Modified-chitosan nanoparticles : application in a new pharmaceutical vector for oral delivery of insulin

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INTRODUCTION

In case of therapeutic applications, drugs are mainly by oral or parenteral route administered. Even if, the oral route represents the most physiological and the most comfortable way for the patient, it can't be used for sensitive pharmaceuticals such as peptides and proteins. Because of their fragility towards the attacks met along the gastro-intestinal tract (pH, enzymes), they will be degraded and not biologically active anymore. That's why, this kind of drugs is often administered by injections. This is especially true for insulin.

All the previous studies have shown that encapsulation is the best suitable strategy for oral administration of insulin. By the way, we propose a patented, original and complex pharmaceutical vector (Frère Y., 2004). This pharmaceutical vector, already described (Callet A., 2008), is divided into 3 biocompatible parts: several insulin-loaded nanoparticles dispersed in pharmaceutical oil, included in a vehicle made of a natural and biocompatible polymer. The different parts of this complex pharmaceutical vector should solve the diverse issues encountered: protection against gastric and intestinal environments (pH, enzymes), enhancement of the intestinal mucus and membrane crossing.

Here, we will only study the insulin-loaded nanoparticles in order to observe their ability to protect insulin against the intestinal media and to transport it from the intestine to blood circulation. They are synthesized by a complex coacervation technique by which a complex coacervat is created between 2 oppositely charged polyelectrolytes. After synthesis, they are physically characterized and *in vivo* experiments are done.

MATERIAL AND METHODS

Insulin is a peptide with an isoelectric point at 5.5 and biologically active between pH 2 and 10. Here, it will be used as a commercial Umuline® solution from Eli Lilly. For nanoparticles synthesis, special attention has been given to chitosan: a non-toxic, biodegradable and biocompatible well-known polymer for therapeutic applications. Considering both chitosan solubility and positively charge (pH < 4) and insulin stability and negatively charge (pH > 5.5), it is evident that native chitosan can't be used to form nanoparticles.

Actually, some chitosan derivatives are both soluble and positively charged at pH > 5.5, permitting the nanoparticles formation. Among them, the N,O-carboxymethylated chitosan (NOCC) is mainly used in therapeutic domain and reported as non-toxic, either *in vitro* in fibroblast culture assays or *in vivo*, in testing with intraperitoneal, oral or subcutaneous treatments (Costain D.J., 1997). NOCC was synthesized per a procedure described in the literature (Chen S.-C., 2004) from chitosan MW 150 kDa, DA 16%.

Preparation of NOCC/Insulin Nanoparticles (Np-NOCC): Nanoparticles are synthesized by complex coacervation (Bayat A., 2008; Lin Y.H., 2007). This technique consists in adding, dropwise, a diluted solution of polyelectrolyte into another diluted solution made of an oppositely charged polyelectrolyte. By this technique, a complex coacervat is created involving electrostatic interactions. Here, the mild synthesis conditions (ambient temperature, no organic solvent and low stirring speed) permits to obtain nanoparticles, not to denature insulin and to keep it biologically active.

RESULTS AND DISCUSSION

Insulin is contained in nanoparticles made in a bioresorbable and metabolisable polymer (NOCC). These nanoparticles, synthesized by complex coacervation, protect insulin in the intestinal environment, make it cross the intestinal barrier (with an inferior 200 nm required size, Desai M.P., 1996) and release it in the blood and/or systemic circulation. The mild synthesis conditions permits insulin to keep its biological activity.

Physical characterization of Np-NOCC: Nanoparticles characterization has been done such as size measurements by static light scattering and microscopic observations. We can observe nanoparticles (figure 1) with an average size of 50-150 nm, adequate size to permit the intestinal barrier crossing. Nanoparticles observations by Transmission Electronic Microscopy and Atomic Force Microscopy confirmed nanoparticles formation and the obtained size by static light scattering (figure 2).

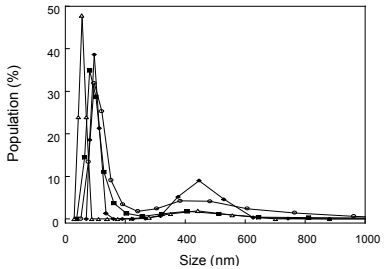

Figure 1: Size distribution of Np-NOCC obtained by static light scattering (in intensity, 5 measurements)

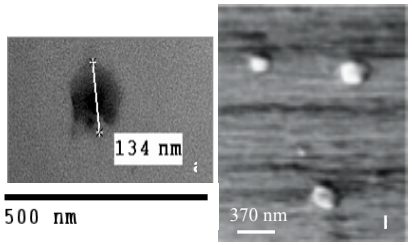

Figure 2: Transmission Electronic Microscopy (a) and Atomic Force Microscopy (b) observations of Np-NOCC

The slightly size variation observed between static light scattering and microscopies is due to interactions between nanoparticles and support and in particular to the drying process required by sample preparation.

***In vivo* studies of Np-NOCC:** *In vivo* experiments have also been done to check that insulin was not denaturated and so, still biologically active. It has been done by glycemic monitoring after subcutaneous and intraduodenal administrations on streptozotocin-induced diabetic Wistar rats.

Subcutaneous administration (figure 3) permits to give information about the insulin biological activity. By considering the biological response of insulin solution versus Np-NOCC both leading

to the glycemia decrease, we can say that insulin is liberated and still biologically active. Its release is fast (in comparison with the non-encapsulated insulin) as the beginning of decreasing takes place at the same moment. We can also conclude that the whole quantity of administrated insulin is released, as the decrease of glycemia leads to a normoglycemia (1.26 g/L) at a time similar for both Np-NOCC and insulin solution and for the same administrated dose (2IU/200g). This preliminary study leads to the proof that insulin remains intact after its encapsulation in nanoparticles and still plays its biological role.

Then intraduodenal administration (figure 4) has been done, given information concerning the nanoparticles absorption. As non-encapsulated insulin is denatured and degraded by the intestinal environment (pH, enzymes), no decrease of glycemia is observed with insulin solution. It confirms that insulin protection by encapsulation is necessary to preserve its activity otherwise it leads to the same biological answer as for no administration (empty stomach). In case of Np-NOCC, the glycemia slowly goes down during the experiment meaning that insulin is released and active. Moreover, the normoglycemia is reached within 250 minutes meaning that the insulin release is relatively fast and that the insulin protection by nanoparticles is efficient in the intestinal conditions.

By this experiment, we can conclude that nanoparticles are efficient, protecting insulin and crossing the intestinal epithelium.

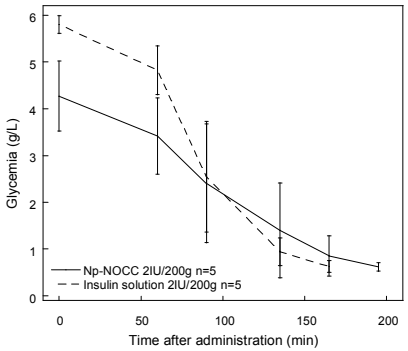

Figure 3: Glycemic monitoring after subcutaneous administration

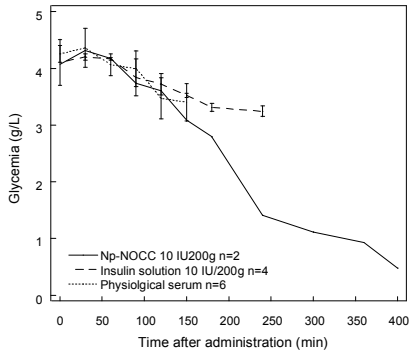

Figure 4: Glycemic monitoring after intraduodenal administration

CONCLUSION

The complexity of this pharmaceutical vector brings, by each of its constituents, a solution to the different problems previously exposed. The nanoparticles are synthesized by complex coacervation from a non-toxic and biocompatible polymer, within insulin is stable and still biologically active. This polymer is a chitosan derivative (NOCC), water-soluble at pH where insulin is both stable and negatively charged.

The obtained nanoparticles have an average size of 150 nm, size permitting the intestinal epithelium crossing. They have demonstrated their ability to encapsulate insulin and to release it without any loss of biological activity (*in vivo* experiments).

In conclusion, this study shows that insulin encapsulation, without denaturation, is possible and that its biologically activity is preserved. *In viv*o studies have demonstrated that:
- insulin solution and Np-NOCC leads to the same response by subcutaneous administration meaning that insulin is still active,
- Np-NOCC, by intraduodenal administration, leads to a glycemia decrease whereas non-encapsulated insulin has no effect meaning that its protection is efficient.

ACKNOWLEDGEMENTS

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