GANTREZ AN NANOPARTICLES ASSOCIATED TO POLYETHYLENEGLYCOL FOR ORAL DRUG DELIVERY

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Introduction

The oral route is one of the preferred ways for drug delivery. However, a large amount of drugs remain poorly available when administered orally, due to their low water solubility, slow dissolution rate and instability in the gastrointestinal tract. To solve these problems, one of the most interesting approaches would be the use of pegylated nanoparticles. Gantrez® AN (PVM/MA) was considered as a nanoparticle carrier due to its low toxicity and excellent biocompatibility and has been proposed to prepare bioadhesive nanoparticles for oral drug delivery (1). On the other hand, pegylation of the nanoparticles may increase the drug loading and modify their gut bioadhesion capabilities (2). However, the main difficulty of the pegylation strategy is the preparation of nanoparticles with a stable PEG-attachment.
In the present study PEGs with different molecular weights (2000, 6000 and 10000 Da) were examined aiming to optimize pegylation degree of the resulting nanoparticles. In addition, the bioadhesive potential of pegylated nanoparticles was evaluated.

Experimental methods

NPs preparation and characterization

Pegylated nanoparticles were prepared by two different procedures based on a modification of the solvent evaporation method. The first procedure consisted of simultaneous dispersion of PEGs in Gantrez® AN 119 organic solution of acetone. In the second process, PEG was incubated with the pre-formed particles. Then the polymers were desolvated with an ethanol/water mixture and the resulting nanoparticles were purified by ultracentrifugation. For bioadhesion studies, nanoparticles were loaded with rhodamine B-isothiocyanate (RBTIC).
Pegylated nanoparticles were characterized by measuring the size, zeta potential, morphology and the amount of PEG associated to the nanoparticles.

In vivo bioadhesion studies

A single dose of 1 ml aqueous suspension containing 10 mg of pegylated nanoparticles or control ones (NP) were orally administered to male Wistar rats. The animals were sacrificed by cervical dislocation at different time points post-administration. Then, the stomach, small intestine and caecum were removed and divided into segments of 2 cm length and digested in 1ml 3N NaOH for 24 h. The RBITC was extracted with methanol and the amount of adhered nanoparticles to the mucosa was assayed by spectrofluorimetry.

Results and discussion

Nanoparticles characterization

The first interesting thing was that, independently on both the MW and concentration of PEG used, higher pegylation degree was always achieved with the incubation between PEG and Gantrez before nanoparticle formation than PEG incubated with pre-formed nanoparticles.
Table 1 summarises that, in all cases, pegylated nanoparticles displayed a similar and homogeneous size of about 150 nm. On the contrary, little differences in the negative zeta-potential of all types of nanoparticles were found.

The association of PEGs to the nanoparticles was dependent on their molecular weight demonstrating higher binding efficiency for PEG 10000 and PEG 6000 compared to PEG 2000.

Table 1. Physico-chemical characteristics of pegylated nanoparticles (mean ± SD, n = 6). Pegylation was carried out with 25 mg PEG.

	Size (nm)	**Zeta potential (mV)**	**Associated PEG (%)**
NP	164.8 ± 9.9	- 43.2 ± 7.1	-
NP2	143.1 ± 6.8	- 49.5 ± 7.7	5.65
NP6	140.7 ± 7.4	- 49.8 ± 2.2	13
NP10	151.4 ± 9.3	- 50 ± 2.06	12.51

In vivo bioadhesion studies

Figure 1 shows the amounts of NP2 and NP formulations in the stomach, intestinal segments and caecum at 0.5, 1, 3 and 8 h after the oral administration of an aqueous dispersion containing 10 mg nanoparticles. NP displayed an initial tropism for the stomach mucosa and the upper regions of the small intestine (Figure 2) which rapidly decreased after 3h. On the contrary, PEG-NP demonstrated longer and more homogenous distribution within the gut with a well pronounced preference to adhere to the distal parts of small intestine. In addition, in vivo studies revealed higher bioadhesive intensity of the pegylated nanoparticles in comparison with the conventional ones – e.g. 1.6-fold increase of AUC_{adh} for the particles modified with PEG 2000. The data also showed longer gastrointestinal residence of the pegylated nanoparticles (MRT_{adh} of about 3.3 h) compared to the non-pegylated (2.77 h). In addition, pegylated nanoparticles have preferably demonstrated high affinity to the small intestine wall during almost 8 hours.

In summary, pegylated nanoparticles possess specific intestinal bioadhesion properties making them suitable carriers for application of drug molecules with poor oral bioavailability.

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References

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Figures

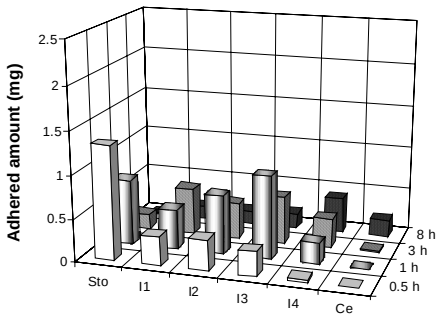

Figure 1. Gut distribution of the PEG-NP2 prepared with 25 mg PEG 2000. The (*x*- axis) is the different gut segments; [Stomach: **Sto**; Intestine portions: **I1, I2, I3, I4**; Caecum: **Ce**], (*y-axis*) represents the adhered fraction of the nanoparticles in the mucosa (mg); (*z*-axis) represents the post administration time.

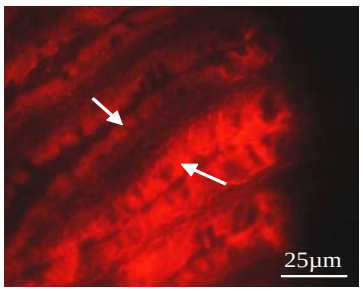

Figure 2. Visualization of the NP in the gut mucosa was performed by fluorescence microscopy. The photographs revealed an intensive localization of RBITC-loaded PEG-NP into the intestinal enterocytes.