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Research paper

Synergistic combination dry powders for inhaled antimicrobial therapy: Formulation, characterization and *in vitro* evaluation

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ABSTRACT

In combination antimicrobial therapy, the desired outcome is to broaden the antimicrobial spectrum and to achieve a possible synergistic effect. However, adverse antagonistic species may also emerge from such combinations, leading to treatment failure with serious consequences. It is therefore imperative to screen the drug candidates for compatibility and possible antagonistic interactions. The aim of this work was to develop a novel synergistic dry powder inhaler (DPI) formulation for antimicrobial combination therapy via the pulmonary route. Binary (ciprofloxacin hydrochloride and gatifloxacin hydrochloride, SD-CIP/GAT) and ternary (ciprofloxacin hydrochloride, gatifloxacin hydrochloride, and lysozyme, SD-CIP/GAT/LYS) combinations were prepared via spray-drying on a BUCHI® Nano Spray Dryer B-90. The powder morphologies were spherical with a slightly corrugated surface and all within the respirable size range. The powders yielded fine particle fractions (of the loaded dose) of over 40% when dispersed using an Aerolizer® at 60 L/min. Time-kill studies carried out against the respiratory tract infection-causing bacteria *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumonia*, and *Acinetobacter baumannii* at 1 × the minimum inhibitory concentration (MIC) over 24 h revealed no antagonistic behavior for both the binary and ternary combinations. While the interactions were generally found to be indifferent, a favorable synergistic effect was detected in the dual combination (SD-CIP/GAT) when it was tested against *P. aeruginosa* bacteria.

1. Introduction

Infectious diseases caused by bacterial pathogens are a major threat to global human health and are also one of the leading causes of human morbidity and mortality [1]. According to the World Health Organization (WHO), infectious diseases accounted for 32% of deaths worldwide, 68% of deaths in Africa and 37% of deaths in Southeast Asia, in all, killing nearly 14 million people in the developing countries each year [2]. The developed world is not spared either. The number of annual deaths due to infectious diseases was estimated at approximately 170,000 in the United States as of the year 2000 [1,3]. Without doubt, infectious diseases have afflicted the world population with a huge socio-economic cost.

Since the introduction of penicillin into clinical practice in the 1940s, antibiotics have always been used as the first line of defense against many infections. However, the widespread use, misuse, and abuse of antibiotics have contributed to the development of multiply antibiotic-resistant bacteria (sometimes referred to as "super bugs"), leading to the emergence of new and the re-emergence of old infectious diseases. In the past two decades, 16 new infectious diseases have been identified and five others have been identified as re-emerging according to the US National Institutes of Health (NIH; Bethesda, MD, USA) [4,5]. Antimicrobial combinatorial therapy offers a powerful means of combating the spread of antibiotic-resistant bacterial pathogens [6]. Quite often, bacteria develop resistance via a variety of different mechanisms, and the probability of a bacterium developing resistance to all the antibiotics employed in the combinatorial therapy is much lower than in the case for a single antibiotic in monotherapy [7]. A combination of antibiotics may provide much broader spectrum coverage than any single antibiotic alone. For example, in tuberculosis treatment, combinatorial therapy has been practiced for over 50 years as it has demonstrated a reduced risk of bacterial resistance during therapy [8].

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Under many clinical circumstances, antimicrobial combinatorial therapy is also advocated for achieving antibiotic synergism (i.e., combined activity is more than the sum of individual activities), to further enhance the bactericidal activity and the rate of killing *in vivo* relative to those attainable with individual agents [7]. One of the best established examples of such synergism is the use of penicillin with an aminoglycoside in bacterial endocarditis due to *Enterococcus faecalis*, *Staphylococcus aureus*, and the viridans group streptococci [9]. Although synergism is often desired, unexpected antagonistic outcomes (i.e., combined activity is less than the sum of individual activities) may be derived from such combinations. There are many examples of antibiotic antagonism, among them, penicillin and aureomycin serves as one of the most classic examples of antibiotic antagonism whereby the survival rate of children treated with penicillin dramatically decreased from 79% to 21% when both penicillin and aureomycin are used together in pneumococcal meningitis treatment [10]. Therefore, scientists and clinicians have to be mindful of the pitfalls of antagonistic combinations. Inappropriate choice of antimicrobial combinations would not only deprive the patient of the therapeutic dose but might also endanger their lives.

In treating respiratory infections, delivery of the antibiotics via the pulmonary route is clearly more advantageous over the more traditional routes as the lung is directly targeted [11]. Since the drugs are delivered directly to the target organ, high drug concentrations can be achieved within the lung compared to systemic administration of the agent thereby avoiding effects of systemic toxicity [12]. Additionally, the high local concentration of the inhaled antibiotics could prevent biofilm formation, hence impeding the emergence of drug resistant bacteria [12].

In the delivery of antibiotics to the respiratory tract, nebulization of aqueous solutions obtained from marketed intravenous preparations has been the most common means of administration since the 1940s [11,13,14]. However, nebulizers are often regarded as hospital or home-setting devices [11], delivering fine particle fractions (FPF) of only around 10% or less to the patient [15,16]. Hence, a longer time is required to achieve the therapeutic dose which inadvertently affects patient compliance. Furthermore, when co-nebulizing two or more antibiotics together, there is also the risk of undesired precipitation in solution [17]. Therefore, all these limitations have reflected the need to develop alternative drug delivery devices like the dry powder inhaler (DPI) to improve patient compliance and ease of administration [11]. The DPI, being portable and quick to use, has successfully enhanced patient compliance and improved formulation stability as the powdered drug is used instead of the drug solution or suspension.

Currently, there is a drought in antibiotic DPI formulations both in the market and in the clinical trials. The only commercial antibiotic DPI formulation known to date is the Novartis® TIP (Tobramycin Inhaled Powder), just recently launched in the UK in September 2011 [11]. Colobreathe® is a DPI formulation of colistin, which has recently completed phase III trials, and is expected to be fully launched soon [11]. Cipro Inhale® (ciprofloxacin inhaled powder) is currently undergoing Phase II development [11]. There is no known combination of antibiotic DPI available commercially. In the academic scene, DPI antibiotic formulations on tobramycin sulfate, gentamicin, azithromycin, and colistin sulfate have been reported [18–21], with some emerging work into combinatorial therapy to enhance the therapeutic dose.

Although Adi et al. [22] had prepared an inhalable co-spray-dried antibiotic (ciprofloxacin hydrochloride and doxycycline hydrochloride) formulation with acceptable FPFs, the formulation was found to be non-synergistic via the qualitative disk diffusion test. The result was in line with an earlier report which mentioned the ciprofloxacin–doxycycline pair as being suppressive (or antagonistic at low concentrations) [6].

About a year after the initial effort by Adi et al. [22], Tsifansky et al. [23] reported the co-encapsulation of ciprofloxacin and ceftazidime in a microparticle system containing dipalmitoylphosphatidylcholine (DPPC), albumin, and lactose and demonstrated the additive anti-pseudomonal activity against a tested strain of *Pseudomonas aeruginosa*. Synergism was not achieved. To date, to the best of the authors' knowledge, no synergistic DPI antimicrobial combination has ever been reported in the literature.

In this work, two inhalable combinatorial DPI antimicrobial formulations, namely, ciprofloxacin hydrochloride/gatifloxacin hydrochloride binary combination (SD-CIF/GAT) and ciprofloxacin hydrochloride/gatifloxacin hydrochloride/lysozyme ternary combination (SD-CIF/GAT/LYS) were developed and quantitatively tested for synergy against the respiratory tract infection-causing bacteria *P. aeruginosa*, *S. aureus*, *Klebsiella pneumonia*, and *Acinetobacter baumannii*. Ciprofloxacin hydrochloride, gatifloxacin hydrochloride, and lysozyme are currently administered either intravenously or orally and have all yet to be developed and tested for the commercial inhalation market.

2. Materials and methods

2.1. Materials

Ciprofloxacin hydrochloride (CIP) and gatifloxacin hydrochloride (GAT) were supplied from Junda Pharmaceutical Co. Ltd. (Changzhou, China). Lyophilized hen-egg white lysozyme (LYS), disodium hydrogen phosphate, phosphoric acid, and trifluoroacetic (TFA) were purchased from Sigma Chemical Co. (Louis, MO, USA). Ultrapure water was used in the experiments. HPLC grade acetonitrile was supplied by Merck (Darmstadt, Germany). The model bacteria used in the study were obtained from the American Type Culture Collection (ATCC) and included *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, and *A. baumannii*, obtained from the National University Hospital (Singapore). Mueller–Hinton broth (Oxoid, Basingstoke, UK) was used as the culture media for the antimicrobial activity test.

2.2. Preparation of spray-dried particles

Powders of ciprofloxacin hydrochloride (SD-CIP), gatifloxacin hydrochloride (SD-GAT), lysozyme (SD-LYS), binary combination powders of ciprofloxacin hydrochloride/gatifloxacin hydrochloride (SD-CIP/GAT) and ternary combination powders of ciprofloxacin hydrochloride/gatifloxacin hydrochloride/lysozyme (SD-CIP/GAT/LYS) were obtained by spray-drying aqueous solutions of the antimicrobial agents on a B-90 Nano Spray Dryer (Büchi Labortechnik AG, Flawil, Switzerland) with operating parameters as detailed in Table 1. All solutions were filtered through a 0.45 μm syringe filter (Millipore, Bedford, MA, USA) prior to spray-drying to minimize blockage due to any undissolved particles at the spray mesh. The composition of spray-dried powders prepared in this study is summarized in Table 2. The spray-dried powders were stored in a desiccator at room temperature for further characterization.

Table 1
Spray drying parameters.

Parameters	
Spray mesh size (μm)	5.5
Feed concentration (w/v %)	0.75
Nitrogen flow rate (L/min)	120
Relative spray rate (mL/h)	4
Inlet temperature (°C)	120
Outlet temperature (°C)	40–45
Yield (%)	70–80