In vitro simulation of *in vivo* performance of oral dosed nanoparticulate insulin

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INTRODUCTION AND OBJECTIVE

At previous BRG meetings, we described the development of nanoparticulate multilayer polymeric structures for oral delivery of insulin. The most effective formulation involves an insulin-loaded alginate-dextran nanoparticulate core, complexed with a chitosan-PEG shell and finally with an albumin coat. The formulation reduced glycaemia in diabetic rats over a 24h period following a single oral administration, in a dose dependent manner. Pharmacological availability was by far the highest (42%) of any reported oral dosage form for insulin, and bioavailability was 30% relative to subcutaneous administration (Reis, 2008).

Nanoparticles and insulin were independently labelled and tracked through the gastrointestinal tract, and following internalization via the intestinal mucosa and enterocytes, into the mesenteric blood, thus through the portal-hepatic route of administration. Co-localization of the labels show that insulin is translocated along with the nanoparticle carriers likely through the transcellular route (Woitiski, 2011).

In vitro simulations were conducted by measuring insulin nanoparticulate permeation through intestinal membrane models. Caco-2/HT29 cell monolayers more closely simulated nanoparticulate permeation through the animal model intestinal mucosa, showing the importance of mucus-secreting cells for nanoparticles constructed of mucoadhesive biomaterials. Improved and intimate contact between the nanoparticles and mucosal layer thus appear to promote nanoparticle permeation, certainly in comparison to insulin alone which was poorly absorbed (Woitiski, 2011) Chitosan as a coating polymer is known to open tight junctions between enterocytes, and the albumin coating was shown to protect insulin from proteolytic enzymes (Reis, 2008).

Since less than half of the insulin was accounted for in the *in vivo* trials, GI simulations were conducted in the present study to examine factors affecting insulin nanoparticulate retention/release and stability.

MATERIALS AND METHODS

Human recombinant insulin was mixed with 2% alginate solution containing 5% ultrafine calcium carbonate, and 0.75% dextran sulphate. The mixture was emulsified in paraffin oil at high speed, facilitated with 2.5% Span 80. After 15 min, gelation was induced by addition of paraffin oil containing acetic acid to adjust the pH from about 7.5 to 6.5 (emulsification/internal gelation through release of soluble Ca^{++}). After separating particles from the dispersion, particle cores were coated in PEG-chitosan, then in albumin solutions. Details are provided in Reis (2008). Insulin assay was conducted by ELISA, and particle sizing by Malvern Zetasizer.

RESULTS AND DISCUSSION

Nanoparticles consisted of an alginate-dextran core containing insulin, complexed with a chitosan-PEG coat, followed by albumin outer layer. The layered structure may be seen in Figure 1. Mean diameter was 10 nm, Zeta potential was -7 mV and encapsulation efficiency was 85%.

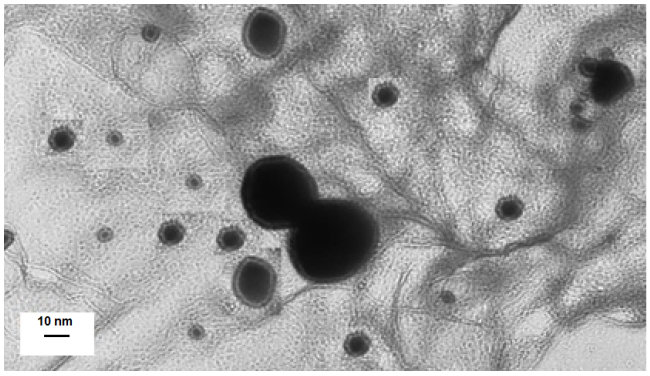

Figure 1: TEM of insulin nanoparticles

A release profile of insulin in gastric (2 h) followed by intestinal (3 h) simulation is provided in Figure 2. Minimal release of insulin is observed in acidic condition, likely due to the collapsed structure of the core polymer alginate, followed by particle swelling in neutral pH medium initiating the release of insulin over the subsequent 3 h period.

Insulin retention/release in neutral pH medium is dependent on medium composition as seen in Figure 3. Buffer solutions tested were those commonly used for GI simulation. It is apparent that the phosphate buffer and high Na:Ca ratio strongly promotes insulin release due to displacement of Ca^{++} from the particle core, and the chelating properties of phosphate, while bicarbonate buffers and lower Na:Ca ratio reduces release by a half over 3h. As insulin assay was based on antibody binding, it is assumed that fully intact insulin is being detected in the release medium.

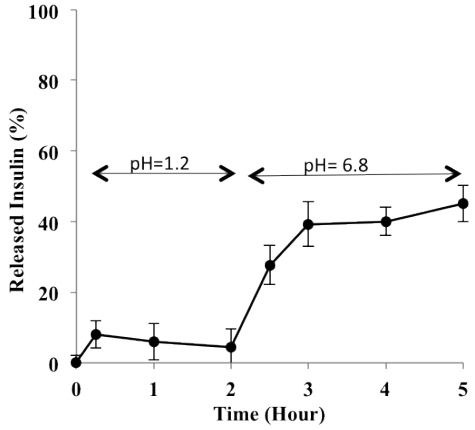

Figure 2: Insulin retention/release in GI simulation

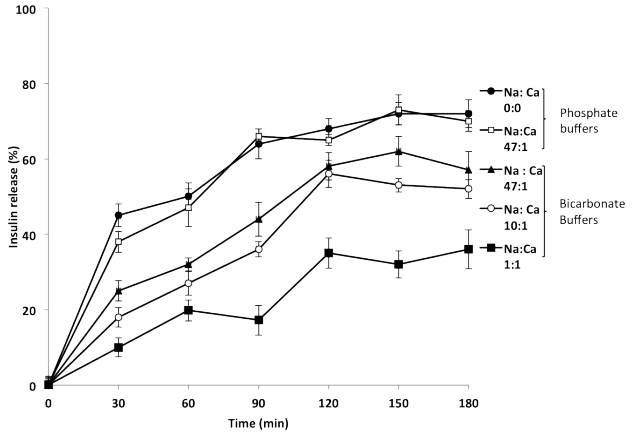

Figure 3: Insulin retention/release in GI-simulating buffers at neutral pH.

We have shown an insulin oral pharmacological availability (PA) of 42% in diabetic rats (Reis, 2008). A simple interpretation is that 42% of the insulin is accounted for, relative to injectable insulin. The *in vitro* simulation presented in figure 2 suggests that just under half of the insulin is potentially released into the GI tract. Subsequent dissolution of the nanoparticles in chelating solution, released the remaining insulin content demonstrating that all of the insulin is accounted for. The question then is whether the 42% PA is due to prematurely released insulin, or insulin loss in gastric, protease-rich GI fluids.

The effect of acid and protease enzymes on insulin stability was then examined. Insulin stability in gastric simulation is presented in Figure 4 and intestinal simulation in Figure 5. It is apparent that while only small amounts of insulin are released in acid medium, it is highly susceptible to both acid and pepsin as seen in Figure 4. Insulin appears subsequently stable in intestinal medium for additional 3h, including in the presence of trypsin. Differences in concentration relative to the control, are likely due to changes in quaternary structures of insulin which are pH dependent (Bryant, 1993) and do not interact stoichiometrically with ELISA antibodies.

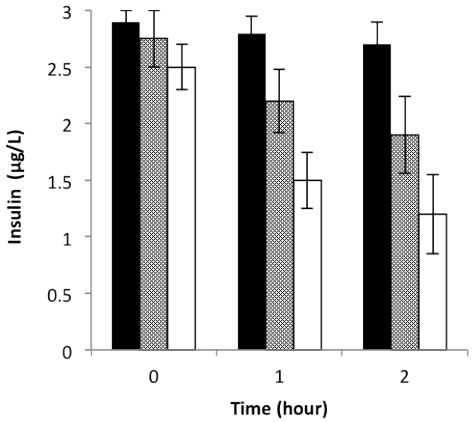

Fig. 4: Insulin stability in neutral (black), gastric (gray) and gastric medium with pepsin (white)

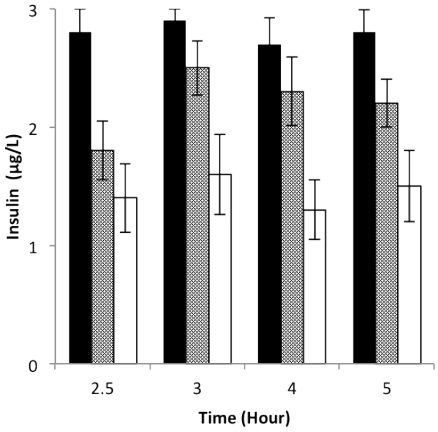

Figure 5: Following gastric simulation, insulin stability in intestinal medium (gray) and with trypsin (white) compared to neutral (black)

The nanoparticulate form thus enables insulin gastric retention/protection and controllable release of stable insulin under GI conditions, in a manner largely consistent with PA results obtained in vivo.

CONCLUSIONS

A nanoparticulate polymer complex containing human insulin is described providing the highest level of PA reported in the literature. Insulin instability in acid (gastric) and neutral (intestinal) medium including in presence of intestinal proteases, is neutralized when in nanoparticulate form. It is noteworthy that the extent of premature release of insulin and demonstrated stability in intestinal simulation is consistent with the level of PA observed *in vivo*.

REFERENCES

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