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Research Paper

Preparation and *in Vivo* Evaluation of a Dry Powder for Inhalation of Capreomycin

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Purpose. To develop an aerosol system for efficient local lung delivery of a tuberculostatic drug.
Methods. The antibiotic, capreomycin sulfate, was spray dried to form a dry powder aerosol. The chemical content and physical properties of resulting particles were assessed under various storage conditions. Plasma concentrations of capreomycin after insufflation into guinea pigs were evaluated at three doses, and compared to IV and IM administration of a capreomycin solution.
Results. Dry powder aerosols containing capreomycin were formulated to enable efficient delivery of large drug masses to the lungs of guinea pigs. Aerosols loaded with 73% CS were shown to possess good aerosolization properties and physical–chemical stability for up to 3 months at room temperature. Upon insufflation into guinea pigs, the amount of CS reaching the bloodstream was significantly lower compared to IV or IM administration, but resulted in a significantly longer drug half-life.
Conclusions. The results indicate that large doses of capreomycin in dry powder form can be efficiently delivered to the lungs of guinea pigs, which may result in high local drug exposure but significantly reduced systemic exposure as suggested by plasma concentrations in the present studies. These systems have considerable potential to provide more effective therapy for MDR-TB

KEY WORDS: aerosols; antibiotics; insufflation; pulmonary drug delivery; tuberculosis.

INTRODUCTION

Tuberculosis (TB) is reemerging as a major public health threat, initiated largely by a rise in the number of people with AIDS and the development of TB strains that are resistant to antibiotics (1,2). TB is the world's leading cause of death from a single infectious organism, killing about 2 million people each year. One-third of the population carries the bacterium, with 10% of the carriers developing active TB and becoming spreaders of the disease. New cases of TB, and therefore death tolls, are expected to rise if further control strategies are not put into place across the globe.

Current treatment for active TB involves prolonged chemotherapy with antibiotics administered orally or via injection. Multidrug resistant tuberculosis (MDR-TB) can result when TB treatment is incomplete, either due to patient noncompliance or when the wrong or an incomplete combination of drugs is prescribed. Cases of multidrug resistant TB (MDR-TB) require upwards of 2 years of chemotherapy with less effective and more toxic second-line drugs (3,4). Although MDR-TB is a systemic disease, the major burden often occurs in the lungs. Therefore, delivering high drug doses of TB antibiotics locally to the lung mucosa may provide rapid sterilization of the lungs, leading to shortened drug therapy. Aerosol and endotracheal administration of antibiotics have been used effectively to treat respiratory infections including *Pneumocystis carinii* pneumonia in HIV/AIDS patients (5–8), *Pseudomonas aeruginosa* in CF patients (9,10), multiple Gram-negative infections in ambulatory settings, and TB disease (11,12). Several small clinical trials of aerosolized anti-TB agents have shown that adjunct administration may hasten conversion of sputum smears, an indicator of eradication of active bacteria in the lung. However, studies of aerosol treatment to date have utilized nebulizers to administer the large drug doses required in antibiotic therapies. While useful in clinical settings, they are inefficient and unsuitable for rugged field environments. As an example, a single dose of TOBI® (aerosolized tobramycin), an FDA-approved product for managing CF patients with *P. aeruginosa*, requires approximately 200 inhalations over 13 min (13).

In the present study, we report the development of dry powder aerosols for the local delivery of capreomycin, a cyclic pentapeptide antibiotic indicated for pulmonary infec-

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tions associated with MDR-TB (14), to the lungs. Excipient concentration, as well as spray drying conditions, were investigated in order to optimize the aerodynamic properties of spray-dried capreomycin powders. Powder stability under various storage conditions was also evaluated. *In vivo* pharmacokinetic studies were conducted in a guinea pig model to assess capreomycin disposition after insufflation of dry powder to the lungs.

MATERIALS AND METHODS

Materials

Capreomycin sulfate was received as a gift from Eli Lilly (Indianapolis, IN). L-leucine was purchased from Sigma-Aldrich (St. Louis, MO). USP grade ethanol was obtained from PharmCo Products Inc. (200 proof, Brookfield, CT); water was from a Milli-Q water purification system (Millipore Corp., Billerica, MA).

Capreomycin is a peptide antibiotic of the aminoglycoside family. It is a white or almost white powder, freely soluble in water, and insoluble in alcohol. L-leucine is a white crystalline powder, soluble in water at ~32 g/l (60°C), and slightly soluble in alcohol (0.72 g/l in 99% alcohol). L-leucine was incorporated into the formulation as a dispersibility enhancer to improve powder aerosolization properties (15–17).

Preparation of Dry Powder Aerosols

Solutions containing various concentrations of capreomycin sulfate and L-leucine were prepared in 50:50//ethanol: water (total solids concentration = 3.6 g/l) and heated to 60°C for complete solute dissolution. Solutions were spray-dried using a pilot scale spray dryer (Niro Inc., Columbia, MD) equipped with a two-fluid nozzle with a 1 mm nozzle diameter (Spraying Systems Co., Wheaton, IL). The inlet temperature was varied from 186–189°C to achieve an outlet temperature of ~65°C. A feed flow rate of 80 ml/min and a process gas flow rate of 79–82 kg/h were used. The nozzle was operated at a pressure of 40–43 psi and a sheath air flow rate of 29–31 g/min. Spray-dried powders were collected via a cyclone. To optimize dry powder aerodynamic properties, the effect of atomizer air flow rate was investigated.

Aerosol Chemical Characterization

Capreomycin sulfate content in each powder was determined by HPLC analysis (1100 Series, Agilent Technologies, Palo Alto, CA) in 22:78//methanol:phosphate buffer with 0.3 % wt heptafluorobutyric acid using a C18 reverse-phase column (Agilent ZORBAX Eclipse XDB-C18) at 1.0 ml/min and 25°C. The water content of each powder was determined using a Karl Fischer Coulometric Titrator (EMD Aquastar AQC 34, Libertyville, IL) coupled with a drying oven (Mettler Toledo DO307, Columbus, OH) set to a temperature of 150°C. A dry air stream was used to transfer moisture from the oven to the titration cell containing the titrant (EMD CombiCoulomat).

Aerosol Particle Geometric Size and Tap Density

Particle mean geometric size and size distribution were measured by laser diffraction using a HELOS diffractometer and a RODOS variable-shearing dry powder disperser (Sympatec, Lawrenceville, NJ) at applied regulator pressures of 0.5, 1, 2, and 4 bar. The geometric standard deviations (GSD) of dry powders were determined from:

$$\mathrm{GSD} = (d_{84\%}/d_{16\%})^{0.5} \quad (1)$$

where d_n is the diameter at the nth percentile of the cumulative distribution (18).

The bulk density of the particles was determined by tap density measurements. Briefly, particles were loaded into 0.3 ml sections of a 1-ml plastic pipette, capped with NMR tube caps, and tapped approximately 600–1,200 times using a Varian tapper (Cary, NC) until the volume of the powder did not change. The tap density was determined from the difference between the weight of the pipette before and after loading, divided by the volume of powder after tapping.

Theoretical aerodynamic diameter (d_{aero}) was calculated from the aerosol geometric size and tap density using the following equation:

$$d_{\mathrm{aero}} = \frac{d_g\sqrt{\rho/\rho_{\mathrm{ref}}}}{\gamma} \quad (2)$$

where d_{g} is the particle geometric diameter, ρ is the particle density, $\rho_{\mathrm{ref}} = 1$ g/cc, and γ is a shape factor (for a spherical particle, $\gamma = 1$; for aerodynamic diameter calculations, the particles in this study were assumed to be spherical). It is noted that tap density measurements underestimate particle bulk densities since the volume of particles measured includes the interstitial space between the particles. The true particle density, and therefore the aerodynamic diameter of a given powder, is expected to be slightly larger than reported.

Imaging of Aerosol Particles by Scanning Electron Microscopy

Aerosol particle surface morphology was evaluated by scanning electron microscopy (SEM) with a LEO 982 Field Emission Scanning Electron Microscope (Zeiss, Thornwood, NY). Particles were attached to SEM mounts using double-sided carbon tape and sputter coated for 60 s using a Pt/Pd target (Cressington 208HR, Valencia, PA). Populations representative of each dry powder sample were photographed.

In Vitro Aerosolization of Dry Powder Aerosols

The aerodynamic properties of the powders were assessed using an eight-stage Mark II Andersen Cascade Impactor (ACI-8, Thermo Electron, Waltham, MA). Capsules (size 3 Shionogi Qualicaps, Madrid, Spain) containing 29.5 ± 7.6 mg of powder were placed in a hand-held, dry powder, breath-activated inhaler device (Plastiape, Osnago Lecco, Italy). The capsule was punctured and the powder drawn through the cascade impactor operated at a flow rate of 28.3 l/min for 4.2 s to simulate an inspiration. Dry powder

Table I. Capreomycin Sulfate (CS) and Water Content of Dry Powder Aerosols as a Function of the Initial Feed Content

CS:Leucine in the Feed (wt%)	CS Content (%)	Water Content (%)
50:50	46.5 ± 2.6	4.0 ± 0.5
60:40	55.7 ± 1.8	4.0 ± 0.4
70:30	63.7 ± 1.3	4.8 ± 0.2
80:20	73.1 ± 1.0	5.3 ± 0.7
90:10	81.6 ± 1.3	7.0 ± 0.9

Values are reported as the mean ± SD.

aerosols deposited on glass fiber filters on each of the nine stages of the impactor. The amount of powder on each stage was measured by gravimetric analysis. Fine particle fractions of the total dose (FPF_{TD}), the percentage of aerosolized particles that reached the lower seven stages of the impactor (corresponding to aerodynamic diameters below 5.8 μm), or the lower five stages (corresponding to aerodynamic diameters below 3.3 μm) were then calculated. Additionally, the mass median aerodynamic diameter (MMAD) was determined from the cumulative mass distribution curve (18).

Disposition of Capreomycin from LPPs in Guinea Pigs

Male Dunkin-Hartley guinea pigs, with cannulas implanted in the jugular vein for continuous blood sampling, were employed to study the disposition of capreomycin. Groups of six to eight animals under light anesthesia received capreomycin particles by endotracheal insufflation (Penn Century, Philadelphia, PA) in three escalating doses: 1.4, 7.2 and 14.5 mg/kg. Capreomycin solution (20 mg/kg) delivered by the intravenous (IV) and intramuscular (IM) routes were used to compare the parameters of capreomycin disposition by the pulmonary route.

After administration of each capreomycin treatment, blood samples were collected from each animal at 0, 0.083, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 6 and 8 h time points. Sterile saline solution was used to replace the blood volume lost through sample collection. Samples were then centrifuged and plasma separated and stored at −80°C until analysis. After collection of the last blood sample, animals were euthanized and bronchoalveolar lavage was conducted to determine capreomycin concentration remaining in the airways of the animal after the blood sampling period.

Capreomycin concentrations in plasma and bronchoalveolar lavage were determined by HPLC employing the method reported by Rossi, *et al.* (19). Drug plasma concentration versus time data was analyzed (WinNonlin, Pharsight Corporation, Mountain View, CA) to obtain key pharmacokinetic (PK) parameters such as area under the curve (AUC), mean residence time (MRT) and half-life ($t_{1/2}$). Maximum capreomycin concentrations (C_{max}) were determined from the non-fitted plasma versus time profiles for each individual animal.

The differences in PK parameters for the different treatments were analyzed with the analysis of variance (ANOVA) and least-squares significant-differences multiple comparison method. A probability level of < 0.05 was considered to be statistically significant.

Stability Study Design

The powder was aliquoted into 15 glass scintillation vials (~200 mg each) in a glove box at 10.5% RH, then tightly capped. Three vials each were placed in four plastic desiccated chambers containing anhydrous calcium sulfate (W.A. Hammond Drierite Co., Xenia, OH). The chambers were stored at room temperature under dark conditions, at room temperature exposed to sunlight, at 4°C (refrigerated), and at 40°C as an accelerated stability condition. The final three vials were placed uncapped at 40°C and 75% RH in a humidity chamber. Powder physical and chemical properties were analyzed at set timepoints of 0, 1, 2 and 6 weeks, 2 months, and 3 months. Statistical significance was determined by regression analysis. *P*-values < 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Effect of Chemical Composition on Aerosol Properties

Dry powder aerosols containing various percentages of capreomycin sulfate (CS) and leucine were formed by spray drying from 50% ethanolic solutions. Analysis of CS content of the powders revealed that the drug is fairly stable during the spray-drying process, with final drug contents of 90–93% ob-

Fig. 1. Scanning electron microscopy images of **a** 50:50, **b** 60:40, **c** 70:30, **d** 80:20, and **e** 90:10//CS:leucine dry powder aerosols. Bar = 10 μm.

Table II. Geometric Size of Dry Powder Aerosols Determined at Dispersion Pressures of 0.5, 1.0, 2.0 and 4.0 bar

CS:Leucine in the Feed (wt %)	Diameter (μm) (0.5 bar)	Diameter (μm) (1.0 bar)	Diameter (μm) (2.0 bar)	Diameter (μm) (4.0 bar)
50:50	5.40 ± 1.83	5.45 ± 1.86	5.54 ± 1.84	5.58 ± 1.82
60:40	4.75 ± 1.76	4.64 ± 1.75	4.74 ± 1.74	4.68 ± 1.74
70:30	4.22 ± 1.68	4.21 ± 1.66	4.18 ± 1.65	4.18 ± 1.66
80:20	3.74 ± 1.64	3.69 ± 1.68	3.81 ± 1.65	3.79 ± 1.63
90:10	3.40 ± 1.75	3.30 ± .66	3.16 ± 1.57	3.14 ± 1.56

Values are reported as the mean ± geometric SD.

tained (Table I). Water content analysis showed that hydration of the particles due to water absorption increased with increasing CS content, as expected for a hydrophilic antibiotic.

Scanning electron microscopy indicated that increasing the CS content of the spray-dried powders led to increasingly spherical and smaller aerosol particles (Fig. 1). As the percentage of CS in the spray-dried solution was increased from 50 to 90%, a decrease in mean geometric diameter from 5.4 to 3.4 μm was observed (Table II). Additionally, when CS concentration was increased from 50 to 70%, denser particle formation was observed (0.36 g/ml for 50% leucine particles compared with 0.68 g/ml for 30% leucine particles). These observations are in line with those presented by Lin *et al.* (20), who reported that higher solubility materials incorporated into drying droplets generally form smaller and denser particles. However, at CS concentrations above 70%, the density of the spray-dried powders began to decrease (0.64 g/ml for 20% leucine particles; 0.32 g/ml for 10% leucine particles). Lin *et al.* also observed that dry particle morphology obtained from drying droplets can be dependent on many factors, including operating conditions and material properties. In the current studies, operating parameters were kept constant during spray drying. Therefore, it is predicted that solute material properties, including CS and leucine solubilities and latent heats of crystallization, played important roles in the final particle structure.

Theoretical estimates of d_{aero} ranged between 1.9 and 3.5 μm, indicating that the powders were of a suitable aerodynamic size for deposition in the alveolar region of the lungs. Comparison of MMAD with the theoretical aerodynamic diameter (d_{aero}), however, indicates that powders did not behave as individual particles, but rather as particle aggregates since the MMAD was significantly larger than theoretical d_{aero}. This aggregation phenomenon is commonly seen with dry powder aerosols (21,22). Despite this aggregation, in vitro simulation of total lung deposition ($FPF_{TD} < 5.8$ μm) was about 50% for the 50–80% CS powders, with no statistically significant differences between the powders (Table III). Estimated deep lung deposition ($FPF_{TD} < 3.3$ μm) for these powders was about 10%, with the 70:30//CS:leucine powder achieving the highest value of 11.5 ± 0.2%. Therefore, based on MMAD, these powders are expected to deposit in both the bronchial and alveolar regions of the lungs. Similar results have been obtained in other recent studies where spray-drying the antibiotic, tobramycin, with excipients led to the formation of highly respirable particles containing large drug concentrations (23,24). Interestingly, although the 90% CS powder had the lowest theoretical d_{aero}, it also had the highest MMAD, indicating significant aggregation. This high MMAD led to a significant drop in total lung deposition (from 50 to 40%) and deep lung deposition (from 10 to 8%). Increased cohesiveness of this powder compared to those powders with higher leucine concentrations on the surface would be expected, since leucine has been shown to reduce surface energy and adhesion of dry powders (15,17). Although these factors were not investigated in these studies, they present opportunities for further research in this area.

Given our intent to deliver a maximum dose of CS to the lungs, and the relatively poor aerosol properties of the 90:10// CS:leucine powder, we chose to optimize the 80:20//CS:leucine powder physical properties as described below.

Optimization of Aerosol Aerodynamic Diameter

To achieve efficient delivery of aerosol to a guinea pig model via passive inhalation (for future pharmacodynamic experiments), an aerodynamic diameter of about 4 μm would be required to allow the powder to bypass the guinea pig nose and enter into the lung spaces. Therefore, optimization of particle aerodynamic diameter was achieved by diminishing the particle mean geometric size from 3.7 to 2.6 μm—maintaining a tapped density of approximately 0.6 g/cc—by adjusting atomizer flow rate from 1.8 to 10.8 kg/h. This optimized CS powder exhibited an estimated total lung deposition (i.e. $FPF_{TD} < 5.8$ μm) of 58.2%, similar to that found for the un-optimized powders. However, deep lung deposition (i.e. $FPF_{TD} < 3.3$ μm) increased from 10.0 to

Table III. Aerodynamic Characterization of Capreomycin-Containing Dry Powder Aerosols

CS:Leucine in the Feed (wt %)	Theoretical d_{aero} (μm)	MMAD (μm)	$FPF_{TD} < 5.8$ μm (%)	$FPF_{TD} < 3.3$ μm (%)
50:50	3.27	4.86 ± 0.06	49.8 ± 1.0	10.7 ± 0.8
60:40	3.38	4.82 ± 0.05	48.7 ± 2.1	10.1 ± 0.3
70:30	3.47	4.70 ± 0.02	52.6 ± 0.8	11.5 ± 0.2
80:20	2.95	4.74 ± 0.07	50.0 ± 2.5	10.0 ± 0.9
90:10	1.87	4.99 ± 0.10	40.9 ± 4.0	7.8 ± 0.2

Values are reported as the mean ± SD.

Table IV. Pharmacokinetic Parameters Obtained After Administration of the Different Capreomycin Formulations by the Different Routes (Average ± SD, $n = 6–8$)

Parameter	IV (20 mg/kg, solution)	IM (20 mg/kg, solution)	Insufflation (14.5 mg/kg powder)	Insufflation (7.2 mg/kg powder)	Insufflation (1.4 mg/kg powder)
AUC (μg h/ml)	49.3 ± 10.7[a]	58.2 ± 11.0	17.0 ± 3.9[b]	8.2 ± 1.5[c]	1.2 ± 0.4[c]
AUC/Dose (μg h/ml)	2.4 ± 0.5	2.8 ± 0.6	1.7 ± 0.4[b]	1.5 ± 0.3[c]	1.2 ± 0.4[c]
C_{max} (μg/ml)	53.6 ± 3.9[a]	32.3 ± 3.8	6.7 ± 1.3[b]	3.3 ± 0.5[c]	0.9 ± 0.4[c]
C_{max}/Dose (μg/ml)	2.7 ± 0.2[a]	1.6 ± 0.2	0.7 ± 0.1[b]	0.6 ± 0.1	0.9 ± 0.4[c]
$t_{1/2}$ (h)	0.8 ± 0.1	1.1 ± 0.3	1.5 ± 0.4[b]	1.7 ± 0.4[b]	1.2 ± 0.6
MRT (h)	0.88 ± 0.09*	1.44 ± 0.30	1.80 ± 0.12[b]	1.72 ± 0.18[b]	1.01 ± 0.31

[a] Significantly different than IM
[b] Significantly different than IV and IM
[c] Significantly different than IV and IM and 14.5 mg/kg powder

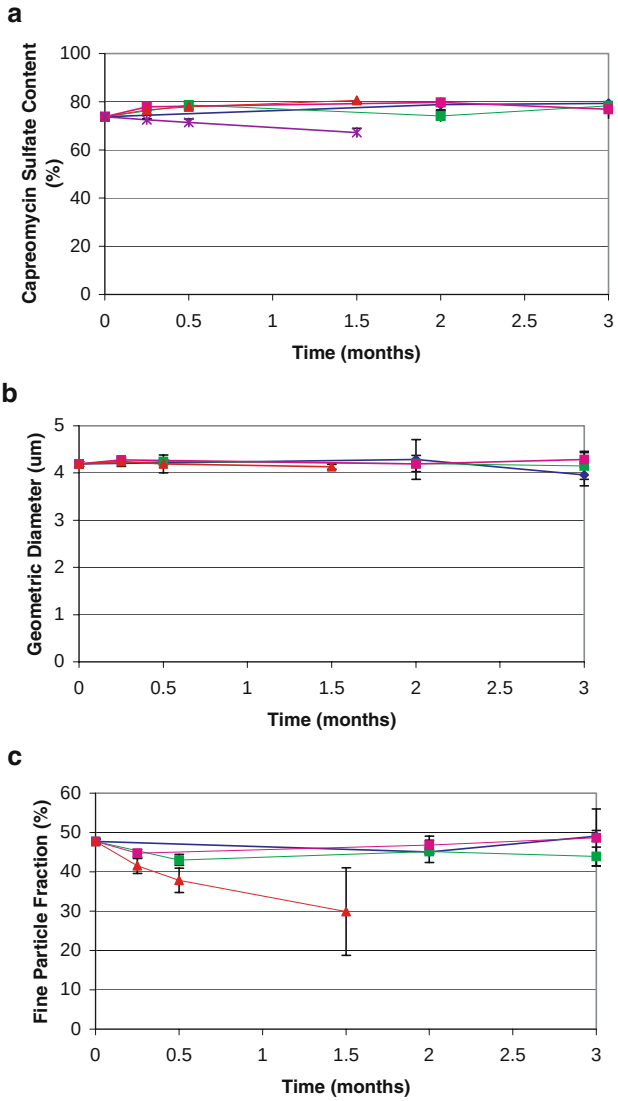

Fig. 2. Time-dependent stability of **a** capreomycin sulfate content, **b** geometric diameter, and **c** fine particle fraction ($FPF_{TD} < 5.8$ μm) of 80:20//CS:leucine dry powder aerosol, stored under various stress conditions. Legend key: *filled diamonds*, 4°C; *light-shaded filled squares*, RT dark; *dark-shaded filled squares*, RT light; *filled triangles*, 40°C; *asterisks*, 40°C/75%RH.

17.0%, representing a 40% improvement. This shift in the deposition pattern within the cascade impactor to lower stages resulted in a decrease in the MMAD to 4.14 μm (compared to 4.74 μm for the un-optimized powder). Decreasing particle size has also been shown by other researchers to improve the aerodynamic properties of a number of different aerosol particles (25,26). To produce gram quantities of powder for animal studies, scale-up on the NIRO spray dryer was performed and produced a powder with similar aerodynamic properties (MMAD = 4.19 μm, GSD = 1.46 μm, $FPF_{TD} < 5.8$ μm = 60.2 ± 0.6% and $FPF_{TD} < 3.3$ μm = 16.2 ± 1.5%) at a collection efficiency of 35%. Higher collection efficiencies could be obtained by either spray drying for longer periods of time between cleanings or by adding an outlet filter to aid in powder collection.

Disposition of Capreomycin from LPPs in Guinea Pigs

No capreomycin was detected in the bronchoalveolar lavage of animals of any of the treatment groups after the last blood sample was taken (at 8 h) indicating that all capreomycin was absorbed or eliminated from the lungs in the case of animals receiving particles by the pulmonary route.

Key pharmacokinetic parameters obtained from the analysis of individual plasma concentrations versus time curves after dosing of capreomycin via different formulations are shown in Table IV. Powders that were insufflated into the lungs were assumed, within the limits of the experimental error, to completely deposit in the lungs. Area under the curve and C_{max} correlated well with the capreomycin dose administered, with IV and IM groups having the largest AUC (49.3 and 58.2 μg h/ml, respectively) and C_{max} (53.6 and 32.8 μg/ml,

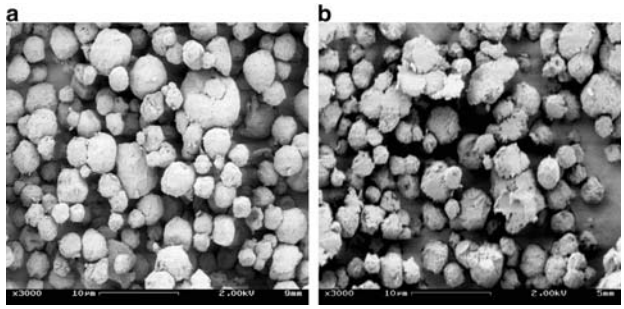

Fig. 3. Scanning electron microscopy image of 80:20//CS:leucine dry powder aerosol after storage under 40°C/75% RH conditions for **a** 1 week and **b** 6 weeks. Bar = 10 μm.

respectively) and animals receiving 1.4 mg/kg capreomycin by insufflation, the smallest ones (AUC = 1.2 μg h/ml; C_{max} = 0.9 μg/ml). For further comparison between these treatments, AUC and C_{max} were corrected by dose in the respective treatment. AUC and C_{max} after IV or IM administration of capreomycin remained statistically different from those after pulmonary administration (insufflation) even after adjusting AUC and C_{max} by dose. Notably, half-life was significantly longer after pulmonary administration of 7.2 or 14.5 mg capreomycin/kg (1.7 and 1.5 h, respectively) than that after any other treatment (IV $t_{1/2}$ = 0.8 h; IM $t_{1/2}$ = 1.1 h), showing the feasibility of these particles, when delivered by the pulmonary route, for the treatment of pulmonary tuberculosis. Thus, pulmonary administration of these particles may have advantages over the conventional IM route for the treatment of pulmonary tuberculosis, because significantly smaller doses of capreomycin will be required which will avoid systemic toxicity.

Storage Stability of 80:20//Capreomycin sulfate:Leucine Aerosols

A three-month physical and chemical stability analysis of the 80:20//CS:leucine aerosols was conducted under refrigerated (4°C), room temperature (RT), and accelerated (40°C) conditions. Figure 2 shows the stability of the CS content, geometric diameter, and fine particle fraction (FPF_{TD} < 5.8 μm) of the aerosol over time. When stored at 4°C or RT, the powder exhibited no significant changes in CS content, geometric diameter, or aerosolization properties ($P > 0.05$ in all cases). Under 40°C conditions, CS content and geometric diameter remained stable for up to 3 months. However, the FPF_{TD} of aerosols stored at 40°C for 6 weeks decreased by 40% ($P = 0.003$; Fig. 2 C), indicating the heat/moisture sensitivity of the powder. When placed in direct contact with a 40°C and 75% RH atmosphere, the aerosols exhibited significant changes in powder morphology (Fig. 3). An apparent reduction in CS content per mass of aerosol ($P = 0.000$) was also observed due to water uptake (CS content data was not corrected for water uptake). No increase in contamination peaks or the appearance of degradation peaks was observed by HPLC. Further experiments have shown that a thin layer of powder placed in a 40°C and 75% RH atmosphere completely liquefies (dissolves in the adsorbed water) within 1 month. These results indicate that protecting the powder from humidity is critical for powder stability.

CONCLUSIONS

Our results indicate that dry powder capreomycin formulations can be efficiently delivered from a breath-activated inhaler (>50% of packaged drug is delivered to an *in vitro* lung model) to achieve high drug concentrations in the lungs. The significant decrease in the systemic exposure to capreomycin, due to a smaller dose delivered (14.5 mg/kg insufflated versus 20 mg/kg IM), indicates a significant advantage of pulmonary delivery over intravenous or intramuscular, where serious side effects can be diminished. These systems remain stable at room temperature conditions, potentially reducing the need for cold chain storage of the dry powders.

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