A new pharmaceutical vector for oral administration of Insulin

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Introduction

In the therapeutic fields, drugs are most often orally, intravenously, intramuscularly, or subcutaneously administered. The oral route represents the most physiological and the most comfortable way for the patient but it can't be used for pharmaceuticals which are sensitive to the gastro-intestinal environment, such as peptides and proteins. In fact, these molecules are very fragile towards the attacks met along the gastro-intestinal tract (pH, enzymes). That's why, they are more often administered by injection. It's specifically the case of insulin.

All the previous studies made in this area have shown that encapsulation is the best suitable strategy for improving the bioavailability of such drugs. However, up to date, none of the solutions given in the literature is really efficient. In fact, several studies have put in evidence that administration by oral way leads to a very weak bioavailability of these active principles because it rarely exceeds 3 % of the initially ingested dose. It's due to their physico-chemicals properties (size, charge and hydrophily) which make their way through the intestinal barrier to the blood circulation more complicated.

However, no one can, at present, give a solution allowing the oral administration of insulin. So, we propose an original and complex pharmaceutical vector (Y. Frère, L. Danicher, A. Belcourt, figure 1).

This pharmaceutical vector is divided into 3 biocompatible parts:

- ***Several nanoparticles*** containing the drug and obtained from natural polymers that can be biologically degraded to prevent any accumulation after the controlled release of insulin in the interstitial liquid and/or in the blood. Their surfaces can be modified to enhance their mucoadhesive properties and facilitate their transport across the intestinal epithelium

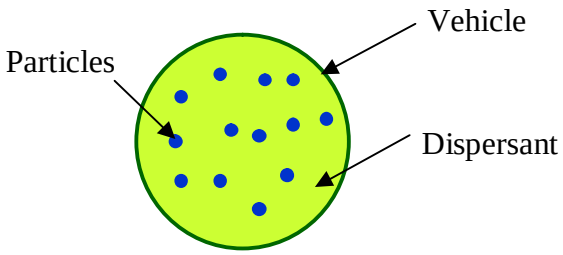

Figure 1: Patented complex pharmaceutical vector for oral administration of drugs

- ***A lipophilic dispersant*** that not only has to prevent the diffusion of insulin (hydrosoluble) out of the nanoparticles during storage, but also to limit the migration of gastric juices them during their passage in the stomach

- ***A vehicle*** made of a natural and biocompatible polymer that protects nanoparticles containing insulin during their migration through the mouth, the oesophagus and the stomach, and releases them in the intestine

The different parts of this complex pharmaceutical vector should solve the diverse issues encountered (protection along the gastrointestinal tract, enhancement of the active compound bioavailability in the interstitial liquid and/or blood). This will lead to a significant increase of the polypeptide drug bioavailability via the oral route administration.

Here, we will only study the nanoparticles (synthesis, characterization and *in vivo* experiments), so that we can first see the nanoparticles functionalities. If the obtained results are convincing, we will then continue with a further study where we will take into account the whole vector.

Material and methods

When designing oral formulations, it has to be taken into account that the active principle will be exposed to several pH environments along the gastrointestinal tract where it varies from acidic in the stomach to slightly alkaline in the intestine. This way along the gastrointestinal tract also leads to enzymatic environment of different compositions, depending on the localisation.

In this study, the active principle (in this case insulin) is a peptide which is, by nature, pH sensitive. It also has a composition which, in presence of enzymes, can leads to its degradation and loss of biological activity. Certainly the materials used to encapsulate insulin have to protect it from the previously exposed environment (intestine media) but it especially have to permit the encapsulation process in conditions where insulin will be then still biologically active.

That's why, the need of a material permitting to form particles in mild conditions (pH, agitation temperature…) is important. It's also needed to use a compound with can bring some specific properties as biodegradability, bioadhesivity, etc…

Special attention has been given to chitosan, which is a well-known polymer for these kinds of applications. It's a poly(aminosaccharide), normally obtained by partial alkaline deacetylation of chitin, the principle component of living organisms such as fungi and crustacean. This naturally occurring polymer has a repeating structural unit of 2-acetamido-2-deoxy-b-D-glucose. Since chitosan itself is non-toxic, biodegradable and biocompatible, several biological applications have been reported for chitosan such as a cholesterol trap or drug carrier. This biological macromolecule gives several advantages for this kind of applications such as bioadhesivity, biotolerance and biodegradability but as the huge disadvantage not to be soluble at physiological pH, where we have to work not to denature insulin. However, chitosan is only soluble in a few dilute acid solutions, which limits its wide applications and especially with insulin which cannot be placed in this acidic environment.

Recently, there has been a growing interest in chemical modification of chitosan to improve its solubility and widen its applications. Chemical modifications are also powerful tools to control the interaction of the polymer with drugs, to enhance the drug loading efficiency as well as tailor the drug release period. Chemically modified chitosans have great utility in controlled release and targeting studies of almost all class of bioactive molecules.

It has been first thought that trimethylated chitosan (TMC) will be usable in this way but the presence of quaternary ammonium shows a very important toxicity for this application. But some modified chitosans are already used in therapeutic domain as for example the case of N,O-carboxymethylated chitosan (NOCC), a chitosan that has been modified by carboxymethylation for repair and regeneration of bone tissues or use as antioxidant agent. NOCC is a chitosan derivative having carboxymethyl substituents on some of both the amino and primary hydroxyl sites of the glucosamine units of the chitosan structure. It was reported that NOCC is non-toxic, either *in vitro* in fibroblast culture assays or *in vivo* in testing with intraperitoneal, oral, or subcutaneous treatments. Additionally, NOCC is suitable as an excipient in ophthalmic formations to improve the retention and bioavailability of drugs.

Preparation of water-soluble CS (NOCC)

NOCC was synthesized per a procedure described in the literature (S.-C. Chen et al, 2004). Chitosan (MW 150 kDa) with a degree of deacetylation of approximately 84 % was acquired from Sigma Aldrich.
Monochloroacetic acid and isopropyl alcohol were purchased from Sigma Chemical Co. All other chemicals and reagents used were of analytical grade.

Characterization of NOCC

Fourier transformed infrared spectroscopy (FT-IR) in ATR diamante mode and proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) were used to confirm substitutions of carboxymethyl groups on the amino and primary hydroxyl sites of the modified chitosan.

The obtained NOCC used for the FT-IR analysis was first dried. Analysis was performed on an FT-IR spectrometer (Bruker Vertex 70). The sample was scanned from 400 to 4000 cm-1. ^{1}H-NMR studies were carried out with deuterium oxide. Analyses of the proton spectra were conducted on an NMR spectrometer (Bruker 400 MHz). The degrees of substitution of carboxymethyl groups on the amino and primary hydroxyl sites of the modified chitosan (NOCC) were estimated by the relative peak intensities between the H on the carboxymethyl groups and the H at C2 of monosaccharide residue in the ^{1}H-NMR spectrum of NOCC per a method reported in the literature (M. Sugimoto et al, 1998).

Preparation of NOCC/Insulin particles

Particles are synthesized by complex coacervation (A. Bayat et al, 2008). 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 agitation speed) permits 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 (modified chitosan : NOCC). These particles, synthesized by complex coacervation, have to protect it in the intestinal environment, to make it cross the intestinal barrier and to release it in the blood and/or systemic circulation. The mild synthesis conditions permits insulin to keep its biological activity. The obtained nanoparticles will be emulsified in a biotolerated oil which plays a double role (prevents the insulin release during storage and prevents the migration of gastric juices during the passage of the stomach). Then, this emulsion will be contained in a vehicle made in a bio-tolerated polymer (alginic acid) which should protect the particles in the stomach and then release them in the intestine.

The nanoparticles characterization has been done such as size measurements by static light scattering and microscopic observations. We can observe nanoparticles with an average size of 50-150 nm, adequate size (inferior to 200 nm) to permit the intestinal barrier crossing.

In vivo experiments have also been done to check that insulin was still biologically active and not denaturated. These experimentations have, by the way of glycemic monitoring after sub-cutaneous

and intra-duodenal administration on rats with streptozotocin-induced diabetic Wistar rats, given the proof that insulin is still active as the glycemia slows down during the experiment.

Conclusions

The complexity of this complex pharmaceutical vector will bring, by each of its constituents, a solution to the different problems previously exposed. Nevertheless, the use of this vector leads to chemical constraints. Chitosan is only soluble in acid solutions, where insulin is neither stable nor biologically active. A chemical modification is obvious to make it soluble at physiological pH. The nanoparticles are then synthesized by complex coacervation from a non-toxic and biocompatible polymer, within insulin is stable and still biologically active. The obtained nanoparticles have an average size of 150 nm. This size permits to cross the intestinal epithelium. These systems have demonstrated their ability to encapsulate insulin and to release it without any loss of biological activity (*in vivo* experiments).
So, this study has shown that we are able to synthesized nanoparticles containing biologically active insulin, with a size permitting the intestinal epithelium crossing.
Surface modifications remain to be done in order to optimize their properties (mucoadhesion, furtive features, stability during storage) to improve intestinal crossing. Once the vehicle synthesis will be optimized, complementary *in vitro* and *in vivo* experiments will be carried out to evaluate the effectiveness of this new pharmaceutical complex vector.

References

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