Elaboration of complex pharmaceutical vector for oral peptid delivery: application to diabetes.

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INTRODUCTION AND OBJECTIVES

General purpose

In therapy, most common way for administration of active compounds are oral, intravenous, intramuscular or subcutaneous injection. The most physiological and more comfortable for the patient is the oral way, however it can't be used for many drugs including peptide nature. Indeed, two major problems are met. First, peptidic drugs undergo many aggressions along the gastrointestinal tract (GIT) as pH and enzymes, which alter their structure and their biological function. Then, their passage through the intestinal wall is limited by their large size and hydrophilicity. This explains the low bioavailability of proteins administered orally (1 to 3%), (Pauletti 1996).

To answer to this, many strategies are proposed to encapsulate drugs. In this context, our team developed a pharmaceutical vector able to answer to these requirements. This vector is based on double encapsulation: first is constituted by nanoparticles (NPs) containing peptide which must promote its passage through the intestinal wall, and second is a vehicle containing NPs and protect them from gastric environment (Frère 2004).

Application of the complex pharmaceutical vector in diabetes

Diabetes is a global health scourge and represents the sixth leading cause of death worldwide. It is characterized by chronic hyperglycemia. To treat it, insulin, hypoglycaemic hormone, is administrated in diabetic patients by subcutaneously injection which are painful and constraint. To remedy this issue, our pharmaceutical vector can be applied.

The NPs can be synthesized from a variety of materials (polysaccharides, synthetic polymers) based on many factors as size, solubility, stability, charge, biocompatibility, toxicity (Kreuter 1994). In this study, NPs are synthesized from biodegradable polymers, poly (D, L-lactide-co-glycolide) acid (PLGA) by the method of the double emulsion and solvent elimination (Kompella 2001).

Vehicle, when to him, is formed from gel. It's obtained by gelation of alginate with a solution of divalent cation (Gombotz 1998) as calcium chloride. Alginate is a natural polysaccharide, non-toxic and biodegradable when given orally. It have been extensively studied for the oral delivery of active ingredients such as insulin sensitive (Gray 1988).

The aim of this study is to characterize NPs of insulin and small vehicle of alginate, alone or together. In order to administrate the whole vector in rats, the alginate vector must be miniaturized.

MATERIALS AND METHODS

Nanoparticles of insulin synthesis

NPs are synthesized by dissolving poly (D, L-lactide-co-glycolide) acid 50/50 from Boehringer Ingelheim in ethyl acetate and mixing it with Pluronic® F68 (BASF) dissolved in same solvent. Then an aqueous solution of insulin (Umuline®, Eli Lilly) is dispersed into polymers solution by ultrasonication (US). This first emulsion, a water/oil emulsion (w/o), is dispersed in a surfactant solution to obtain by US a second emulsion (w/o/w). This multiple emulsion is added to a diluted solution of surfactant used in the second emulsion and then the organic solvent is evaporated overnight by slow magnetic stirring (130rpm).

Alginate vector synthesis

Alginate vector is synthesized by an encapsulation unit VarJ30 (Nisco Engineering) equiped with a simple head. A dispersion of oil (Mygliol) containing NPs, in 2% of low molecular weight of alginate aqueous solution is sprayed at pressure of 50mbars for 5 minutes in solution of 2% of calcium chloride and then stirred at 150rpm for 1 hour.

Characterisation of NPs and vehicle

Granulometric analysis were done by dynamic light scattering in Zetasizer nano zs apparatus (Malvern Instrument) at 1/500 in ultra pure water with disposable cuvette for NPs and in LS 13320 (Beckman Coulter) for alginate vehicle. Zeta potential of NPs was determined in Zetasizer (1/100 in 1mmol/L of NaCl) in special cuvette. Three lectures of each sample are done and each condition is done in triplicate.

NPs were observed in TEM (Tecnai G2 sphera) and vehicle on basic optical microscope.

Resistance of alginate vehicle in cannulae

To administrate the whole vehicle in rat by oral way, resistance of alginate beads was tested through rigid dosing cannulae. For this, beads solutions was injected at different speeds through cannulae either automatically with syringe pumps (Leagto 200,

KDScientific) or manually. Size and aspect were analyzed by granulometric measurement and microscopic observations.

RESULTS AND DISCUSSION

NPs of insulin

By double emulsion and evaporation of ethyl acetate, NPs of PLGA/Pluronic® F68 with insulin, obtained, presented a size less than 200nm, a monodisperse population and a negative surface charge. TEM observation showed rounded NPs (Figure 1).

	NPs insulin
Size (nm)	184.2 ± 2.5
Polydispersity index	0.092
Zeta potential (mV)	-6.6 ± 3.3

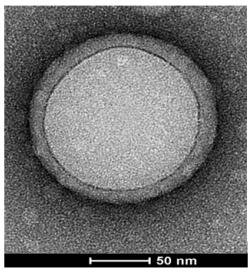

Figure 1: Characterisations of NPs insulin by granulometric test (left) and TEM (right).

Effect of NPs on biological activity

Results obtained with NPs insulin in diabetic rats show that encapsulated insulin is significantly more active than insulin alone at the same concentration (figure 2): normoglycemia is reached faster (9h).

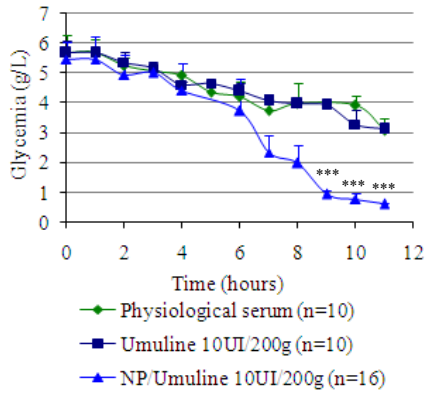

Figure 2: Effect of nanoparticles/Umuline synthesized with US and administrated by intra-duodenal injection on glycemia of diabetic rats.

Pharmaceutical vector

Alginate beads containing NPs in oil presented a size of 200µm and a rounded aspect (Figure 3).

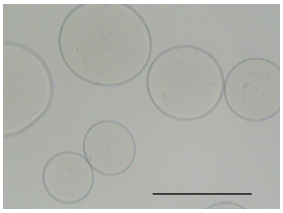

Figure 3: Miscroscopic observation of alginate vector (bar scale: 200µm)

Resistance of alginate vehicle in cannulae

Alginate beads injected manually or automatically at different speeds (low, medium or fast) through cannulae, presented the same aspect in microscopic observation and the same size of 200µm (figure 4).

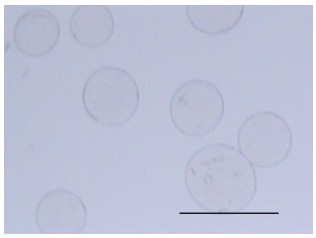

Figure 4: Miscroscopic observation of alginate beads through cannulae (bar scale: 200µm)

CONCLUSIONS

In this study, NPs obtained are functional and decrease glycemia of diabetic rats. To form our pharmaceutical vector, NPs were encapsulated in alginate vehicle. The vector presents a form and size expected and isn't destroyed or deformed through cannulae for rat gavage. Preliminary results of their stability in different media and their administration in diabetic rats are in progress. Glycemia is followed over time to validate the whole vector.

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