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Research paper

Engineering hydrophobically modified chitosan for enhancing the dispersion of respirable microparticles of levofloxacin

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ABSTRACT

The potential of amphiphilic chitosan formed by grafting octanoyl chains on the chitosan backbone for pulmonary delivery of levofloxacin has been studied. The success of polymer synthesis was confirmed using FT-IR and NMR, whilst antimicrobial activity was assessed against *Pseudomonas aeruginosa*. Highly dispersible dry powders for delivery as aerosols were prepared with different amounts of chitosan and octanoyl chitosan to study the effect of hydrophobic modification and varying concentration of polymer on aerosolization of drug. Powders were prepared by spray-drying from an aqueous solution containing levofloxacin and chitosan/amphiphilic octanoyl chitosan. L-leucine was also used to assess its effect on aerosolization. Following spray-drying, the resultant powders were characterized using scanning electron microscopy, laser diffraction, dynamic light scattering, HPLC, differential scanning calorimetry, thermogravimetric analysis and X-ray powder diffraction. The in vitro aerosolization profile was determined using a Next Generation Impactor, whilst in vitro antimicrobial assessment was performed using MIC assay. Microparticles of chitosan have the property of mucoadhesion leading to potential increased residence time in the pulmonary mucus, making it important to test the toxicity of these formulations. *In-vitro* cytotoxicity evaluation using MTT assay was performed on A549 cell line to determine the toxicity of formulations and hence feasibility of use. The MTT assay confirmed that the polymers and the formulations were non-cytotoxic. Hydrophobically modifying chitosan showed significantly lower MIC (4-fold) than the commercial chitosan against *P. aeruginosa*. The powders generated were of suitable aerodynamic size for inhalation having a mass median aerodynamic diameter less than 4.5 μm for formulations containing octanoyl chitosan. These highly dispersible powders have minimal moisture adsorption and hence an emitted dose of more than 90% and a fine particle fraction (FPF) of 52%. Powders with non-modified chitosan showed lower dispersibility, with an emitted dose of 72% and FPF of 20%, as a result of high moisture adsorption onto the chitosan matrix leading to cohesiveness and subsequently decreased dispersibility.

1. Introduction

Chronic respiratory infections are difficult to eradicate leading to high rates of morbidity and mortality and high healthcare costs [1]. Lower respiratory tract infections (LRTIs) are responsible for the highest number of deaths in low-income countries, placing a considerable strain on their health economies [2]. LRTIs lead to 3.2 million deaths annually worldwide, accounting for 6.7% of the global disease burden, and they were the 3rd leading cause of death in 2011 [2]. A major concern with conventional antibiotic treatment of LRTIs is the requirement of a high dose to be delivered for effective eradication of the organism [1]. Hence, in the past decade increasing attention has been given to developing systems for delivery of antibiotics by means of inhalation directly to the respiratory epithelium [3,4].

There are very few antibiotic formulations marketed for the treatment of pulmonary infections, e.g. Tobramycin and Aztreonam inhalation solutions for nebulization. However, much research is being conducted into engineering inhalable antibiotic carrying nanoscale carriers such as liposomes [5,6], polymeric nanoparticles [7,8], solid lipid nanoparticles [9,10] and polymer

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nanoparticle aggregate particles (PNAPs) [11], in particular to target the biofilm pulmonary infections associated with cystic fibrosis (CF) or chronic obstructive pulmonary disease (COPD).

CF and COPD are characterized by the presence of a thick viscoelastic mucus layer which has mesh with average spacing of 100–400 nm, and is responsible for failure of many treatment modalities using the pulmonary route for local and systemic delivery [12,13]. *Pseudomonas aeruginosa* has been reported to be the major causal pathogen associated with approximately 80% of CF cases [14]. Another hindrance to antibiotic penetration is the bacterial generated multicellular surface associated biofilm composite of exopolymeric substances (EPS), such as polysaccharide, proteins and extracellular DNA which forms a strong, sticky network housing bacterial cells and serves as a barrier delaying penetration of antibiotics [14,15]. Polymer/lipid nanoscale systems have been proposed to be more effective than conventional antibiotic formulations as they can penetrate the biofilm due to a decrease in direct interactions between EPS and antibiotic [12,14]. Carrier-associated delivery of antibiotic also aids in decreasing the degradation caused by antibiotic-inactivating enzymes, such as β-lactamase present in the biofilms secreted by *P. aeruginosa* [14]. However, delivery of nanoparticle systems directly to the pulmonary region is problematic due to their instability resulting from particle–particle interaction, and poor deposition properties within the airways, resulting in loss during exhalation [16]. Tsapis et al. [17] have described a strategy whereby nanoparticles are incorporated into microscale structures, embracing the advantages of both nanoscale and micrometre-scale particles, enabling efficient delivery using a simple dry powder inhalation (DPI) system. Microparticles formed by aggregated nanoparticles of mass median aerodynamic diameter (MMAD) 1–5 μm, when inhaled, deposit efficiently in the peripheral lung at the site of pulmonary infection. Exposure to the humid/moist conditions of the lungs results in liberation of nanoparticles. On reaching the vicinity of the biofilm colonies, the matrix/polymer dissolves and releases the antibiotic leading to high localized exposure [18,12].

Drugs employed in inhalation products require a size less than approximately 5 μm. This is usually achieved by micronization/jet milling [19]. Powders produced by such high energy processes exhibit strong inter-particulate attractions, leading to agglomeration. Moreover, micronization does not permit precise control of particle morphology, size distribution, particle density or surface composition leading to variations, and produces defects and/or amorphous regions on crystal surfaces, resulting in altered aerosolization properties and posing considerable formulation challenges [20–22]. This has led to alternative ways of controlling particle size distribution, particle porosity, surface roughness, particle density, etc. Spray-drying has emerged as an attractive, one-step technique for producing powders suitable for pulmonary delivery [23]. This method also provides potential for incorporating a wide range of excipients into the spray-drying feedstock, including dispersibility enhancers, such as L-leucine [4,24–26]; drug-release modifiers, such as glyceryl behenate [27], hydroxypropyl cellulose [28] and poly lactic acid poly lactides [29,30], providing a convenient means of manipulating the properties of the end product [31].

Chitosan, a naturally occurring linear copolymer of β-(1 → 4)-2-acetamido-D-glucose and β-(1 → 4)-2-amino-D-glucose processed by partial deacetylation of chitin has been extensively investigated for transmucosal drug delivery, for instance for pulmonary, nasal and vaginal mucosal administration [32–34] due to its low toxicity, biodegradation, biocompatibility, mucoadhesiveness and enhancement of transcellular permeation [4,16,30]. Additionally, the potential benefits of chitosan and its derivatives include the following: immune enhancing effects [35], hypercholesterolemic effects [36], antimicrobial properties and antitumor properties [37]. Furthermore, the highly reactive primary amino groups and primary/secondary hydroxyl groups of chitosan provide opportunities for derivatization [38,39]. For instance, polymeric amphiphiles formed by hydrophobic modification of chitosan, following acylation using fatty acid chains, such as stearoyl, octanoyl and palmitoyl, have been widely investigated due to the potential of forming nanosized micelles for drug and gene delivery [40]. Acylated chitosan has also been proposed to possess anti-microbial properties against the gram-negative bacteria *P. aeruginosa* and *Escherichia coli*, which are commonly associated with lung infections [41].

Varied strategies have been employed to engineer particles with improved flowability and aerosolization of dry powders and subsequently powder performance. These include modification of particle surface [42,43], porous low density particles [44] and inclusion of coarser carrier particles and excipients. Current excipients used for pulmonary delivery are confined to classes such as sugars viz. lactose, trehalose, and mannose and amino acids viz. L-leucine, trileucine and phenylalanine [45,46]. These carrier/excipients help to reduce the interparticulate forces between drug molecules, alter density of particles, improve surface activity and subsequently reduce the dependence of FPF on flow rate and inhaler type hence improving aerosolization [46]. L-leucine a hydrophobic amino acid in particular has been shown to possess the capability of migration to the surface of the particle droplet in the rapid atomization phase of spray-drying hence preventing water from being entrapped onto the surface producing pitted particles on drying, which have a reduced contact area and consequently reduced cohesion.

However, previous studies have demonstrated that L-leucine enhances the growth of bacterial associated biofilms in *P. aeruginosa* models and hence limiting its use in CF and COPD making it absolutely necessary to find a replacement dispersibility enhancer for dry powders [47]. None of the previous literature studies have shown the dispersibility enhancement effects of hydrophobically modified chitosan. In this study, we have hydrophobically modified chitosan and investigated the effects on the aerosolization of the fluoroquinolone antibiotic levofloxacin, and the antimicrobial activity of polymer and formulations. It would be interesting to study the effect of hydrophobic modification of chitosan on the dispersibility of the dry powders as L-leucine is a model dispersibility enhancer which is a hydrophobic amino acid. These formulations, optimized for levofloxacin content, were prepared by spray-drying, and were investigated for their thermal, physicochemical, antimicrobial, aerosolization and toxicological properties in order to explore the potential of this formulation approach to enhancing the delivery of levofloxacin as a therapeutic inhalation aerosol for the treatment of lung infections.

2. Materials and methods

2.1. Materials

Levofloxacin ⩾98.0% purity, octanoyl chloride 99% purity and L-leucine ⩾98.0% purity were purchased from Sigma–Aldrich Life Sciences (Poole, UK); (1 → 4) 2-amino-2-deoxy-β-D-glucose (Chitooligosaccharide) 1–3 K was purchased from Kitto Life Co. Ltd (Kyongki, Korea); Spectra/Por® pre-wetted dialysis tubing with Molecular Weight Cut off (MWCO) 1 kDa; sodium bicarbonate 99.7% purity and HPLC grade methanol, absolute ethanol, acetonitrile, trifluoroacetic acid (TFA), acetone and chloroform were purchased from Fisher Scientific Ltd. (Loughborough, UK).

2.2. Synthesis of water soluble N,O-octanoyl chitosan 1–3 K

N,O-octanoyl chitosan was synthesized as shown in Fig. 1. Chitosan oligosaccharide 1–3 K (10.0 g) was dispersed in 100 mL