Double encapsulation of insulin: a new pharmaceutical vector for oral route

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INTRODUCTION

Diabetes, a chronic disease, can be defined as a loss of glycaemia control. Usually, insulin, a protein, prevents glycaemia from rising in the blood (glycaemia< 1.2 g/L). Among the various types of diabetes, the most known is the type 1 diabetes says insulino-depending, obliging the patients to inject, by subcutaneous way, some insulin and this, several times a day. It leads to the glycaemia decrease but entails a daily discomfort for patients. A most physiological way could be used to deliver insulin: the oral route.

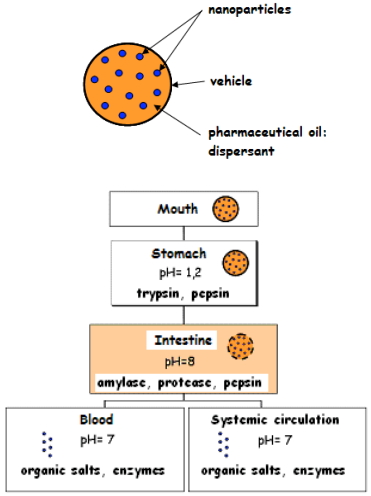

Figure 1: Becoming of our vector during its migration across the gastro-intestinal tract

In this optics, we elaborate a patented complex pharmaceutical vector (Frère Y., 2004) composed of three components by a double encapsulation technique: the nanoparticles are dispersed in pharmaceutical oil contained in a vehicle. The vehicle would carry insulin-loaded nanoparticles.
Our biodegradable vector prevents insulin-loaded nanoparticles migration in the gastro-intestinal tract. Its role, after the mouth, the oesophagus and the stomach crossing, is to be degraded in the intestine to liberate nanoparticles entrapping insulin (figure 1).
The lipophilic dispersant (pharmaceutical oil) that not only has to prevent the diffusion of insulin solution out of the nanoparticles during storage, but also to limit the migration of gastric juices during their passage in the stomach,
The nanoparticles can be hollow or full and have to protect insulin inside a biodegradable container and favor its administration under its biologically active form. These nanoparticles have to cross the intestinal barrier to reach blood and/or systemic circulation in order to liberate active insulin.

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MATERIALS AND METHODS

In this preliminary study, we focussed on the synthesis of hollow insulin-loaded nanoparticles according to a double emulsion process: type water/oil/water says W/O/W (figure 2). We use only biodegradable polymers (PLGA 50/50) and stabilizers (Pluronic F68, PVA 18-88) to form biocompatible nanoparticles for our biological application (Delle F. et al., 2005).

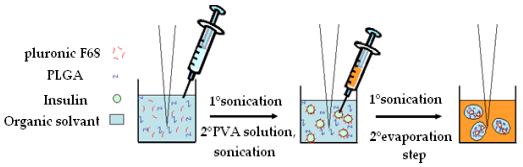

Figure 2: Process of synthesis of insulin-loaded nanoparticles

The W/O/W process is divided into three steps:
-the first dispersion (W/O) permits to make aqueous nanodroplets containing insulin dispersed in an organic phase with PLGA and stabilized by Pluronic F68,
-the second dispersion (W/O/W) permits to obtain aqueous nanodroplets containing insulin dispersed in PLGA nanodroplets stabilized by PVA 18-88,
-the final step consists in organic solvent elimination by an evaporation technique which leads to solid hollow insulin-loaded nanoparticles.

RESULTS AND DISCUSSION

The nanoparticles size was analysed by static light scattering and confirmed by microscopic observations (AFM, TEM and SEM). The average size of our nanoparticles is 160-190 nm in diameter. With this size (Desai M. P., 1996), the insulin-loaded nanoparticles should be able to cross the intestinal barrier (figure 3).

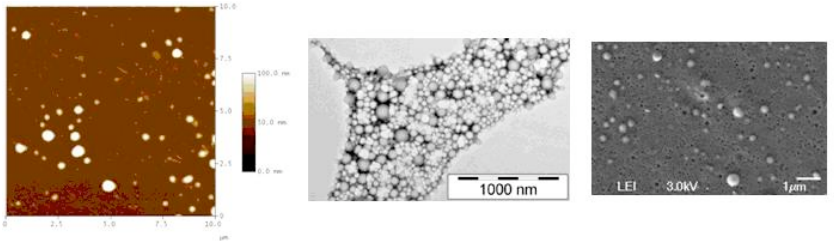

Figure 3: Analysis of PLGA insulin-loaded nanoparticles by AFM (A), TEM (B) and SEM (C)

To check if our carrier is effective, *in vivo* experiments were done on diabetic Wistar rats (streptozotocin-diabetic rat model). Different kinds of administrations (intravenous and intraduodenal) permit to reduce glycaemia of these animals.

It first confirms that insulin-loaded nanoparticles keep insulin active and then, that it permits its release in blood or in the systemic circulation to reduce glycaemia (figure 4 and 5).

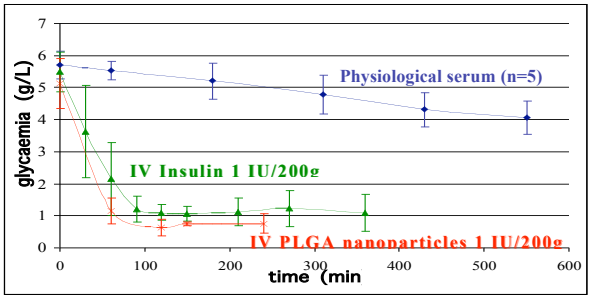

Figure 4: Intravenous injections of insulin (1 IU/200g) and PLGA insulin-loaded nanoparticles (1 IU/200g) to diabetic Wistar rats

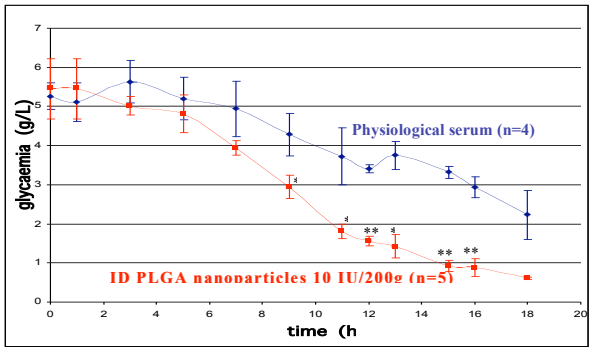

Figure 5: Intraduodenal administrations of PLGA insulin-loaded nanoparticles (10 IU/200g) to diabetic Wistar rats

The first obtained biological results are encouraging meaning that diabetic rats receiving an administration via intravenous or intraduodenal way return to a normoglycaemia after the administration of our insulin-loaded nanoparticles.
Today, new works are in progress to quantify the release profile of our nanoparticles, their internal structure...

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CONCLUSION

With these first results, we can conclude that our carrier is effective: it permits the insulin protection by nanoparticles, which can release it after the intestinal barrier crossing.
In the future, different experiments to modify the nanoparticles surface (addition of sugars, amino acids…), to facilitate their adhesion to the intestinal mucus, to obtain a better intestinal crossing and finally to get a better blood furtivity have to be done. These modifications aim at increasing their probability to cross the intestinal barrier and to improve their expected biological response.
To continue, several other studies would concern the vehicle optimization and especially concerning:
-the nanoparticles loading to reach the best insulin entrapment rate,
-the synthesis of the whole pharmaceutical vector
-oral administration experiments on the whole vector.

BIBLIOGRAPHY

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