SALMONELLA-LIKE NANOPARTICLES FOR ORAL TARGETING

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Introduction

Mucoadhesive polymers and conventional nanoparticles display a low site-specificity within the gastrointestinal tract after their oral administration. In order to overcome this drawback, ligands (i.e. lectins and vitamin B12 derivatives) able to bind to specific receptors within the gut have been proposed to enhance the nanoparticles binding specificity [1, 2]. Therefore, the aim of this work was to design and evaluate a polymeric vector by the association between Gantrez® AN [poly (methyl vinyl ether-co-maleic anhydride)] nanoparticles and flagellin-enriched *Salmonella* Enteritidis extract in order to obtain promising nanoparticles able to mimic the *Salmonella* invasion and colonization in the gastrointestinal tract.

Materials and methods

Materials

Gantrez® AN 119 was gifted from ISP (Spain). Rhodamine B isothiocyanate (RBITC), 1,3 diaminopropane and fluorescein isothiocyanate (FITC) were purchased from Sigma (Spain). All the other chemical reagents were supplied from Fluka (Switzerland). *Salmonella* extract enriched in flagellin was prepared according to a method previously described [3].

Nanoparticles design and characterizations

Gantrez nanoparticles containing the *Salmonella* extract enriched in flagellin (SE-NP) were prepared by solvent evaporation method [1], and then fluorescently labelled with RBITC. SE-NP were characterized by determining the size, zeta potential, yield of the nanoparticles' fabrication process, RBITC content and flagellin loading.

Bioadhesion study

The bioadhesion study was carried out using a protocol described previously [1]. Briefly, an aqueous suspension containing 10 mg of the nanoparticles fluorescently labelled with RBITC were administered perorally to male Wistar rats fasted overnight. The animals were sacrificed by cervical dislocation 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). Each mucosa segment was opened lengthwise, rinsed with PBS and digested with NaOH 3 M for 24 h. RBITC content was assayed by spectrofluorimetry.

Competitive tissue distribution study

In order to investigate the specificity of adhesive interactions, competition studies between SE-NP and *Salmonella* cells were performed. For this purpose, rats were orally administered with both 10 mg SE-NP and 5 mg of fluorescently-labelled *Salmonella* (FITC-SAL) previously dispersed in 1 mL water. In the first set of experiments, both formulations were mixed and administered together. In the second one, SE-NP was administered 30 min before FITC-SAL. In all cases, the animals were sacrificed 2 h post-administration and the ileum was removed and washed with PBS. Then, sections of 5 μm were cut and examined by confocal laser scanning microscopy.

Results and Discussion

Nanoparticles characterization

The yield of the nanoparticles was of about 70%. The resulting SE-NP have a homogeneous size of about 280 nm containing about 14.5 µg flagellin/mg nanoparticles.

Bioadhesion study

Figure 1 describes the distribution of the adhered amounts of nanoparticles in the gut mucosa as a function of time. From these results it is clear the higher ability of SE-NP to adhere within the gut when compared with control nanoparticles. In addition, SE-NP appeared to show an important tropism for the proximal ileum (I3 portion). In fact, about 30% of the given dose was found in this I3 region 1-h post administration. Similarly, this high ability of SE-NP to target the ileum was also observed 3 h post administration ($P > 0.05$).

Competitive tissue distribution study

Visualisation of FITC-labelled *Salmonella* by fluorescence green spots (Fig. 2a, b) confirmed the interaction of the bacteria with the enterocytes, their colonization in the tissue of the ileum (Fig. 2a) and uptake by Peyer's patches (Fig. 2b). When SE-NP and FITC-SAL were administered together by the oral route, both the bacteria and the nanoparticles displayed a similar distribution within the intestinal mucosa (Fig. 2c). However, the ability of SE-NP to be taken up by Peyer's patches appeared to be negatively affected by the presence of the bacteria (Fig. 2d). When SE-NP was administered by the oral route 30 min before FITC-SAL, both bacteria and nanoparticles were observed in the ileum mucosa (Fig. 2e) with a similar distribution as described before. However, in the Peyer's patches tissue, SE-NP were found broadly distributed, whereas the bacteria were neither able to adhere to nor penetrate this lymphoid tissue (Fig. 2f).

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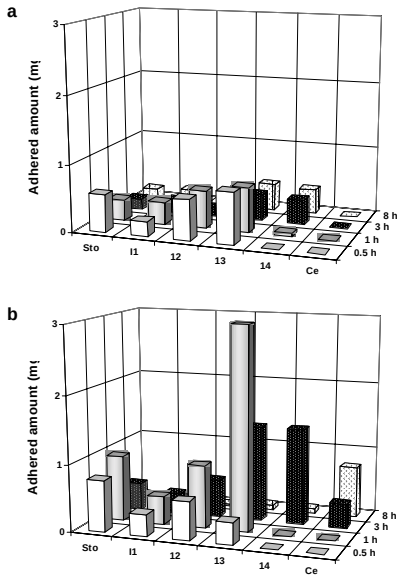

Figure 1. Distribution of nanoparticles formulation in the gastrointestinal tract of rats after the oral administration of 10 mg RBITC-loaded nanoparticles at different time intervals. (**a**) Control nanoparticles, C-NP; (**b**) Flagellin-loaded nanoparticles, SE-NP

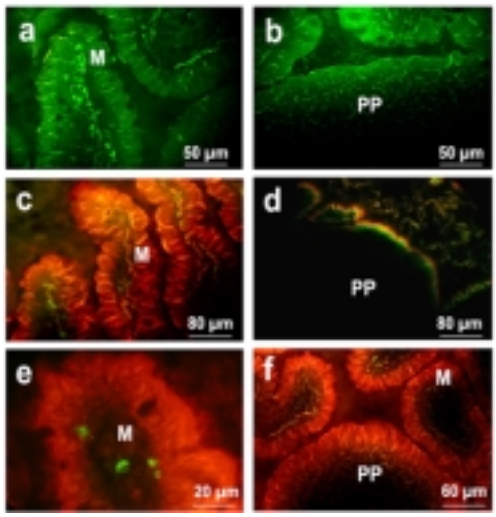

Figure 2. Competitive tissue (ileum) distribution between both of SE-NP and FITC-SAL visualized by fluorescence microscopy (FM) and confocal laser scanning microscopy (CLSM). FM for FITC-SAL in the mucosa and in Peyer's patches respectively when administrated alone (**a,b**). CLSM for both SE-NP and FITC-SAL in the mucosa and in Peyer's patches respectively when administrated together (**c,d**). CLM for both NP-SE and FITC-SAL in the mucosa and Peyer's patches respectively, when NP-SE were administrated 30 min before the FITC-SAL (**e,f**). (**M**: Mucosa; **PP**: Peyer's patches).