Colistin Powders with High Aerosolisation Efficiency for Respiratory Infection: Preparation and *In Vitro* Evaluation

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ABSTRACT: In many respiratory infections caused by multi-drug-resistant Gram-negative bacteria, colistin is often the last-line drug for treatment despite its nephrotoxicity when administered parenterally. Inhalation therapy of colistin has great potential to improve the efficacy while reducing adverse effects. In this study, inhalable powder formulations of colistin (sulphate) were produced via spray drying. The colistin powders were found to have intact antimicrobial activity against A*cinetobacter baumannii* measured by broth micro-dilution. Both the raw material and spray-dried formulations were amorphous and absorbed significant amount of water up to 30% (w/w) at relative humidity (RH) of at least 70%. The spray-dried formulations were physically stable in the amorphous form at 60% RH and 25°C, having a high aerosol efficiency (emitted dose >86% and fine particle fraction total >83%) which remained unchanged after a 3-month storage. Storage at an elevated RH of 75% resulted in the aerosolisation performance significantly decreased, and at RH 90%, the formulation particles fused together (but without re-crystallisation). Although spray drying has been extensively used for generating inhalable drug particles, this is the first report that colistin powder can be physically stable in the amorphous form at ambient conditions, indicating that spray-drying approach is suitable for producing inhalable colistin powder formulation. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3736–3747, 2013

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INTRODUCTION

Infections caused by multi-drug-resistant Gram-negative bacteria are becoming critical challenges worldwide because they are often associated with a high mortality rate, and no effective new antibiotics against these very problematic pathogens will be available for many years to come.[1] In many cases, colistin (also known as polymyxin E) is used as the last-line therapy.[2] Two forms of colistin are clinically available: colistin sulphate (refers to colistin below) and colistin methanesulphonate sodium (CMS), an inactive prodrug of colistin.[3] Only a very small proportion of CMS converts to colistin *in vivo* after intravenous administration.[3]

For treatment of respiratory infections caused by multi-drug-resistant Gram-negative bacteria, parenteral administration of colistin is commonly used[2]; however, the currently recommended dosage regimens for parenteral use may not produce sufficient colistin concentration in the respiratory tract because of its pharmacokinetic characteristics.[2,4] Consequently, higher parenteral doses are required to generate drug concentrations well above its minimum inhibitory concentrations (MICs) in the respiratory tract. Unfortunately, high doses of CMS via parenteral administration can lead to nephrotoxicity in 45%–55% patients.[5,6] An alternative approach is to deliver active colistin directly to the respiratory tract, thereby minimising the systemic exposure, nephrotoxicity and potential development of bacterial resistance while maximising its anti-bacterial killing.[4] Pharmacokinetic study in cystic fibrosis patients demonstrated that, when compared with intravenous administration (serum C_{max} 2.93 mg/L, $T_{1/2}$ 7.4 h),[7] the inhaled CMS solution via nebulisation produced much higher concentrations of the formed colistin in the sputum (C_{max} 40 mg/L) for a longer period (i.e., drug concentrations maintained well above MICs for 12 h), whereas negligible systemic drug exposure (C_{max} 0.17 mg/L) was observed.[8]

Nebulisation of CMS solutions into the respiratory tract is a complementary hospital practice to parenteral administration for the treatment of respiratory infections.[9] However, nebulisation requires hospital or home setting device, well-trained professional oversight, long administration time and, more importantly, low drug delivery efficiency.[10] For example, only less than 15% of total drug deposited in lungs after the nebulisation treatment in an *in vivo* study with intrapulmonary percussive ventilation or jet nebulisation.[11] In contrast, dry powder inhalers (DPIs), which consist of drug (alone or with excipients) powder and inhaler device, are easier to carry and operate, which would lead to better patient compliance. DPIs also provide more rapid drug action and higher drug delivery efficiency.[12] Very few studies to date have reported the development of colistin DPI formulations even after a commercial DPI product of CMS (Colobreathe®) was approved to market in Europe.[13,14] In these limited number of studies, the traditional DPI formulations consisting of jet-milled colistin and lactose carrier generally have a moderate aerosolisation efficiency (i.e., fine particle fraction [FPF] ~40%).[13] This issue is common for jet-milled powders, caused by the strong attractive forces between jet-milled drug particles which have high surface energy introduced by milling process.[15] A recent preliminary study suggested particle engineering via spray drying may produce the colistin formulations which are more suitable for inhalation therapy.[16]

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Although spray drying has been extensively employed to produce inhalable powders, there is a concern that the spray-dried drug particles may exist as amorphous form and be physically unstable.[17] However, there has been no report of the physico-chemical properties of colistin powder in the previous literature, leaving this issue unaddressed. Furthermore, physico-chemical properties, including particle size, morphology, crystallinity and water absorption, play a critical role in the aerosol performance of powder formulation.[18] The lack of such information causes serious uncertainty over stability issues that may arise during manufacturing, transportation and storage, which becomes particularly important after a commercial DPI product of CMS (Colobreathe®) was approved to market by European Medicines Agency for treatment of cystic fibrosis. Hence, the aim of this study was to develop and characterise dry powder formulations of colistin with high aerosolisation efficiency, engineered via spray drying without addition of any external excipient. Although colistin exists in two different forms of colistin and CMS, the former was chosen in this study because CMS is an inactive prodrug of colistin.[3]

EXPERIMENTAL

Chemicals

Colistin sulphate was purchased from Sigma–Aldrich (for high-performance liquid chromatography [HPLC] standard curve construction; Castle Hill, New South Wales, Australia) and Zhejiang Shenghua Biology Company, Ltd. (as the supplied formulation; Hangzhou, Zhejiang, China). Methanol (HPLC grade) was purchased from Fisher Scientific (Fair Lawn, New Jersey) and trifluoroacetic acid from Sigma–Aldrich.

Bacterial Strain

Acinetobacter baumannii (ATCC 19606) was purchased from the American Type Culture Collection (Manassas, Virginia), and maintained in tryptone soya broth (Oxoid Australia, Adelaide, South Australia, Australia) with 20% glycerol at −80°C.

Spray Drying

Colistin was dissolved in either water or co-solvent of ethanol–water (1:1) to prepare the feed solutions (6 mg/mL) for spray drying. Spray drying was performed using a B-290 mini spray dryer (Büchi Labortechnik AG, Falwil, Switzerland). The following operating conditions were selected and modified based on previous preliminary study[16]: inlet temperature, 80°C; atomiser setting, 700 L/h; aspirator, 40 m^3/h and feed rate, 2 mL/min. Two powder formulations spray dried from either water or co-solvent solutions were kept in a glass desiccator containing silica gel at 20°C until used.

MICs Against A. *Baumannii*

Acinetobacter baumannii was chosen to test susceptibility as this Gram-negative bacterium can cause nosocomially acquired respiratory infections,[19] and for many multi-drug-resistant strains, colistin is the only effective antibiotic.[3] MICs of the colistin powder formulations against *A. baumannii* were measured by broth micro-dilution.[3] Briefly, an aliquot of an overnight culture was diluted in cation-adjusted Mueller-Hinton broth (Oxoid, Hampshire, England) giving approximately 10^6 cfu/mL as the inocula. Sterile drug solutions were prepared in Milli-Q water to achieve a concentration of 5.12 mg/mL. An aliquot of the drug solution was added into broth and diluted (1:2) to achieve the drug concentrations of 0, 0.125, 0.25, 0.5, 1, 2 and 4 mg/L. Micro-plates were incubated at 35°C in a humidified incubator for approximately 20h. MICs were determined as the lowest concentration without visible bacterial growth.

Particle Sizing

Particle size distribution of the powder formulations were measured by laser diffraction with a Scirocco dry powder module (Mastersizer 2000; Malvern Instruments, Worcestershire, UK). The powder formulations were dispersed through a measurement window by compressed air with air pressure of 4 bar.[20] The X_{10} (diameter at 10% undersize), X_{50} (diameter at 50% undersize) and X_{90} (diameter at 90% undersize) were calculated from size distribution data. All measurements were conducted with three replicates.

Particle Morphology

Scanning electron microscopy (SEM) was employed to investigate the morphology of the supplied and two spray-dried formulation powders. Each formulation powder was sprinkled on a carbon sticky tape and mounted on a SEM stub, followed by sputter coating with gold (15 nm thick) using a K550X sputter coater (Quorum Emitech, Kent, UK). The images were captured with a field emission Zeiss Ultra Plus SEM (Carl Zeiss SMT AG, Oberkochen, Germany) at 5 kV.

Dynamic Water Vapour Sorption

The moisture sorption behaviour of the formulation powders was investigated using dynamic vapour sorption (DVS) (DVS-1; Surface Measurement Systems Ltd., London, UK). Each formulation sample was subject to drying at 0% relative humidity (RH) at the start of the measurement and then exposed to the RH ranging from 0%–90% at 10% RH increments. The environmental RH was increased from 0% to 90% for the sorption cycle and decreased from 90% to 0% for the desorption cycle. Equilibrium moisture content at each testing RH was determined by a dm/dt of 0.002% per minute.

Crystallinity

Powder X-ray diffraction (PXRD) (Shimadzu XRD-6000; Shimadzu Corporation, Kyoto, Japan) was employed to evaluate the crystallinity of the formulations. Cu-Kα radiation at a generator voltage of 40 kV and a current of 30mA was employed. The data collection was performed by the 2θ scan method with 1° as incident beam angle and a scan speed of 2° per minute at 0.02° step angle in the range of 5°–80°.

In Vitro Aerosolisation Performance

A multi-stage liquid impinger (Apparatus C; British Pharmacopeia 2012; Copley Scientific Limited, Nottingham, UK) with a USP induction port (USP throat) was used to determine the *in vitro* aerosolisation performance. Dispersion was performed in a controlled environment cabinet: temperature, 20 ± 3°C; and RH, 50 ± 3%. The cut-off diameters for stages