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Dry powder pulmonary delivery of cationic PGA-co-PDL nanoparticles with surface adsorbed model protein

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ABSTRACT

Pulmonary delivery of macromolecules has been the focus of attention as an alternate route of delivery with benefits such as; large surface area, thin alveolar epithelium, rapid absorption and extensive vasculature. In this study, a model protein, bovine serum albumin (BSA) was adsorbed onto cationic PGA-co-PDL polymeric nanoparticles (NPs) prepared by a single emulsion solvent evaporation method using a cationic surfactant didodecyldimethylammonium bromide (DMAB) at 2% w/w (particle size: 128.64 ± 06.01 nm and zeta-potential: +42.32 ± 02.70 mV). The optimum cationic NPs were then surface adsorbed with BSA, NP:BSA (100:4) ratio yielded 10.01 ± 1.19 μg of BSA per mg of NPs. The BSA adsorbed NPs (5 mg/ml) were then spray-dried in an aqueous suspension of L-leucine (7.5 mg/ml, corresponding to a ratio of 1:1.5/NP:L-leu) using a Büchi-290 mini-spray dryer to produce nanocomposite microparticles (NCMPs) containing cationic NPs. The aerosol properties showed a fine particle fraction (FPF, dae < 4.46 μm) of 70.67 ± 4.07% and mass median aerodynamic diameter (MMAD) of 2.80 ± 0.21 μm suggesting a deposition in the respiratory bronchiolar region of the lungs.The cell viability was 75.76 ± 03.55% (A549 cell line) at 156.25 μg/ml concentration after 24 h exposure. SDS-PAGE and circular dichroism (CD) confirmed that the primary and secondary structure of the released BSA was maintained. Moreover, the released BSA showed 78.76 ± 1.54% relative esterolytic activity compared to standard BSA.

1. Introduction

An increase in mortality and morbidity associated with pulmonary diseases has led to the exploration of pulmonary drug delivery as a non-invasive approach for the treatment and management of these diseases and also for administration of therapeutics for systemic delivery (Carlotta et al., 2011; Yang et al., 2008). The lung as a delivery route offers a large surface area (80–90 sq m), extensive vasculature, a thin alveolar epithelium (0.1–0.5 μm) leading to rapid absorption (Scheuch et al., 2006). It is also believed that compared to any other entry portal in the body, the pulmonary epithelia appears to be more permeable to macromolecules (Patton, 1996). Advancements in biotechnology in the last decade have led to the development of new therapeutics such as peptides, proteins and other macromolecules (Sullivan et al., 2006). Despite barriers such as the respiratory mucus, mucociliary clearance, macrophages, enzymes and basement membrane that limit absorption (Agu et al., 2001), several macromolecules have been extensively investigated such as insulin (Al-Qadi et al., 2012; Kling, 2014), bovine serum albumin (BSA) (Jiang et al., 2010; Kunda et al., 2014), calcitonin (Yamamoto et al., 2005) for delivery via the pulmonary route.

Biodegradable nanoparticles (NPs), are being explored for the delivery of macromolecules as they offer improved bioavailability, controlled or sustained release and biocompatibility (Kumari et al., 2010; Tawfeek et al., 2011). Several factors such as polymer properties, size and charge of NPs, and the stabilizer(s) employed in the preparation of NPs play a vital role in determining their uptake, biodistribution, drug loading and fate after administration,

Abbreviations: BSA, bovine serum albumin; CD, circular dichroism; DMAB, didodecyldimethylammonium bromide; L-leu, L-leucine; NPs, nanoparticles; NCMPs, nanocomposite microparticles; PGA-co-PDL, poly(glycerol adipate-co-ω-pentadecalactone); PVA, polyvinyl alcohol; SD, spray-drying; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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all of which affect the therapeutic efficacy (Bhardwaj et al., 2009; Peetla and Labhasetwar, 2009). Herein, we used a cationic surfactant, quaternary ammonium salt didodecyldimethyl ammonium bromide (DMAB) to produce positively charged NPs. It is established that the cationic surfactant DMAB produces small, stable NPs and prevents particle agglomeration (Bhardwaj et al., 2009; Chen et al., 2010; Hariharan et al., 2006; Kwon et al., 2001). The particle size and surface charge of NPs are known to play an important role in determining the cellular uptake, and cationic NPs compared to anionic NPs (negatively charged) or neutral NPs, have better interactions with the negatively charged cell membrane thereby improving their cellular uptake (Hariharan et al., 2006; Peetla and Labhasetwar, 2009). NPs and protein can be attached together either by simple physical adsorption based on charge or hydrophobic interactions (Mody et al., 2013; Wendorf et al., 2006), or complex processes; such as chemical conjugation and encapsulation (Zhao et al., 2014). The encapsulation of proteins may present some problems such as low loading and loss of protein activity due to harsh formulation conditions; such as interaction with organic solvents and the higher stirring speed employed in the NP preparation process (Bramwell and Perrie, 2006; Jiang et al., 2005). Alternatively, an adsorption process avoids protein contact with the harsh conditions offering enhanced stability over encapsulated proteins hence providing a promising alternative for encapsulation (Bramwell and Perrie, 2006; Florindo et al., 2010).

Due to their small size and low inertia NPs are exhaled after inhalation resulting in low doses in the lungs, and the high surface energy promotes aggregation making them difficult to handle (Stevanovic and Uskokovic, 2009; Yang et al., 2008). Therefore, the NPs to be used in pulmonary delivery are required to be formulated into microcarriers for ideal aerosolisation properties (Sinsuebpol et al., 2013). This can be achieved using excipients, such as lactose, mannitol, trehalose and L-leucine (L-leu) to produce nanocomposite microparticles (NCMPs) that encompass NPs in a microcarrier (Li et al., 2005; Seville et al., 2007). The NCMPs are formulated using manufacturing techniques such as freeze-drying, spray-drying (SD), spray-freeze drying or supercritical fluid technologies (Al-fagih et al., 2011; Kunda et al., 2013). The aim of this study was to produce cationic NPs with surface adsorbed BSA, and formulate the NPs into NCMPs via SD using L-leu as a carrier for dry powder inhalation.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA, MW 67 KDa), didodecyl dimethyl ammonium bromide (DMAB), phosphate buffered saline (PBS, pH 7.4) tablets, poly(vinyl alcohol) (PVA, MW 9–10 K, 80%), RPMI-1640 medium with L-glutamine and $NaHCO_3$, thiazoly blue tetrazolium bromide (MTT), tween 80® and 4-nitophenyl acetate were obtained from Sigma–Aldrich, UK. L-leucine (L-leu) was purchased from BioUltra, Sigma, UK. Tissue culture flasks (75 cm^2) with vented cap, 96-well flat bottom plates, acetone, acetonitrile (ACN, HPLC grade), antibiotic/antimycotic Solution (100X), dichloromethane (DCM), dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific, UK. Divinyladipate was obtained from Fluorochem, UK. Fetal calf serum (FCS) heat inactivated was purchased from Biosera UK. Micro BCA™ protein assay kit was purchased from Thermo Scientific, UK. Poly(glycerol adipate-co-ω-pentadecalactone) (PGA-co-PDL, MW of 14.7 KDa was synthesized in our laboratory at LJMU as previously published by Thompson et al. (Thompson et al., 2006) and human adenocarcinomic alveolar basal epithelial cell line, A549, was purchased from ATCC.

2.2. Preparation of nanoparticles

The cationic NPs were prepared using a previously established oil-in-water (o/w) single emulsion solvent evaporation method (Kunda et al., 2014). Briefly, PGA-co-PDL, (200 mg), (Nile Red, NR, 0.5 mg for confocal microscopy) and DMAB (0, 1 and 2% w/w of polymer) were dissolved in 2 ml DCM and upon addition to 5 ml of 5% w/v poly(vinyl alcohol) (PVA) was probe sonicated (20 microns amplitude) for 2 min under ice to obtain an emulsion. This was immediately added drop wise to 20 ml of 0.75% w/v PVA under magnetic stirring at a speed of 500 RPM. This mixture was left stirring at room temperature for 3 h to facilitate the evaporation of DCM. The NP suspension was collected using centrifugation (78,000 × *g*, 40 min, 4 °C), washing twice to remove unbound and excess surfactant. The NPs were then surface adsorbed with protein as described in Section 2.3.

2.3. Protein adsorption and quantification

The NP suspension (equivalent to 10 mg) was resuspended in 4 ml of BSA (or FITC-BSA for confocal microscopy) at different NP: BSA ratios (100:4–100:20) corresponding to 100–500 μg/ml BSA. After 1 h of rotation at 20 RPM on a HulaMixer™ Sample Mixer (Life Technologies, Invitrogen, UK) the NP suspensions were centrifuged and the supernatant analysed for protein content using a micro BCA protein assay kit. The amount of BSA adsorbed per milligram of NPs ($n = 3$) was calculated using Eq. (1):

$$\text{Adsortion}(\mu\text{per mg of NPs}) = \frac{(\text{Initial protein conc} - \text{Supernatant protein conc})}{\text{Amount of NPS}} \quad (1)$$

2.4. Characterization of nanoparticles

Morphological analysis of NPs was performed by transmission electron microscopy (TEM) using a FEI Morgagni Transmission Electron Microscope (Philips Electron Optics BV, Netherlands) at an acceleration voltage of 100 kV. Approximately 50 μl of the NP suspension was stained with 2% ammonium molybdate and placed on a carbon coated copper grid. Digital images were taken at magnification of 44,000 and 110,000.

Particle size, poly dispersity index (PDI) and surface charge (zeta potential) were measured by dynamic laser scattering (DLS) using a laser particle size analyser (Zetasizer Nano ZS, Malvern Instruments Ltd., UK). An aliquot of 100 μl of the NP suspension was diluted with 5 ml of deionized water loaded into a cuvette and the measurements recorded at 25 °C ($n = 3$).

2.5. Preparation of nanocomposite microparticles

The NPs were incorporated into NCMPs using L-leu as a carrier at a weight ratio of 1:1.5 (NP:L-leu). The empty NPs, BSA adsorbed NPs or FITC-BSA adsorbed Nile Red NPs (NR NPs for confocal microscopy) were dispersed in distilled water containing L-leu at a concentration of 12.5 mg/ml (5 mg/ml NPs and 7.5 mg/ml L-leu). The resultant suspension was then spray-dried using a Büchi B-290 mini spray-dryer (Büchi Labortechnik, Flawil, Switzerland) at a feed rate of 10%, an atomizing air flow of 400 L/h, aspirator capacity of 100% and an inlet temperature of 100 °C (outlet temperature approximately 45 ± 2 °C). The dry NCMPs were collected from the cyclone (Büchi Labortechnik) and stored in a desiccator at room temperature until further use.