Poster Session 3 – Biopharmaceutics

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In-vivo evaluation of insulin nasal gel spray in rabbits

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The nasal route has been extensively investigated as an alternative method for systemic peptide and protein drug delivery. A combination of high molecular weight and polar character as well as significant degree of enzymatic degradation and rapid transport away from the site of absorption by the mucociliary clearance system, is thought to restrict passage across the nasal epithelium (Chandler *et al* 1991). A sprayable gel formulation that causes longer residence time of drug in the nasal cavity would be a potential way to improve effectiveness for peptides and proteins, Rouholamini Najafabadi *et al* (2002).

The purpose of this study was to investigate the nasal absorption of insulin from a carbopol-based gel spray in rabbits.

Gel formula was prepared with Carbopol 934P (0.4% w/w) in distilled water containing 30 IU mL^{-1} insulin, neutralized to pH of 7.4 $\pm$ 0.2. Viscosity was adjusted to 2000 cps by adding proper amount of NaCl. The prepared gel was filled into the bottles with spray pumps (Pfeiffer, Germany). Male white rabbits weighing about 3.0 kg were fasted for 16 h prior to the experiments. A 100-μL dose of the following preparations were delivered to each nostril: [A] Insulin gel spray (30 IU mL^{-1}). [B] Insulin solution (30 IU mL^{-1}). [C] Placebo gel spray. Insulin solution also was administered intravenously to compare the results [D]. Blood samples were taken periodically from the marginal ear vein. Plasma glucose determinations were carried out within 4 h sampling using an ACCU-CHECK glucose analyzer (Roche Diagnostics, Germany) (Table 1).

Table 1 Blood glucose variations following administration of different preparations (n = 6)

Time (min)	% Blood glucose variations			
	A	B	C	D
15	96.5 $\pm$ 4.3	104.7 $\pm$ 6.8	102.8 $\pm$ 6.0	90.9 $\pm$ 3.3
30	86.9 $\pm$ 9.3	99.8 $\pm$ 6.0	105.0 $\pm$ 8.6	68.1 $\pm$ 8.1
45	70.6 $\pm$ 6.6	93.5 $\pm$ 8.4	108.2 $\pm$ 8.4	53.5 $\pm$ 4.4
60	61.2 $\pm$ 7.1	101.4 $\pm$ 6.1	108.1 $\pm$ 9.2	39.1 $\pm$ 10.5
90	68.0 $\pm$ 6.9	104.4 $\pm$ 7.7	114.0 $\pm$ 15.4	56.9 $\pm$ 4.0
120	78.5 $\pm$ 8.5	100.5 $\pm$ 4.0	111.7 $\pm$ 12.9	66.8 $\pm$ 7.6
180	89.5 $\pm$ 7.8	98.3 $\pm$ 5.8	107.8 $\pm$ 10.4	96.3 $\pm$ 4.4
240	93.6 $\pm$ 6.6	96.0 $\pm$ 3.0	104.5 $\pm$ 7.8	104.1 $\pm$ 2.7

Data are means $\pm$ s.d.

Comparing A and B formulations, 40% decrease in blood glucose level was achieved after 1 h. The administration of placebo gel spray did not cause any modification in blood glucose levels, indicating that the animals were not stressed by the administration procedure and blood sampling. The absolute bioavailability obtained was about 27%.

The results of this study suggest that the carbopol gel spray appear to increase the permeability of the nasal mucosa, and thereby promote the nasal absorption of a high-molecular weight polypeptide.

Chandler, S. G., Illum, L., Thomas, N. W. (1991) *Int. J. Pharm.* **76**: 61–70

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The design and construction of a biomimetric pilus for the targeting of nanospheres through the cell glyocalyx

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Recent studies have demonstrated that the epithelial glycocalyx inhibits the uptake of DNA and protein via viral and non-viral particulate vehicles (Pickles *et al* 2000). In order to address this, the aim of this project is to engineer a stalk which projects from the surface of a nanoparticle that has the potential to penetrate the glycocalyx, and hence facilitate receptor-mediated endocytosis and internalisation. This rationale is modelled on the cell invasion mechanism of the type VI pili of *Pseudomonas*, *Neisseria* and *Vibrio* (Lee *et al* 1994). In this project, the candidate stalk is derived from Tetrabrachion, a right-handed tetrameric coiled coil surface protein of *Staphylothermus marinus* (Peters *et al* 1996). This stalk will be engineered to terminate in streptavidin in order to allow rapid screening of biotinylated ligands, such as invasin, placed at the tip of the 'pilus'.

Four different lengths of the coiled coil from 5 to 50 nm were derived in order to assess the relationship between coiled coil length and glycocalyx thickness. The DNA sequences of the four polypeptides of Tetrabrachion — Pro1160-Ser1196, Pro1160-Asp1300, Pro1160- Thr1404 and Pro1160-Asn1494 — were amplified from *Staphylothermus marinus* genomic DNA (DSM3639) by PCR. Sequence analysis demonstrated several codon changes in the PCR amplicons compared with published sequences suggesting strain specific codon sequences. The resultant amino acid substitutions — S1321T, G1324E, N1327K and H1357T — were not predicted to be involved in hydrophobic contacts stabilising the tetramer and therefore were not altered. The core structure of Streptavidin (G40-P159) was amplified from *Streptavidin avidinii* genomic DNA by PCR. The antiparallel arrangement of the streptavidin tetramer imposes a geometric constraint for positioning against the tetrabrachion tetramer. However, simple molecular modelling suggested that a 15 residue glycine linker would be sufficient to avoid inappropriate contact. Therefore, the Tetrabrachion DNA sequences and streptavidin core, separated by a 15 glycine linker, were cloned into pBluescript and sub-cloned into glutathione-S-transferase- (pGEX-4T3) and polyhistidine-fusion (pQE80L and pRSETa) expression vectors, in order that the proteins may be expressed in *E. coli* and isolated by affinity chromatography. The DNA sequence of the integrin-binding D4-D5 domain pair of invasin has been amplified by PCR from *Yersinia psuedotuberculosis* genomic DNA (DSM8992) and cloned into pMAL-c2x. Expression in *E. coli* DH5α and purification on maltose-amylose resin has yielded 15 mg L^{-1} of the expected maltose binding protein-invasin fusion of 18 kDa.

Tetramerisation and alpha-helical structure for the tetrabrachion-streptavidin chimera will be verified using gel permeation chromatography/PAGE and circular dichroism. Further studies will involve the coupling of the coiled coil- streptavidin complex to nanospheres, focusing on the binding and internalisation of the nanospheres to cell lines, which exhibit a glycocalyx.

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Peters J., *et al.* (1996) *J. Mol. Biol.* **257**: 1031–1041

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In-vitro release of deferoxamine mesylate from multivesicular liposomes

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Liposomes can act as a depot from which the entrapped compound is slowly released over time. Such a sustained release process can be exploited to maintain therapeutic drug levels in the blood or at the local administration site for prolonged periods of time. Thus an increased duration of action and a decreased frequency of

administration are beneficial consequences. Various types of liposomal formulations have been utilized as drug delivery vehicles for sustained release of drugs (Ye *et al* 2000). Multivesicular liposomes (Depofoam particles) have a highly characteristic physical structure, distinguishing them from other types of liposomes and other lipid-based drug delivery systems. Depofoam particles are microscopic and spherical, and each particle encloses multiple non-concentric aqueous chambers bounded by a single bilayer lipid membrane with a foam-like appearance under the microscope. Multivesicular liposomes (MVL) have advantages over other types of liposomes in that the composition and structure results in good stability during storage, drug release rate is slow and highly efficient entrapment of hydrophilic molecules including a variety of therapeutic proteins, analgesics, anticancer, antimicrobial and antiviral agents (Katre *et al* 1998; Ramprasad *et al* 2002).

Deferoxamine mesylate (DFO) remains the only first-line iron-chelating agent. Unfortunately because it has a short half-life and is poorly absorbed by the gastrointestinal tract, DFO must be administered parentrally, usually by daily subcutaneous continuous infusion (Franchini *et al* 2000). The administration is uncomfortable for patients. Therefore, a slow-release depot preparation of DFO is needed.

This study describes the preparation and characterization of depofoam particles as a slow-release delivery system for subcutaneous administration of DFO. Multivesicular liposomes containing deferoxamine mesylate were prepared by modification of the method of Kim *et al*. All of the studies were set up in three independent experiments. One millilitre of DFO (100 mg mL^{-1}) in 1% sucrose (w/v) was emulsified with an equal volume of chloroform solution containing 20.5 mg phosphatidyl cholin (ScPc-1, Sigma Type IV-S), 20.5 mg Soy lecithin Lipoid S75 (ScPc-2), 19 mg cholesterol, 26 mg triolein at ambient temperature (23–28°C) for 30 min at 3500 rev min^{-1}. Then each half of resulting "water-in-oil" emulsion was expelled rapidly through a narrow-tip Pasteur pipette into 25 mL aqueous solution containing dextrose 3.2% and L.lysine (40 mM), and then was shaken for 20 s at 3500 rev min^{-1}. The resulting emulsions were added to 1 L baffled flask containing 25 mL of aqueous solution containing dextrose 3.2% (w/v) and L.lysine (40 mM). Chloroform was removed by flushing the surface of mixture over a 15-min period at 37 $\pm$ 2°C with nitrogen gas at 8 L min^{-1}. The resulting multivesicular liposomes were then isolated by centrifugation at 600 *g* for 5 min and washed with 0.9% NaCl solution three times.

The Depofoam particles were characterized by their morphology, particle size and capture volumes. The effects of various concentrations of components on capture volumes of these particles were studied. The results showed that the particles were smooth, multivesicular without any debris. The particle size of these liposomes was 15–35 μm with capture volumes about 27%. The multivesicular particles (Depo-DFO) released DFO in a sustained manner without a rapid initial release. The in-vitro studies in 5% dextrose at 37°C showed that the multivesicular liposomes released DFO slowly over several days, and 57% DFO was released in 9 days.

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Expression and functionality of carrier-mediated transporters within an immortalised brain microvascular cell line model of the blood–brain barrier

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Robust in-vitro models of the blood–brain barrier (BBB) are a valuable tool for studies of CNS drug delivery (Gumbleton & Audus 2001). In order to further characterise the utility of an immortalised brain microvascular cell line (mouse b.End3) as a model system to study BBB transport mechanisms (Omidi *et al* 2002), a comprehensive RT-PCR study was first undertaken to confirm the presence of RNA transcripts for carrier systems recognised to be present within either the in-vivo BBB itself or indeed the choroid epithelium of the blood–CSF barrier. The transporters characterised included: the glucose transporter GLUT1; the organic anion transporters OAT1 and Oatp 1/2; the concentrative nucleoside transporters cNT 1/2/3; the equilibrative nucleoside transporters eNT 1/2/3; the monocarboxylic transporters MCT 1/2; the oligopeptide transporters PepT 1/2; the multidrug resistance transporters mdr 1a/1b and MRP 1/3/45/6; and the carrier systems for amino acids including system y^+L, system A, system $b^{0,+}$ and system L.

System L is the major Na^+-independent bidirectional neutral amino acid transporter, and among nutrient transporter systems within the BBB this system displays the highest permeability (min cm^{-1}) although the K_m value is considered relatively low (~10–30 μM). It is therefore an important tranporter for further functional assessment since the design of CNS drugs to mimic neutral amino acids and hence exploit system L transport is attractive, such drugs may suffer significant competition for transport from dietary neutral amino acids. Both the LAT1/4F2hc and the LAT2/4F2hc system L transporter systems were expressed in b.End3 cell monolayers mediating transport of large and small neutral amino acids, respectively (Wagner *et al* 2001). Modulation of these carrier systems by astrocyte-derived factors was apparent with changes in L-phenylalanine transport (K_m 16.7 μM) via LAT1/4F2hc resulting in decreased affinity (K_m of 48.3 $\pm$ 19.6 μM) with respect to apical or luminal uptake, but not in the polarised transport of L-phenylalanine via LAT1 which nevertheless gave a basal to apical permeability at least 2-fold greater than the apical to basal permeability.

The b.End3. cell line appears to be a useful model system as for the study of carrier mediated transport processes for solutes entering or exiting the CNS. The cell line is commercially available from European Collection of Animal Cell Cultures. (ECACC).

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