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Human serum albumin as a potential nanocarrier for the oral delivery of therapeutic proteins

Pangua Irigaray Cristina*[1], Espuelas Millán Socorro[1], Martínez Ohárriz Cristina[2], Irache Garreta Juan Manuel[1]

[1] Pharmaceutical Technology and Chemistry Department, University of Navarra.
[2] Chemistry Department University of Navarra.
*Correspondencia: cpangua@alumni.unav.es

1. Introduction

The oral delivery of therapeutic proteins faces different barriers (pH, enzymes, peristalsis, mucus, etc.) that highly hampers their stability and absorption [1].

In addition, protein drugs have poor oral bioavailability due to their large size and physicochemical characteristics. In order to solve these drawbacks, one alternative may be the combination of nanocarriers with permeation enhancers in order to promote the bioavailability of these drugs. Within this aim, particularly interesting are those permeation enhancers with ionizable groups that may interact with hydrophobic molecules (i.e., proteins) by hydrophobic ion pairing (HIP) resulting in hydrophobic complexes [2]. Furthermore, the encapsulation of these complexes into nanoparticles may protect the loaded protein from its premature degradation and facilitate its release in the epithelium surface.

In this context, the aim of this work was to design nanocarriers capable of encapsulating lipophilic complexes of a model protein with hydrophobic counter ions and reaching the surface of the intestinal epithelium.

2. Materials and Methods

2.1. Hydrophobic ion-pairing

The hydrophobic ion pairing (HIP) complexes were prepared by mixing the aqueous solution of a model protein (MP) with two different counterions, sodium deoxycholate (DS) and sodium docusate (DOCU). To obtain the highest complexation efficiency (CE%), different protein-to-counterion molar ratios and pH conditions were evaluated. The dissociation of the complexes in gastric and intestinal simulated fluid, as well as the solubility of HIP complexes in aqueous media were also studied.

2.2. Preparation of nanoparticles

The HIP complexes were encapsulated in human serum albumin (HSA) nanoparticles coated with poly(ethylene glycol) 35,000 (PEG) following a desolvation method [3]. The physicochemical characterization of the nanoparticles was evaluated by measuring the mean particle size and zeta potential. The model protein quantification was measured by microfluidic electrophoresis.

2.3. Ex vivo mucus diffusion evaluation

In order to evaluate the mucus permeating properties of the resulting nanocarriers, their diffusion in pig intestinal mucus was determined by Multiple Particle Tracking (MPT).

2.4. In vivo biodistribution evaluation

The biodistribution of the nanoparticles labeled with a fluorescent marker (Lumogen Red) was evaluated in rats. For this purpose, four hours after the oral intake of the nanoparticles, slices of different portions of the gastrointestinal tract were obtained and visualized by fluorescence microscopy.

3. Results and discussion

3.1. HIP characterization

HIP complexes between the model protein and either DS or DOCU were successfully formed at a ratio of either 1:200 or 1:150 respectively. FTIR spectrum confirmed the formation of ion pair bonds between the protonable aminoacids of the protein and the negative ionized groups of the counterions. In all cases the solubility of the complexes in water was lower than 10 %.

3.2. Nanoparticles characterization

These complexes were successfully loaded in human serum albumin nanoparticles (Table 1). For DS-containing nanoparticles (DS-NPA), the mean size was of about 255 nm and the zeta potential was close to -30 mV. For nanoparticles containing the HIP complex with DOCU (DOCU-NPA), the resulting nanocarriers displayed a mean size of 330 nm and a negative zeta potential of -26 mV. In both cases, the encapsulation efficiency of the protein complex was higher than 70 %.

Table 1. Physicochemical characterization of nanoparticles. Data expressed as mean ± SD (n=3).

	Particle size (nm)	PDI	Z Potential (mV)	EE (%)
NPA	260	0.13	-28	70
DOCU-NPA	330	0.19	-26	72
DS-NPA	255	0.13	-29	90

3.3. *Ex vivo mucus diffusion of nanoparticles*

The capability of nanoparticles to diffuse in pig mucus was evaluated by MPT (Figure 1). Compared with control nanoparticles (NPA), DS-NPA displayed a similar ability than their control nanoparticles to diffuse in mucus. On the contrary, the relative diffusion of DS-NPA was significantly higher than for DOCU-NPA.

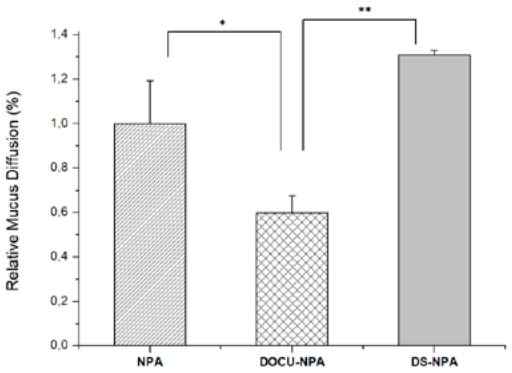

Fig 1. Relative diffusion of nanoparticles through pig intestinal mucus.

3.4. In vivo biodistribution of nanoparticles

After administration to rats, both DS-NPA and DOCU-NPA were capable of reaching the epithelium surface of the distal small intestine (Figure 2). Nevertheless, this ability to reach the epithelium appeared to be higher for DS-NPA than for DOCU-NPA.

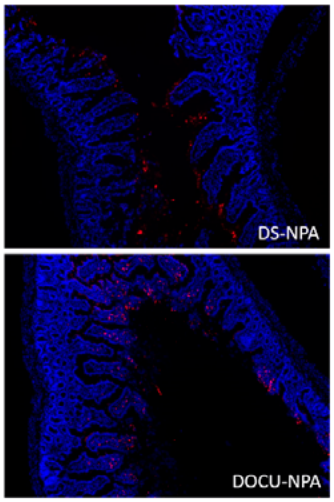

Fig 2. Fluorescence microscopy images of jejunum 4 hours after the oral administration of DS-NPA and DOCU-NPA.

4. Conclusions

In summary, albumin-based nanoparticles can adequately incorporate lipophilic complexes of a model protein. The resulting nanocarriers displayed mucus-permeating properties and the capability of reaching the surface of the distal intestinal epithelium. All together may be of interest to promote the oral bioavailability of therapeutic proteins.

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References

1. Muheem A, Shakeel F, Jahangir MA, Anwar M, Mallick N, et al. A review on the strategies for oral delivery of proteins and peptides and their clinical perspectives. Saudi-Pharm J. 2016;24:413-28.
2. Cao J, Wang H, Ling Y, Dong J, Xia R, Sun X. Nanoparticles: Oral delivery for protein and peptide drugs. AAPS PharmSciTech. 2019;20:190.
3. Luis de Redín I, Boiero C, Martínez-Ohárriz, C, Agüeros M, Ramos R, et al. Human albumin nanoparticles for ocular delivery of bevacizumab. Int J Pharm. 2018;541:214-23.

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