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Preparation of coated nanoparticles for a new mucosal vaccine delivery system

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Abstract

It has been found that the adsorption of antigens onto chitosan particles is an easy and unique mild loading process suitable to be used with vaccines. In order to increase the stability of this particles and to prevent an immediate desorption in gastrointestinal fluids, a coating process with sodium alginate was developed. One of the challenges of this developing process was to keep the particles in the nanosized range in order to be taken up by M-cells of the Peyer's patches. The observed inversion of the particles' zeta potential values after coating suggested the presence of an alginate coating layer. These results were confirmed by FTIR and DSC techniques. Additionally, in vitro release studies showed that the presence of the alginate layer around the particles was able to prevent a burst release of loaded ovalbumin and to improve the stability of the nanoparticles in simulated intestinal fluid at 37 °C. The optimisation of the coating process resulted in 35% (w/w) for the loading capacity of the coated particles. SEM investigations confirmed a suitable size of the coated nanoparticles for the uptake by M-cells.

Keywords: Chitosan; Sodium alginate; Ovalbumin adsorption; Coated nanoparticles; Mucosal vaccination

1. Introduction

In recent years, mucosal vaccination is being considered as a subject of great interest due to its advantages above the i.m. or s.c. application. The presence of specific antibodies in mucosal surfaces has long been recognized as the first barrier against pathogens entrance. The most effective way to induce

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mucosal immunity (i.e., secretory IgA) is to administer a vaccine directly to the mucosal surface. Additionally, the existence of a common mucosal immune system allows successful targeting of vaccines to inductive compartments within mucosa-associated lymphoid tissues, inducing local humoral responses in lymphoid tissues at distant mucosal loci (Alpar et al., 1998). Both intranasal and oral routes have been used in several studies to achieve this goal. Particularly, the oral administration permits targeting of a suitable vaccine-loaded delivery system to the ports of entry (so-called M-cells) of the largest inductive lymphoid tissue in the body, the intestine. The oral route is well accepted and easily allows the vaccination of large populations. However, the acidic environment of the stomach and the presence of enzymes make the oral delivery of vaccines a challenge where is difficult to achieve high and reproducible effects. In order to solve these difficulties, a considerable number of polymeric microparticulate systems are under investigation to deliver vaccines to the intestine while protecting them from adverse conditions that could affect their bioactivity (Singh and O'Hagan, 1998). Another important aspect is that these delivery systems could act as imunostimulants or adjuvants, increasing the immunogenicity of poor immune response antigens (Jabbal-Gill et al., 1999; Singh and O'Hagan, 1999).

Nevertheless, from a pharmaceutical perspective, it became evident that further advances in the formulation of delivery platforms needs to be introduced in order to increase both the stability of the antigens in the gastro-intestinal tract and the uptake of antigen-containing particles by the M-cells. One of the parameters that should be addressed is the size of the particles. It is well known that the size of the particles should be below 10 μm in order to be taken up by M-cells of the Peyer's patches in the gut (Eldridge et al., 1991; Jani et al., 1992). Moreover, the preservation of antigen stability during encapsulation is also essential for the development of a successful controlled release vaccine delivery platform.

Chitosan microparticles as an oral and intranasal vaccine delivery system were already used in our group showing promising capabilities (Van der Lubben et al., 2001a,b, 2003; Bivas-Benita et al., 2003). In these studies, the vaccine was loaded by a mild and simple but effective adsorption method. By this method, deleterious preparation conditions, like elevated temperatures, high shear rates or the presence of organic solvents were avoided. This method has also been described by other groups that reported good adsorption capacities for different substances (Mi et al., 1999; Hejazi and Amiji, 2002). In the case that the chitosan particles are not very porous, the antigen will be preferentially adsorbed to the particle surface. This can cause stability problems because processes like desorption or the attack of the antigens by enzymes or acidic substances from the body fluids may occur. These obstacles may be overcome by coating those particles with an acid resistant polymer, like sodium alginate.

The two chosen polymers chitosan and sodium alginate, for this novel delivery platform are naturally occurring polysaccharides. They are polyelectrolyte polymers of opposite charges, biocompatible and biodegradable, and show a good safety profile. Furthermore they have been used as pharmaceutical excipients. Chitosan is the deacetylated form of chitin comprising copolymers of glucosamine and *N*-acetyl glucosamine linked by β-(1-4) linkages. The primary amino groups lead to special properties that make chitosan very interesting for pharmaceutical applications. Sodium alginate is also a hydrophilic polymer and comprises D-mannuronic (M) and L-guluronic acid (G) residues joined linearly by 1,4-glycosidic linkages (Johnson et al., 1997). The wide pharmaceutical applicability of alginates is, to a large extent, associated with their gel-forming capacity. Di- or polyvalent cations (calcium being the most widely studied example) can induce the gelation by cross-linking of the guluronic acid units (Rajaonarivony et al., 1993; Johnson et al., 1997). Sodium alginate has been used for preparing nanoparticles (Rajaonarivony et al., 1993; Gonzalez Ferreiro et al., 2002), microspheres (Wu et al., 1997; Fundueanu et al., 1999; Takka and Acarturk, 1999; Kulkarni et al., 2001; Chan et al., 2002; Coppi et al., 2002), microcapsules (Esquisabel et al., 2000) and beads (Kulkarni et al., 2001), for oral delivery. In particularly, the use of alginate microparticles as an antigen delivery system has been described in several publications and there are some indications that they are able to induce a mucosal and systemic immune response in a variety of animal species by both oral and intranasal administration (Cho et al., 1998; Bowersock et al., 1999; Rebelatto et al., 2001).

Over the last years, sodium alginate has also been used as a coating material for cells with some