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Nanocrystals embedded in chitosan-based respirable swellable microparticles as dry powder for sustained pulmonary drug delivery

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ABSTRACT

In this study, nanocrystals embedded in microparticles were designed to achieve sustained pulmonary drug delivery of hydrophobic drugs. Chitosan based microparticles were engineered to allow sustained drug release via swelling and mucoadhesive properties of the polymer. Taking cinaciguat as a hydrophobic model drug, drug nanocrystals were prepared by high pressure homogenization and then encapsulated in chitosan microparticles via spray drying. Through various in vitro characterizations, it was shown that drug loaded microparticles had a high drug loading with promising aerosolization characteristics (mean volume diameter (Dv50) 3–4 μm, experimental mass mean aerodynamic diameter (MMADe) 4–4.5 μm, fine particle fraction (FPF%) 40–45%, emitted dose (ED%) 94–95%). The microparticles showed high swelling capacity within 5 min, with various sustained drug release rates depending on chitosan concentration and molecular weight. Furthermore, aerosolization performances under various inhalation conditions were investigated. It was found that both inspiratory flow rate and volume had an influence on the aerosolization of developed microparticles, indicating actual inhalation efficiency might be compromised under disease conditions. Taken together, in vitro data indicate that chitosan based swellable microparticles could potentially be useful as nanocrystal carrier to achieve sustained pulmonary delivery. To complete the feasibility assessment of this formulation principle, future in vivo safety and efficacy studies are needed.

1. Introduction

The pulmonary route has aroused a great interest during the last few years for the administration of a number of therapeutic molecules, aiming to achieve both local and systemic effects. This attraction of pulmonary drug delivery is due to the numerous advantages over many other delivery routes, including low thickness of the epithelial barrier, extensive vascularization, large alveolar surface area, low enzymatic metabolic activity and the absence of first-pass effect, which are beneficial for drug absorption (Courrier et al., 2002; Lu and Hickey, 2007; Patton and Byron, 2007). Further development of pulmonary sustained or controlled release dosage forms was studied to cut down drug administration frequency, extend drug residence time, maximize drug efficacy and reduce systemic exposure, especially for toxic drugs (Liang et al., 2015; Smyth and Hickey, 2011).

However, due to the inherent lung clearance mechanisms of exogenous substances, it is still a challenge to successfully develop respirable carrier systems with adequate aerodynamic properties that can confer sustained release effect. Generally, particles targeted to the deep lung should be small enough with an aerodynamic diameter of ~0.5–5 μm, but should not be too small (<0.5 μm) otherwise the particles would fail to deposit and be exhaled again (Chow et al., 2007; Grenha et al., 2007; Musante et al., 2002; Telko and Hickey, 2005). The appropriate particle size for inhalation is also the ideal size range for the uptake by alveolar macrophages, therefore, even for hydrophobic drugs, sustained pulmonary drug release can't be achieved despite of their limited dissolution in the lung fluid. Although it was shown that increasing microparticle size could reduce macrophage phagocytosis (Ahsan et al., 2002; Makino et al., 2003), it is unpractical for the objective of pulmonary drug delivery due to the inefficient deposition of fine particle fraction to the targeted region. Therefore, development of particles that can reduce or even escape macrophage phagocytosis and in the meantime have respirable aerodynamic size is the major challenge.

Among the several technologies currently under investigation, swellable microparticles are one of the promising strategies to achieve this goal, as this kind of particles is small enough to be inhaled and meanwhile it has swelling capacity of becoming larger in size after deposition upon getting into contact with the lung fluid (El-Sherbiny et al., 2010; El-Sherbiny and Smyth, 2012; Selvam et al., 2011). For the design of swellable microparticles, selection of an appropriate polymer is

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of special importance. Chitosan (CS) is a cationic polysaccharide with many beneficial properties such as biodegradability, low toxicity, low immunogenicity, excellent biocompatibility as well as mucoadhesion and a permeation enhancer (Ding et al., 2012; Hirano et al., 1990; Mao et al., 2010). Chitosan has been used as an absorption enhancer in the nasal mucosa (Mei et al., 2008; Na et al., 2010) and in pulmonary tissues (Jae et al., 2006), attributed to its transient opening of the intercellular tight junctions. A further consideration when delivering particulates for sustained release is the pulmonary mucociliary clearance mechanism which can move particles from the lower lung regions to the throat (Patton and Byron, 2007). Therefore, a suitable mucoadhesive component is essential. Thus, CS is an ideal candidate for sustained pulmonary drug delivery as it can not only act as a drug release modifier but also shows mucoadhesive properties (Dang and Leong, 2006; Harikarnpakdee et al., 2006; Martinac et al., 2005). However, how to incorporate hydrophobic drugs into chitosan microparticles within respirable size is still a big challenge. To guarantee even drug distribution in the microparticles, nanoparticles were prepared and encapsulated (El-Sherbiny and Smyth, 2012). Low drug loading and organic solvent remaining are the main shortcomings related to the traditional nanoparticles. With the well-established nanocrystal technology (Jacobs and Müller, 2002; Keck and Müller, 2006; Sun et al., 2011a,b), our hypothesis is that, by encapsulating nanocrystals into chitosan based microparticles, respirable microparticles with sustained pulmonary drug delivery can be obtained.

Therefore, in this study, cinaciguat, a nitric oxide (NO)-independent activator of soluble guanylate cyclase for the treatment of pulmonary hypertension, was used as a hydrophobic model drug, and chitosan as matrix, were used to prepare respirable swellable microparticles. The resulting formulations were characterized in terms of particle size, drug loading efficiency, powder crystallinity, water content, morphology, in vitro release, dynamic swelling behavior, powder flowability and in vitro release profile. The in vitro aerodynamic performance was investigated using the next generation impactor (NGI) under various conditions by loading these microparticles in capsules (Vcaps®) and aerosolizing them with a breath-actuated inhaler device (Cyclohaler®).

2. Materials and methods

2.1. Materials

Chitosan (Molecular weight 400 kDa, deacetylation degree ≥ 85% and moisture content ≤ 10%) was purchased from Jinan Haidebei Marine Bioengineering Co., Ltd., (China) and degraded as reported previously to get chitosans with molecular weight of ~50 and ~100 kDa (Mao et al., 2004). Cinaciguat (BAY 58-2667) was provided by Bayer Healthcare (Wuppertal, Germany). Polyvinyl pyrrolidone (PVP-k12), Pluronics F68 were donated by International Specialty Products Inc. (USA) and BASF in China respectively. Tweens (20 and 80) of injection grade were from Sigma-Aldrich in China. HPLC grade acetonitrile, dichloromethane (DCM), 1-Methyl-2-pyrrolidone (NMP) and acetic acid were supplied by Shandong Yuwang Co., Ltd. (China). All other reagents, unless otherwise specified, were of analytical grade.

2.2. Preparation of cinaciguat nanosuspension

The cinaciguat nanosuspension was prepared by High Pressure Homogenization (HPH) method as described previously (Sun et al., 2011a,b). Briefly, 250 mg of cinaciguat coarse powder were dispersed in 50 mL aqueous solution containing 0.1% (w/v) Pluronic F68. The dispersion was then processed through a high pressure homogenizer AH100D (ATS Engineering Inc., Shanghai, China) at 150 bars for 20 cycles. Thereafter it was homogenized at 300, 600 and 900 bars for 3 cycles respectively followed by 15 cycles at 1200 bars. The exact drug concentration in the nanosuspension was analyzed by HPLC.

2.3. Preparation of cinaciguat nanocrystals embedded in swellable microparticles

Cinaciguat loaded swellable microparticles were prepared using spray drying technique. Briefly, 1.5–3.0 g chitosan powder (MW, ~50 or ~100 kDa) were dissolved in 500 mL diluted acetic acid solution and pH of the final solution was adjusted to 6.0. For drug loading, a specific volume of freshly prepared cinaciguat nanosuspension was added to the chitosan solution. For all the formulations prepared, chitosan concentration and theoretical drug loading (%) were calculated according to the following equation:

$$\text{CS concentration}\ (\%) = \left[M_{CS}\ (g)/\left(V_{\text{aqueous solution}} + V_{\text{drug nanosuspension}}\right)\ (mL)\right] \times 100\% \quad (1)$$

$$\text{Theoretical drug loading}\ (\%) = \left[M_{\text{cinaciguat}}\ (g)/\left(M_{CS} + M_{\text{cinaciguat}}\right)(g)\right] \times 100\% \quad (2)$$

After adding cinaciguat nanosuspension, the whole suspension was subsequently spray-dried using a spray-dryer equipped with a high performance cyclone and a 0.5 mm two-fluid nozzle (SD-1000, Tokyo Rikakikai Co., Ltd., Japan.). The optimized operation conditions and parameters were: inlet temperature 110 °C, atomizing pressure 190 kPa, drying air flow rate 0.7 m^3/min and feeding rate 3.0 mL/min.

2.4. Characterization of cinaciguat nanocrystals loaded swellable microparticles

2.4.1. Particle size analysis

Particle size of cinaciguat nanosuspension was measured by Malvern Zetasizer Nano (Malvern, UK) and the Z-average value was used to evaluate the size of the nanosuspension and the size distribution was expressed using Polydispersity index (PDI). Particle size distribution of the drug loaded swellable microparticles was measured using a Beckman-Coulter LS 230 particle size analyzer (Beckman-Coulter LS 230, USA). Approximately 20 mg of the dry powder was re-dispersed in absolute alcohol and then measured. The volume mean diameter (D_v) was used to evaluate the geometric diameter of the microparticles. Each sample was measured in triplicate.

2.4.2. Drug loading efficiency determination

Actual drug loading efficiency was determined according to the following procedures. Briefly, 10 mg of the swellable microparticles were weighed precisely and 5.0 mL of 1.0% (w/v) acetic acid solution was used to dissolve the chitosan carrier, then *N*-methylpyrrolidone was added to 50.0 mL (±0.05 mL) to dissolve cinaciguat nanocrystals. 10.0 μL of the solution was subjected to RP-HPLC analysis on a Phenomenex Gemini column with pre-column (150 mm × 4.6 mm, particle size: 5 μm) at 40.0 °C. Gradient elution method was used. The detection wavelength was 230 nm and drug retention time was in the range of 14.70–14.80 min.

2.4.3. Powder crystallinity analysis

Thermodynamic analysis of the particles was performed using differential scanning calorimeter (DSC-1, Mettler-Toledo, Switzerland). Powder samples (~3 mg) were weighed and placed in hermetically sealed aluminum pans. The samples were scanned at a heating rate of 10 °C/min from 25 °C to 350 °C in nitrogen atmosphere. The melting temperature was determined from the endothermic peak of the DSC curve recorded.

The solid state form of the drug within swellable microparticles was further validated by X-ray powder diffraction (X-RPD) using an X-ray diffractometer (X'pert PRO, PANalytical B.V., The Netherlands) with Cu-Kα radiation generated at 40 mA and 35 kV. Samples were analyzed in a 2θ range of 4.5° to 40° with a step size of 0.033° and a counting time of 0.6 s per step.