P-045	**Comparison of two chitosan based nanoparticles to predict intestinal absorption**	

Antunes F. [1], Andrade F. [1], Ferreira D. [1] and Sarmento B. [1,2 *]
[1] Pharm Technol Dpt, Fac of Pharm, University of Porto, Portugal [2]Pharm Sc, Instituto Superior de Ciências da Saúde-Norte, Gandra, Portugal
[*]bruno.sarmento@ff.up.pt

INTRODUCTION

Oral administration of proteins like insulin has some limitations, including low oral bioavailability due to degradation in the stomach, inactivation and digestion by proteolytic enzymes in the luminal cavity, poor permeability across intestinal epithelium because of its high molecular weight and lack of lipophilicity strategies have been utilized to increase permeability of proteins by intestinal mucoadhesion of carriers and junctional modulation. Nanoparticles are under consideration for oral delivery of proteins like insulin by stabilizing, by ensuring biological activity during transit through the gastrointestinal tract and by facilitating absorption and delivery to the target site (Yih 2006). Solid lipid nanoparticles (SLN) are generally well tolerated by the body and do not have most of the disadvantages of colloidal carriers, what make them an alternative to the polymers used in the production of drug delivery systems (Rieux 2006). Chitosan (CS) is a natural polymer that is biologically safe, non-toxic, biocompatible and biodegradable polysaccharide. CS nanoparticles have gained more attention as drug delivery carriers because of their better stability, low toxicity, simple and mild preparation method and providing versatile routes of administration. Dextran sulfate (DS) is a biodegradable and biocompatible polyanion, with a branched carbohydrate backbone and negatively charged sulfate groups able to strongly interact with positively charged proteins. CS-DS nanoparticles can be formed under mild processing conditions and are formulated by employing the oppositely charged polymers CS and DS under aqueous conditions. In this context, the aim of this study was to compare two chitosan based nanoparticles, SLN and DS, to predict intestinal absorption of insulin through an *in vitro* cellular model of Caco-2:HT29:Raji B cells to partially reproduce the characteristics of intestinal enterocytes, mucus-secreting cells and M-cells, respectively.

MATERIALS AND METHODS

Cell culture

Caco-2 and HT29 cells were grown separately in flasks in DMEM supplemented with 10% foetal bovine serum, 1% nonessential aminoacids, 1% L-glutamine and 1% PEST, at 37 ºC under a 5% CO_2 water saturated atmosphere and the predetermined cell numbers of each type were mixed prior to seeding to yield cell ratios of 9:1 for Caco-2 ($3x10^5$) to HT29 cells, respectively. Co-cultures from Caco-2 and HT29 cells were seeded into (3 µm pore Transwell filters, Corning, NY) inserts and were maintained under identical conditions with medium changes every other day. Raji B cells were cultivated in the same conditions of the other cells. $5x10^5$ Raji B cells were added to the basolateral chamber of 14-day-old Caco-2:HT29 co-culture and the cultures were maintained for 4–6 days (Rieux 2007). Co-cultures of Caco-2 cells, cultivated as above except for the presence of HT29 cells, were used as controls. Co-cultures from Caco-2 and HT29 cells were seeded on the lower face of filters and culturing them overnight. The filters were then transferred in the Transwell device with the epithelial cells facing the lower chamber of the plates. Epithelial cells were cultured until they were fully differentiated (14 days). Raji B cells were added to the apical compartment of 14-day-old Caco-2:HT29 co-culture and the cultures were maintained for 4–6 days (Kernéis 1997).

Preparation of nanoparticles

Witepsol E85 SLN were prepared by a modified solvent emulsification-evaporation method using a sonicator probe based on a w/o/w double emulsion with Tween 80 as surfactant. To coat SLN with chitosan it was used chitosan solution as secondary aqueous solution and physical adsorption on the mucoadhesive polymer promoted under magnetic stirring until solvent removal. Insulin was determined by HPLC. DS/CS nanoparticles were prepared as previously described by Sarmento et al. (2006). The amount of insulin associated with the nanoparticles was calculated by HPLC determining the difference between the initial and remained insulin in the supernatant after nanoparticle removal by centrifugation.

Permeability studies

All cell monolayers were used after 21-28 days in culture. During this time the development of the monolayer was monitored by Transepithelial Electrical Resistance (TEER) measurements using the EVOM equipment. Transwell® insulin absorption experiments were run at 37 ºC from apical to basolateral chamber, at initial apical concentration of 100 µg/ml. Transport experiments were run in Hanks' Balanced Salt Solution (HBSS) at 37 ºC. At different times, basolateral samples were collected and insulin determined by HPLC as described previously (Yih 2006).

Apparent permeability coefficient (P_{app}) was calculated from the measurement of the flow rate of insulin form the donor to the acceptor chambers: P_{app} (cm/s) = dQ/dt (A x C_0), where, dQ is the total amount permeated insulin (µg), A is the diffusion area (cm^2), C_0 is the initial concentration of insulin (µg/ml), and dt is the time of

experiment (s). The coefficient *dQ*/*dt* represents the steady-state flux of insulin across the monolayer.

RESULTS AND DISCUSSION

Insulin absorption by mucoadhesive nanoparticles was higher on DS/CS than in SLN/CS nanoparticles and the TEERs were slightly lower on the membranes where it was tested DS/CS nanoparticles than those of SLN/CS (Figure 1). This is in agreement with the results of Merwe (2004) and Ward et al. (2007), where they described that chitosan nanoparticles are attractive protein carriers as the polymer is mucoadhesive and enhances paracellular permeability by opening the tight junctions between epithelial cells and as the tight junctions open, the TEER of Caco-2 cell monolayers significantly reduced, due to ion passages through the paracellular route. Actually the results demonstrate that while insulin absorption increase, TEER measurements decrease, which is indicative of the reversible opening of tight junctions to enhance permeability. Chitosan coating demonstrated absorption enhancing (data not shown). This may occur due to mucoadhesion and opening of the tight junction between the epithelium cells that can improve the insulin permeability into the triple co-culture monolayer.

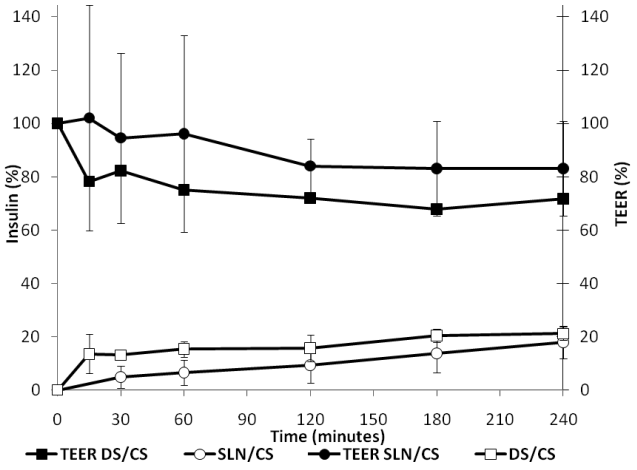

Figure 1 : Cumulative insulin transport and TEER cell monolayer measurements from insulin loaded DS/CS and SLN/CS nanoparticles across Caco-2:HT29:Raji B triple co-culture seeded at Transwell® membranes.

Insulin *in vitro* transport increased after encapsulation into nanoparticles (SLN and DS) and mainly into chitosan-coated nanoparticles (data not shown), justified by insulin release from nanoparticle attached to cell monolayer and through nanoparticle transcellular transport pathway. Table 1 shows P_{app} of insulin facilitated by both chitosan based nanoparticles evaluated (DS/CS and SLN/CS). The cumulative amount of insulin permeated through Caco-2:HT29:Raji B monolayer facilitated by nanoparticles was enhanced as compared with insulin free-form.

Table 1 : Apparent permeability coefficient (P_{app}) of insulin permeability across triple co-culture.

	P_{app} x 10^{-6} cm/s triple co-culture
Insulin free-form	0.15 ± 2.72
Dextran sulfate nanoparticles coated with chitosan	2.61 ± 1.23
Solid lipid nanoparticles coated with chitosan	2.13 ± 5.04

CONCLUSIONS

One of the most important factors in defining oral drug absorption of proteins should be drug permeability across the intestinal membrane. Therefore, it is crucial to improve nanoparticulate systems that protects proteins and peptidic drugs in order to increase its bioavailability. This study was a first attempt to compare two chitosan based nanoparticles using an *in vitro* cell model composed of Caco-2:HT29:Raji B cells for drug permeability assessment. Results demonstrate that DS/CS nanoparticles seem to be better in terms of insulin absorption than SLN/CS nanoparticles. Nevertheless, both chitosan based nanoparticles tested in this study are promising alternatives for the development of formulation for oral insulin administration.

REFERENCES

- Merwe S. (2004) *Trimethylated chitosan as polymeric absorption enhancer for improved peroral delivery of peptide drugs* European Journal of Pharmaceutics and Biopharmaceutics 58 225-235.
- Rieux A. et al. (2006) *Nanoparticles as potential oral delivery systems of proteins and vaccines: A mechanistic approach* Journal of Controlled Release 116 1-27.
- Rieux A. et al. (2007) *An improved in vitro model of human intestinal follicle-associated epithelium to study nanoparticle transport by M cells* European Journal of Pharmaceutical Sciences 30 380-391.
- Sarmento B. et al. (2006) *Development and characterization of new insulin containing polysaccharide nanoparticles* Colloids and Surfaces B: Biointerfaces 53 193-202.
- Ward P. (2000) *Enhancing paracellular permeability by modulating epithelial tight junctions* Pharmaceutical Science and Technology Today 3 346-358.
- Yih T. et al. (2006) *Engineered nanoparticles as precise drug delivery systems* Journal of Cellular Biochemistry 97 1184-1190.