Simultaneous particle size reduction and homogeneous mixing to produce combinational powder formulations for inhalation by the single-step co-jet-milling

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Abstract

Homogeneous mixing of two cohesive jet-milled drug powders is a challenge for pharmaceutical manufacturing because of their cohesive nature resulting in the formation of strong and random agglomerates. In this study, colistin and ciprofloxacin were co-jet-milled to develop combinational antibiotic dry powder formulations for inhalation. The properties of particle size, morphology, content uniformity and in-vitro aerosolization were evaluated. The distribution of two drugs in the co-jet-milled powders was assessed using Time-of-flight – secondary ion mass spectrometry (ToF-SIMS). The co-jet milled powders demonstrated an acceptable content uniformity indicating homogeneity. In general, ToF-SIMS images showed relatively homogeneous distributions of ciprofloxacin and colistin in the co-milled formulations. Importantly, the two drugs generally had the similar FPF and deposition behavior in each combinational formulation supporting that the particle mixtures were relatively homogenous and could maximize the antimicrobial synergy. In conclusion, co-jet-milling could be a viable technique to produce the combination powders for inhalation.

Keywords

Dry powder inhaler; aerosol performance; jet milling; uniformity; mixing

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Introduction

Delivering antibiotics to the lungs i.e., the site of infection at an optimal drug concentration is central to effective treatment of respiratory infections. However, for many antibiotics only a small fraction of oral and parenterally administered antibiotics reaches the infection sites in the lungs. In addition, there is a considerable risk of emergence of resistance and severe side effects of orally/systemically administered antibiotics which are grand challenges to the clinical management of chronic lung infections. Inhalation is a promising route for administering appropriate dosages of drugs directly to the lungs. Compared with systemic/oral therapy, inhalation therapy ensures rapid clinical responses with relatively lower doses while reducing the risk of systemic side effects and the risk of emergence of resistance. Spray drying and jet milling are two commonly employed techniques to develop dry powder inhaler formulations. Spray drying has been employed to produce combination formulations, however some spray-dried drugs may generate relatively less thermodynamically stable amorphous forms. The unstable amorphous particles may convert into more thermodynamically stable crystalline form, which could alter the aerosolization behavior.

Although the jet-milled particles are in general very cohesive with poor flowability and unsatisfactory aerosolization behavior, jet milling is still the mainstay approach to manufacture inhalable drug particles in the industrial practice. Studies have also shown that co-jet milling with lubricants may potentially facilitate simultaneous particle size reduction and particle surface coating, which resulted in improved aerosolization performance. However, homogeneous mixing of two cohesive jet-milled drug particles is a grand challenge for pharmaceutical manufacturing because of their cohesive nature that forms strong and random agglomerates. Addition of coarse lactose carriers may be an option to improve homogeneity of two drugs for low-dose inhalation medications; but for high-dose inhalation medicines such as antibiotics, large amounts of lactose carriers should be avoid to reduce the total powder load. Here our hypothesis is that co-jet milling two drugs can generate a homogeneous dry powder inhaler formulation. Colistin and ciprofloxacin are selected as two model drugs here as these two antibiotics have shown synergistic antimicrobial activities against resistant Pseudomonas aeruginosa. Colistin DPI (Colobreathe, in the form of colistimethate sodium) has been approved in the Europe and ciprofloxacin DPI is under development. Combining colistin and ciprofloxacin into a single DPI product may improve patients’ compliance and maximize the antimicrobial activities.

Materials and Methods

Materials

Ciprofloxacin hydrochloride monohydrate and colistin sulphate were supplied by Beta Pharma (Wujiang City, China). Acetonitrile (HPLC grade) was supplied by Merck (Fair Lawn, New Jersey).
Jet milling

An initial blend of ciprofloxacin and colistin was prepared manually by stirring two powders for 5 min in a mortar and pestle. The resultant blend (500 mg) was co-milled using a jet-mill (Piconizer spiral jet mill, Hosokawa Alpine AG, Germany), at 1 g/min feed rate, grinding pressure 5 bar and feeding pressure of 6 bar. Each pure drug was jet-milled at the same processing parameters.

Scanning electron microscopy (SEM)

A scanning electron microscope (NOVA nanoSEM, FEI Company, Hillsboro, Oregon, USA) was applied to assess particle morphology. Briefly, the powder sample was coated with a platinum film at 40 mA for 1 min using a sputter coater (208 HR, Cressington Sputter Coater, England, UK) and images were taken by the built-in software.

Particle size

The particle size analysis was conducted with a Mastersizer 3000 equipped with Aero-S for dry powder dispersion (Malvern Instruments, Worcestershire, UK), using the laser diffraction method. The compressed air of 4 bar was used to produce powder dispersion for each sample (5 - 10 mg), and it was measured for 5 s. The feed rate of 50–60% was used for keeping the suitable laser obscuration level at 2–6%.

Powder x-ray diffraction (P-XRD)

A Rigaku Smartlab™ diffractometer (Rigaku Americas, Texas, USA) with a Cu-Kα radiation source was used to evaluate powder crystallinity. The diffraction patterns were determined from 5 to 40° 2θ at a scan speed of 5°/min at 40 kV.

Dynamic vapor sorption

A dynamic vapor sorption equipment (DVS, Surface Measurement Systems Ltd., London, UK) was applied to evaluate moisture sorption property of the powder samples. Each measurement has a sorption cycle and a desorption cycle between 0 and 90% RH at the 10% RH increment. Powders were considered to reach equilibrium, when the change in mass with respect to time i.e., dm/dt was less than 0.002% per minute.

Time-of-flight – secondary ion mass spectrometry (ToF-SIMS)

Distribution of two drugs in each combinational formulation was determined using a ToF-SIMS (nanoToF instrument, Physical Electronics Inc., Chanhassen, MN, USA) at 30 kV. ToF-SIMS data were collected from 4 areas (50 × 50 μm each) per sample. The fragments of m/z ~332 atomic mass unit (amu) were selected as the exclusive element for ciprofloxacin, which is corresponding to the \([C_{17}H_{19}FN_3O_3]^+\). The fragments of m/z ~30 atomic mass unit (amu) and ~86 amu were chosen for colistin, which is corresponding to \([CH_4N^+]\) and \([C_5H_12N^+]\)

Drug quantification

Contents of ciprofloxacin and colistin were measured by an established HPLC method. Briefly, an HPLC system and an Eclipse Plus column (5 μm C18 150 × 4.60 mm; Agilent,
Waldbronn, Germany) were used to detect ciprofloxacin and colistin both at 215 nm. The mobile phase consisted of 76% w/w 30 mM sodium sulfate solution (adjusted to pH 2.5 with H₃PO₄), and 24% v/v acetonitrile was running at 1.0 mL/min. The standard curves were linear (r² > 0.999) in the concentration ranges of 0.01–0.5 mg/mL for colistin and 0.004–0.125 mg/mL for ciprofloxacin.

In-vitro aerosol efficiency

A next-generation impactor was used to determine in-vitro aerosolization performance (NGI, Copley, Nottingham, UK) ²⁸. Each powder sample (10 ± 2 mg) was filled into the Size 3 HPMC capsules (QualiCaps, Whitsett, NC, USA). The capsules were loaded in a RS01 DPI device (Plastiape S.p.A., Osnago, Italy), which has a similar design to the Osmohaler. Briefly, 4 L of air was drawn through the inhaler by a vacuum pump to generate two airflow rates of 60 L/min for 4 s and 100 L/min for 2.4 s, which are corresponding to pressure drop values of ~1.6 kPa and ~4 kPa across the RS01 DPI device, respectively. Fine particle fraction (FPF) was calculated as the fraction of drug with an aerodynamic diameter < 5 μm over the total recovered drug. Triplicates were conducted for each formulation.

Content Homogeneity

The content uniformity of ciprofloxacin and colistin in the resultant combination formulations were quantified. Briefly, ten samples of 10 ± 0.5 mg for each formulation were weighed and dissolved in mobile phase, which was then diluted to an appropriate concentration for quantification of ciprofloxacin and colistin. The drug quantification methods for content homogeneity and dispersion tests are provided above.

Acceptance value (AV) was calculated by the following equation (1):

\[ | M - \bar{X} | + ks \] (1)

where M is the reference value, \( \bar{X} \) is the mean of individual contents%, k is the acceptability (if n=10, then k=2.4), and s is the standard deviation (SD). The target content is 100.0% in this study, and the values of M are like follows: If 98.5 % \( \leq \bar{X} \leq 101.5 \% \), then \( M = \bar{X} \), if \( \bar{X} \leq 98.5 \% \), then \( M=98.5\% \), and if \( \bar{X} \geq 101.5 \% \), then \( M=101.5\% \).

Statistical analysis

One-way analysis of variance (ANOVA) with post hoc Tukey test was employed to determine the statistical difference for three groups and more using a Prism software (GraphPad Software, Inc., La Jolla, CA, USA). It was deemed as significantly different if \( p < 0.05 \).
Results

Scanning electron microscopy (SEM)

The jet milled ciprofloxacin and colistin particles have irregular shapes (Figure 1). Some small particles were sticking on the relatively large ones. There was no apparent difference between different jet-milled particles.

Particle sizing

All jet-milled formulations had similar size distributions with $D_{50} < 2.1 \mu m$ and $D_{90}$ was $< 5.4 \mu m$ indicating that most particles were very small (Table 1).

Content Homogeneity

In general, it is challenging to obtain homogeneous mixtures of two very cohesive particles because the cohesive particles stick to each other strongly $^{29,30}$. The formulations produced via co-jet milling demonstrated an acceptable content homogeneity i.e., contents within 90-110% and $AV \leqslant 15\%$ (Table 1).

Powder x-ray diffraction (P-XRD)

P-XRD patterns of the jet-milled ciprofloxacin showed some crystalline peaks (Figure S1). The P-XRD patterns of colistin had no peaks indicating the amorphous nature (Figure S1). The co-jet-milled samples consisting of colistin and ciprofloxacin also showed peaks corresponding to ciprofloxacin. Previous studies have shown that the spray-dried ciprofloxacin is amorphous, which tends to crystallize upon storage and affects the aerosolization $^{12}$. Since the jet-milled ciprofloxacin was crystalline, it is likely to have better physical stability than the spray-dried amorphous ciprofloxacin particles.

Dynamic water vapor sorption

The jet-milled colistin absorbed substantial amounts of water (up 30% w/w) at the elevated RH due to its amorphous nature $^{31}$. In contrast, the jet-milled ciprofloxacin absorbed substantially lower amounts of water ($< 2%$ w/w) at all humidity levels compared with the jet milled colistin (Figure S2). The moisture absorption levels for the co-jet-milled formulations are between jet-milled ciprofloxacin and jet-milled colistin. In addition, the water uptake was completely reversible indicating no apparent crystallization events. There was hysteresis between sorption and desorption profiles for the colistin-containing formulations. This is due to the hygroscopic nature of amorphous colistin with moisture trapped into the invaginations or cores of particles during sorption, while a slower removal of water during the desorption process. $^{32,33}$

Distribution of two drugs by ToF-SIMS

We expect no coating shall occur during the co-jet-milling process; surface characterization technique of ToF-SIMS was used to qualitatively evaluate distributions of two drugs in the co-jet-milled powder formulations. Our ToF-SIMS measurement had a relatively high spatial resolution of $\approx 250 \text{ nm}$. Figure 3 showed that in general ciprofloxacin and colistin particles are relatively homogeneous in the co-jet-milled powders.

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**In-vitro aerosolization**

In Figure 2A, the data at 0% colistin concentration are for ciprofloxacin jet-milled alone formulation, 25% colistin concentration are for Colistin-Ciprofloxacin 1:3 formulation, 50% colistin concentration are for Colistin-Ciprofloxacin 1:1 formulation, 75% for Colistin-Ciprofloxacin 3:1 formulation and 100% for colistin jet-milled alone formulation. The results showed that the jet-milled ciprofloxacin alone powder showed a relatively lower FPF of 57.5 ±1.9, while the colistin alone powder had a relatively higher FPF of 80.2 ±1.7 at the flow rate of 100 L/min (Figure 2A). FPF values of the co-jet milled powders was significantly higher than the jet-milled ciprofloxacin alone powder but significantly lower than that of jet-milled colistin alone formulation at both 60 L/min and 100 L/min (p < 0.05). It is noteworthy that for each co-jet-milled formulation, no significant difference was measured in FPF between colistin and ciprofloxacin (p > 0.05).

Deposition data also showed that in general two drugs have similar deposition patterns for each formulation except for those in the throat, S2, S4 and S5 (Figure 2B). Such similar FPF and deposition patterns in each co-jet-milled formulation confirmed the formation of relatively homogeneous mixtures, despite very different aerosolization behavior of two jet-milled pure drugs; though such similarity in deposition patterns for two drugs does not reach the same level to the co-spray-dried particles, which incorporate two drugs in a single particle. Another possibility is that two jet-milled drugs form preferential agglomerates (such as colistin-ciprofloxacin agglomerates over colistin-colistin and ciprofloxacin-ciprofloxacin agglomerates) according to the loaded drug ratios.

**Conclusion**

Mixing two cohesive powders into a uniform blend is extremely challenging due to formation of strong and random agglomerates. Majority of current combinational low-dose DPI products are blended mixtures of coarse carriers and separately jet-milled drug particles, or made of packing two drug formulations in two separate blisters. However, for high-dose medications such as antibiotics, large amount of coarse carriers should be avoided. Our study has shown that co-jet-milling of colistin and ciprofloxacin achieved simultaneous size reduction and homogeneous mixing of two drugs, as supported by content uniformity, ToF-SIMS and aerosol deposition data. The potential limitations of co-jet-milling two drugs could be difficult in controlling the particle size of each drug, specifically when the mechanical properties of two drugs are different. Understanding such relatively homogeneous mixtures are critical to produce inhalation products with superior quality.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:
Representative SEM images of (A) jet-milled ciprofloxacin, (B) jet-milled Colistin, (C) Colistin-Ciprofloxacin_1-3 (D) Colistin-Ciprofloxacin_1-1, and (E) Colistin-Ciprofloxacin_3-1. Scale bars represent 1 μm.
Figure 2:
Dispersion data of the jet-milled formulations: (A) FPF (%) and (B) deposition.
Figure 3:
Distributions of ciprofloxacin (green) and colistin (red) on the surfaces of Colistin-Ciprofloxacin_1-3 (A), Colistin-Ciprofloxacin_1-1 (B) and Colistin-Ciprofloxacin_3-1 (C). The scale bar represents 10 μm.
Table 1:
Particle sizes of the co-jet milled formulations (mean ± SD, n=3) and content of ciprofloxacin and colistin in the formulations (mean ± SD, n=10) and their acceptance values (AV).

| Formulations       | Particle Size (μm) | Content Uniformity (%) | 
|--------------------|--------------------|------------------------|
|                    | D_{10}             | D_{50}                 | D_{90}                 | 
|                    | Content            | AV                     | Content               | AV               |
| Ciprofloxacin      | 0.91±0.02          | 1.97±0.03              | 5.38±0.22              | -                  |
| Colistin-Ciprofloxacin_1-3 | 0.89±0.04          | 2.02±0.03              | 4.95±0.17              | 101.7±1.7          | 4.2               | 102.5±1.4          | 4.4               |
| Colistin-Ciprofloxacin_1-1 | 0.95±0.02          | 1.94±0.02              | 4.75±0.42              | 98.3±2.4           | 5.9               | 101.9±1.8          | 4.7               |
| Colistin-Ciprofloxacin_3-1 | 0.96±0.03          | 1.98±0.02              | 4.31±0.16              | 98.1±1.3           | 3.6               | 104.5±1.5          | 6.5               |
| Colistin           | 0.92±0.01          | 1.86±0.01              | 3.75±0.03              | -                  | -                 | -                 | -                 |

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