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Tailored Antibiotic Combination Powders for Inhaled Rotational Antibiotic Therapy

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ABSTRACT

Respiratory lung infections due to multidrug-resistant (MDR) superbugs are on a global upsurge and have very grim clinical outcomes. Their MDR profile makes therapeutic options extremely limited. Although a highly toxic antibiotic, colistin, is favored today as a "last-line" therapeutic against these hard-to-treat MDR pathogens, it is fast losing its effectiveness. This work therefore seeks to identify and tailor-make useful combination regimens (that are potentially rotatable and synergistic) as attractive alternative strategies to address the rising rates of drug resistance. Three potentially rotatable ternary dry powder inhaler constructs (each involving colistin and 2 other different-classed antibiotics chosen from rifampicin, meropenem, and tigecycline) were identified (with distinct complementary killing mechanisms), coformulated via spray drying, evaluated on their aerosol performance using a Next-Generation Impactor and tested for their efficacies against a number of MDR pathogens. The powder particles were of respirable size (d_{50}, 3.1 ± 0.3 μm-3.4 ± 0.1 μm) and predominantly crumpled in morphology. When dispersed via a model dry powder inhaler (Aerolizer®) at 60 L/min, the powders showed concomitant *in vitro* deposition with fine particle fractions of ~53%-70%. All formulations were successfully tested in the laboratory to be highly effective against the MDR pathogens. In addition, a favorable synergistic interaction was detected across all 3 formulations when tested against MDR *Pseudomonas aeruginosa*.

Introduction

The increasing occurrence of multidrug-resistant (MDR) bacteria, otherwise known as the "superbugs," is an alarming public health care threat. Of particular concern are the carbapenem (β-lactam class of antibiotics)-resistant Gram-negative bacteria, which acquire resistance through the enzymatic hydrolysis of carbapenems via carbapenemases.[1-3] Infections involving these MDR bacteria are often dire in terms of therapeutic options due to the lack of antibiotics that are active against these pathogens. At present, respiratory lung infections due to MDR bacterial pathogens or "superbugs" have very grim clinical outcomes [4] with mortality rates as high as 80% for nosocomial and community-acquired pneumonia.[5,6] Hence, the development of alternative treatment regimens is a pressing need.

Colistin (also known as polymyxin E) is a 50-year-old cationic polypeptide antibiotic that is experiencing clinical revival. During the early years of its clinical use, nephrotoxicity and neurotoxicity [7] issues diminished its popularity. Its current resurgence as a "last-line" therapeutic against hard-to-treat MDR respiratory pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae*[8] arises as many of these organisms still retain susceptibility toward it.

However, in recent years, sporadic worldwide reports of colistin resistance have emerged (e.g., the United States,[3] Singapore,[9] Greece,[10,11] the United Kingdom,[12] and South Korea[13]).[3] Therefore, in an effort to extend the therapeutic life of colistin, combination regimens based on colistin as the "anchor" drug as opposed to monotherapy is proposed as a strategy for combating drug resistance. Combinatorial therapy has several benefits. First, from the

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microbiological standpoint, it thwarts the emergence of bacterial drug resistance as the simultaneous development of resistance toward multiple drug classes is remote[14] and second, from a patient management standpoint, it has the potential to reduce colistin toxicity by allowing a lowered therapeutic dose. This is achieved via a synergistic or additive interaction of colistin with its partner antibiotics.[8,15,16]

When route of administration of antibiotics is considered, inhaled delivery of colistin combinations is more effective in eradicating MDR pathogens as it directly targets the foci of infection. Elevated drug concentrations can be achieved locally at the site of lung infection without the adverse effects of systemic toxicity.[17] A dry powder inhaler (DPI) formulation is superior over the other aerosol delivery modes (e.g., nebulizer or metered dose inhaler) in terms of stability, delivery efficiency, portability, ease-of-use, and the avoidance of undesired precipitation in solutions.[18-21]

To date, to the best of the authors' knowledge, there has been no commercially available inhaled DPI product for the eradication of respiratory "superbugs." In addition, no DPI combination involving a synergistic ternary mechanism of action on resistant bacteria (with actual successful testing on resistant species) has ever been reported in the academic literature (previous efforts at best were on binary synergism and tested on nonresistant strains[15]). Commercially, the only antibiotic DPI therapies that are currently available or in the pipeline have been limited to just the single species (i.e., tobramycin, colistin, and ciprofloxacin monotherapies),[15,21-24] and these are clearly not indicated for the specific treatment of MDR pathogens.

In this work, we thereby sought to identify, formulate, and then evaluate the performance of 3 ternary colistin DPI combinations (i.e., colistin/tigecycline/rifampicin [COL/TIG/RIF], colistin/meropenem/rifampicin [COL/MEM/RIF], colistin/meropenem/tigecycline [COL/MEM/TIG]) that could potentially be useful in the fight against respiratory "superbugs." Colistin was the "anchor" drug, while the other 2 species were chosen from distinct antibiotic classes (conferring distinct killing mechanisms) to make up the "multiple-classed" ternary cocktails (i.e., 3 derived permutations).

Materials and Methods

Materials

Meropenem trihydrate and tigecycline were purchased from Afine Chemical (Hangzhou, Zhejiang, and China). Rifampicin was obtained from Leshan Sanjiu-Longmarch Pharmaceuticals Corporation, Ltd. Colistin sulfate, ammonium acetate, phosphoric acid, and trifluoroacetic were purchased from Sigma Chemical Corporation (St. Louis, MO). Ultrapure water was used in the experiments. HPLC-grade acetonitrile was supplied by Merck (Darmstadt, Germany). Clinical isolates of MDR bacteria used in the study were obtained from the National University Hospital, Singapore, and included *P aeruginosa* PSE2 (resistant to ciprofloxacin, aminoglycosides, and carbapenems), *K pneumoniae* ENT443 (resistant to ciprofloxacin and carbapenems), *Escherichia coli* EC424 (resistant to ciprofloxacin and carbapenems), and *Acinetobacter b* AB1 (resistant to ciprofloxacin, aminoglycosides, and carbapenems). Mueller–Hinton broth (Oxoid, Bashingstoke, UK) was used as the culture media for the antimicrobial activity test. Etest strips were purchased from bioMérieux SA (Marcy-l'Etoile, France).

Preparation of Spray-Dried Powders

Powders of colistin (SD-COL), meropenem (SD-MEM), rifampicin (SD-RIF), tigecycline (SD-TIG), ternary combination powders of colistin/meropenem/rifampicin (SD-COL/MEM/RIF) in weight ratio of 5:4:12, colistin/meropenem/tigecycline (SD-COL/MEM/TIG) in weight ratio of 5:4:4, and colistin/tigecycline/rifampicin (SD-COL/TIG/RIF) in weight ratio of 5:4:12 were prepared by spray drying the ethanol–water cosolvent feedstock of antimicrobial agent/s on a B-90 Nano Spray Dryer[15,25-27] (Büchi Labortechnik AG, Flawil, Switzerland) with operating parameters as detailed in Table 1. The operating parameters for spray drying were adapted from Lee et al.[27] A smaller spray mesh size (i.e., 5.5 μm) and more dilute solute concentration (i.e., 0.65 wt/vol %) were applied instead, so as to maximize the production of respirable-size particles (i.e., <5 μm). Higher inlet temperature (i.e., 120°C) was also used to generate drier and hence more aerodynamic powders. Colistin[28,29] and meropenem[29,30] are both hydrophilic drugs, whereas rifampicin[30,31] and tigecycline[28,32] are lipophilic species. Therefore, appropriate cosolvent choice at a suitable ratio is vital toward the production of uniform ternary combination particles. A water–ethanol mix was selected as the desired cosolvent system in view of the solvents' low toxicity profiles and the drugs' mutual miscibilities in those solvents. The water–ethanol cosolvent volume ratio was adjusted to 2:1 to ensure good solubility of all antibiotics in the cosolvent system.

In this work, *in vitro* susceptibility of control and MDR strains against each individual drug of the different combinations was first determined (Table 2). The minimum inhibitory concentration (MIC) values obtained experimentally were further guided by European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical susceptibility breakpoints[33] (Table 2) to obtain ratios that would be "universally" potent (Table 2). The spray-dried powders were stored in a desiccator at room temperature for further characterization. In this work, colistin sulfate (instead of colistimethate sodium) is chosen as colistin sulfate is more stable than colistimethate sodium in human plasma.[34]

Surface Morphology

The morphology of the powder particles was examined by a field emission scanning electron microscopy (JEOL JSM-6700; JEOL, Tokyo, Japan) at 5 kV. Before imaging, the samples were dispersed onto carbon sticky tabs and coated with gold for 80 s using a sputter coater (Cressington 208HR, Watford, UK).[15]

Particle Size Analysis

The particle size distribution of the spray-dried powders was determined by laser diffraction on the Malvern Mastersizer 2000 (Malvern Instruments, UK) using the Scirocco dry dispersion unit. The powders were dispersed in triplicates at 3 bars of pressure using refractive indices of 1.575 for SD-COL and SD-COL/MEM/TIG, 1.639 for SD-MEM, 1.675 for SD-TIG, and 1.613 for SD-RIF, SD-COL/MEM/RIF, and SD-COL/TIG/RIF.[16] For the combination formulations, the refractive indices were based on the major component in the formulation.

Table 1
Spray-Drying Parameters

Parameter	Value
Spray mesh size (μm)	5.5
Feed concentration (w/v %)	0.65
Cosolvent (DI water:ethanol) ratio (v/v)	2:1
Nitrogen flow rate (L/min)	120
Relative spray rate (mL/h)	4
Inlet temperature (°C)	120
Outlet temperature (°C)	60-65
Yield (%)	80-90