Steroid-Decorated Antibiotic Microparticles for Inhaled Anti-Infective Therapy

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ABSTRACT: Despite advances in vaccination and antimicrobial therapy, community-acquired pneumonia (CAP) remains as a leading cause of morbidity and mortality worldwide. As the severity of CAP has been linked to the extent of inflammation in the body, adjunctive therapeutic measures aimed at modulating the immune response have therefore become increasingly attractive in recent years. In particular, for CAP patients with underlying medical conditions such as chronic obstructive pulmonary disease (COPD), a steroid–antibiotic combination will no doubt be a useful and timely therapeutic intervention. Unfortunately, no combined steroid–antibiotic dry powder formulation is available commercially or has been reported in the academic literature. The aim of this work was hence to develop a novel steroid–antibiotic dry powder inhaler formulation [ciprofloxacin hydrochloride (CIP) and beclomethasone dipropionate (BP)] for inhaled anti-infective therapy. The spray-dried powder was of respirable size (d_{50} of ~2.3 μm), partially crystalline and had BP preferentially deposited on the particle surface. Favorably, when formulated as a binary mix, both CIP and BP showed much higher drug release and fine particle fractions (of the loaded dose) over their singly delivered counterparts, and had robust activity against the respiratory tract infection-causing bacteria *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus aureus*. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:1115–1125, 2014

Keywords: dry powder inhaler; community-acquired pneumonia (CAP); chronic obstructive pulmonary disease (COPD); corticosteroids; combinatorial therapy; spray drying; aerosols; pulmonary drug delivery; formulation; antiinfectives

INTRODUCTION

Community-acquired pneumonia (CAP) is an inflammatory condition of the lungs due to an infection arising from sources external to a hospital or extended-care facility (e.g., nursing home) setting. Although CAP can be brought about by bacteria, viruses, or fungi, the most common cause of CAP in adults is still via a bacterial infection. The severity of CAP has been linked to the extent of inflammation in the body, as inflammation is the body's natural response to the invading infection-causing pathogens.[1,2] Despite advances in vaccination and antimicrobial therapy, CAP remains as a leading cause of human morbidity and mortality, and has afflicted the world population with a huge socio-economic cost.[1–4]

Chronic obstructive pulmonary disease (COPD) is a pulmonary condition where the airways become chronically inflamed and irreversibly narrowed, hence leading to a shortness of breath. Furthermore, patients with COPD appear to be at a higher risk of developing CAP than patients in the general population.[5,6] Recent studies have reported on the impaired immunological mechanisms in COPD patients brought about by the defective alveolar macrophage phagocytosis of bacteria. This consequently increases the susceptibility of COPD patients to infections.[7,8] Moreover, COPD patients tend to develop persistent bacterial colonization in the lower airways through the inflammatory mechanism, hence leading to exacerbations of COPD and progression of airway obstruction.[9,10] Therefore, CAP patients with COPD frequently experience poorer clinical outcomes than patients without COPD. The former has been reported to exhibit higher 30- and 90-day mortality than the latter.[11] As these risks tend to increase with COPD severity, it is vital for CAP patients with COPD to seek medical intervention early to prevent COPD exacerbations and limit disease progression.

Currently, antibiotic treatment remains the mainstay of CAP therapy. However, patients with obstructive airways disease complicated by CAP could also benefit from the inclusion of inhaled corticosteroids into their treatment regimen in view of the drug's potent immunomodulatory and anti-inflammatory properties.[3] Although the release of inflammatory mediators from alveolar macrophages is useful for eliminating invading pathogens from pulmonary infections, excessive releases are on the contrary, harmful to the lungs. Hence, modulation of the

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inflammatory response is vital to creating a balance between the beneficial and harmful effects.[12–14]

Corticosteroids are the most commonly used physiological inhibitors for inflammation. They interfere with the inflammatory expression and action that are associated with COPD and pneumonia. The inflammatory response in the lung is a complex process that involves the coordinated expression of both proinflammatory and anti-inflammatory cytokines.[1] Corticosteroids can switch off genes that encode proinflammatory cytokines such as tumor necrosis factor alpha, interleukin-1 beta, and interleukin-6, and switch on those that encode anti-inflammatory cytokines such as interleukin-10.[15–17] For CAP patients with COPD, the downregulation of the unwanted excessive cytokine response by corticosteroids has the beneficial effect of reducing excessive pulmonary inflammation and accelerating clinical recovery. In addition, bronchospasms (wheezing), a common condition found in these patients, could also be treated with the corticosteroids.[3,18,19]

Understandably, clinicians have therefore applied the use of corticosteroids as an adjunctive therapy (i.e., anti-inflammatory properties) for different infectious diseases as early as the 1950s.[20] The use of corticosteroids in combination with antibiotics has been proven to be effective and safe in several randomized controlled trials in patients with bacterial and tuberculous meningitis,[21–23] tuberculous pericarditis,[24] septic arthritis,[25] and septic shock.[26] In CAP–COPD patients, coadministration of corticosteroids with antibiotics has been shown to be safe and efficacious, and is therefore frequently administered by the physician for treatment.[2,3] On a further note, for smokers who are predisposed to COPD and bacterial infections (i.e., tobacco smoke compromise the antibacterial function of leukocytes, including neutrophils, monocytes, T cells, and B cells),[6] treatment via the steroid–antibiotic combination will no doubt be a useful and timely therapeutic intervention as well. Unfortunately, no combined steroid–antibiotic dry powder formulation is available commercially or has been reported in the academic literature.

Hence, the objective of this study is to pioneer, via state-of-the-art nanospray drying,[27,28] a novel inhalable dry powder formulation incorporating both an antibiotic and a corticosteroid for the treatment of bacterial infections in CAP–COPD patients. A dry powder inhaler (DPI) formulation is favored over the other aerosol delivery modes [e.g., nebulizer or metered dose inhaler (MDI)] because of the improved formulation stability associated with the powdered drug (as compared with the solution or suspension in the MDI and nebulizer), improved delivery efficiency, portability, ease-of-use, and the avoidance of undesired precipitation in solutions (e.g., in the nebulizer).[29–32]

Ciprofloxacin hydrochloride (CIP) and beclomethasone dipropionate (BP) were selected as the model antibiotic and corticosteroid in view of their broad-spectrum activity against both gram-negative and gram-positive bacteria (especially in CAP[4]), and robust anti-inflammatory activity,[33] respectively. Among the members of the fluoroquinolone family (a class of broad-spectrum antibiotics), the antibiotic ciprofloxacin has been known for its clinical efficacy and low potential for adverse effects.[34–36] In addition, the efficacy, safety, and tolerability of ciprofloxacin DPI in human subjects had been successfully evaluated under Phase I and II clinical trials.[36,37] Phase III efficacy studies are currently underway.[38] Inhaled BP, being a robust anti-inflammatory agent, was previously demonstrated to be an effective treatment for patients with nonasthmatic chronic airflow obstruction.[39]

The feasibility of combining the antibiotic and the corticosteroid as an inhalable dry powder formulation for direct concomitant delivery to the lung was investigated. The spray-dried binary formulation that contained CIP and BP (SD-CIP/BP), as well as their single counterparts (SD-CIP and SD-BP), was evaluated for their physicochemical characteristics, aerosol performance, and antimicrobial properties.

Table 1. Spray-Drying Parameters

Parameters	
Spray mesh size (μm)	7
Feed concentration (w/v, %)	0.7
Cosolvent (water–methanol) ratio (v/v)	1:3.5
Nitrogen flow rate (L/min)	120
Relative spray rate (mL/h)	4
Inlet temperature (°C)	75
Outlet temperature (°C)	38–42
Yield (%)	80–90

MATERIALS AND METHODS

Materials

CIP and BP were supplied from Junda Pharmaceutical Company Ltd. (Changzhou, China). Disodium hydrogen phosphate and phosphoric acid were purchased from Sigma Chemical Company (St. Louis, Missouri). Ultrapure water was used in the experiments. High-performance liquid chromatography (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, Manassas, Virginia) and included *Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Staphylococcus aureus* obtained from the National University Hospital (Singapore). Mueller-Hinton broth (Oxoid, Bashingstoke, UK) was used as the culture media for the antimicrobial activity test.

Preparation of Spray-Dried Particles

Powders of CIP (SD-CIP), BP (SD-BP), and binary combination powders of BP/CIP in a weight ratio of 1:32.5 (SD-CIP/BP) were prepared by spray drying CIP alone, BP alone, and CIP with BP from a methanol–water cosolvent feedstock using a B-90 Nano Spray Dryer (Büchi Labortechnik AG, Flawil, Switzerland)[27,28] with operating parameters as detailed in Table 1. All solutions were filtered through a 0.45-μm syringe filter (Millipore, Bedford, Massachusetts) prior to spray drying to minimize blockage due to any undissolved particles at the spray mesh. The spray-dried powders were stored in a desiccator at room temperature for further characterization.

Surface Morphology

The morphology of the powder particles was examined by field emission scanning electron microscopy (FESEM; JEOL JSM-6700; JEOL; Tokyo, Japan) at 5 kV. Prior to imaging, the samples were dispersed onto carbon sticky tabs and coated with gold for 100 s using a sputter coater (Cressington 208HR; Cressington, Watford, UK).

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