O8-2 **Oral absorption enhancement of insulin-loaded chitosan-coated solid lipid nanoparticles**

Fonte P. [1,2 #] **Andrade J.C.**[2] **Seabra V.**[2] **Ferreira D.**[3] **and Sarmento B.** [2,3 *]
[1] CICS, RGandra, Portugal [2] Instit Sup Ciências da Saúde – Gandra, Portugal [3] Dep Pharm Technol, Univ of Porto, Portugal * Supervisor # pedro.fonte@cespu.pt

INTRODUCTION AND OBJECTIVES

Insulin has usually been administered subcutaneously in the treatment of diabetes mellitus. Oral delivery of insulin is expected to be an alternative route but has some limitations, including low bioavailability due to degradation in the stomach, inactivation and digestion by proteolytic enzymes in the luminal cavity, and poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity. Solid lipid nanoparticles (SLN) show several advantages as drug delivery systems, such as good tolerability, biodegradation, and the possibility of large industrial scale production. We have previously demonstrated the feasibility of SLN to improve the oral absorption of insulin in diabetic animals (Sarmento et al. 2007). Enhancing mucoadhesion properties of nanoparticles, namely by using Chitosan coatings, has been a successful strategy used to promote the contact of carriers with the intestinal epithelium, thus increasing the therapeutic proteins concentration at the site of absorption.

The purpose of this work was to develop a new nanoparticulate carrier intended for the oral administration, composed of a lipid core aimed to protect and to control the release of insulin and coated by the mucoadhesive chitosan to improve insulin absorption.

MATERIALS AND METHODS

Preparation and characterization of chitosan-coated solid lipid nanoparticles

The nanoparticles were prepared by a modified solvent emulsification-evaporation method based on a w/o/w double emulsion technique (Zhang et al. 2006). Witepsol 85E (Sasol, Germany) was the lipid matrix and Chitosan (50 KDa, degree of deacetylation of 85%; Sigma, Portugal) was the coating biopolymer of the produced nanoparticles (Fonte et al. 2011).

Particle size and zeta potential were analyzed by photon correlation spectroscopy and laser doppler anemometry, respectively, with a Malvern Zetasizer 5000 (Malvern Instruments, UK). Nanoparticle morphology was observed using scanning electron microscopy (SEM) on a JEOL JSM-840 SEM (Japan). The insulin association efficiency (AE) and loading capacity (LC) were determined by the following equations:

AE % = Total amount of insulin−Free insulin in supenatantTotal amount of insulin ×100

LC % = Total amount of insulin−Free insulin in supenatantTotal weight of nanoparticles ×100

The free insulin in supernatant was determined, after SLN isolation by filtration through a 0.2 µm filter (Schleicher and Schuell, Germany), by a HPLC-UV method previously developed and validated by our group (Sarmento et al. 2006).

In vitro and in vivo studies

Caco-2 cells (ATCC, USA), HT29 cells (ATCC, USA) and the mixture of Caco-2/HT29 cells (90:10 ratio) were used. All cell monolayers were used after 21 days in culture. At different times, basolateral samples were collected and insulin determined by HPLC-UV (Sarmento et al. 2006).

Diabetes was induced in rats by a single intraperitoneal injection of streptozocin. SLN and chitosan-coated SLN dispersions were administered intragastrically by gavage needle to diabetic rats at insulin dose of 25 IU/kg, based on the total insulin content of the SLN. Control rats were similarly administered with equivalent volumes of insulin oral solution. Also, a control group treated with subcutaneous insulin (2.5 UI/kg) was included in the experiment. Blood glucose level was determined using the Medisense Precision Xceed Kit, (Abbot, Portugal, range 10–600 mg/dL), and expressed as a percent of the baseline plasma glucose level.

RESULTS AND DISCUSSION

The physical-chemical properties of the developed insulin-loaded SLN are shown in Table 1. The increase of the mean particle size in the Chitosan-coated SLN is attributable to Chitosan coating. Moreover, the changing of the surface charge also confirmed the deposition of the positively charged chitosan on the surface of SLN, which make it able to further interact with the negatively charged intestinal membrane. The AE and LC obtained for SLN and Chitosan-coated SLN, may be considered a positive achievement regarding the hydrophilic character of the protein and the hydrophobic nature of the lipid matrix. The slightly higher AE for chitosan-coated SLN may be due to the deposition of non-encapsulated insulin, which interacts with the positive chitosan on the particles surface.

Table 1 : Physical-chemical properties of developed insuli-loaded SLN (*n*=3, mean±SD)

Formulation	Size (nm)	PdI	Zeta Potential (mV)	Insulin AE (%)	Insulin LC (%)
SLN	243±10	0.33±0.03	−25.1±0.3	43.6±2.2	2.1±0.4
Chitosan-coated SLN	470±32	0.63±0.02	+34.2±3.4	52.2±5.3	1.4±0.2

SLN exhibited irregular shape, most probably due to the deformation during the beam incision for microphotograph collecting, and dense lipid matrix (Figure 1a). After coating, a smooth surface layer and some aggregation was noted due to chitosan deposition (Figure 1b), which may justify the increase of the mean particle size for chitosan-coated SLN reported above.

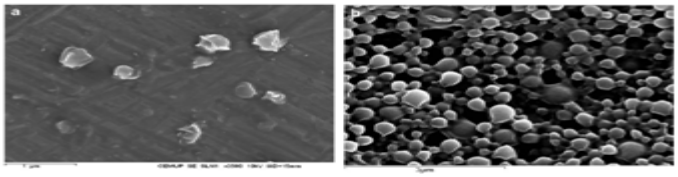

Figure 1 : Micrographs of SLN containing insulin (a) and Chitosan-coated SLN containing insulin (b)

Although only with a later onset, observed after 1 h of contact with Caco-2 cell monolayer, probably due to delayed insulin release from nanoparticle matrix, chitosan-coated SLN demonstrated better absorption-enhancing properties compared with the uncoated SLN (Figure 2). This may occur due to mucoadhesion and opening of the tight conjunctions between the epithelium cells that can improve the insulin permeability. Simultaneously, insulin is presumably released from the nanoparticle attached to the cell monolayer or transported whithin nanoparticles by the transcellular pathway.

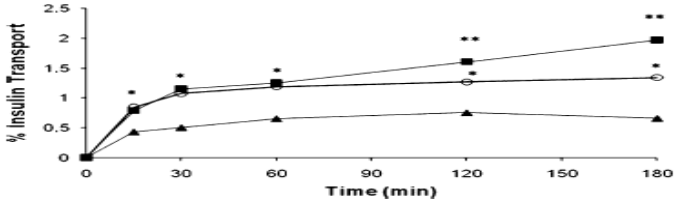

Figure 2 : Cumulative transport of insulin across Caco-2 monolayer encapsulated into SLN (*empty circles*) and into chitosan-coated SLN (*filled squares*) compared with free soluble insulin (*filled triangles)* (*n*=3, mean±SD)

Insulin absorption was higher on the Caco-2/HT29 co-culture monolayer model when compared with the Caco-2 model (Figure 3). The difference between both was the presence of HT29 mucus-producing goblet cells, whose mucus causes the retention of nanoparticles and the protein itself on the intestinal epithelium. Moreover the difference of the percentage of insulin transport for chitosan-coated SLN after 3h, in the both models give rise that the mucoadhesion properties of these nanoparticles promotes the contact of proteins with the intestinal epithelium, increasing the concentration at the site of absorption, enlarging its permeation either paracellularly or transcellularly.

The reduction of the initial glucose levels versus time after intragastric insulin-loaded SLN, insulin-loaded chitosan-coated SLN, insulin solution and subcutaneous administration of insulin is depicted in Figure 4. The mean plasma glucose baseline value was taken as 100% level.

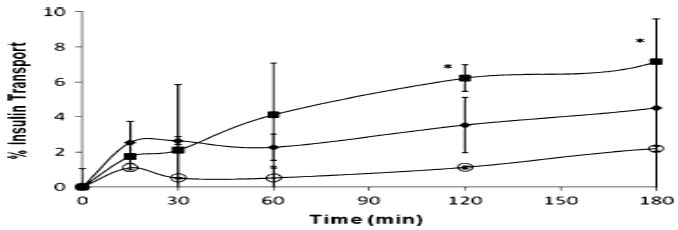

Figure 3 : Cumulative transport of insulin across Caco-2/HT29 co-culture monolayer encapsulated into SLN (*filled diamonds*) and into chitosan-coated SLN (*filled squares*) compared with free soluble insulin (*circles)* (n=3; mean±SD)

Insulin-loaded SLN decreased glycemia by comparison with rats treated with oral insulin solution. This hypoglycemic effect occurred in a biphasic way, which can originate an immediate release of insulin but also retain a significant fraction of the drug entrapped into the lipid matrix during prolonged time. These overall results suggested that SLN was able to protect insulin from degradation and enhance its intestinal absorption, which is highly boosted when SLN are further coated with chitosan.

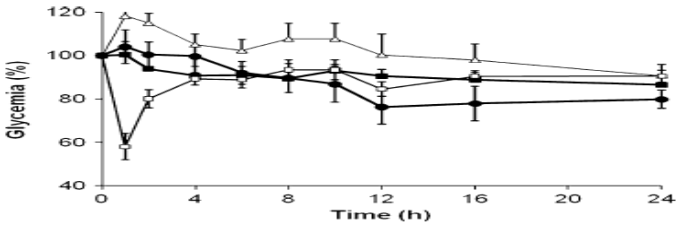

Figure 4 : Percentage reduction of plasma glucose concentration in diabetic rats after administration of subcutaneous injection of insulin, 2.5 UI/Kg (*empty squares*); oral insulin solution, 25 IU/kg (*empty triangles*); insulin-loaded SLN, 25 IU/Kg (*filled squares*); and insulin-loaded chitosan-coated SLN, 25 IU/Kg (*filled circle*; n=6; mean±SEM)

CONCLUSIONS

Chitosan coating was found to improve the stability and intestinal absorption properties of SLN containing insulin, which may contribute for the development of an optimized oral insulin formulation.

REFERENCES

- Fonte et al. (2011) *Chitosan-coated solid lipid nanoparticles enhance the oral absorption of insulin*. Drug Deliv and Transl Res 1-10.
- Sarmento et al. (2006) *Development and validation of a rapid reversed-phase HPLC method for the determination of insulin from nanoparticulate systems*. Biomed Chromatogr 20(9): 898-903.
- Sarmento et al. (2007) *Oral insulin delivery by means of solid lipid nanoparticles*. Int J Nanomedicine 2(4): 743-749.
- Zhang et al. (2006) *Lectin-modified solid lipid nanoparticles as carriers for oral administration of insulin*. Int J Pharm 327(1-2): 153-159.