed only filtered air), 0.4, and 1.2 mg/L (Table 1). The 0.4 mg/L exposure (30 min) was expected to yield a dose of about 0.2 mg/kg and the 1.2 mg/L (30 min) about 2- to 3-fold higher (0.65 mg/kg). Additionally, the 0.65 mg/kg dose is approximately equal to the high end dose that was shown to be effective when delivered via intra-tracheal administration (0.8 mg/kg)[13]. Actual deposited doses for the 0.4 and 1.2 mg/L aerosol concentrations exposures were calculated to be 0.22 and 0.65 mg/kg, respectively.

Blood collection

At each time point, mice were euthanized and blood was collected via cardiac puncture with heparinized syringes. Whole blood was spun down and the plasma was transferred to sample vials and stored frozen (−80 °C) until analysis.

Tissue collection

The lungs, trachea, larynx, tracheobronchial lymph nodes, and upper gastrointestinal (GI) tract (i.e. oropharynx and esophagus) were harvested, placed into tubes, and flash-frozen in liquid nitrogen and stored frozen (−80 °C) until analysis.

BALF collection

After tissue was collected, the lungs of all animals were lavaged two times with 0.7 mL of Dulbecco's phosphate buffered saline (D-PBS) (Sigma Aldrich®, St. Louis, MO). The rinsate from the two washes per animal were combined and frozen (−80 °C) until analysis. Analysis for PYY(3–36) was corrected for the recovery volume prior to data modeling.

Description of bioanalytical method

PYY(3–36) concentrations in mouse plasma, lung tissue (i.e. homogenate), and BALF were determined using a PYY(3–36) enzyme-linked immunosorbent assay (ELISA) kit (Millipore Biopharma Services, Billerica, MA [formerly Linco Research Inc., St. Charles, MO]) with capture (Catalog No. CAB66-2) and detect (Catalog No. DAB66-2) probe monoclonal antibodies (mAbs) specific for human PYY(3–36). Standard curves along with quality control (QC) and dilution quality control (DQC) samples at three concentrations across the range of the curves were prepared separately for each matrix with a dynamic range of 0.05–50 ng/mL in plasma and BALF and 0.1–100 ng/mL in lung tissue. The LLOQ in plasma and BALF were 0.05 ng/mL and 0.1 ng/mL in lung tissue. The precision and accuracy of the curve and control samples were within ± 25% of nominal concentrations.

Determination of deposited dose

Respiratory minute volume (RMV) was calculated in liters per minute using the equation:

$$\mathrm{RMV} = 0.499\mathrm{BW}^{0.809},$$

where BW = average body weight in kilograms on the exposure day[14].

Deposited dose was calculated based on this equation:

$$\text{Deposited dose} = (\mathrm{C} \times \mathrm{RMV} \times \mathrm{T} \times \mathrm{DF})/\mathrm{BW},$$

where C = the average PYY(3–36) concentration in the exposure atmosphere during the exposure period, T = exposure time, and DF = the deposition fraction (assumed to be 2.5%)[15]. Note that for both the PK and PD studies, the PYY(3–36) concentration was 70% of the total mass of powder; therefore, the calculation for deposited dose of PYY(3–36) has also been corrected for this (Table 1).

Pharmacokinetic analysis

Pharmacokinetic parameters were calculated using WinNonLin® software (Phoenix Version 6.2, Pharsight Corp., Sunnyvale, CA) from average concentration ($n = 3$ at each timepoint) versus time profiles as follows. The maximum plasma, lung, and BALF concentrations (C_{max}) were determined from the experimental data. T_{max} is defined as the time of the first occurrence of C_{max}. The area under the curve (AUC_{0-t}) was calculated using the log-linear trapezoidal approximation, where t equals the last time point of collection. Specifically IP at $t = 2$ h, SC and inhalation at $t = 4$ h.

Pharmacodynamic testing methods

To determine the pharmacodynamic relationship of the inhaled PYY(3–36), a pre-weighed food jar was placed inside each rodent cage. At 0.5, 1, 2, and 4 h after dosing, each jar was removed and weighed. In this manner, food consumption was determined as a function of time for each of the three dose groups.

Results

Physical characterization of spray-dried particles

D10 and PYY(3–36) were completely dissolved in deionized water and then spray-dried to produce solid 70:30 PYY(3–36):D10 particles. The spray drying process and equipment were designed to manufacture and collect respirable-sized particles (i.e. 1–5 μm) with a relatively narrow particle size distribution.

Table 2 shows the average aerodynamic particle-size distributions measured by NGI for three separate manufacturing batches of the spray-dried 70:30 PYY(3–36):D10 formulation to demonstrate the robust nature of the formulation and manufacturing process. The impaction data show that particles have a MMAD of ~2.0 μm with a GSD of ~2.1 and a FPF of more than 80%. These data confirm the robust nature of the process and the reproducible aerosol behavior of the dry-powder and is further shown by the

similarity of the particle sizes measured here with those of the powders aerosolized for the rodent studies.

The spray-dried powder was also characterized by size-exclusion HPLC. The dry-powder formulation was confirmed to be 70% PYY(3–36). The chromatograms showed no detectable change in the baseline or peaks – such as higher or lower molecular weight – indicates the absence of degradation or aggregation during the spray dry process or reconstitution of the dry-powder. SEM images (Figure 1) highlight the shriveled-raisin morphology expected, using spray drying film-forming materials such as those that contain D10.

Dry-powder aerosol development

For both rodent inhalation studies, aerosol methods were developed to achieve the target aerosol concentration. Based on the development work, the target aerosol concentration could be achieved with particles that had an MMAD of 1.8–2.0 μm and a GSD between 1.5 and 1.8. These data correlate well to the impaction data discussed earlier (Table 2).

Table 2. Aerodynamic-particle size and particle-size distribution, showing low test variability of the three PYY(3–36):D10 manufacturing batches.

Attribute	Average value	Standard deviation
FPF % <4.6 (μm)	81	0.05
MMAD (μm)	2.0	0.15
GSD	2.1	0.22

Pharmacokinetic results

After PYY(3–36) was administered to mice via IP injection, SC injection or inhalation, active concentrations were measured in plasma. Additionally, PYY(3–36) was measured in the lung tissue, and BALF of the animals dosed by inhalation. For all analysis of pharmacokinetics, it should be noted that the endogenous plasma levels of PYY(3–36) have been reported to be in the 0.1–0.2 ng/mL levels[16]. The resultant pharmacokinetic parameters are shown in Table 3.

The plasma versus concentration–time profile for the three SC doses is shown in Figure 2. The plasma concentrations indicate rapid absorption from the SC dose with apparent first-order elimination. Over the range of doses tested the half-life and T_{max} are similar. The C_{max} and AUC were found to be proportional to dose (R^2 for linear regression of correlation >0.99 for both).

The results from the inhalation PK study are shown in Figures 3, 4, and 5 for PYY(3–36) concentrations in plasma, lung tissue, and BALF, respectively. The pharmacokinetic parameters are shown in Table 3. The C_{max} of PYY(3–36) in plasma, lung tissue, and BALF was observed at the first time point after each dose was administered (at 5 min) following completion of the 30 min exposure time, except for the 0.006-mg/kg dose in lung tissue where T_{max} was reached at 15 min post-dose. The half-lives for IP and SC administration were similar, however the half-life following inhalation doses of 0.02 and 0.21 mg/kg (0.006 mg/kg had insufficient data to model half-life) was increased when

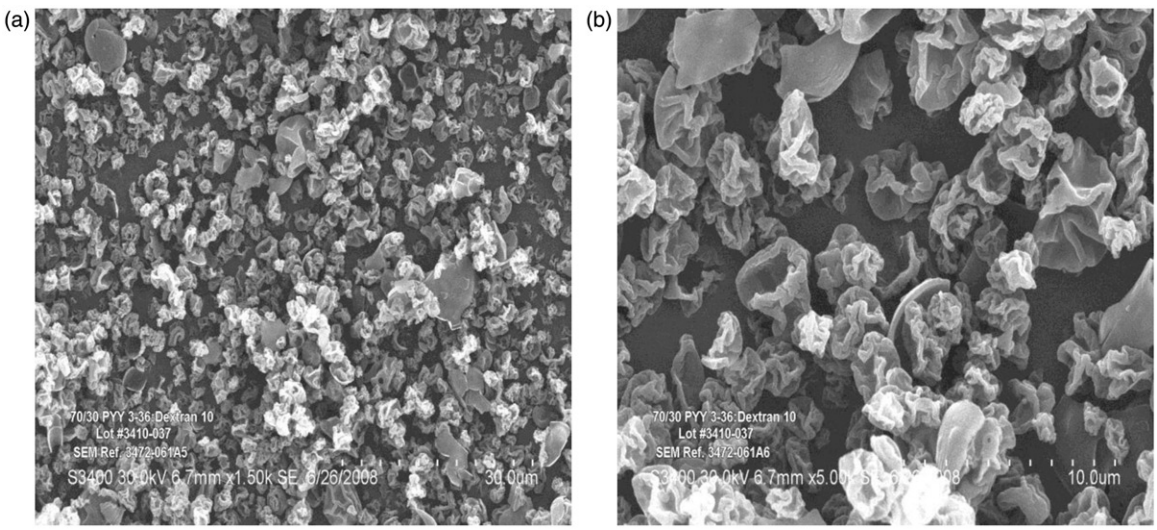

Figure 1. SEM images of the PYY(3–36):D10 particles after spray drying, showing 1.5K – (a) and 5K-fold (b) magnification.

Table 3. Mean PYY(3–36) pharmacokinetic parameters in mice following SC, IP and inhalation dosing.

	IP – 0.1 mg/kg	SC – 0.03 mg/kg	SC – 0.1 mg/kg	SC 0.3 mg/kg	IH – 0.006 mg/kg			IH – 0.02 mg/kg			IH – 0.21 mg/kg		
PK parameter	Plasma	Plasma	Plasma	Plasma	Plasma	Lung	BALF	Plasma	Lung	BALF	Plasma	Lung	BALF
C_{max} (ng/mL)	126.0	37.8	103.8	237.7	2.83	19.0	47.4	15.0	77.9	400	237	1377	16879
T_{max} (h)	0.25	0.25	0.25	0.50	0.08	0.25	0.08	0.08	0.08	0.08	0.08	0.08	0.08
AUC_{0-t} (ng*h/mL)	73.53	33.80	96.2	234.4	1.05	19.8	31.8	9.62	60.4	173	86.0	583.7	11496
$t_{1/2}$ (h)	0.38	0.56	0.49	0.40	NA*	0.46	0.55	1.65	0.59	0.44	1.10	0.31	0.49

C_{max} = the maximal concentration in each matrix. T_{max} = the time of maximum concentration. AUC_{0-t} = the area under the curve from time zero to last time point (IP = 2 h, SC and IH = 4 h).
*Insufficient number of data points for IH 0.006 mg/kg to model half-life.

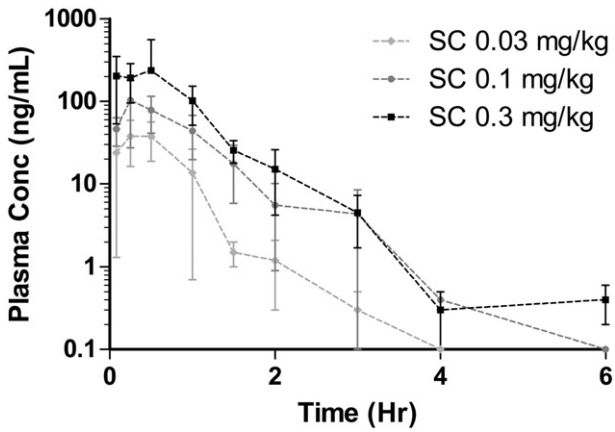

Figure 2. PYY(3–36) concentration in mouse plasma after SC dosing. Shown with error bars as the standard error of the mean.

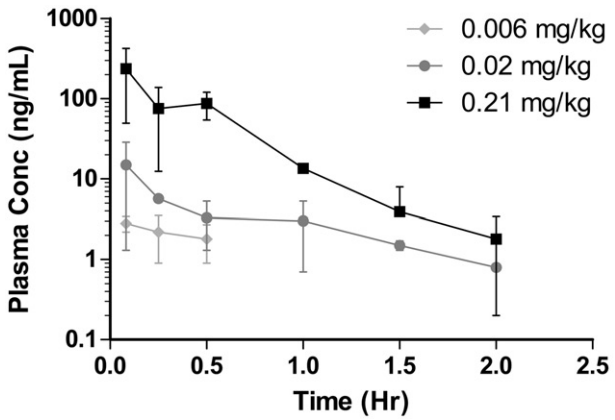

Figure 3. PYY(3–36) concentration in mouse plasma tissue after nose-only exposure. Shown with error bars as the standard error of the mean.

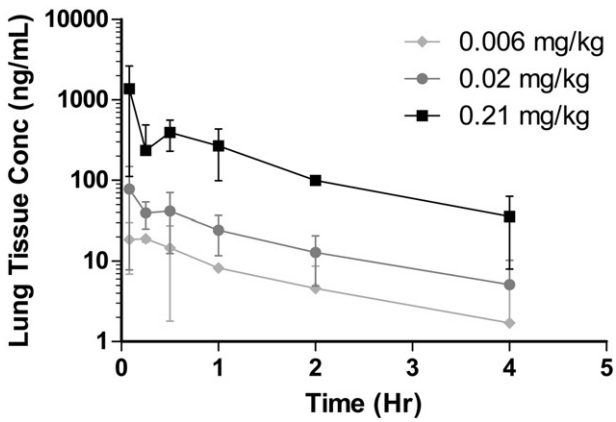

Figure 4. PYY(3–36) concentration in mouse lung tissue after nose-only exposure. Shown with error bars as the standard error of the mean.

compared to SC and IP. The plasma, lung tissue and BALF exposure (AUC) where found to be proportional to pulmonary deposited dose over the range tested (R^2 of linear regression all >0.99).

For the inhalation doses tested, the lowest C_{max} of PYY(3–36) was observed in plasma. The exposure ratios (C_{max} and AUC) were compared for each inhalation dose to determine the increase

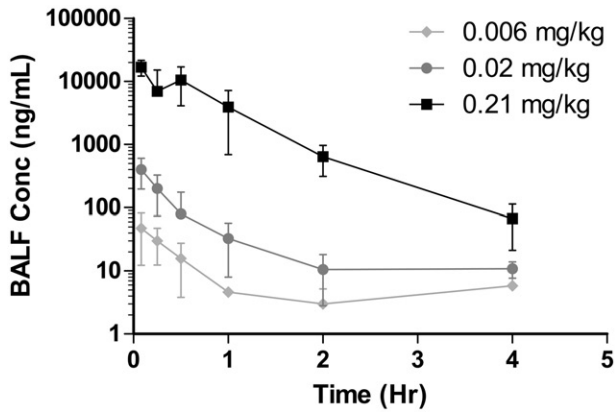

Figure 5. PYY(3–36) concentration in mouse BALF for the IP, SC and inhalation doses that resulted in similar AUC. Shown with error bars as the standard error of the mean.

Table 4. PYY(3–36) exposure enhancement in respiratory tract (BALF and lung tissue) versus plasma in mice following inhaled doses of 0.006–0.21 mg/kg.

Dose (mg/kg)	Enhancement in BALF over plasma	Enhancement in lung tissue over plasma
0.006	17/30	7/19
0.02	27/18	5/6
0.21	71/134	6/7

Exposure multiples calculated using C_{max} /AUC values shown in Table 4.

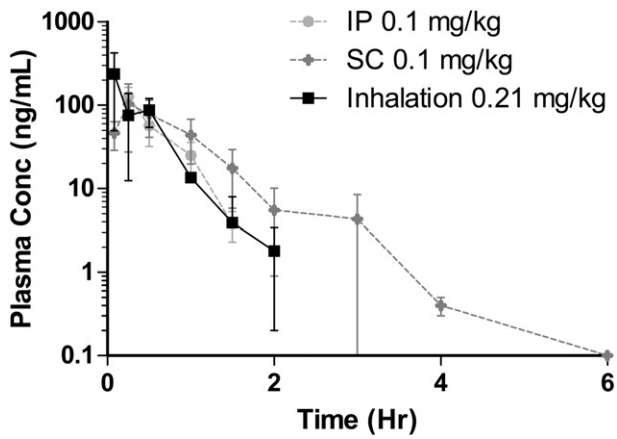

Figure 6. PYY(3–36) concentration in mouse plasma for the IP, SC and inhalation doses that resulted in similar AUC. Shown with error bars as the standard error of the mean.

in exposure to the pulmonary region via inhalation delivery. The relative increase in exposure over systemic plasma in lung tissue ranged from 5 to 19 fold, Table 4.

Equivalence for the different routes of administration and selection of PD study doses was based on systemic exposure (for plasma AUC, see Table 3) to PYY(3–36). These data indicated that the 0.1 mg/kg IP, 0.1 mg/kg SC and 0.21 mg/kg inhalation doses were equivalent, as shown in Figure 6.

At the completion of the inhalation PD study, after 4 h, animals were sacrificed and BALF, lung tissue and plasma samples taken to determine PYY(3–36) levels. The results, summarized in Table 5, show similar trends as observed in the PK study and

Table 5. PYY(3–36) concentration (ng/mL) in mice following inhaled doses of 0.22 and 0.65 mg/kg, 4 h post-inhalation drug delivery.

	0.22 mg/kg			0.65 mg/kg		
	Plasma	Lung	BALF	Plasma	Lung	BALF
Concentration (ng/mL)	0.62	25.9	2030	3.6	214	16 700

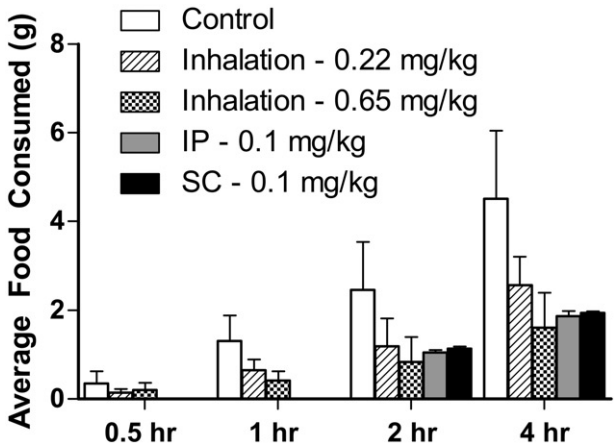

Figure 7. Average food consumed by mice as a function of time after dosing via IP, SC or inhalation. Shown with error bars as the standard error of the mean.

correlate elevated PYY(3–36) levels to the PD responses observed and discussed below.

Pharmacodynamic results in food consumption study

Figure 7 summarizes the food consumption results from the PD study. These results are overlaid with the previously conducted PD studies with IP and SC dosing.

As the data show, the control group consumed an average of 4.51 g of food, inhalation low dose group consumed 2.57 g, and inhalation high dose group consumed 1.61 g over the 4-h period after dosing and before necropsy. The SC and IP doses resulted in similar food consumption (IP: 1.87 g and SC: 1.95 g) over the 4-h period.

Discussion

The focus of this study was to utilize pharmacokinetic data from common delivery routes for proteins and peptides (IP injection and SC injection), merge them with pharmacokinetic data from inhalation delivery, and determine whether similar pharmacodynamics would be observed. In order to test our hypothesis, the engineered particles were prepared by spray drying an aqueous solution of PYY(3–36) and Dextran having a molecular weight of 10 000 daltons (D10). D10 was selected from a list of other potential stabilizing excipients based on its precedence for use in parenteral and inhalation delivery[17–25]. D10 is important for the physical stability of these powders because it (a) stabilizes the amorphous dispersion and (b) maintains discrete particles of a respirable size during storage. D10 has a high glass transition temperature (T_g), which helps to kinetically stabilize the amorphous nature of the spray-dried powders. D10 can be spray-dried alone with a single drug or with several compounds. In addition, D10 is not composed of reducing sugar groups and is therefore compatible with primary amine containing actives, including many biotherapeutics.

The spray-dried PYY(3–36) materials for this study were found to be highly respirable and reproducible in both *in vitro* testing and in the preclinical exposure systems. While rigorous thermal analysis and stability was not conducted on this formulation, the water uptake and thermal characterization of D10 is optimal for stabilizing amorphous powders with a high Tg and relatively low hygroscopicity. The thermal properties and water uptake for D10 has been reported elsewhere[25–27]. For proteins and peptides such as PYY, which cannot be micronized, the combination of spray drying and the use of a stabilizing excipient can be an enabling strategy for producing dry-powder formulations[23,25,26].

Regardless of route delivered (IP, SC or inhalation), matrix (systemic plasma, lung tissue, BALF) or dose, PYY(3–36) is rapidly absorbed with T_{max} values all less than 0.5 h. Similarly for both SC and inhalation delivery, the C_{max} and AUC were highly proportional to dose. The enhancement of exposure in the lung tissue ranged from 5- to 19-fold over plasma when PYY(3–36) was delivered via inhalation. Interestingly, the inhalation doses all resulted in more rapid absorption (shorter T_{max}) when compared to both IP and SC, owning to the rapid absorption of spray-dried formulation and the highly respirable particle-size distribution of the developed formulation.

For all inhalation doses, the PYY(3–36) concentrations track the expected diffusional concentration gradient of a low-permeability molecule with high water solubility in the lung through all three compartments (Figures 3, 4, and 5). Specifically, the highest levels of PYY(3–36) are observed in the BALF where the powder is deposited, followed by the lung tissue and finally ending up in the plasma. The shape of the PK curves may suggest a second input rate into the plasma, such as nasal deposition, but future experiments will be necessary to show if these shapes are due to real physiologic phenomena or experimental sampling challenges.

Prior to the development of the novel inhalation formulation, efficacy in the PD model was observed with a 0.1 mg/kg SC and IP dosing. Therefore, dose selection was based on equivalent systemic exposure (plasma AUC) from the inhalation route, 0.21 mg/kg pulmonary deposited dose. The measured systemic exposure for the doses used in the PD study shows similar concentration versus time profiles (Figure 6) and therefore were predicted to have similar PD response. As the literature in the area of protein and peptide dose proportionality and scaling is lacking, an additional higher pulmonary deposited dose (0.65 mg/kg) was included in the inhalation PD study.

The inhalation PD studies show that a similar decrease in food consumption was seen for both pulmonary deposited doses, with the 0.65 mg/kg nearly matching the IP and SC doses. While there is a pulmonary deposited dose–response for decrease in food consumption, it deviates from linearity, which is in contrast to the PK parameters that were found to be highly proportional to dose. Two potential causes for the PK/PD responses not being proportional to dose include: (1) the 0.65 mg/kg pulmonary deposited dose was not evaluated in the PK model and the PK may be non-linear in this dose range and (2) the PD response may have been saturated at this pulmonary deposited dose.

Conclusion

The hypothesis tested in these studies was that a similar pharmacodynamic endpoint could be achieved for spray-dried engineered particles delivered to the mouse lung using a nominally similar dose, determined by systemic pharmacokinetics. Determination of a nominally similar dose for a protein/peptide between IP, SC and inhalation is an area of literature that is lacking. Therefore, pharmacokinetic studies were performed to determine the appropriate dose(s) for inclusion in a PD study.

The PK data indicated that an inhalation pulmonary deposited dose 2 × greater (0.21 mg/kg) than IP and SC delivery (0.1 mg/kg) resulted in similar systemic plasma exposure (plasma AUC). When tested in the PD model, the 2 × increased pulmonary deposited dose resulted in a decrease in food consumption; however, a 6 × increased pulmonary deposited dose was required to match that of the IP and SC delivery. Based on these results, respirable engineered particles of PYY(3–36) and D10 show promise as a non-invasive therapeutic for appetite suppression. Future work may be centered on potentially understanding PK/PD response for PYY(3–36).

These results substantiate our initial hypothesis of demonstrating similar PD response for a spray-dried powder of a peptide delivered to the lung relative to an IP or SC, albeit with a total pulmonary deposited dose of 2 × to 6 × higher dose than the IP or SC dose.

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Declaration of interest

Authors declare no conflicts of interest.

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