Effective oral delivery of insulin by a nanoplex composite delivery system containing alginate as core material

Speaker: Ronald J. Neufeld
Graduate students conducting the research: Catarina Pinto Reis (Univ. of Coimbra/Queen's Univ.), Bruno Sarmento (Univ. of Porto/Queen's Univ.), Burak Erdinc (Queen's Univ.), Kristen Bowey (Queen's Univ.), Camile Woitiski (Univ. of Coimbra)
Research supervisors and collaborators: António Ribeiro, Francisco Veiga (Univ. of Coimbra), Domingos Ferreira (Univ. of Porto), Christiane Damgé (Univ. Louis Pasteur), Ronald J. Neufeld (Queen's Univ.)

Introduction

An international collaboration has led to the development of nanoencapsulated insulin and demonstration of its oral pharmacological bioavailability.

Challenges

The following objectives set for the program have been met.

An *in vitro* cell based assay was developed involving measuring the phosphorylation status of protein kinase B following insulin receptor binding with rat myoblasts, for routine monitoring of insulin bioactivity. Nanoparticulate insulin was shown active *in vitro* and *in vivo* following subcutaneous injection and oral administration to diabetic rats.

Nanoparticulate insulin was protected from acid pH and protease attack during passage through stomach and gastrointestinal tract following oral administration.

Biopolymers use in formulation of nanoparticles were non-toxic, biodegradable and had mucoadhesive and permeation enhancing properties.

A nanoparticulate delivery system was developed, suitable for uptake by intestinal enterocytes, and in particular, by the Peyer's patches which take up particles less than 5 μm in diameter.

Bioactive insulin, was delivered across the intestinal lumen in diabetic rats.

Insulin pharmacological bioavailability was demonstrated using the diabetic rat model.

Nanoparticles were tracked through the GI tract using labelled insulin and adhesion and uptake of the nanoparticles/insulin demonstrated.

Formulations developed and tested

The following nanoparticulate formulations containing insulin have been tested for oral delivery with diabetic rats. In each case, the insulin was entrapped within in the core of the particle with high yield:

core	**coat**
alginate	-
alginate	chitosan-pectin
alginate	chitosan-casein
alginate	chitosan-albumin
alginate-dextran	chitosan-polyethylene glycol-albumin
dextran	chitosan

While all formulations demonstrated varying degrees of bioavailability following oral administration, entrapped insulin showed high sensitivity to pepsin (in vitro) and protease (in vivo) attack. Thus, coating strategies were developed to improve enzymatic resistance and thus improve insulin oral absorption. Chitosan was selected as coating material as it is biocompatible, can increase the stability of alginate nanospheres, is mucoadhesive, and is also an effective permeability enhancer. However, chitosan-coated alginate-dextran sulfate core nanospheres were still highly sensitive to pepsin. Albumin was then added as a second coating material and PEG as a stabilizing polymer. Albumin was selected as a protection from proteolytic enzymes and PEG is known to increase stability of polymeric nanoparticles and improve transport of large proteins across nasal and intestinal barriers.

Formulation methods

Two formulation methods have been developed, and a third is in process of being developed. The three methods will be fully described during the oral presentation.

1. Nanoemulsion dispersion/triggered *in situ* alginate polymer gelation followed by polyelectrolyte complex coating
2. Ionotropic alginate-Ca pre-gel followed by polyelectrolyte complex coating
3. Nanospray drying is at an advanced stage of development

Assay methods used to characterize insulin loaded nanoparticles

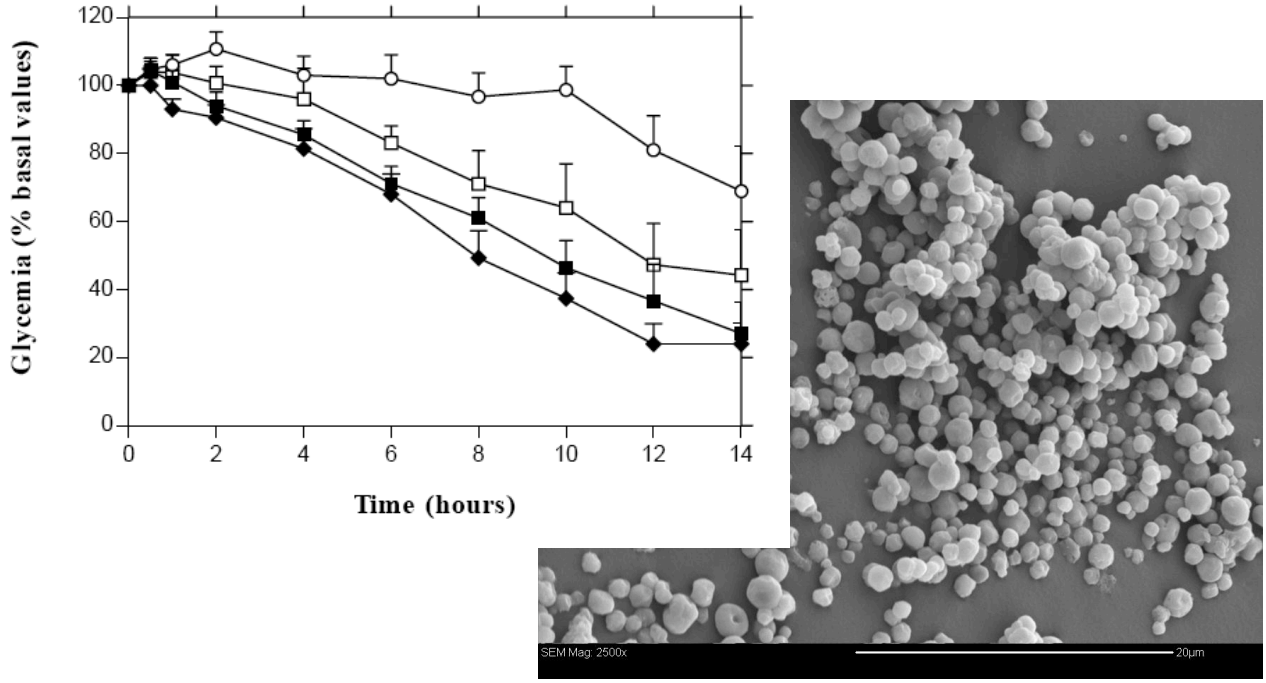
The following assay methods have been used to characterize the insulin loaded nanoparticles and will be described in the oral presentation.

- *in vitro* bioassay using rat myoblasts (described at a previous BRG meeting)
- HPLC and ELISA detection of insulin
- diabetic rats dosed subcutaneously with nanoparticulate insulin to demonstrate bioactivity
- diabetic rats dosed perorally with nanoparticulate insulin
- glycemic response of diabetic rats monitored, and pharmacological activity and insulinemia determined
- response to glucose challenge determined with oral insulin dosed diabetic rats

- metabolic/toxicologic studies have been conducted, both short and long term, monitoring hyperphagia, polydipsia, polyuria, albuminuria, and proteinuria
- tracking of FITC-insulin nanoparticles through the GI tract reveals site of adhesion/absorption of insulin/nanoparticles.
- nanoparticle and insulin characterization through DSC, FTIR and CD

Glycemic response to oral dosage of insulin-loaded nanoparticles

Following figure shows glycemic response for insulin-loaded nanoparticles containing 25 (❑), 50 (■) and 100 IU insulin/kg (◆), compared to blank nanoparticle controls (❍), dosed orally to fasting diabetic rats.



Opportunities

Oral delivery of nanoparticulate insulin with high bioavailability offers the potential for greatly improved patient convenience and compliance, and offers the potential for the oral delivery of other peptide or protein based drugs.

References

Recent references related to delivery of nanoparticulate insulin, based on this collaboration.

Pinto Reis, C., F. Veiga, A.J. Ribeiro, R.J. Neufeld and C. Damgé. Oral delivery of insulin by an alginate-dextran nanoparticulate system. In submission.

Pinto Reis, C., A.J. Ribeiro, R.J. Neufeld, C. Damgé. Polyelectrolyte biomaterial interactions provide nanoparticulate carrier for oral insulin delivery. In submission.

Sarmento, B., A. Ribeiro, F. Veiga, D. Ferreira and R.J. Neufeld. Oral insulin bioavailability contained in polysaccharide nanoparticles. Biomacromolecules, in press, July 31, 2007.

Sarmento, B., A. Ribeiro, F. Veiga, P. Sampaio, R.J. Neufeld and D. Ferreira. Alginate-chitosan nanoparticles are effective for oral insulin delivery. Pharmaceutical Research, in press, June 1, 2007.

Pinto Reis, C., A.J. Ribeiro, S. Houng, F. Veiga and R.J. Neufeld. Nanoparticulate delivery system for insulin: design, characterization and *in vitro/in vivo* bioactivity. Eur. J. Pharmaceut. Sci., in press, 29 Dec., 2006.

Sarmento, B., A.J. Ribeiro, F. Veiga, D.C. Ferreira and R.J. Neufeld. 2007. Insulin-loaded nanoparticles are prepared by alginate ionotropic pre-gelation followed by chitosan polyelectrolyte complexation, J. Nanosci. Nanotechnol., 7: 1-9.

Pinto Reis, C., A.J. Ribeiro, R.J. Neufeld and F. Veiga. 2007. Alginate microparticles as a novel carrier for oral insulin delivery. Biotechnol. Bioeng., 96: 977-989.

Pinto Reis, C., R.J. Neufeld, A.J. Ribeiro and F. Veiga. 2006. Nanoencapsulation II. Biomedical applications and current status of peptide and protein nanoparticulate delivery systems. Nanomedicine: Nanotechnology, Biology and Medicine, 2: 53-65.

Pinto Reis, C., Ronald J. Neufeld, A.J. Ribeiro and F. Veiga. 2006. Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine, 2: 8-21.

Reis, C.P., R.J. Neufeld, A.J. Ribeiro and F. Veiga. 2006. Design of insulin-loaded alginate nanoparticles: Influence of the calcium ion on polymer gel matrix properties. Chem. Ind. Chem. Eng. Quarterly, 12: 47-52.

Sarmento, B., S. Martins, A. Ribeiro, F. Veiga, R.J. Neufeld and D. Ferreira. 2006. Development and comparison of different nanoparticulate polyelectrolyte complexes as insulin carriers. Intern. J. Peptide Research and Therapeutics, 12: 131-138.

Pinto Reis, C., R.J. Neufeld, S. Vilela, A.J. Ribeiro and F. Veiga. 2006. Review and current status of emulsion/dispersion technology using an internal gelation process for the design of alginate particles. J. Microencapsul., 23: 245-257.