ADJUVANT PROPERTIES OF MANNOSYLATED NANOPARTICLES FOR ORAL VACCINATION

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Introduction

The association of microorganisms' derived adhesive factors (i.e. flagellin, invasin) to the surface of nanoparticles has been considered as a promising strategy to increase their gut bioadhesive capacity and to enhance their capture by Peyer's patches [1,2]. In fact, many microorganisms express surface glycoconjugates enriched in mannose residues that can promote their interaction with mucosal tissue of the gastrointestinal tract [3]. This adhesive mechanism is mediated by the high affinity of mannose binding to the so-called Mannose-Binding Lectins (MBL) which are expressed on the enterocytes and lymphoid cells of the small intestine. For that reason, we have designed mannosylated nanoparticles (M-NP), and then investigated their mucosal bioadhesive properties and uptake by Peyer's patches. Finally, we have studied the applications of mannosylated nanoparticles as a non-live vector in oral vaccination strategy using ovalbumin-loaded M-NP.

Materials and methods

Materials

Gantrez® AN 119 (gift from ISP, Spain). Rhodamine B isothiocyanate (RBITC), 1,3 diaminopropane (DP) and Ovalbumin (grade V) and mannosamine were purchased from Sigma (Spain). All the other chemical reagents were supplied from Fluka (Switzerland).

Nanoparticles preparation and characterization

Gantrez® AN nanoparticles were prepared by a solvent evaporation method previously described [4]. Then, these nanoparticles were incubated with 5 mg mannosamine for 1 h, at RT. The resulting mannosylated nanoparticles (M-NP) were cross-linked by DP, purified by centrifugation, and finally lyophilized using sucrose (5%). Ovalbumin -loaded mannosylated nanoparticles (M-NP) were prepared by sonication of 5 mg OVA and 1 mg mannosamine in 5 mL of acetone containing 100 mg of Gantrez® AN . The polymer was desolvated by 10 mL ethanol, and then 3 mL water containing 5 mg mannosamine were added. The organic solvents were evaporated under reduced pressure, and the nanoparticle suspensions were incubated for 1 h and then purified described above. The resulting mannosylated nanoparticles (M-NP and OVA-M-NP) have been characterized by measuring the size, zeta potential, mannosamine loading, OVA encapsulation and concanavalin A (Con A) affinity.

Bioadhesion study

The bioadhesion study was carried out using a protocol described previously [4]. Briefly, 10 mg of the nanoparticles fluorescently labelled with RBITC were administered perorally to male Wistar rats fasted overnight. The animals were sacrificed at 0.5, 1, 3 and 8 h post-administration. Then, the gut was removed and divided into six anatomical regions: stomach (Sto), intestine (I1, I2, I3 and I4) and caecum (Ce). RBITC content was assayed in each segment by spectrofluorimetry. In parallel, the mucosal distribution of the nanoparticles and their uptake by Peyer's patches were visualized by fluorescence microscopy

Oral immunization study

A single dose of 100 µg OVA was orally administered in the form of either OVA-loaded mannosylated nanoparticles (OVA-M-NP) or free OVA solution. OVA-loaded uncoated nanoparticles (OVA-NP) were used as control. Systemic (IgG1 and IgG2a) and intestinal

mucosal (IgA) antibody responses were determined by ELISA till 6 weeks post-administration.

Results and discussion

Nanoparticles characterization

Mannosylated nanoparticles displayed a size of about 300 nm and loaded with approximately 35 µg mannosamine per mg nanoparticles. In vitro agglutination assay using Con A confirmed the successful coating of nanoparticles with mannosamine as well as the stability of that sugar on the surface of mannosylated nanoparticles after their incubation in simulated gut fluids.

Bioadhesion study

The bioadhesion profile described for M-NP displayed a higher bioadhesive capacity than non-mannosylated nanoparticles at 1 and 3 h post-administration (Fig.1). Interestingly, 1 h post-administration, M-NP clearly showed an important tropism to the ileum, in which around 25 % of the given dose was found adhered. Moreover, fluorescence microscopy corroborated the stronger interactions of M-NP with the normal mucosa and indicated their strong uptake by Peyer's patches (Fig.2).

Immunization study

After oral administration of a single small dose (100 µg) of OVA-loaded mannosylated nanoparticle (OVA-M-NP) a much higher and balanced systemic specific antibody response [IgG1 (Th2-response) and IgG2a (Th1-response)] were noted compared to OVA-NP. In addition, OVA-M-NP were able to elicit a significant intestinal secretory IgA (S-IgA) at least for 6 weeks.

Acknowledgements

This work received financial support from CICYT (grant SAF 2001-0690-C03), AECI (Agencia Española de Cooperación Internacional), Asociación de Amigos University of Navarra, "Fundación Roviralta" and "Fundación Universitaria de Navarra".

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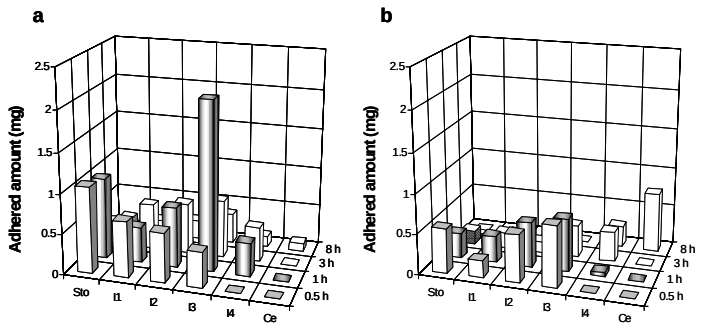

Figure 1. Bioadhesion study described the gut distribution of the nanoparticles formulations; M-NP (**a**) and control ones (**b**). The (*x*-axis) is the different gut segments; [Stomach: **Sto**; Intestine portions: **I1, I2, I3, I4**; Caecum: **Ce**], *y-axis* represents the adhered fraction of the nanoparticles in the mucosa (mg); (*z*-axis) represents the post administration time.

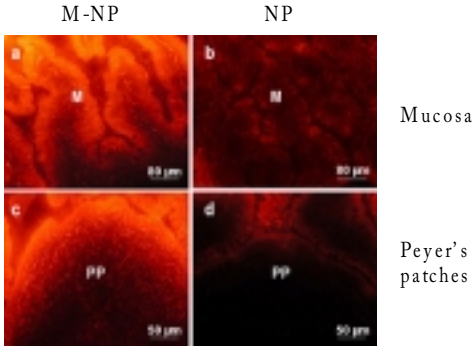

Figure 2. Visualization of M-NP and NP in the normal mucosal cells (M) and Peyer's patches (PP) of the ileum by fluorescence microscopy.