Dry powder nitroimidazopyran antibiotic PA-824 aerosol for inhalation

by

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Running title: Inhaled PA-824 for TB treatment
Abstract

We formulated PA-824, a nitroimidazopyran with promise for the treatment of tuberculosis, for efficient aerosol delivery to the lungs in a dry powder porous particle form. The objectives of this study were to prepare and characterize a particulate form of PA-824, assess the stability of this aerosol formulation at different environmental conditions and determine the pharmacokinetic parameters of the powder after pulmonary administration. The drug was spray dried into porous particles containing a high drug load and possessing desirable aerosol properties for efficient deposition in the lungs. Physical, aerodynamic and chemical properties of the dry powder were stable at room temperature for six months and refrigerated conditions for at least one year. Pharmacokinetic parameters were determined in guinea pigs after pulmonary administration of the PA-824 powder formulation at three doses (20, 40, 60 mg/kg) and compared to intravenous (20 mg/kg) and oral (40 mg/kg) delivery of the drug. Oral and inhaled delivery of PA-824 achieved equivalent systemic delivery at the same body dose within the first twelve hours of dosing. However, animals dosed by the pulmonary route showed drug loads remaining locally in the lungs 32 hours post-exposure whereas those given oral drug cleared more rapidly. Therefore, we expect from this pharmacokinetic data that pulmonary delivery may achieve the same efficacy as oral at the same body dose, with a potential improvement in efficacy related to pulmonary infection. This may translate into the ability to deliver lower body doses of this drug for the treatment of tuberculosis by aerosol.
Introduction

The global epidemic caused by the infectious disease tuberculosis (TB) has garnered attention recently due to the growing concern over the emergence of multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB), which are caused by *Mycobacterium tuberculosis* that show resistance to multiple first-line drugs or that are virtually untreatable by existing TB antibiotics (6). An investigational nitroimidazopyran, PA-824, is one of the most promising TB drug candidates in thirty years. PA-824 appears to have bactericidal activity against both active replicating and persisting or latent *M. tuberculosis* with the potential of treating MDR-TB (21). It has been proposed that this activity against static bacilli could help sterilize TB lesions faster (26). Therefore, reduced treatment times may be possible with drug regimens that include PA-824. Any advance in TB therapy that addresses drug resistant tuberculosis and reduces frequency of dosing, dose required and duration of treatment has the potential to improve patient treatment.

Preclinical evaluation in animal models of PA-824 alone and in combination with moxifloxacin, rifampicin, isoniazid and/or pyrazinamide indicated potential for the treatment of TB and MDR-TB (9, 13-15, 21, 22, 25). Drugs were delivered orally in these studies, with likely only a fraction of the administered dose reaching the lung tissue, the primary infection site of TB. Thus, direct delivery of PA-824 to the lungs could improve the effectiveness of treatment by increasing the local concentration of drug in the lungs thereby targeting pulmonary TB, while still
delivering drug systemically for non-pulmonary TB. It is also possible that this non-invasive route of administration may require smaller doses compared to standard oral treatment, thus, potentially reducing side effects and shortening time of treatment. Inhaled antibiotics have been demonstrated to improve treatment of TB (18) and other diseases such as cystic fibrosis (19). However, these treatments were given by nebulization, a process which requires lengthy treatment times and sterile water to achieve relatively low delivery efficiencies (8).

The dry powder inhaler is an alternative method for pulmonary drug delivery, with the ability to deliver stable formulations easily from simple inhalers. The porous particle system, a dry particle formulation, is well suited for efficient delivery to the lungs (1). This novel particulate aerosol has also been explored for TB treatment with the tuberculostatic agents para-aminosalicylic acid (PAS) and capreomycin (2, 3, 23). PAS, delivered as a dry powder, was shown to achieve greater lung exposure in rats at a lower total body drug dose than the typical oral dose (23). Pulmonary delivery of capreomycin gave promising efficacy results in a guinea pig model of tuberculosis, with lower wet organ weights and bacterial burden in the lungs than animals given intravenous or intramuscular treatment (2, 3). Thus, pulmonary administration of PA-824 in a porous particle form may provide an additional therapeutic advantage for this promising new drug. In the present study, PA-824 was formulated for aerosol delivery into a porous particle form and characterized for physical and aerosol properties that would be
desirable for deposition in the lungs. The powder was tested for physical, aerodynamic and chemical stability at a variety of environmental conditions over a period of one year.

The disposition of PA-824 after oral delivery was determined previously in mice (10, 13), rats (20), dogs and monkeys (16), but not after pulmonary delivery to guinea pigs. The TB infected guinea pig model is a clinically relevant animal model with respect to human disease because the pathogenesis and lung pathology in guinea pigs mimics that in humans with active TB (12, 17, 24). It is likely that drug disposition will be different in TB infected animals than in healthy animals. As the initial step to determine PA-824 disposition in guinea pigs, the present studies were conducted in healthy animals to define baseline pharmacokinetic parameters of PA-824 after intravenous administration of solution and pulmonary administration of the dry powder formulation, and compared to those obtained by the oral standard route of delivery. In addition, local PA-824 concentrations were determined in the lungs after the drug concentration became undetectable in systemic circulation to evaluate the efficiency of delivery by both routes of administration.
MATERIALS AND METHODS

Materials

L-leucine was obtained from Spectrum Chemicals & Laboratory Products (Gardena, CA) and the phospholipid 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) from Genzyme Pharmaceuticals (Cambridge, MA). PA-824 was received from the Global Alliance for TB Drug Development (New York, NY). Acetonitrile, ethanol USP grade and methanol were purchased from Pharmco Products Inc. (Brookfield, CT). Water from a Millipore Corp. (Billerica, MA) Milli-Q water purification system was used.

Preparation of Porous Particles

The spray drying solution was prepared by dissolving L-leucine in pure water at 55 ºC. PA-824 and DPPC were then added to ethanol with heating and stirring. The aqueous solution was added to the ethanol mixture immediately before spray drying for a final solids concentration of 4 g/L and solvent ratio of 70% ethanol and 30% water (v/v). The final formulation consisted of a ratio of 75:20:5, PA-824: L-leucine:DPPC (wt %).

Porous particles were prepared using a Niro, Inc. Mobile Minor spray dryer (Columbia, MD). The inlet temperature was set at 107 ºC and the solution feed rate at 60 ml/min. The solution was pumped into the two-fluid nozzle of the spray dryer with a gas flow rate of 25 g/min. Spray dried powders were collected in a container at the outlet of the cyclone.
Particle Characterization

Characterization of Dry Powders

The volume diameter of the spray dried powder was measured by laser diffraction using a HELOS system with RODOS dry dispersing unit (Sympatec Inc., Lawrenceville, NJ). Each measurement was performed in triplicate at an applied pressure of 2 bar.

The aerodynamic properties and particle distribution of the powder (fine particle fraction, FPF; mass median aerodynamic diameter, MMAD; geometric standard deviation, GSD) were determined with standard methodology using an eight-stage Andersen Cascade Impactor (ACI; Copley Scientific Limited, Nottingham, UK). The fine particle fraction of the total dose of powder less than or equal to an effective cut-off aerodynamic diameter of 5.8 µm (FPF_{TD}) was calculated by dividing the powder mass recovered from stages 1-7 of the impactor by the total particle mass.

The morphology of the dry particles was evaluated using a 982 field emission scanning electron microscope (SEM, LEO, Carl Zeiss, Inc., Thornwood, NY) after coating powder samples with a Platinum/Palladium layer (208HR sputter coater, Cressington Scientific Instruments Inc., Watford, UK).
Drug Load of Powders

The PA-824 load of the spray dried powder was determined by a reverse-phase high performance liquid chromatography (HPLC) method using an Agilent 1100 Series HPLC system with Zorbax columns and ChemStation software (Agilent Technologies Inc., Palo Alto, CA). The mobile phase was run on a linear gradient from 20% acetonitrile and 80% water to 60% acetonitrile and 40% water over 30 minutes with 5 minutes of equilibration time. Analysis was performed on a 50 µL injection at a flow rate of 1.5 ml/min through an Agilent Zorbax Eclipse XDB-C18 (4.6 x 150 mm) column and absorbance recorded at 330 nm. An Agilent Zorbax Eclipse XDB-C18 analytical guard column was also used.

Powder Stability Study

A spray dried PA-824 formulation of 75:20:5 // PA-824: L-leucine:DPPC (wt %) was stored at different environmental conditions to assess physical, aerodynamic and chemical stability over time. Powder aliquots were transferred to glass vials in a controlled environment held at 10% relative humidity and then placed at temperatures of 4°C, 25°C and 40°C, representing refrigerated, room temperature and accelerated storage conditions. Sealed sample vials were stored protected from light in polyethylene jars with anhydrous calcium sulfate desiccant (W.A. Hammond Drierte Co., Xenia, OH). Samples were removed from storage at 0, 0.5, 1, 3, 6 and 12 months and allowed to equilibrate to room temperature before analysis. The powders were analyzed in triplicate for particle size, fine particle fraction of the total dose and drug content. Statistical
significance was determined by linear regression analysis, where p-values < 0.05 were considered to be statistically significant.

Pharmacokinetic Studies

Experimental Design

Male guinea pigs weighing 463.86 ± 65.11g were employed in the pharmacokinetic (PK) studies. Animals were housed in a 12 h light/12 h dark cycle and constant temperature environment of 22 °C. A standard diet and water were supplied ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of North Carolina. Twenty-four hours before the study, each animal underwent cannulation of the right external jugular vein for continuous blood sampling (4, 5).

Animals were randomly divided into five groups (n = 6-7) receiving PA-824 as follows: intravenously (IV) in solution at a dose of 20 mg/kg, oral gavage of the drug in a mixed micelle suspension at 40 mg/kg, and as dry powder via intratracheal insufflation by the pulmonary route as porous powders at nominal doses of 20, 40 and 60 mg/kg. For the insufflation procedure, animals were anesthetized and the trachea visualized with a laryngoscope. A small animal insufflator (Penn Century, Philadelphia, PA) was inserted into the trachea and placed at a distance of 1 cm from the carina. PA-824 powders were dispersed with the help of 5 mL of air from an empty syringe. The insufflator chamber and tube containing the dose of PA-824 powder were weighed carefully before and
after delivery to the animal to estimate the actual delivered dose to be used for
the PK analysis for each animal.

PA-824 was formulated into a micelle suspension for oral dosing with 10%
hydroxypropyl-β-cyclodextrin and 10% lecithin as described previously (15). PA-
824 doses were based on results from an efficacy study of oral administration of
PA-824 in a guinea pig model of tuberculosis where a dose of 40 mg/kg delivered
daily for 30 days showed a statistically significant reduction of bacilli in lungs and
spleen (21). After drug administration, blood samples (0.35 ml) were collected
from each animal into heparinized tubes at 0, 0.08, 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8,
12 and 24 hours. Additional samples were collected at 28 and 32 hours in
animals dosed with powder at 60 mg/kg. Sterile saline solution was used to
replace the blood volume lost through sample collection. Plasma was separated
and stored at -80 °C until analysis. After collection of the last blood sample,
animals were euthanized by exanguination and bronchoalveolar lavage (BAL)
was conducted (5 ml sterile saline). The bronchoalveolar lavage sample was
centrifuged and the pellet and supernatant separated.

**Sample Analysis**

Plasma and BAL samples were analyzed by a validated HPLC assay for drug
content. Serum concentrations of PA-824 were determined using a system
consisting of a ThermoFinnegan P4000 HPLC pump (San Jose, CA) with model
AS1000 fixed-volume autosampler, a model UV2000 ultraviolet detector, a
Gateway Series e computer (Poway, CA), and the Chromquest HPLC data management system. The plasma standard curve for PA-824 ranged from 0.20 to 50 µg/ml. The absolute recovery of PA-824 from plasma was 88.2%. The overall validation precision across all standards was 0.67 to 5.38%. Sample preparation for the BAL supernatant was an adaptation of the plasma method, with identical HPLC method.

Pharmacokinetic Analysis

The pharmacokinetic parameters area under the curve (AUC), elimination rate constant (K), mean residence time (MRT), half-life (t_{1/2}) and bioavailability (F) were obtained by non-compartmental methods (WinNonlin, Pharsight Corporation, Mountain View, CA). Maximum PA-824 concentrations (C_{max}) and time to obtain the maximum PA-824 concentration (T_{max}) were determined from the non-fitted plasma versus time profiles for each individual animal. Bioavailability (F) was calculated by the following equation:

\[ F = \frac{AUC_{\text{lung, oral}}}{AUC_{\text{IV}}} \times \frac{D_{\text{IV}}}{D_{\text{lung, oral}}} \]

where D is the dose. The subscripts indicate the routes of administration: intravenous (IV), pulmonary (lung) and gastrointestinal (oral) delivery.
Statistical Analysis

Data for the pharmacokinetic study was subjected to analysis of variance (ANOVA) and least-squares significant-differences multiple comparison method. A probability level of 5% (p < 0.05) was considered to be statistically significant.

RESULTS

Particle manufacture and characterization

Porous particles with a load of approximately 75% (w/w) PA-824 were prepared by spray drying. The resulting dry powder formulation consisted of thin-walled porous particle structures as shown in Figure 1. The median volume diameter of the particles was 4.14 ± 0.04 µm, and had desirable aerosol properties for human pulmonary delivery (7, 11) indicated by an MMAD of 4.74 ± 0.08 µm, GSD = 1.53 ± 0.02 and FPF_{TD} = 53.3 ± 2.7 %. The PA-824 content of the powder was 74.8 ± 0.5% of the total mass as estimated by HPLC with UV detection.

Powder stability study

The stability of a PA-824 powder formulation composed of 75:20:5, PA-824: L-leucine:DPPC (wt %) was studied for one year at refrigerated (4 °C), room temperature (25 °C) and accelerated conditions (40 °C). The results for volume median diameter, FPF_{TD} and PA-824 content analysis over time are shown in Figure 2. The powder showed no statistically significant differences (p > 0.05) in physical or aerodynamic characteristics or chemical content for one year at refrigerated conditions and for at least six months at room temperature. Powder
at the accelerated condition (40 °C) maintained stability for at least three months and only showed a significant decline in aerosol properties at six months ($p = 0.000$) and PA-824 content at one year ($p = 0.039$).

**Pharmacokinetic studies**

Average PA-824 plasma concentration versus time curves are shown in Figure 3. The terminal phases of these curves appear to be linear for all treatments. A dose dependency was observed among groups receiving particles by the pulmonary route, with animals receiving the largest dose having the highest plasma concentration and PA-824 remaining detectable for a longer period of time. Interestingly, the plasma concentration versus time profiles for animals dosed by the oral and pulmonary routes with 40 mg/kg of PA-824 appeared almost superimposable for the first 8 hours, but plasma concentrations in animal receiving an oral dose were detectable only 12 hours after dosing, whereas those of animals dosed by the pulmonary route remained detectable 24 hrs after dose administration.

The local PA-824 concentrations remaining in the lungs 32 hours after drug administration to the different groups are shown in Figure 4. PA-824 was detectable at levels well above the detection threshold of the analytical method in the lungs of the three groups of animals dosed by the pulmonary route in a dose dependent manner, whereas no drug was detected in the lungs of animals receiving PA-824 by the intravenous or oral routes.
PK parameters obtained by non-compartmental methods are presented in Table 1. AUC and $C_{\text{max}}$ after PA-824 administration by the pulmonary route increased linearly with the dose ($R^2 = 0.999$) with statistical differences between the three values. AUC was statistically comparable in animals receiving 40 mg/kg by the oral and pulmonary routes. However, PA-824 was eliminated ($K$) at a slower rate when given at doses of 40 and 60 mg/kg by the pulmonary route, correlating with significantly longer half-lives ($t_{1/2}$) and mean residence time (MRT). Notably, among animals receiving 40 mg/kg PA-824, $t_{1/2}$ by the pulmonary route was almost double that of those receiving the drug by the oral route. $C_{\text{max}}$ and $T_{\text{max}}$ were statistically comparable in groups of animals receiving the 40 and 60 mg/kg doses. Bioavailability was approximately 60% and statistically comparable in all treatment groups.

DISCUSSION

The continuing TB epidemic, including a growing number of cases of MDR/XDR-TB and the increasing co-incidence of HIV and TB synergy, requires new and improved treatment as part of the strategy for containment and reduction of the infection. The first drug demonstrating promise in treating both active and latent tuberculosis is the nitroimidazopyran PA-824, typically administered orally. Our goal was to develop PA-824 in a form suitable for delivery as an aerosol directly to the lungs, the primary site of TB infection.
We have shown the potential for the antibiotic PA-824 to be formulated into a dry powder porous particle form that can be delivered efficiently by the pulmonary route. This high drug load formulation maintained physical, aerodynamic and chemical stability at refrigerated conditions (4 °C) for over a year and room temperature (25 °C) for more than six months. This room temperature stability may reduce the need for a cold chain for storage and distribution. At accelerated conditions (40 °C), chemical stability was maintained throughout the 12 month study duration, while aerodynamic properties showed significant decline at six months at this elevated temperature. This heat sensitivity is typical for dry powders to be delivered by inhalation (2). In summary, the stability of the formulation is temperature dependent, with chemical and physical stability maintained during the 12 month study duration at refrigerated conditions and for more than six months at room temperature conditions, while likely allowing for short-term storage excursions at higher temperatures.

Pharmacokinetic parameters after oral administration of PA-824 in guinea pigs were determined in the present study, revealing that the disposition of PA-824 differed from literature reports for other different species. Half-life after oral administration in guinea pigs (2.43 h) was comparable to that in monkeys and rats (2-5 h) (16), but slightly longer than that in dogs (1-2 h), confirming the observation that systemic absorption in dogs may be low due to poor absorption and rapid metabolism (16). T_{max} after oral administration to guinea pigs (4 h) was also similar to that in monkeys (3.33 h), but significantly shorter than that in rats.
which may indicate a shorter absorption time in guinea pigs. $C_{\text{max}}$ values 14- to 17-fold higher than MIC values of PA-824 against *M. tuberculosis* (0.015 - 0.25 µg/ml) (21) were observed in guinea pigs after pulmonary and oral administration of a 40 mg/kg dose, respectively. In contrast, Nuermberger et al. (13) reported $C_{\text{max}}$ values of 80- to 110-fold higher than the MIC in mice after oral administration of a 100 mg/kg dose. These observations may be the result of administering different doses, but also variation in the disposition of this drug by the two species. Therefore, caution should be exercised when selecting pharmacokinetic parameters from one species to identify a dose or dosing regimen including PA-824 for the treatment of TB in another species.

In the present study, $C_{\text{max}}$ and AUC showed a linear dose dependency for powders administered to guinea pigs by the pulmonary route, as was previously reported in mice after oral administration of PA-824 (10).

Differences in the disposition of PA-824 were observed in guinea pigs when administered by different routes at the same dose. Systemic PA-824 levels appeared similar following delivery by the pulmonary and oral routes within the first 12 h after dosing of 40 mg/kg. However, the drug was not detectable in plasma after 12 h in animals receiving oral doses, whereas systemic drug levels remained detectable in animals dosed by the pulmonary route until 24 h. This observation is consistent with the slower elimination rates and longer half-lives and MRT obtained for animals dosed by the pulmonary route. Most notably, PA-
824 concentrations were sustained locally in the lung 32 h after dosing, even after systemic concentrations were undetectable. Tissue concentrations 3 to 8-fold higher than in plasma have also been reported after oral administration of PA-824 to rats (20). However, these observations were made at much earlier time points (4-6 h) than in the present study (32 h). Thus, we show that delivering drug directly to the lungs confers the advantage of increasing tissue concentrations of drug at the primary site of TB infection for longer periods of time than drug delivered orally.

Pharmacokinetic data reported for guinea pigs in the present study, indicate that aerosol administration to the lungs achieves either comparable or lower systemic exposure than the oral route, with elevated local lung concentrations. Therefore, we propose that pulmonary delivery may achieve the same efficacy as oral at the same body dose, with a potential improvement in effectiveness related to pulmonary infection. This may translate into the ability to deliver lower body doses with less frequency than standard dosing regimens.
Acknowledgements

This work was supported by a grant from the National Institute of Health/NIAID under Grant Number 5 U01 61336 and a National Science Foundation Graduate Research Fellowship.

The authors acknowledge the Global Alliance for TB Drug Development for PA-824 material used in this study and Dr. Melvin Spigelman and Dr. Doris Rouse for their assistance. We also thank Plastiape for their generous donation of inhaler devices.
References


Table 1. Pharmacokinetic parameters obtained by non-compartmental analysis after administration of PA-824 formulations by different routes and at different doses (Mean ± standard deviation, n=6-7).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IV 20 mg/kg</th>
<th>Oral 40 mg/kg</th>
<th>Insufflation 20 mg/kg</th>
<th>Insufflation 40 mg/kg</th>
<th>Insufflation 60 mg/kg</th>
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<tbody>
<tr>
<td>AUC_{0-t} (µgh/ml)</td>
<td>26.54±2.20²</td>
<td>25.77±6.40²</td>
<td>14.80±3.84³</td>
<td>32.34±16.79²</td>
<td>50.96±9.53¹</td>
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<tr>
<td>K (h⁻¹)</td>
<td>0.37±0.04¹</td>
<td>0.30±0.05²</td>
<td>0.24±0.01²</td>
<td>0.17±0.05³</td>
<td>0.13±0.05³</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>1.91±0.24³</td>
<td>2.43±0.56³</td>
<td>2.83±0.10²</td>
<td>4.36±1.06²</td>
<td>5.91±2.51¹</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>2.69±0.31³</td>
<td>5.37±0.53²</td>
<td>5.17±0.45¹</td>
<td>7.52±1.91³</td>
<td>8.26±1.80¹</td>
</tr>
<tr>
<td>Cmax (µg/ml)</td>
<td>9.19±1.54¹</td>
<td>4.14±0.78²</td>
<td>2.01±0.55³</td>
<td>3.42±1.14²</td>
<td>4.58±2.49²</td>
</tr>
<tr>
<td>T_{max} (h)</td>
<td>0.11±0.07²</td>
<td>4.00±0.63¹</td>
<td>4.33±1.03¹</td>
<td>3.25±2.09¹</td>
<td>3.60±2.88¹</td>
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<tr>
<td>F_{(0-∞)}</td>
<td>-</td>
<td>0.56±0.12¹</td>
<td>0.59±0.16¹</td>
<td>0.63±0.32¹</td>
<td>0.62±0.10¹</td>
</tr>
<tr>
<td>F'</td>
<td>-</td>
<td>-</td>
<td>2.11±0.56¹</td>
<td>1.12±0.57²</td>
<td>0.74±0.12²</td>
</tr>
</tbody>
</table>

AUC = Area under the curve; K = Elimination rate constant; t₁/₂ = half-life; MRT = Mean residence time; C_{max} = Maximum concentration; T_{max} = Time at which C_{max} occurs; F = Absolute bioavailability; F' = Relative bioavailability compared to oral treatment.

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

**Figure 1.** Scanning electron micrograph of spray dried PA-824 porous particles (PP). [Scale bar represents 2 µm.]

**Figure 2.** PA-824 porous powder stability data for (a) volume median diameter, (b) fine particle fraction of the total dose and (c) PA-824 content as a percentage of the initial value at 4 °C (○), 25 °C (△) and 40 °C ( ■) over a period of 12 months.

**Figure 3.** Average PA-824 plasma concentration versus time curves (log scale) after administration of PA-824 at the following treatments and doses: intravenous solution (IV; 20 mg/kg), oral mixed micelle suspension (Oral; 40 mg/kg), and insufflated PA-824 dry powder porous particles (Insuff; 20, 40, 60 mg/kg). (Mean ± standard deviation, n = 6-7).

**Figure 4.** PA-824 concentrations in bronchoalveolar lavage (BAL) fluid 32 hours after dosing. While no PA-824 was found in the lung fluid for drug given as intravenous solution or oral mixed micelle suspension, powders given by insufflation showed sustained levels of PA-824 in the lungs after 32 hours. There also appears to be a dose dependency on the drug levels remaining in the lungs (Mean ± standard deviation, n = 6-7).
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