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Research paper

Microspheres containing lipid/chitosan nanoparticles complexes for pulmonary delivery of therapeutic proteins

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Abstract

Chitosan/tripolyphosphate nanoparticles have already been demonstrated to promote peptide absorption through several mucosal surfaces. We have recently developed a new drug delivery system consisting of complexes formed between preformed chitosan/tripolyphosphate nanoparticles and phospholipids, named as lipid/chitosan nanoparticles (L/CS-NP) complexes. The aim of this work was to microencapsulate these protein-loaded L/CS-NP complexes by spray-drying, using mannitol as excipient to produce microspheres with adequate properties for pulmonary delivery. Results show that the obtained microspheres are spherical and present appropriate aerodynamic characteristics for lung delivery (aerodynamic diameters around 2–3 μm and low apparent tap density of 0.4–0.5 g/cm^3). The physicochemical properties of the L/CS-NP complexes are affected by the phospholipids composition. Phospholipids provide a controlled release of the encapsulated protein (insulin), which was successfully associated to the system (68%). The complexes can be easily recovered from the mannitol microspheres upon incubation in aqueous medium, maintaining their morphology and physicochemical characteristics. Therefore, this work demonstrates that protein-loaded L/CS-NP complexes can be efficiently microencapsulated, resulting in microspheres with adequate properties to provide a deep inhalation pattern. Furthermore, they are expected to release their payload (the complexes and, consequently, the encapsulated macromolecule) after contacting with the lung aqueous environment.

Keywords: Chitosan nanoparticles; Dry powders; Microspheres; Phospholipids; Pulmonary delivery; Spray-drying

1. Introduction

The pulmonary administration of therapeutic macromolecules is currently receiving increased attention, and the design of adequate carriers appears as the limiting factor to succeed. In this respect, microspheres have been proposed, since they can be tailored to exhibit appropriate aerodynamic properties [1] and they should possess a very narrow range of aerodynamic diameter, usually accepted to vary between 1 and 5 μm, although some authors restrict this range to 2–3 μm [1–4].

Liposomes have been presented as an interesting alternative for administration of biomolecules through several mucosal surfaces [5], since they are versatile and tend to be relatively innocuous (produced with natural and biodegradable compounds), and also provide protection to the encapsulated material [3,6–8]. Their organised structure (an aqueous core encapsulated within one or more phospholipid bilayers) permits the association of drugs to both the aqueous and lipid phase and drug release can usually be controlled, depending on the bilayers number and composition [3,6]. In order to achieve an improved controlled release, the incorporation of a drug-loaded vesicle inside a second vesicle, the encapsulation of particulate matter inside lipid vesicles, or even the adsorption of lipid bilayers onto polyelectrolyte-coated capsules have been reported [9–13]. Moreover, we have recently reported the preparation of, a new drug delivery

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system consisting of lipid/chitosan nanoparticles (L/CS-NP) complexes, intended for gastric delivery as a first approach that permitted the protection of chitosan nanoparticles from the acidic gastric environment [14]. Similar structures have been reported as promising carriers in biotechnology, as drug, antigen or gene delivery systems [15–18].

The application of liposomes has been suggested for sustained lung release of several drugs and their interaction with the endogenous phospholipids was proposed as a contribution to the prolonged retention of peptides within the lung. Furthermore, enhanced drug absorption provided by phospholipids similar to those composing the pulmonary surfactant was also reported, although the mechanism of absorption enhancement is still unknown [19–21]. One of the major problems concerning the pulmonary administration of particulate systems is the rapid capture by the alveolar macrophages [3,22], a process known to be affected by several factors such as particle size, surface properties, composition and local concentration [23–25]. In this respect, the macrophagic capture of PLGA microparticles upon interaction of microparticles with alveolar macrophages in culture was reported to be reduced by the inclusion of phosphatidylcholine, -serine and -ethanolamine in the formulation [26].

Nanoparticles, which can be produced with a wide variety of polymers and nanotechnologies [27,28], have also been recently proposed as delivery systems for peptides and proteins through the pulmonary route [29–32]. In this respect, chitosan is a very attractive polysaccharide due to its reported low toxicity, biodegradability and mucoadhesivity [33–35]. In fact, chitosan has been demonstrated to induce low or absent toxicity in cell lines representative of the pulmonary route (16HBE14o- and Calu-3) [36,37]. Our group has introduced the preparation of chitosan/tripolyphosphate (CS/TPP) nanoparticles by an extremely mild and rapid ionotropic gelation procedure between chitosan and its counterion TPP [38], which show an excellent capacity for protein association (as high as 95%), as well as an improvement of peptide absorption through several epithelia, such as the nasal, ocular and intestinal [39–42]. Furthermore, we recently reported the production of microspheres as carriers for protein-loaded chitosan nanoparticles to the lung, with the aim of improving their aerosolisation patterns. These nanoparticle-loaded microspheres, obtained by spray-drying a suspension of nanoparticles in mannitol, exhibited adequate aerodynamic properties for lung delivery [32] and demonstrated to be biocompatible with respiratory epithelial cell layers (Calu-3 and A549) [43].

The spray-drying of liposomes has been reported to not compromise their stability [44], and a work on the spray-drying of solid lipid nanoparticles demonstrated that the presence of carbohydrates like mannitol, lactose and trehalose provided an increased stability to the spray-dried product, because the sugar layer around the particles prevented the lipids coalescence [45].

In this work, the production of microspheres containing lipid/chitosan nanoparticles complexes, intended for the pulmonary administration of macromolecules, using a spray-drying technique is reported. For this purpose, mannitol, which is known for its non-toxic and degradable properties [2], was chosen as microencapsulation excipient and insulin as the model protein. Microspheres, aerodynamic properties were characterised, as well as their ability to deliver *in vitro* the lipid/nanoparticles complexes. Moreover, the effect of different lipid compositions on the complexes physicochemical characteristics and on the nanoparticles release profile was investigated.

2. Materials and methods

2.1. Chemicals

Chitosan (CS) in the form of hydrochloride salt (Protasan® 213 Cl, deacetylation degree: 86%, viscosity: 95 mPa) was purchased from FMC Biopolymer AS (Norway). Dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidyl glycerol (DMPG) were supplied by Lipoid (Germany). Pentasodium tripolyphosphate (TPP), glycerol, D-mannitol (Ma), phosphate buffered saline tablets (PBS) pH 7.4 and bovine insulin were supplied by Sigma Chemicals (USA). Ultrapure water (MilliQ Plus, Millipore Ibérica, Spain) was used throughout. All other chemicals were of reagent grade.

2.2. Preparation of chitosan nanoparticles (CS-NP)

CS-NP were prepared according to the procedure developed by our group, based on the ionotropic gelation of CS with TPP, in which the positively charged amino groups of CS interact with the negatively charged TPP [38]. Briefly, CS and TPP were dissolved in purified water in order to obtain solutions of 1 mg/mL and 0.42 mg/mL, respectively. The spontaneous formation of nanoparticles occurred upon incorporation of 1.2 mL of the TPP solution in 3 mL of the CS solution (final CS/TPP ratio of 6:1 (w/w)), under mild magnetic stirring (Plate A-13 Serie D, SBS, USA) at room temperature.

The insulin loaded CS-NP were obtained following the protein dissolution in NaOH 0.01 M (0.9 mg insulin/0.6 mL NaOH) and its consequent incorporation in the TPP solution (pH 11.6; 0.6 mL TPP solution + 0.6 mL insulin solution). The insulin concentration in the TPP solution was calculated in order to obtain CS-NP with a theoretical content of 30% (w/w) insulin respective to CS.

CS-NP were concentrated by centrifugation at 16,000*g* on a 10 μL glycerol bed for 30 min at 15 °C (Beckman Avanti 30, Beckman, USA). The supernatants were discarded and nanoparticles were re-suspended in 100 μL of purified water.

CS-NP were also prepared on a large scale, adding 12 mL of the TPP solution to 30 mL of the CS solution and maintaining the stirring conditions. They were