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Nanoparticle agglomerates of fluticasone propionate in combination with albuterol sulfate as dry powder aerosols

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Abstract

Particle engineering strategies remain at the forefront of aerosol research for localized treatment of lung diseases and represent an alternative for systemic drug therapy. With the hastily growing popularity and complexity of inhalation therapy, there is a rising demand for tailor-made inhalable drug particles capable of affording the most proficient delivery to the lungs and the most advantageous therapeutic outcomes. To address this formulation demand, nanoparticle agglomeration was used to develop aerosols of the asthma therapeutics, fluticasone or albuterol. In addition, a combination aerosol was formed by drying agglomerates of fluticasone nanoparticles in the presence of albuterol in solution. Powders of the single drug nanoparticle agglomerates or of the combined therapeutics possessed desirable aerodynamic properties for inhalation. Powders were efficiently aerosolized (~75% deposition determined by cascade impaction) with high fine particle fraction and rapid dissolution. Nanoparticle agglomeration offers a unique approach to obtain high performance aerosols from combinations of asthma therapeutics.

Keywords

Fluticasone; albuterol; combination therapy; dry powder; aerosols

1. Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are currently treated using either nebulizers, pressurized metered dose inhalers or dry powder inhalers (Dalby and Suman, 2003; Murnane et al., 2008b; Yang et al., 2008a). A major determinant of aerosol deposition in the respiratory tract is the aerodynamic size of particles and the polydispersity

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(Louey et al., 2004; Pilcer and Amighi, 2010; Pritchard, 2001). Inhaled drugs should ideally possess an aerodynamic diameter less than 5 μm for delivery into the 'deep' lung for local therapy or systemic absorption (Weers et al., 2010). Nanoparticles (<0.5 μm) are more likely to be exhaled, which may lead to dose variability (Shi et al., 2007). If delivered as a suspension, such small particles are also prone to particle growth due to Ostwald ripening and can suffer from uncontrolled agglomeration (Berkland, 2010). A major obstacle to inhaled therapeutics is the inability to efficiently deliver large quantities of a drug to the deep lung (Gillian, 2010).

Natural aerosols, in particular, spores from molds and fungi as well as soot and asbestos, have a size and structure that allows them to aerosolize efficiently into the lungs. They are composed of underlying nanostructures that join together to form microparticles. Following this rationale, nanoparticle agglomerates were designed by formulating nanometer-sized drug particles, then assembling them to micron-sized clusters with the desired aerodynamic diameter (e.g., 1 μm for treating distal airways or 3–5 μ m for treating upper airways) (Bailey et al., 2008; El Gendy et al., 2009; Plumley et al., 2009). By agglomerating nanoparticles under controlled process conditions, nanoparticle agglomerate dry powders can be tailored to the desired physical and chemical characteristics for aerosol delivery and dissolution (Aillon et al., 2010; El-Gendy and Berkland, 2009).

Asthma is a disease that is commonly treated with two types of aerosolized agents; bronchodilators (β_2 agonists) and anti-inflammatory agents (steroidal compounds). Apart from acute asthma attacks, which are primarily treated with short acting β_2 agonists, there is a strong need for chronic therapy to reduce inflammation and to avoid asthma exacerbations (Barnes, 2002). Therapeutic interventions using combinations of a β_2 agonist and a glucocorticoid have emerged as an effective asthma management strategy to control persistent asthma (Rajeswari et al., 2006). The use of β_2 agonists to prevent bronchial spasm and glucocorticoids to decrease inflammation is widely accepted (Westmeier and Steckel, 2008). Combination formulations have also been suggested to be more effective than a single drug due to synergistic effects in the same target cell in the lung epithelia. It appears rational, therefore, to combine both substances in one particle instead of formulating a combination product containing both drugs in a physical mixture (Adi et al., 2008; Nelson et al., 2003; Papi et al., 2007).

Combination products such as Advair and Symbicort are currently marketed. Advair combines fluticasone propionate and salmeterol xinafoate into one inhaler (Michael et al., 2000). Salmeterol (long acting β_2 agonists) does not replace the need for rescue inhalers, such as albuterol, which are still necessary for immediate relief of asthma symptoms (Kamin et al., 2007; Salpeter et al., 2006). Symbicort is another combination product containing budesonide and formoterol. Fluticasone propionate is a synthetic corticosteroid used to treat asthma, allergic rhinitis (hay fever) and eosinophilic esophagitis (Murnane et al., 2008a; Rehman et al., 2004; Vatanara et al., 2009). Albuterol sulfate is a short-acting β_2 adrenoreceptor agonist used for the relief of bronchospasm in conditions such as asthma and COPD, and is currently one of the most prescribed bronchodilators for the treatment of bronchial asthma (Ahmad et al., 2009; Xu et al., 2010).

Development of dry powder aerosols for delivering fluticasone and/or albuterol nanoparticle agglomerates as single anti-asthmatic therapies or in combination to achieve synergistic effect is herein described. The study illustrates the formulation of fluticasone nanoparticles using potentially acceptable surfactants that control the size and surface charge of the prepared nanoparticles. Also, albuterol nanoparticles free of excipients were engineered using different techniques. The nanoparticle suspensions were destabilized via ionic charge interactions using L-leucine. Combination drug formulations were prepared by adding albuterol aqueous solution to the fluticasone nanoparticle suspension followed by addition of L-leucine. The aerosol performance of these nanoparticles agglomerate formulations were fully characterized and compared to micronized stock drug.

2. Materials and methods

2.1. Materials

Fluticasone propionate (Flu) and albuterol sulfate (Albu) were generously provided by 3M. L-α-phosphatidylcholine (lecithin; Lec), cetyl alcohol (CA), L-leucine (Leu) and polyvinylpyrrolidone K90 (PVP) were purchased from Sigma Chemical Co., USA. Pluronic F-127 (PL, Mw ~12,220) was purchased from BASF, USA. Ethanol, acetone, potassium dihydrogen phosphate (KH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) and sodium chloride (NaCl) were purchased through Fisher Scientific, USA. Floatable dialysis membrane units (MWCO=10 kDa) were obtained from Spectrum Laboratories Inc., USA. Amicon Ultra Centrifugal filter units (MWCO=5 kDa) used for dissolution were purchased from Millipore, Co (Billerica, MA). Double-distilled water was used throughout the study, provided by an EASYpure® RODI (Barnstead International, USA).

2.2. Nanoparticle formulation

2.2.1. Preparation of fluticasone nanoparticle suspension—Nanoparticle suspensions of fluticasone propionate were prepared using antisolvent precipitation. Solutions of the drug in organic solvent (acetone or ethanol) were prepared at different concentrations and directly injected into water at a rate of 2.5 mL/min. A variety of solvent/non-solvent ratios were precipitated under ultrasonication (probe-type sonicator, Fisher Scientific, Sonic Dismembrator) operating with an amplitude of 48% in an ice bath or under homogenization (probe-type homogenizer, Tissue tearor, Biospec Products, Inc.). Hydrophobic surfactants (cetyl alcohol and lecithin) were added to the drug solution while hydrophilic surfactants (PL F127, PVA and PVP K90) were dissolved in the aqueous phase.

2.2.2. Formulation of combination therapy—The combined formulation was prepared by adding albuterol sulfate dissolved in water to the precipitated fluticasone propionate nanosuspension during homogenization at 25,000 rpm. The two drugs were combined, at a ratio of 2:1 w/w, fluticasone propionate: albuterol sulfate (Papi et al., 2007; Westmeier and Steckel, 2008).

2.2.3. Fabrication of albuterol nanoparticle suspension—Albuterol sulfate nanoparticles were prepared by precipitation or by a top-down (attrition) method. Concerning the precipitation technique, solutions of albuterol in water were prepared and

directly injected into ethanol or acetone at a rate of 2.5 mL/min. Various solvent/ non-solvent ratios were used under ultrasonication operating with an amplitude of 48% or under homogenization. In the top-down method, albuterol nanoparticles were prepared by ultrasonicating or homogenizing a suspension of albuterol in acetone or ethanol. The concentration of the drug in the anti-solvent was varied between 0.2 and 1 mg/mL. The ultrasonication or homogenization time was also varied.

2.3. Characterization of nanoparticle suspensions

The average size and polydispersities of the nanoparticle suspensions were determined by dynamic light scattering (Brookhaven, ZetaPALS, SA). The same instrument was used to determine the zeta potential of the nanoparticles in 1 mM potassium chloride solution. Three runs of 15 cycles were acquired, and the mean zeta potential was recorded. Measurements were taken at an angle of 90° to the incident light source. Some samples were frozen at −80 °C and lyophilized (FreeZone 1) for ~36 h at a temperature of −50 °C under vacuum (~0.02 millibar). Lyophilized powder was stored at room temperature for further characterization.

2.4. Agglomeration of nanoparticle suspensions

Nanoparticles were agglomerated via addition of an agglomerating agent. L-Leucine solution in water (2.5 mg/mL) was slowly injected into nanoparticle colloids during homogenization at 25,000 rpm for 30 s. The amount of L-leucine added was adjusted to a fluticasone: L-leucine ratio equal to 1:1 for agglomerating the fluticasone suspension and the combination suspension. An albuterol: L-leucine ratio of 1:1.5 was used for agglomerating the albuterol suspension.

The agglomerated suspensions were incubated with the agglomerating agent for three hours. Then, the size of the prepared nanoparticle agglomerates was measured in Isoton diluent using a Coulter Multisizer 3 (Beckman Coulter Inc.) equipped with a 100 μm aperture. The suspensions were kept overnight at room temperature to allow evaporation of organic solvent and then frozen at −80 °C. The frozen suspensions were transferred to the freeze dryer where drying lasted for ~3 days. Lyophilized powder was stored at room temperature for further characterization.

2.5. Particle size and morphology by transmission electron microscopy (TEM)

Lyophilized powders were resuspended in Isotonic solution and the particle size and size distribution was detected using a Coulter Multisizer 3. In addition, the size and morphology of the lyophilized nanoparticles and nanoparticle agglomerate powders were evaluated using JEOL 1200 EXII transmission electron microscope. Prior to imaging, carbon-coated grids (Electron Microscopy Sciences) were placed on a droplet of the suspensions on a glass microscope slide to permit the adsorption of the particles onto the grid. After this, the grid was blotted with a filter paper and air dried for 1 h.

2.6. Powder flow characteristics

Nanoparticle agglomerate dry powders were poured through a glass funnel from a height of 4 cm onto a level bench top. The angle that the side of the conical heap made with the horizontal plane was recorded as the angle of repose ($\tan\theta$ = height / radius). In addition,

bulk and tapped densities were determined. Then, the Hausner ratio (tapped density / bulk density) and Carr's compressibility index (C_i) [(tapped density – bulk density)/ tapped density X 100%] were calculated (Kumar et al., 2001; Louey et al., 2004).

2.7. Evaluation of Aerosol performance of nanoparticle agglomerate dry powders

2.7.1. Measurement of theoretical mass mean aerodynamic diameter—The geometric particle size and tap density measurements were used for calculating the theoretical mass mean aerodynamic diameter (d_{ae}) of the nanoparticle agglomerates (El-Gendy et al., 2010b; Fiegel et al., 2008).

2.7.2. Aerodynamic size distribution by time-of-flight analysis—The aerodynamic diameter and size distributions of the nanoparticle agglomerate powders were determined by time-of-flight measurement (TOF) using an Aerosizer LD (Amherst Instruments, Hadely, MA, USA) equipped with a 700 μm aperture operating at 6 psi. For these studies, ~1 mg of the powder was added to the instrument disperser and data were collected for ~60 s under high shear force (~3.4 kPa). The instrument size limits were 0.10–200 μm and particle counts were above 100,000 for all measurements.

2.7.3. *In vitro* aerosol deposition of nanoparticle agglomerates by cascade impactor—An eight-stage Mark II Andersen Cascade Impactor (ACI, Tisch Environmental, Inc.) had stages with particle aerodynamic diameter specifications at a flow rate of 28.3 L/min as follows: pre-separator (10.00 μm), stage 0 (9.00 μm), stage 1 (5.80 μm), stage 2 (4.70 μm), stage 3 (3.30 μm), stage 4 (2.10 μm), stage 5 (1.10 μm), stage 6 (0.70 μm), stage 7 (0.40 μm) and the final filter (< 0.40 μm). Aerodynamic behavior of nanoparticle agglomerate dry powders was assessed using the ACI and compared with that of the two drugs as received.

The powder was delivered into the cascade impactor by placing capsules (gelatin type, size 3, generously provided from Capsugel®, NJ, USA) containing 5 ± 0.5 mg of powder into a Plastiape Monodose Inhaler RS01 Model 7. The capsule was punctured and the powder was drawn through the cascade impactor which was operated at a flow rate of 28.3 L/min for 4 s. Dry powder aerosols deposited on each of the nine stages of the impactor were quantified by HPLC. After actuation, the device, capsule, adapter, throat, all plates, stages and filter were washed into separate volumetric flasks using ethanol (for fluticasone alone or Flu/Alu combination) or phosphate buffered saline (pH 7.4 for albuterol). Appropriate sample dilutions were made prior to testing by HPLC. Each sample was tested in triplicate.

Concerning the combination formula, the powder deposited on stages was suspended in ethanol and was ultrasonicated in a bath-type sonicator (Branson 3510) for 30 min. Then, the solution was centrifuged (Beckman, Avanti) at ~15,000 rpm for 30 min and the amount of fluticasone in the supernatant was determined using a reversed-phase HPLC method. As albuterol has a very slightly solubility in ethanol, the drug content in both supernatant and precipitate were detected by HPLC.

All ACI experiments were performed under controlled conditions (21 ± 2 °C, 50 ± 5% RH) in triplicate. The emitted dose (ED) was defined as the mass of drug delivered from the

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