Chitosan as bioavailability enhancer of nanoparticle containing biopharmaceutics

Neto, A.[1], Gehm, C.[1], Teixeira, J.[1], Martins, S.[1], Ferreira, D.[1], Sarmento, B.[1,2]
[1] University of Porto, Porto, Portugal
[2] Centro de Investigação em Ciências da Saúde (CICS), Gandra, Portugal
anapatricianeto@gmail.com

INTRODUCTION

For several reasons, oral delivery is considered the preferred route of drug administration. However, the transport of drugs across the intestine is a complex and dynamic process that includes the passage across several functional pathways. This process becomes more complex for biopharmaceutic drugs. 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. Some pharmacological properties of conventional drugs, such as pharmacokinetics and biodistribution, can be improved with the incorporation of those in nanoparticles (Allen, T.M., 2004), among them the carriers with lipid nature. 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.
One of the advantages of nanoparticles, when administered orally, is that they can be absorbed transcellularly, not only through the membranous epithelial cells (M-cells) of the Peyer's patches in the gut-associated lymphoid tissue (GALT), but also through enterocytes (Rieux, A. 2006). The uptake of nanoparticles carrying proteins by enterocytes has been demonstrated to be a limited but capable process. Enhancing mucoadhesion properties of nanoparticles by chitosan are usually explored with efficiency to promote the contact of proteins with the intestinal epithelium, increasing the concentration at the site of absorption (Sarmento, B. 2007). Chitosan has been considered due to its biodegradability, mucoadhesivity and protein absorption enhancement (Artursson, P. 1994).
The purpose of this work was to develop a new nanoparticulate carrier intended for the oral administration of therapeutic proteins. The new carrier is composed of a lipid core aimed to protect and to control the release of insulin and coated by the mucoadhesive chitosan to improve retention of insulin into the absorption window. In vitro Caco-2 cell model was used to predict the degree of efficacy of the developed nanoparticles before *in vivo* assays in diabetic animal model.

MATERIAL AND METHODS

Witepsol 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.
The particle size, zeta potential and association efficiency (AE) of insulin-loaded SLN and chitosan-coated SLN were determined by photon correlation spectroscopy, laser doppler anemometry and HPLC, respectively.
Caco-2 cells were seeded on Snapwells and after 21 days, monolayers with transepithelial electrical resistance (TEER) > 250 **Ω** X cm2 were washed with PBS and mounted in a NaviCyte Vertical

Ussing/Diffusion Chamber interfaced with computer-based data acquisition software and a multichannel voltage-current clamp. Voltage pulses were passed across the monolayer and TEER values were monitoring throughout the entire experiment. Silver–silver chloride electrodes in saturated KCl in glass barrels terminating in ceramic tips were used to clamp the voltage and measure the current. Formulations were dispersed in 8 ml of HBSS (final insulin concentration 200 µg/mL) and placed in the apical chamber. The basolateral chamber was filled with 8 mL of HBSS. Samples of the medium from the donor and receiving chambers were collected at the start, and at different times of the experiment for analysis of insulin content. The assay was performed at 37ºC and cells were continuously bubbled with 5% CO2/95%O2.
Insulin pharmacological activity was determined in streptozocin induced diabetic animals. After two weeks, rats with fasted blood glucose levels above 250 mg/dL were used for experiments. These rats were fasted for 12 h before experiments and remained fasted for 24 h during the experiment, but had free access to water ad libitum. SLN dispersions (1.0 mL) were administered intragastrically by gavage needle to rats at insulin dose of 25 IU/kg, based on the total insulin content of the SLN. Pharmacological availability (PA) of peroral insulin-loaded SLN was determined based on a 100% availability of the control solution administered subcutaneously to the diabetic rats at a dose of 2.5 IU of insulin/kg. Plasma glucose level was determined using the Medisense Precision Xceed Kit.

RESULTS AND DISCUSSION

SLN and chitosan-coated SLN containing insulin were successfully produced by a modified solvent emulsification-evaporation method based on a w/o/w double emulsion. They are considered to be stable carriers for oral administration. Tween 80 was used as surfactant in the aqueous phase when preparing the insulin-loaded nanoparticles to increase their stability. As summarized in Table 1, insulin-loaded SLN formulation possessed homogenous size distribution around 250 nm and negative zeta potential values. Size increased after chitosan coating mostly probably due to nanoparticle aggregation due to sticking effect of chitosan. The chitosan coating was also confirmed by the positive charge on the surface of SLN. The AE of insulin was around 45%, and is noteworthy that being a hydrophilic molecule a much lower association efficiency of insulin within the lipid matrix of SLN was expected.

Formulation	SLN	Chitosan-SLN
Size (nm)	243 (± 10)	1630 (± 32)
PdI	0.62 (± 0.02)	0.63 (± 0.02)
Zeta Potential (mV)	-25.1 (± 0.3)	34.2 (± 0.5)
Insulin AE (%)	43.6 (± 2.2)	47.2 (± 5.3)

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

The microscopic appearance and the structural characterization of SLN showed that these particles exhibited spherical shape and dense lipid matrix. After coating, fluffy surface layer due to chitosan deposition was noted and some aggregation (Figure 1), which may justify the increase of mean particle size for chitosan-coated SLN reported before. Nevertheless, nanoparticles were easily ressuspended after sedimentation, indicating that aggregation is reversible. Further investigations on nanoparticles freeze-drying are under evaluation.

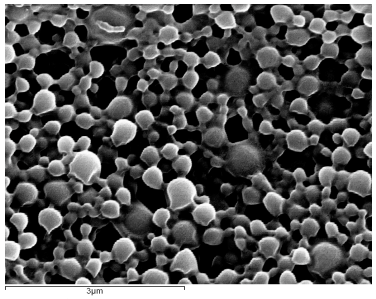

Figure 1: Photomicrographs of chitosan-coated SLN

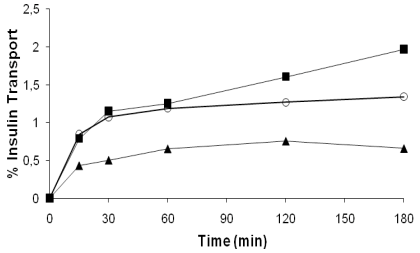

Figure 2: Cumulative transport of insulin across Caco-2 monolayer to basolateral Ussing chamber encapsulated into SLN (o) and into chitosan-coated SLN (■) compared with free soluble insulin (▲).(n=3, mean ± SD)

Insulin in vitro transport increased after encapsulation into SLN and mainly into chitosan-coated SLN (Figure 2), justified by insulin release from the nanoparticle attached to cell monolayer and through nanoparticle transcellular transport pathway. TEER values were measured during the experiment to evaluate the integrity of the cell monolayer. During all experiments, TEER values were constant, indicating that the integrity of the cells was maintained. The effect of chitosan coating on insulin permeability is depicted in Figures 2 and 3. Although latter onset, probably due to delay of release from nanoparticle matrix, chitosan demonstrated absorption enhancing. This may occur due to mucoadhesion and opening of the tight conjunction between the epithelium cells that can improve the insulin permeability into the co-culture monolayer. Also, despite mass balance between Figure 2 and 3 is not 100%, insulin can be retained inside enterocytes or attached to Ussing cells.

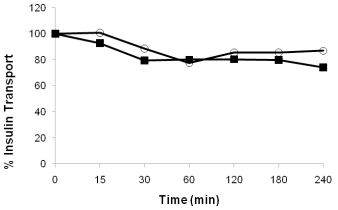

Figure 3: Cumulative transport of insulin across Caco-2 monolayer from apical Ussing chamber encapsulated into SLN (o) and into chitosan-coated SLN (■).(n=3, mean ± SD)

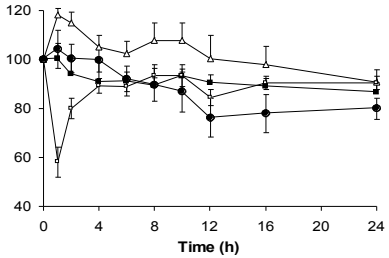

Figure 4. Percentage reduction of plasma glucose concentration in diabetic rats after administration of subcutaneous injection of insulin 2.5 UI/Kg (□), oral insulin solution 25 IU/Kg (Δ), insulin-loaded SLN 25 IU/Kg (■) and insulin-loaded chitosan-coated SLN (●). (n=6, mean ± SEM)

Insulin-loaded SLN decreased glycemia by comparison with animals treated with oral insulin solution (Figure 4). This hypoglycemic effect was observed to occur 4 h after administration for insulin-loaded SLN but faster onset was observed for chitosan-coated insulin-loaded SLN. Moreover, hypoglycemic effect was observed to be more sustained when SLN were coated with chitosan, once more highlighting the main role of chitosan to the absorption enhancing of insulin. SLN were able to partially protect insulin against chemical degradation in the gastrointestinal tract and to promote the intestinal absorption of insulin.

The relative pharmacological bioavailabilities indicate that was around 8% when administered into SLN and 17% after chitosan coating. These overall results suggested that SLN could protect insulin from degradation and enhance intestinal absorption of insulin, which is highly boosted if SLN are further chitosan stealth. SLN undergo physiological degradation and the insulin enter into the blood circulation. It is generally accepted that nanoparticles with hydrophobic surfaces such as SLN, are taken up more extensively by the intestinal epithelium than those with hydrophilic surfaces. Thus, the uptake of nanoparticles with lipid matrix is potentially facilitated. Also, the bioadhesive properties of lipids can lead to a gradient diffusion of insulin from the high concentrations in the SLN matrix towards the intestinal cells. The association of both bioadhesive characteristics of lipids and chitosan, and the adhesion of chitosan at the site of insulin gastrointestinal absorption may offer various advantages for its uptake.

CONCLUSIONS

In conclusion, new chitosan-coated SLN were found to be suitable carrier systems for the administration of insulin through the oral route. This study may contribute for the development of an optimized oral insulin formulation.

REFERENCES

Allen, T.M. *et al.* Drug Delivery Systems: Entering the Mainstream, Science (2004) 303: 1818-1822.

Artursson, P. (1994) Effect of Chitosan on the Permeability of Monolayers of Intestinal Epithelial cells (Caco-2). Pharmaceutical Research 11: 1358-1361

Sarmento, B. *et al*. (2007) Alginate-chitosan nanoparticles are effective for oral insulin delivery. Pharmaceutical Research 24: 2198-2206

Sarmento, B. *et al*, (2007) Oral bioavailability of insulin contained in polysaccharide nanoparticles Biomacromolecules 8: 3054-3060

Rieux, A. *et al*. Nanoparticles as potential oral delivery systems of proteins and vaccines: A mechanistic approach (2006) Journal of Controlled Release (2006), 116:1-27.