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Carrier-based dry powder formulation for nasal delivery of vaccines utilizing BSA as model drug

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ABSTRACT

Dry powder formulations for nasal vaccine delivery offer versatile advantages compared to liquid formulations, such as increased storage stability and simplified administration. The objective of the present study was the development of a dry powder nasal vaccine formulation making use of antigen-loaded chitosan microparticles. Special emphasis was put on the development and characterization of a formulation which can realistically be used in humans by means of a nasal dry powder sprayer. Microparticles of chitosan with bovine serum albumin as model antigen were produced by spray drying and showed a particle size of about 3 μm. In order to improve nasal deposition and dispersibility, powder blends with low separation tendency were prepared. A range of sugar alcohols (mannitol, sorbitol, maltitol) was evaluated as carriers. Among the tested carriers, blends with spray-granulated mannitol showed the most adequate deposition profile without simulated nasal inspiration and were very well dispersible by a nasal dry powder device. In conclusion, this study illustrates a simple and effective strategy for the development of a dry powder vaccine formulation for nasal administration.

1. Introduction

Vaccination has been one of the most successful inventions in the prevention and amelioration of diseases like smallpox, measles and polio [1], but there are still infections where the development of vaccines is desperately needed such as HIV [2]. Increasing interest is also put on the development of therapeutic vaccines in the field of cancer therapy [3,4] and autoimmune diseases [5]. Up to today, vaccines are mainly administered parenterally, which limits the variety of dosage forms to injectable sterile liquids [6]. This has two main disadvantages: parenteral administration requires medical personnel for administration, who are moreover always at risk for needle-stick injuries and infections [7,8]. Further, liquid formulations of biopharmaceuticals often face stability problems because they constantly encounter stress due to changes in temperature, pH or salt concentration [9]. Hence, it is challenging to formulate a stable liquid preparation and these formulations frequently require a cold chain. These disadvantages arise the need for vaccine preparations which are easy and safe to administer and stable without continuous cold chain. Easy and safe administration, possibly by the patient himself, is achieved by the use of alternative administration routes such as mucosal administration. Targeting the mucosal route for vaccination aims at using the same route as many pathogens when entering the body [10]. Hence, mucosal epithelia are well equipped with immune competent cells and vaccination via the mucosa associated lymphoid tissue (MALT) does not only elicit systemic immunization, but also a local immune response including the secretion of immunoglobulin A (sIgA) which can neutralize pathogens at a very early stage of infection [11]. Formulation-wise, mucosal vaccines are not limited to sterile liquids, but can be extended to solid dosage forms which allow increased thermo stability [12]. Therefore, special interest has been put on the use of micro- or nanoparticulate carrier systems for the formulation of antigens as they may protect the antigen, allow controlled delivery and release and can be functionalized on the surface to enhance uptake and immune recognition [13,14].

In this work, a dry powder microparticulate formulation for nasal administration has been developed with chitosan as matrix and bovine serum albumin (BSA) as model antigen utilizing a spray drying step. The approach employs spray dried chitosan microparticles to enable protein delivery to the nose as dry powder by a nasal dry powder device. BSA is not a realistic antigen for vaccination, thus it has only been employed as model in this study as used oftentimes in literature [15–18]. Spray drying is a comparably moderate process which has its benefits in the usability for sensitive molecules such as proteins. Chitosan has already been studied for the preparation of micro- and nanoparticles serving as antigen carriers in nasal and inhalative vaccine formulations [19–21]. The biopolymer is biodegradable and shows an adjuvant effect boosting the immune reaction elicited by the antigen [22]. Further, chitosan has mucoadhesive properties which have shown to be beneficial to prolong nasal residence of the formulation and mediate intense interaction between particles and epithelium [23,24]. Chitosan is a perfect material for the preparation of particulate antigen formulations for mucosal delivery, especially due to its pH-dependent solubility. This

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renders chitosan insoluble at physiological pH as present on the nasal epithelium. Hence, particles can interact and be taken up from dendritic cells or in the lymphoid structures of the nasopharynx as insoluble particle. This is an important prerequisite for local processing of antigens following mucosal delivery and in turn is needed to obtain the favorable local immune response. Upon cellular uptake particles may get into endosomal compartments where they face a decrease in pH which would mediate particle dissolution and release of incorporated protein. Formulation development includes preparation of a dosage form which can be administered via a nasal device to allow deposition in the nasal cavity and mediate uptake via the nose associated lymphoid tissue (NALT). Microparticles (MP) of a size below 5 μm are suitable for uptake by immune cells [25] but are also in the respirable size range. As first experiments revealed excellent aerodynamic behavior, a second step to prepare interactive mixtures with low separation during dispersion was included in the formulation approach to ensure deposition in the nasal cavity upon nasal administration as determined in a nasal cast model.

2. Materials and methods

2.1. Materials

Bovine serum albumin was purchased from Merck (Merck KGaA, Darmstadt, Germany) and chitosan (Chitoclear FG 95) having a degree of deacetylation of >95% and a molecular weight of about 102 kDa was purchased from Primex (Primex, Siglufjordur, Iceland). Following sugar alcohols were selected as carriers: mannitol (Pearlitol 200 SD and Pearlitol 160 C, Roquette, LeStrem, France), maltitol (SweetPearl P90, Roquette, LeStrem, France; C*Maltidex, Cerestar AG, Krefeld, Germany) and sorbitol (C*PharmSorbidex, Cargill GmbH, Krefeld, Germany; Neosorb P 100 T, Roquette, LeStrem, France). Mean particle size, shape and surface characteristics are summarized in Table 1.

2.2. Delivery device

To disperse the formulations, the PowderJet (RPC Formatec, Mellrichstadt, Germany), a novel nasal dry powder device, was used (Fig. 1). It is a multi-dose reservoir system which is intuitive to use and which is equipped with an innovative metering and ejection mechanism with automatic recharging.

2.3. Experimental methods

2.3.1. Preparation of microparticles by spray drying

Chitosan (1% *w*/*w*) and BSA (0.3% *w*/*w*) were dissolved in 0.01 N hydrochloric acid (HCl) at pH 2.6. The solution was spray dried through a 1.2 mm nozzle using a Büchi B-290 laboratory spray dryer (Büchi, Flawil, Switzerland) equipped with a high performance cyclone at an inlet temperature of 120 °C (outlet: 50–53 °C). Microparticles (MPs) were then stored in a desiccator until further use.

2.3.2. Preparation of powder blends

The carrier materials were sieved on a laboratory sieve shaker (Retsch GmbH & Co. KG, Haan, Germany) using 90 and 45 μm analytical sieves and a fraction of 45–90 μm was used for preparation of powder blends. For this, the carrier material and 2%, 5% and 10% microparticles, respectively, were weighted into small plastic vessels using the sandwich method (batch size 2.0 g). For blending the vessel was placed in a Turbula mixer (W.A. Bachofen, Switzerland) for 3 × 5 min with sieving steps in-between (sieve 300 μm).

For determination of homogeneity five samples of 50 mg were taken randomly from the powder blends. The average protein content and relative standard deviation (RSD) were calculated and powder blends were accepted for further experiments when the RSD was below 5%.

Table 1
Characteristics of carriers, n = 3.

Carrier	Sugar alcohol	Shape	x_{50} of 45–90 μm fraction ± SD, μm	Surface
Pearlitol 200 SD	Mannitol	Irregular	53.9 ± 2.0	Rough
Pearlitol 160 C		Irregular	54.3 ± 1.8	Rough
Neosorb P 100 T	Sorbitol	Spherical	65.3 ± 2.9	Needle-like
C*PharmSorbidex		Platelet	44.9 ± 4.3	Smooth
C*Maltidex	Maltitol	Oblong	77.8 ± 4.5	Smooth
SweetPearl P90		Irregular	28.3 ± 0.8	Smooth

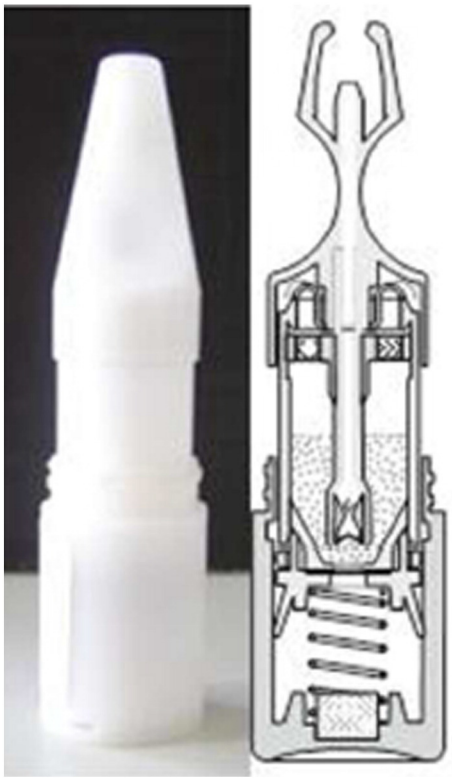

Fig. 1. The PowderJet, the dry powder reservoir nasal sprayer used to disperse the formulations: outer appearance (left) and schematic cross section (right).

2.3.3. Protein quantification

For quantification of protein, samples were dissolved in 0.01 N HCl. Chitosan was precipitated by a pH shift from 2.6 to 10 and removed by centrifugation (30 min, 6000 *g*). Protein content was then determined with a BCA-assay (Micro BCA-assay protein kit, Thermo Scientific, Rockford, IL, USA) using a standard calibration.

2.3.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was performed using a Mini Protean Tetra cell system (BIO-RAD Laboratories Inc., USA) with hand casted 10% polyacrylamide gels (90 × 70 × 1 mm). Samples were dissolved in 0.01 N HCl pH 2.6 and incubated with loading buffer containing 5% mercaptoethanol at 90 °C for 5 min. 10 μL sample volume was transferred to the gels resulting in 4 μg of BSA per lane. A molecular weight marker (PageRuler Prestained Protein ladder, Thermo Scientific, Rockford, IL, USA) and pure protein were used as reference. The electrophoresis was run for 50 min at 200 V. Gels were stained with Coomassie Brilliant Blue (Carl Roth GmbH + Co. KG, Karlsruhe, Germany) and destained with a mixture of acetic acid and isopropanol until protein bands were visible clearly.