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Caenorhabditis elegans as a model under hyperglicemic conditions to evaluate insulin-loaded nanoparticles

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1. Introduction

The oral bioavailability of peptides therapeutics (e.g. insulin) is hampered by both the harsh gastrointestinal environment and poorly penetrating of biological barriers such as mucus layer. In order to solvent these issues and thus enhance the oral absorption of these macromolecules, one possible strategy may be their encapsulation into polymeric nanoparticles with mucus-penetrating properties [1].

In this context, the aim of this work was to explore the potential of mucus-permeating nanoparticles based on the coating of zein nanoparticles (NP) with a polymer conjugate containing PEG (PPA), as carriers for the oral delivery of insulin. For this purpose, the resulting nanoparticles were characterized and their biodistribution and efficacy evaluated in rats and Caenorhabditis elegans (C. elegans), respectively.

2. Materials and Methods

2.1. Preparation of nanoparticles

Nanoparticles were prepared from a hydroalcoholic solution of zein and insulin, by desolvation with water. Then, nanoparticles were incubated with the polymer conjugate PPA, purified by tangential filtration and, finally, dried.

2.2. Characterization of zein nanoparticles

The mean diameter, polydispersity index (PDI) and zeta potential of nanoparticles were determined by photo correlation spectroscopy and electrophoretic laser Doppler anemometry, respectively, using a Zetaplus apparatus. The insulin loading and encapsulation efficacy in zein nanoparticles were calculated by RP-HPLC [1]. To evaluate the surface hydrophobicity of the NP the Rose Bengal test was carried out [2]. The diffusion of nanoparticles through pig intestinal mucus, as an in vitro measurement of their mucus-permeating properties, was assessed by Multiple Particle Tracking (MPT) technique [3].

2.3. Biodistribution of nanoparticles in the gastrointestinal mucosa

The fate of nanoparticles in the gut of male Wistar rats (average weight 225 g) was visualized by fluorescence microscopy.

2.4. In vivo evaluation using C. elegans

The therapeutic efficacy of oral insulin nanoparticles was evaluated in C. elegans grown under high glucose conditions (50 mM). For this purpose, the lifespan, oxidative stress responses, and lipid metabolism of the worms was assessed. The fat content determination in the worm was realized by the fixative-based Nile red method [4]. Intracellular ROS in C. elegans was quantified using the molecular probe H2DCF-DA [4] and the lifespan assay was monitored at 20 °C [5].

3. Results and Discussion

3.1. Characterization of nanoparticles

The influence of coating on the properties of nanoparticles is shown in Table 1. The incorpora-

tion of insulin led to a significant increase of the mean size of the nanoparticles ($p < 0.001$) while the coating with PPA significantly decreased the negative zeta potential of the nanoparticles ($p < 0.001$). The insulin loading was calculated to be about 75 μg/mg NP, with an encapsulation efficiency of 78 %. Besides, the coating with PPA conjugate modified the surface of zein nanoparticles, reducing their hydrophobicity (2.5-fold lower).

Table 1. Physico-chemical characteristics of empty (NP and PPA-NP) and insulin-loaded nanoparticles (I-NP and I-PPA-NP).

	Size (nm)	PDI	Z-potential (mV)	Insulin content (μg/mg)
NP	217	0.17	-45	-
I-NP	253	0.15	-42	74.33
PPA-NP	225	0.10	-34	-
I-PPA-NP	260	0.18	-32	76.68

The MPT studies also showed that PPA-NP exhibited not only much less obstructed Brownian motion in mucus compared to NP, but also 20-fold improved diffusion properties in porcine intestinal mucus (Figure 1). This greater diffusivity of PPA-NP in the mucus was corroborated in the biodistribution study with fluorescently labelled nanoparticles.

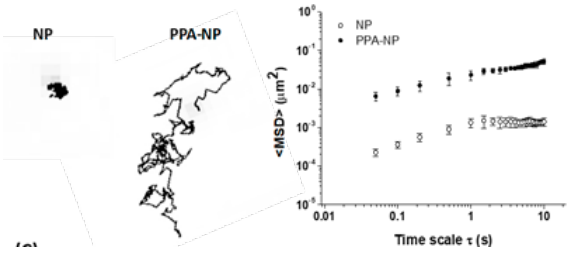

Fig. 1. Transport of bare and PPA-coated nanoparticles in porcine intestinal mucus.

3.2. In vivo evaluation using C. elegans

The in vivo efficacy of insulin-loaded nanoparticles was evaluated in C. elegans grown under high glucose conditions, comparable with the blood glucose levels observed in diabetic patients [5]. The high glucose diet shortened the lifespan of C. elegans, while insulin protected from damage by extending the lifespan of the worms (figure 2), also reducing intracellular glucose (46 %), ROS levels (44 %) and fat accumulation (25 %). The effect was significantly greater when insulin was nanoencapsulated in PPA-NP than in NP or formulated in solution. The effects involved daf-2, daf-16, sod-3 and skn-1, all of them genes that code for products implicated in the insulin-signaling pathway of C. elegans.

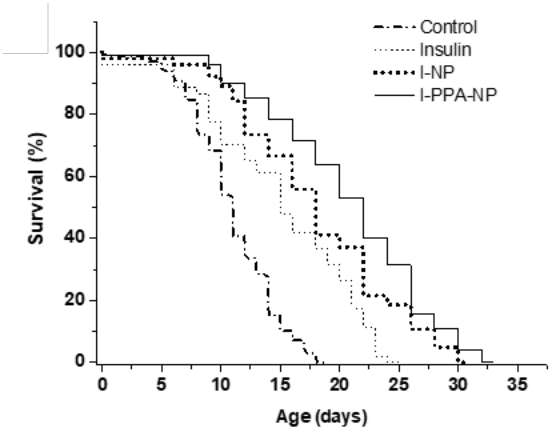

Fig. 2. Effect of insulin (free or encapsulated in bare or PPA-coated nanoparticles) on lifespan in C. elegans grown under high glucose conditions.

4. Conclusions

The coating of zein-based nanoparticles with a PPA conjugate resulted in mucus-permeating nanocarriers, without affecting their capability to encapsulate insulin. In the C. elegans model, this I-PPA-NP induced a significant reduction in the formation of ROS and the fat accumulated in the body. Besides, I-PPA-NP increased the lifespan of worms by interaction with at least DAF-2 receptor. These results suggest that C. elegans offers promising characteristics and valuable tools for evaluating oral insulin delivery systems before moving to more complex model organisms.

References

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