Impact of Formulation and Methods of Pulmonary Delivery on Absorption of Parathyroid Hormone (1–34) from Rat Lungs

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ABSTRACT: The aim of this work was to optimize the absorption of parathyroid hormone 1–34 (PTH) from the lungs by determining factors favoring its transport from the air spaces into the bloodstream. We simultaneously conducted pharmacokinetic and regional lung deposition studies in vivo in the rat following intratracheal administration of PTH in solution or dry powder form. Dry powders of PTH or albumin were prepared by spray-drying using lactose and dipalmitoylphosphatidylcholine (DPPC). Deposition in the trachea, peripheral, and central lobe sections was assessed after tissue grinding using albumin as a marker. The method of intratracheal instillation had a significant impact on PTH absorption from the lungs, and the deeper the deposition within the respiratory tract, the higher the absorption. Inhalation of the PTH powder resulted in high systemic bioavailability despite deposition of the formulation principally in upper airways. We demonstrated that the increased absorption resulted from DPPC that had permeation enhancer properties even though it was abundantly present locally in pulmonary surfactant. Optimization of PTH absorption from the lungs could be attained by targeting the peripheral lungs as well as codelivering DPPC.

INTRODUCTION

Parathyroid hormone is an endogenous polypeptide of 84 amino acids that is synthesized in the chief cells of the parathyroid glands and regulates calcium homeostasis and bone turnover. The 1 to 34 N-terminal fragment (4118 Da) of the hormone (PTH) exhibits full biological activities and has unique anabolic effects on bone when delivered once-daily by subcutaneous (s.c.) injection at low doses. PTH stimulates formation of new bone and improves bone growth and density.1,2

Inhalation aerosols have been shown a promising noninvasive alternative to injection, which could help PTH to attain its full therapeutic potential. Intratracheal instillation in rats and nebulization in monkeys reached absolute bioavailability values of 40 and 29%, respectively.3,4 An absolute bioavailability of 34% was obtained in rats after insufflation of a dry powder of PTH, optimized for aerosolization properties and composition.5

The efficiency of drug absorption from the lungs depends on a number of factors related to drug, formulation, and administration method.6,7 Small lipophilic molecules diffuse within minutes across the trachea, airways, and alveolar tissue, and the dose deposited in the lungs can totally absorb into the bloodstream.8,9 Macromolecules, like albumin...
(65 kDa) or IgG (150 kDa), remain for hours in the air spaces, exhibit a low penetration in the pulmonary tissue, and absorb into the bloodstream with bioavailabilities that can be lower than 5%. Depending on the particular peptide or protein, protease cleavage in the lungs can markedly compete with transport into the systemic circulation and formulation compositions that decrease the rate of enzymatic degradation or accelerate the rate of absorption can significantly increase bioavailabilities. For instance, dipalmitoylphosphatidylcholine (DPPC), the most abundant component of lung surfactant, has been shown to increase absorption of insulin when formulated as admixture or liposomes. An additional increase in absorption of peptides from the lungs can result from methods of administration that target the large and highly vascularized alveolar region.

The aim of this study was to optimize the pulmonary delivery of PTH by defining factors favoring its transport from the airspaces into the bloodstream. We simultaneously conducted pharmacokinetic and regional lung deposition studies in vivo in the rat following intratracheal administration of PTH in dry powder or solution form. Former and recent techniques of pulmonary administration to small laboratory animals were used to deliver the formulations. These experiments showed that PTH absorption correlated with the depth of deposition of the formulation within the respiratory tract and pinpointed a substantial enhancement in absorption caused by DPPC that was used to prepare the aerosol powder of PTH.

MATERIALS AND METHODS

Chemicals

The 1–34 fragment of human parathyroid hormone (pI 8.3), referred to as PTH in the remainder of the text, was obtained from UCB-Biotechnology (Braine-l’Alleud, Belgium). Human serum albumin (fraction V, 96–99% albumin, not purified of fatty acids), biotin-labeled bovine albumin, unconjugated goat antibiotin antibody, 96 vol % ethanol, xylazine, and sulforhodamine 101 were obtained from Sigma-Aldrich (Bornem, Belgium). α-Lactose monohydrate was purchased from Acros Organics (Fair Lawn, NJ) and DPPC (734.05 Da) from Lipoid GMBH (Ludwigshafen, Germany). Streptavidin–horseradish peroxidase was obtained from PharMingen (Becton Dickinson, Aalst, Belgium). 3,3',5,5'-Tetramethylbenzidine (TMB) and high-performance liquid chromatography (HPLC) grade acetonitrile were purchased from VWR (Leuven, Belgium). Sodium pentobarbital, ketamine (Ketalar), and Thalamonal were purchased from Certa (Medeva Pharma, Braine-l’Alleud, Belgium), Warner-Lambert (Zaventem, Belgium), and Janssen-Cilag (Berchem, Belgium), respectively.

Animals

Eleven- to 12-week-old male Wistar rats (424 ± 4 g; Charles River Laboratories, Saint Germain les Arbréles, France) were used for the pharmacokinetic studies and 10- to 12-week-old male Wistar rats (417 ± 6 g; Elevage Janvier, Le Genest St. Isle, France) were used for the lung deposition studies. Animals had free access to tap water and laboratory diet (pelleted commercial standard diet, no. A04, Usine Alimentation Rationnelle, Epinay-sur-Orge, France) during the experimental period. All experimental protocols in rats were approved by the Ethical Committee for Animal Care and Use of the Faculty of Medicine of the Université catholique de Louvain (Brussels, Belgium).

Formulation of the Dry Powders

Dry powders were prepared by spray-drying as previously described, using PTH, human serum albumin, lactose, and/or DPPC, and sulforhodamine 101 or biotin-labeled bovine albumin as markers. PTH, lactose, sulforhodamine, and/or albumin were dissolved in 0.5 mM phosphate buffer pH 7.4. DPPC was dissolved in 96% ethanol. The two solutions were combined to form a 70% ethanolic solution of 0.1% w/v total concentration and spray-dried at 100°C. The powders were collected and stored in a dessicator (at 4°C and 25% relative humidity). The fluorescent marker was incorporated at a low load (0.2% w/w) in the formulations characterized in vitro to allow easy quantification of the powder deposited in the impactor stages. Free and biotinylated albumin were incorporated in the powders administered in vivo for the lung distribution studies because it allowed to quantify the amount of powder deposited in each lung section. Albumin, in contrast to PTH, has a long residence time in the air spaces, and is poorly absorbed in the systemic circulation due to its high molecular weight. This limited losses during dissection and allowed accurate estimation of formulation deposition within the lungs.
Particle Size and Density

The mass median primary particle diameter was measured by laser diffraction (HELOS, Sympatec GmbH, Clausthal-Zellerfelg, Germany) in the wet state mode, as previously described. The powder density was determined by tap density measurements, i.e., following 1000 taps, which allowed the density to plateau.

Aerosolization Properties of the Powders In Vitro

The pulmonary deposition of the dry powders was estimated in vitro using a Multi-Stage Liquid Impinger (MSLI) equipped with a USP induction port (Copley Scientific, Nottingham, UK) at an airflow of 60 L/min under controlled relative humidity (30–40%), as previously described. After dissolution of the particles, the fluorescence of each solution, due to sulforhodamine incorporated in the dry powder, was determined using a Perkin-Elmer Luminescence Spectrometer LS50B ($\lambda_{em} = 586$ nm, $\lambda_{em} = 602$ nm). Measurements were performed in duplicate.

The emitted dose was determined as the percent of total powder mass exiting the capsule. The cumulative mass of powder less than the stated size of each stage of the impactor was calculated and plotted on a log probability scale, as percent of total mass recovered in the impactor against the effective cutoff diameter. The experimental mass median aerodynamic diameter (MMAD) of the particles was defined from this graph as the particle size at which the line crosses the 50% mark. The fine particle fraction was calculated by interpolation from the same plot as the fraction of powder emitted from the inhaler with an aerodynamic diameter ≤5 μm.

HPLC

PTH content in the powders and concentrations in solutions were measured by reverse-phase (RP) HPLC with the Hewlett Packard series 1100 system (Agilent Technologies, Palo Alto, CA) using an RP C18 Jupiter column (250 × 4.60 mm i.d., 5 μm, 300 Å, Phenomenex, Torrance, CA) maintained at 35°C. Isocratic separation was achieved using a water/acetonitrile/trifluoroacetic acid (67:33:0.1) solvent system at a flow rate of 1 mL/min. The column effluent was directed to a variable wavelength detector set to monitor absorbance at 214 nm. Samples were prepared either by suspending the powder in the mobile phase, sonication for 2 min and centrifugation at 3000 rpm and 4°C for 10 min or, by diluting the PTH/DPPC suspensions in the mobile phase and centrifugation at 10,000 rpm and 4°C for 5 min. PTH extraction from the powder allowed recovery of 94 ± 1% of PTH. The injection volume was 100 μL. The limit of quantification was 2.5 μg/mL. The method was linear between 2.5 and 50 μg/mL and the intraassay relative standard deviation (RSD) was 0.4–1.3% over this range.

Pharmacokinetic Studies

Catheters for blood sampling and intravenous (i.v.) injection (CBAS-C30 Solo-Cath 3 Fr × 80 cm, Instech Solomon, Plymouth Meeting, PA) were implanted in the jugular veins of the animals 24 h before the pharmacokinetic study. Rats were anesthetized with 700 μL of Thalamonal and then received a dose of PTH of approximately 20 μg by i.v. bolus injection and various modes of pulmonary delivery (Table 1). All PTH solutions were prepared in phosphate buffer (0.5 mM at pH 7.4) and the volume administered was adjusted to the rat weight to keep the dose per kilogram constant, except for the spray-instillator, which delivered a fixed volume of 0.1 mL. The i.v. and intratracheal solutions of PTH were administered in approximate volumes of 0.5 and 0.1 mL, respectively.

Two methods commonly used to deliver solutions and powders to rat lungs following tracheotomy were first tested. Intrapulmonary instillation was carried out using a truncated needle (23 gauge) inserted between two cartilaginous rings of the trachea. The solution was delivered using a 1-mL standard syringe connected to the needle, and an air bolus of 1 mL was then insufflated using a similar syringe. A PTH powder made of PTH/lactose/DPPC 10:30:60 (w/w/w) was administered after tracheotomy using a Harvard ventilator (Harvard Apparatus, Ltd., Edendridge, UK). A truncated needle (18 gauge) was inserted between two cartilaginous rings of the trachea, and the reservoir tip containing the powder sample was connected to the needle and to the ventilator through a flexible plastic tube. The ventilator insufflated an air bolus of 2 mL through the needle into the lungs, dragging the powder into the respiratory tract. The powder reservoir was weighed before, after powder filling and after administration, to know the exact dose insufflated. 238 ± 12 μg of powder were delivered, which corresponded to a mean PTH dose per rat of 20 μg, given the actual PTH content in the powder.
Intratracheal instillation of PTH was then carried out using more recent techniques in which the delivery tubes were placed directly in the trachea close to the carina through the mouth (and therefore without surgery). The rats were placed and secured in a supine position on a board inclined at an angle of 45°. To correctly put the delivery tube in the trachea, the optical-fiber light of a laryngoscope was positioned in the mouth of the animal, the tongue being pulled out. A solution of PTH or a suspension of PTH/DPPC (1:7) was directly instilled in the trachea using a curved high-precision microsyringe (100-μL Hamilton syringe; Alltech, Lokeren, Belgium). After instillation, a 3-mL air bolus was immediately insufflated in the trachea using a powder insufflator (Model DP-3; Penn-Century, Inc.; Philadelphia, PA), which did not contain any powder sample in its reservoir. The 3-mL air bolus was contained in a syringe connected to the insufflator, and was pushed into the lungs by hand actuation. The solution of PTH was also instilled using a spray-insufflator (MicroSprayer model IA-IB, Penn-Century Inc.; Philadelphia, PA). The spray-insufflator is an aerosol generator consisting of a subminiaturized atomizer located in the tip of a long, thin, stainless steel tube that is attached to a hand-operated, high-pressure syringe. The delivery tube of the device was inserted in the trachea and the instillation of a precise, fixed 100-μL volume of solution was aerosolized by hand actuation of the piston. Spray instillation was or not followed by a 3-mL air bolus delivered with the insufflator (empty from any powder).

The PTH powder was also administered directly into the trachea via the mouth using the powder insufflator, which is a more recent device than the Harvard ventilator system. The delivery tube of the insufflator containing the powder sample was placed close to the carina. Administration of the powder was carried out by hand actuation of a syringe connected to the device and filled with 3 mL of air. The insufflator containing the powder was weighed before, after powder filling, and after administration, to know the exact dose insufflated. 243 ± 19 μg of powder were delivered with the insufflator, which corresponded to a mean PTH dose per rat of 22 μg/kg with the actual PTH content in the powder.

The changes in plasma immunoreactive PTH levels after administration were determined in four to eight rats/group. Blood samples (0.3 mL) were collected from the jugular vein into lithium-heparinized tubes (Microtainer brand tubes lithium heparin, Becton Dickinson, Aalst, Belgium) before and at 5, 10, 15, 25, 40, 60, (90), 120, and 180 min after i.v. and powder delivery. Additional samples were taken at 2.5 min after instillation of PTH and at 1 and 3 min after instillation of PTH/DPPC suspension. Blood was replaced by the administration of 0.3-mL saline to compensate the decrease in volume. The plasma was separated by centrifugation (15 min at 3300 × g and 4°C), stored at −20°C and analyzed within 1 month by a 1–34 PTH radioimmunoassay (Bachem, former Peninsula Laboratories, Merseyside, UK), as previously described. PTH immunoreactive concentrations were calculated by curve-fitting using the PTH plasma standards.

### Table 1. Pharmacokinetic Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>i.v.</th>
<th>Microsyringe</th>
<th>Microsyringe</th>
<th>Spray</th>
<th>Spray</th>
<th>Insufflation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(No DPPC)</td>
<td>(with DPPC)</td>
<td>(No Air-Bolus)</td>
<td>(with Air-Bolus)</td>
<td>(with DPPC)</td>
</tr>
<tr>
<td>Number of rats</td>
<td>4</td>
<td>7</td>
<td>5</td>
<td>5</td>
<td>3</td>
<td>8</td>
</tr>
<tr>
<td>Rat weight (g)</td>
<td>428 ± 10</td>
<td>418 ± 5</td>
<td>407 ± 6</td>
<td>426 ± 3</td>
<td>435 ± 3</td>
<td>416 ± 12</td>
</tr>
<tr>
<td>PTH dose/rat (μg)</td>
<td>19.0 ± 0.88</td>
<td>18.6 ± 0.24</td>
<td>18.1 ± 0.3</td>
<td>20</td>
<td>20</td>
<td>20 ± 1</td>
</tr>
<tr>
<td>C₀ or C_max (ng/mL)</td>
<td>58 ± 5</td>
<td>20 ± 2</td>
<td>51 ± 5</td>
<td>5 ± 1</td>
<td>10 ± 1</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>t_max (min)</td>
<td>6 ± 1</td>
<td>4 ± 1</td>
<td>7 ± 2</td>
<td>5 ± 0</td>
<td>7 ± 1</td>
<td></td>
</tr>
<tr>
<td>AUC (ng · min/mL)</td>
<td>1367 ± 39</td>
<td>507 ± 70</td>
<td>892 ± 76</td>
<td>137 ± 42</td>
<td>222 ± 11</td>
<td>511 ± 64</td>
</tr>
<tr>
<td>t½ (min)</td>
<td>14 ± 1</td>
<td>18 ± 2</td>
<td>14 ± 2</td>
<td>14 ± 2</td>
<td>14.0 ± 0.7</td>
<td>24 ± 2</td>
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<tr>
<td>F_abs (%)</td>
<td>100</td>
<td>37 ± 6</td>
<td>65 ± 7</td>
<td>10 ± 3</td>
<td>16 ± 1</td>
<td>34 ± 5</td>
</tr>
</tbody>
</table>

a Intravenous injection; b Intratracheal instillation of a PTH solution with the microsyringe; c Intratracheal instillation of a PTH/DPPC (1:7) suspension with the microsyringe; d Intratracheal instillation of a PTH solution with the spray-insufflator; e Intratracheal instillation of a PTH solution with the spray-instillator; f Maximal plasma concentration; g Time to peak; h Area under the plasma concentration–time curve; i Elimination half-life; j Absolute bioavailability; Data are presented as mean ± SEM.
The areas under the plasma concentration–time curve (AUC) were calculated using the linear trapezoidal rule. The maximal plasma concentration ($C_{\text{max}}$) and the time to peak ($t_{\text{max}}$) were obtained from the individual concentration–time curves. The absolute bioavailability ($F_{\text{abs}}$) was calculated as ($AUC_{\text{iv}}$ - $AUC_{\text{po}}$) - ($Dose_{\text{iv}}$ - $Dose_{\text{po}}$) · 100 and the plasma elimination half-life ($t_{1/2}$) of $D_{\text{tot}}$ as $\ln 2/k$. The elimination rate constant ($k$) was estimated by linear regression of the last time points of the log concentration versus time curve.22

**Regional Lung Deposition Studies**

To assess the impact of the method of pulmonary administration on the site of deposition of the formulations within the lungs and thereby on PTH absorption, the proportion of deposition in the trachea, central, and peripheral lung sections was investigated using biotin–albumin as a distribution marker. The animals (five rats/group) were anesthetized with 700 µL of ketamine/xylazine (50/5.56 mg/mL). Only the more recent techniques of administration (described in details in the methods for the pharmacokinetic studies) were investigated.

A solution of biotin–albumin (7.8 µg/0.1 mL phosphate buffer) was administered using the 100-µL precision microsyringe (followed by the 3-mL air bolus) and the spray instillator with or without insufflation of a 3-mL air bolus. A fourth group of rats was intratracheally instilled with a suspension of biotin–albumin/DPPC (1:18 w/w; in 0.1 mL of phosphate buffer, sonicated for 2 min beforehand) using the microsyringe, followed by insufflation of a 3-mL air bolus. Approximately 440 µg of a powder formed of biotin–albumin, albumin, lactose, and DPPC in proportion 2.5:7.5:30:60 (w/w/w/w) were administered using the insufflator (average of 11 µg of biotin–albumin per rat) to a fifth group of rats.

The rats were sacrificed immediately after administration by a lethal injection of pentobarbital and the lung was removed and divided into trachea (with main bronchi) and pulmonary lobes. Each lobe was cut in two equal parts by mass: one central part cut round in shape around the bronchus hilum, and one peripheral part. The subdivision was made visually and allowed to obtain a relatively constant weight ratio of the central to the peripheral part of 1.07 ± 0.04. The different tissue samples were finely minced, ground for 2 min in 10 mL of ultrapure water with a tissue-grinder Potter (VWR, Leuven, Belgium) for release of the marker, and centrifuged for 10 min at 15,000 rpm, 4°C. Each sample was assayed by enzyme immunoassay. Plates were coated with an antibiotin antibody, were incubated with dilutions of samples, and were developed with streptavidin–horseradish peroxidase that oxidized TMB (in the presence of H$_2$SO$_4$) to a yellow diimine measured at 450 nm.

To compare the efficiency of administration of the different devices to the lung, several parameters were measured. (1) The deposition in the trachea (T), central (CS), and peripheral (PS) lobe sections was expressed as percentages of total deposition within the lungs. (2) Because the proportion of lung parenchyma (the preferential site of absorption to the systemic circulation) to conducting airways is larger in the peripheral lobe section compared to the central lobe region, the ratio of deposition in the peripheral to central lung (which included the central lobe section and the trachea; P/C ratio) was used as an index of deep lung deposition and was calculated as [PS/(CS+T)]. A P/C ratio close to 1.0 indicates a homogeneous deposition within the lung lobes and limited deposition in the trachea, whereas a ratio close to 0.0 indicates a preferential deposition in the trachea and the central lobe section. (3) The concentration of albumin in each entire lobe was normalized to the average concentration of albumin in the lobes; a ratio of 1 for the five lobes indicates perfect deposition homogeneity between the lobes.

The recovery of albumin was 98 ± 2% when added in vitro to lobe sections just before grinding, 62 ± 15% when intratracheally instilled ex vivo in the excised lung, 67 ± 3% after instillation in vivo and 63 ± 7% after powder insufflation with the insufflator in vivo. The larger losses of albumin ex vivo compared to in vitro might be due to losses during mincing of the lung sections on microscope slides.

**Binding of PTH to DPPC**

A possible binding of PTH to DPPC was assessed by equilibrium dialysis, using Teflon dialysis cells (Dianorm System, München, Germany), under a constant stirring at 20 rpm, at 25°C. The dialysis was performed using cellulose membranes (Spectra/Por2 Membrane Discs, Spectrum Medical Industries, Los Angeles, CA) with a molecular weight cutoff of 12–14 kDa. One milliliter of isotonic phosphate buffer containing 200 µg of PTH and 1.4 mg of spray-dried DPPC, sonicated
2 min beforehand, was dialyzed against 1 mL of isotonic phosphate buffer, to reproduce the 1:7 PTH/DPPC proportion of the powder and suspension formulations used in the pharmacokinetic studies. Measurements were performed in triplicate. Intact PTH was assayed by HPLC in both compartments.

Statistics
The data were validated by the Dixon test. All results are expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) test and the Tukey test were performed to demonstrate statistical differences ($p < 0.05$), using the software Sigma-stat for Windows (SPSS Inc., San Rafael, CA).

RESULTS

Physical Characteristics of the Dry Powders
The dry powders used for the pharmacokinetic and lung deposition studies were made of PTH/lactose/DPPC (10:30:60 w/w/w) and albumin/lactose/DPPC (10:30:60 w/w/w), respectively. These compositions were selected because they allowed to attain high fine particle fractions in impactors in vitro as well as high systemic PTH absorption after inhalation in rats.\(^5\) The PTH and albumin powders presented similar physical characteristics. The primary geometric particle diameters were, respectively, 4.34 and 4.67 μm, and the tap densities of both powders were low with values of 0.063 and 0.045 g/cm\(^3\), respectively. The emitted doses were 90.5 and 85.9%, the fine particle fraction 54 and 52%, and the experimental mass median aerodynamic diameter (MMAD) 4.6 and 4.8 μm, respectively, in the multistage liquid impinger operated at 60 L/min using a Spinhaler inhaler device.

Absorption of PTH from the Lungs
To assess the impact of the methods of pulmonary administration and type of formulation on PTH absorption from the lungs, plasma PTH levels were measured following various modes of intratracheal instillation, insufflation of the PTH powder, and i.v. injection in rats.

We first tested two common methods of pulmonary administration of solutions and powders to small laboratory animals that involved tracheotomy.\(^{21,23}\) Conventional instillation using a standard 1-mL syringe led to a bioavailability of PTH relative to i.v. injection ($F_{abs}$) of 11 ± 4%. Intratracheal inhalation of the PTH powder using a ventilator resulted in an $F_{abs}$ of only 3 ± 1%. Both methods led to a high variability between rats, as shown by the relative standard deviations (RSD) of 72 and 55%, respectively (data not shown). Uneven distribution of the liquid between the lobes, the nonuniform distribution within each lobe, and the varying degree of expectoration of liquid have been demonstrated the major causes of lack of dose reproducibility of this intratracheal instillation method.\(^{21}\) The ventilator method led to important powder losses in the truncated needle, and an unknown quantity of powder was lost in the atmosphere because of the lack of airtight connections. The delivered dose was therefore overestimated, which could explain the low bioavailability of PTH. The low reproducibility and/or low control on the dose delivered made us drop the conventional methods for more accurate and recent techniques, which also did not need tracheotomy but where the delivery tubes were placed directly in the trachea through the mouth.

Intratracheal instillation of a PTH solution using a 100-μL precision microsyringe inserted through the mouth, followed by the administration of a 3-mL air bolus, yielded an $F_{abs}$ of 37 ± 6% (Table 1 and Fig. 1). The use of a spray instillator instead led to an $F_{abs}$ of 10 ± 3%. To push the sprayed droplets further into the lungs, a 3-mL air bolus was administered directly after the spray, as after instillation using the microsyringe. This led to an increase in $F_{abs}$ from 10 to 16 ± 1% ($p < 0.05$). The RSDs were 37, 34, and 9%, for respective methods, indicating increased homogeneity of delivery between animals.

Delivery of the PTH powder to rat lungs using an insufflator resulted in a high and reproducible $F_{abs}$ of 34 ± 5% (RSD = 37%; Table 1 and Fig. 1). This bioavailability was as high as the highest $F_{abs}$ value obtained following instillation. To assess a potential involvement of DPPC incorporated in the dry powder in the efficiency of PTH absorption from the lungs, we coinstilled PTH and DPPC using the microsyringe and measured plasma PTH levels.\(^{13,14}\) The addition of DPPC to the instillate accelerated the rate of PTH transport from the airway lumen to the bloodstream and $F_{abs}$ increased from 37 to 65% (Table 1 and Fig. 1; $p < 0.05$).

Regional Lung Deposition of the Formulations
The absorption of PTH from the lungs greatly varied with the method of pulmonary delivery or
type of formulation (Table 1 and Fig. 1). To further understand these variations, we measured the regional deposition of the solutions and dry powder containing albumin within the lungs. The percentages of tracheal, central, and peripheral deposition relative to total recovery of the poorly diffusible marker albumin are depicted in Figure 2 for each recent mode of delivery utilized in the pharmacokinetic studies.

The percentage of peripheral deposition relative to total deposition reached 34, 23, and 27% when the solution of albumin was delivered by the microsyringe, the spray instillator without and with an air bolus, respectively (Fig. 2). The corresponding P/C ratios were 0.52 ± 0.06, 0.30 ± 0.05, and 0.39 ± 0.06, respectively. A correlation was therefore observed between the P/C ratio and the absolute bioavailability of instilled PTH: the deeper the deposition, the higher the systemic absorption (Fig. 3). Regional deposition was not altered by adding DPPC to the instillate delivered by the microsyringe, showing that the highest PTH bioavailability attained with the PTH/DPPC suspension did not result from a deeper deposition (Fig. 3).

The instillation methods also resulted in differences in distribution of deposition between pulmonary lobes. The albumin solution distributed more homogeneously between the five pulmonary lobes after spray instillation than instillation using the microsyringe (Fig. 4). However, the distribution among lobes was not affected when an air bolus was insufflated after spray instillation or when DPPC was added to the instillate (Fig. 4).

The dry powder deposited primarily in the trachea, that is, 60% of the dose. Twenty-nine percent was recovered in the central region and 11% in the peripheral region, and a P/C ratio of only 0.12 ± 0.04 was obtained. The bioavailability of PTH formulated as a dry powder was therefore far higher than expected in view of the correlation between bioavailability and deposition obtained for the instillation methods (Fig. 3). This suggests that effective PTH absorption following dry powder insufflation principally resulted from the presence of DPPC in the dry powder rather than from an optimized delivery to the deep lungs. The homogeneity of deposition among lobes was intermediate between that of the spray instillator and the microsyringe (Fig. 4).

Two hypotheses were considered to explain the effect of DPPC on absorption: an interaction between DPPC and the PTH molecule, or a direct action of DPPC on the airways, and/or alveolar epithelia. The first explanation was eliminated as no binding of PTH to DPPC was measured by equilibrium dialysis, in contrast to the binding observed between PTH and dimyristoylphosphatidylcholine24 or insulin and DPPC.25 The second hypothesis was therefore considered the most likely to explain the effect of DPPC on PTH absorption.

DISCUSSION

The purpose of this work was to optimize the pulmonary administration of PTH by understanding factors that affected its absorption from the lungs in rats. We showed that the method of intratracheal instillation greatly influenced PTH absorption from the lungs, and that the bioavailability of the hormone correlated with the depth of deposition of the liquid formulation within the respiratory tract. We also showed that inhalation of a powder made of PTH, lactose, and DPPC led
to high systemic absorption of the peptide despite a deposition principally in the trachea and upper airways. Effective absorption from the dry powder resulted from permeation enhancer properties of DPPC even though the phospholipid was already present locally in pulmonary surfactant.

Inhalation of the PTH powder and intratracheal instillation of a PTH solution using a 100-μL precision microsyringe resulted in a similar absolute bioavailability of 34 and 37%, respectively, even though the peripheral to central deposition ratio was respectively 0.12 and 0.52 (Table 1 and Fig. 3). Incorporating DPPC in the solution instilled produced 1.8-fold increase in PTH bioavailability (Fig. 1 and Table 1), confirming the permeation enhancer effect of DPPC. It has been estimated that the lungs of rats contained 3 mg/kg body weight of DPPC. The quantity of DPPC that we delivered to the rat lungs (330 mg/kg) was therefore only 11% that local amount. Absorption of insulin from rat lungs was previously reported to be enhanced by codelivering insulin with a physical dispersion of DPPC in saline, but the mass of DPPC instilled, 6.8 mg/kg, was much larger. The ultimate practical utility of DPPC as permeation enhancer requires demonstration of efficacy in humans at doses representative of therapeutic applications. Assuming a daily dose of 5 mg of powder aerosol and a percentage of

Figure 2. Regional deposition of albumin in the trachea, central, and peripheral lungs after intratracheal instillation of an albumin solution or an albumin/DPPC suspension using the microsyringe, after intratracheal instillation of an albumin solution using the spray-instillator followed or not by an air bolus, and after administration of an albumin/lactose/DPPC powder using the insufflator. Error bars are standard errors of the means (n = 5).

Figure 3. Correlation between peripheral to central lung (P/C) ratio and absolute PTH bioavailability after instillation using the spray-instillator without and with air bolus and using the microsyringe (in increasing order; r² = 0.98). Insufflation of the PTH powder containing DPPC and instillation of the PTH/DPPC suspension (1:7) using the microsyringe did not fit into the correlation, indicating the permeation enhancer effect of DPPC.
DPPC in the powder of 50% by weight, the amount of DPPC added to the local pool size would be approximately 35 mg/kg for a 70 kg person, that is, approximately 2.5% the local amount of 1.4 mg/kg found in humans. It is to be noted that infants with respiratory distress syndrome or adults with acute respiratory distress syndrome are treated with doses of 100 to 200 mg surfactant/kg, in support of the safety of inhaling DPPC.

We assume that exogenous DPPC would enhance the permeability of the airway and/or alveolar epithelia due to transient alterations to local DPPC concentrations and/or surfactant organization. Preferential deposition of the formulation in the upper tract implies, for instance, large DPPC concentrations per unit surface area due to the small contribution the airways make to the total lung surface. The DPPC-induced increase in pulmonary insulin absorption was previously suggested to result from a binding of the peptide to DPPC hydrocarbon chains. Yet, we did not measure any binding between PTH and DPPC in equilibrium dialysis experiments. The use of natural lung surfactant as a drug vehicle for pulmonary administration has been shown to allow deeper and more uniform deposition of instillates within the lungs. However, no modification to overall deposition of the PTH solution was observed when incorporating pure DPPC in the instillate (Figs. 2–4). Administration of the PTH powder formed of DPPC led to high absorption of the hormone despite that deposition principally occurred in large airways (Figs. 1–3). This further indicates that improved pulmonary deposition was an unlikely mechanism for the enhancement in absorption obtained, and rather suggests that DPPC might increase the permeability of the airways epithelial membrane. Phospholipids are known to penetrate cell membranes, decrease bilayers stability, and thereby induce changes in the cytoskeleton that can affect tight junctions and accelerate paracellular passage of hydrophilic drugs.

Various techniques were employed for intratracheal instillation of a PTH solution, and each resulted in a different PTH bioavailability due to a different regional deposition within the lungs (Figs. 1–2 and Table 1). Spray instillation generates 25–30 μm droplets that deposited in the central tract, uniformly between lobes (Figs. 2 and 4). Insufflating an air bolus immediately after spray instillation increased the fraction.
of solution that deposited in peripheral lungs (Fig. 2). Surprisingly, the use of the microsyringe led to the deepest deposition (Fig. 2). The liquid instilled likely formed plugs in upper airways, which were then pushed in the distal tract by the propelling force of the additional air bolus. The deeper the deposition within the respiratory tract, the larger the extent of PTH absorption (Fig. 3). In airways, molecules must traverse a 5–10 μm mucous layer, the 50 μm thick ciliated columnar epithelium and connective tissue for an additional average distance of 100 μm before reaching capillaries. Dependence of absorption on deposition depth has similarly been observed in humans where, for instance, the bioavailability of an aerosolized anti-inflammatory protein, soluble interleukin-4 receptor, was shown proportional to peripheral lung deposition measured by gamma scintigraphy. Previous investigations on peptide and protein absorption from animal lungs have reported bioavailability values that could vary by one order of magnitude between studies, for the same peptide or protein and in the same animal species. Our results imply that interstudy variabilities could originate in part from the varying modes of instillation employed, and that comparison of systemic absorption between formulations (e.g., varying composition) should ensure identical deposition within the lungs.

The high fine particle fractions of the dry powders measured in vitro translated into significant respirable fractions in vivo in the rat. The fraction of particles with an aerodynamic size ≤5 μm was 52–54% in the four-stage liquid impinger using a Spinhaler inhaler device at an airflow rate of 60 L/min. The total fraction of the delivered dry powder mass that was recovered from the lung lobes reached a relatively close value of 40% (Fig. 2). It is noteworthy that dry powder dispersion and penetration in the lungs were not dramatically impeded by the high relative humidity of the respiratory tract and by the increased deposition efficiencies of particles of a given size in the upper respiratory tract of rodents compared to humans.

In conclusion, a method of administration that favored deposition deep into the lungs was of primary importance to optimize pulmonary PTH absorption. Yet, despite substantial deposition in upper airways, high systemic PTH bioavailabilities could be attained by incorporating DPPC in the formulation. We had previously shown that albumin decreased PTH absorption from the lungs, as a consequence of PTH binding to albumin. Albumin and DPPC are present in large amounts in the epithelial lining fluid of the lungs, and the expected safety of their pulmonary administration had motivated their selection for the preparation of dry powder aerosols. Our studies indicate that a compound, even endogenous to the lungs, can markedly impact on pulmonary drug absorption and that selection of excipients should therefore include the consideration of these biological effects.

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