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Bioadhesive starch microspheres and absorption enhancing agents act synergistically to enhance the nasal absorption of polypeptides

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Abstract

This paper investigates the effect of starch microspheres on the absorption enhancing efficiency of various enhancer systems in formulations with insulin after application in the nasal cavity of sheep. The enhancers studied were lysophosphatidylcholine, glycodeoxycholate and sodium taurodihydroxyfusidate, a bile salt derivative. The enhancers were selected on the basis of their perceived or proven mechanism of action and worked predominantly by interacting with the lipid membrane. The bioadhesive starch microspheres were shown to increase synergistically the effect of the absorption enhancers on the transport of the insulin across the nasal membrane. Dependent on the potency of the enhancer system the increment in absorption enhancement was shown to be from 1.4 times to 5 times that obtained for the absorption enhancer in solution.

Keywords: Lysophosphatidylcholine; Glycodeoxycholate; Sodium taurodihydroxyfusidate; Starch microspheres; Nasal delivery; Absorption enhancers

1. Introduction

It has been established that bioadhesive systems based on microspheres are able to increase significantly the systemic absorption of conventional drugs as well as polypeptides across the nasal membrane without the use of absorption enhancing agents that have the potential for irritation or damage (Illum, 1998). Hence, Illum et al. (1994) investigated the use of hyaluronic acid ester microspheres as a nasal delivery system for insulin and found that the mean relative bioavailability obtained in sheep was 11%, compared to 1.2% for a simple nasal insulin solution. Pereswetoff-Morath and Edman (1995, 1996) and Pereswetoff-Morath et al. (1996) studied the effect of particle size and swellability of dextran microspheres for the improved nasal absorption of insulin in rats. They also showed that the microspheres were non-immunogenic and non-toxic, and conducted

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measurements of cilia beat frequency (rat tracheal rings) and histology studies (rat nasal mucosa). Similar microspheres were used by Cornaz et al. (1996) for improving the nasal absorption of nicotine. El-Shafy et al. (2000) studied the nasal absorption of the model material FITC-dextran in combination with various bioadhesive formulations to include chitosan and carbopol.

A range of studies has been performed on starch microspheres as a nasal delivery system for polar small molecules and for peptides and proteins. Illum et al. (1988) found that the bioavailability of gentamicin was increased ten fold when administered nasally in combination with starch microspheres as a freeze dried powder formulation in sheep. Similarly, the nasal absorption of insulin was increased five fold when given with starch microspheres as compared to a simple insulin solution in sheep (Farraj et al., 1990) and the bioavailability of the same drug was increased 30 fold in rats over a solution control when using the microsphere option (Bjork and Edman, 1988, 1990). Morphological examination of the rabbit nasal mucosa after the nasal administration of starch microspheres for up to 8 weeks has shown that the system can be considered to be biocompatible and not induce serious histopathological changes in the nasal mucosa (Bjork et al., 1991).

The mechanism of action of the bioadhesive starch microsphere system can be explained as follows: Firstly, deposition of the starch microsphere formulation in the anterior part of the nasal cavity where none or only few cilia are present. The drug-microsphere system is then presented slowly to the absorptive regions of the nose. Secondly, the formulation is retained in the nasal cavity for an extended time period due to its bioadhesive nature. Thirdly, the gelled system provides a local high drug concentration in close contact with the epithelial absorptive surface. Finally, the absorption of water by the microspheres from the mucus layer as it hydrates and gels could affect the passage of the drug through the paracellular tight junctions (Illum, 1991; Edman et al., 1992; Bjork et al., 1995).

For polypeptides, in excess of 6000 Da, the absorption enhancing effect of the bioadhesive system is not normally sufficient to provide clinically relevant plasma levels but Illum et al. (1988) and Farraj et al. (1990) have shown that the bioadhesive microsphere system can be combined with biological absorption promoters such as lysophospholipids to form effective nasal delivery systems. Hence, L-α-lysophosphatidylcholine when administered nasally in combination with insulin in sheep gave a bioavailability of 5.9% (relative to subcutaneous administration) but this increased to 31.5% when administered with insulin in a freeze dried starch microsphere formulation (Farraj et al., 1990). Lysophospholipids are metabolites of phospholipids and are natural constituents of cell membranes (Stafford & Dennis, 1988). They are surfactant-like molecules and their mode of action in enhancing the transport of drugs across the nasal membrane are considered to be a combination of a mucolytic effect, changes in the physicochemical properties of the cell membrane lipid bilayer and possibly the opening of tight junctions between the cells (Martin et al., 1978, 1992; El-Hariri et al., 1991; Ropke et al., 1996; Marttin et al., 1995). Dependent on their nature (eg the chain length of the fatty acid side chains) the lysophospholipids can be categorised as mild enhancers with little or no toxic effect on the nasal membrane in vivo (Chandler et al., 1991).

The purpose of the present work was to evaluate the influence of the bioadhesive microsphere system on the nasal absorption enhancing effect of lysophosphatidylcholine (LPC) and two other well known absorption enhancing agents, the bile salt sodium glycodeoxycholate (GDC) and the bile salt derivative sodium taurodihydroxyfusidate (STDHF). The last two enhancer systems have been shown to enhance greatly the nasal absorption of polypeptides such as insulin, growth hormone and calcitonin in animal models and in man (Duchateau et al., 1986; Deurloo et al., 1989; Moses et al., 1983; Wearley, 1991), whereas LPC has been shown to be a less effective enhancer when given nasally as a solution with insulin (Farraj et al., 1990) or with human growth hormone (Fisher et al., 1991). For both GDC and STDHF the mode of action is considered to be a combination of enzyme inhibition, mucolytic activity, an interaction with the lipid bilayer of the

cell membrane and possibly the opening of tight junctions (Duchateau et al., 1986, 1987; Ennis et al., 1990; Shao and Mitra, 1992; Marttin et al., 1995, 1996).

In the present work, the different enhancer systems were administered nasally to sheep with insulin either in solution or as a freeze-dried microsphere formulation on an identical mg/kg body weight basis. Insulin was chosen as a model polypeptide drug since it has a suitable molecular weight, it is a good candidate for nasal delivery and the effect of the drug on the blood glucose level and the plasma insulin levels are readily detectable.

2. Materials and methods

2.1. Materials

Semi-synthetic sodium-human insulin (SHI) was obtained from Novo-Nordisk A/S, Denmark. Sodium glycodeoxycholate and lysophosphatidylcholine were purchased from Sigma Chemical Company (Poole, Dorset, UK) and used as supplied. Sodium taurodihydrofusidate was kindly provided by California Biotechnology (CA). All other chemicals were of reagent grade. Degradable starch microspheres 24/45 (DSM) manufactured by emulsion polymerisation of hydrolysed potato starch was obtained from Kabi Pharmacia A/B (Uppsala, Sweden).

The size of the microspheres used in these studies was 48 μm (swollen state, mean volume diameter).

2.2. Formulation preparations

Stock solutions of insulin 200 IU/ml were prepared in phosphate buffer (13.3 mM) at pH 7.3. All solutions were filtered through 0.22 μm sterile filters (μSTAR, Costar, Cambridge, MA) and used immediately. The insulin stock solution was used for intranasal (IN) administration either alone or in combination with each absorption enhancer.

The stock solution was also used for the preparation of the lyophilised starch microsphere formulations. The required quantity of starch microspheres was dispersed in an appropriate amount of the insulin stock solution together with any required enhancer. The mixture was stirred for 1 h at room temperature, and then freeze-dried on an Edwards Modulyo 4K freeze dryer. The loading of the starch microspheres used in these experiments was 0.8 IU of insulin per mg of starch microspheres. The enhancers STDHF or GDC were present at 0.032 mg/mg and LPC was present at between 0.002 and 0.04 mg/mg of starch microspheres.

2.3. Sheep studies

Cross-bred sheep (Suffolk & Texel) were housed indoors on straw beds as a flock. They were fed on a nut concentrate with ad libitum hay and water. The animals were not fasted prior to insulin administration. For blood sampling, the animals were cannulated in an external jugular vein on the first day of the study. The cannula was kept patent by flushing with heparinised normal saline. For intranasal administration the sheep were sedated by an IV dose of ketamine hydrochloride 2 mg/kg to prevent sneezing during administration. The sedation lasted for about 3 min. The methods of intranasal administration of liquid and powder formulations have been described previously (Illum et al., 1988; Farraj et al., 1990).

Thirty-eight sheep were divided into 10 groups of three sheep and two groups of four sheep. Aqueous insulin formulations were administered at 2 IU/kg (200 IU/ml) insulin, 0.08 mg/kg STDHF or GDC and from 0.005 mg/kg to 0.1 mg/kg LPC. Similarly, the lyophilised insulin formulations were administered intranasally at 2 IU/kg insulin in combination with 2.5 mg/kg starch microspheres and similar doses of the enhancers were used as for the solution formulations. The various formulations administered to the sheep are listed in Table 1. Blood samples of 5 ml were collected on crushed ice from the cannulated jugular vein of the sheep at 15 and 5 min prior to the insulin administration and at 5, 10, 20, 30, 40, 50, 60, 75, 90, 120, 150, 180 and 240 min post-administration. Each blood sample was divided into two

Table 1
Composition and quantity of formulations administered to sheep

Nasal formulation	Insulin (IU/kg)	DSM (mg/kg)	GDC (mg/kg)	STDHF (mg/kg)	LPC (mg/kg)
1	2.0	–	–	–	–
2	2.0	–	0.08	–	–
3	2.0	2.5	0.08	–	–
4	2.0	–	–	0.08[a]	–
5	2.0	2.5	–	0.08[a]	–
6	2.0	–	–	–	0.005
7	2.0	–	–	–	0.02
8	2.0	2.5	–	–	0.02
9	2.0	–	–	–	0.05
10	2.0	2.5	–	–	0.05
11	2.0	–	–	–	0.1
12	2.0	2.5	–	–	0.1

[a] $n = 4$.

parts. For insulin analysis, the blood collected (3 ml) was mixed gently in 5 ml heparinised (Li Heparin) tubes for glucose analysis, the blood collected (2 ml) was mixed gently in 5 ml fluoride oxalate tubes. The plasma was separated by centrifugation at 4 °C and 3000 rpm, and then stored awaiting insulin and glucose analysis. The analyses were performed as described previously using a Yellow Springs Instrument 23AM Glucose Analyser and a double-antibody insulin radioimmune assay (Farraj et al., 1990).

2.4. Statistics

The results were analysed using a one-way analysis of variance (ANOVA) by an InStat 2.03 computer programme and an unpaired t-test.

3. Results and discussion

The effect of GDC at a dose of 0.08 mg/kg on the transport of insulin across the nasal membrane after administration as a solution and as a bioadhesive starch microsphere powder formulation in sheep in terms of the resultant plasma glucose levels is shown in Fig. 1. The decreases in glucose levels were 40% for the GDC solution formulation and 55% for the bioadhesive GDC microsphere formulation. For both systems the minimum glucose levels were reached within 50 min. Hence, the biological effect obtained for the two insulin formulations containing GDC, identical in terms of both insulin dose and GDC dose on a mg/kg basis, was found to be more pro-

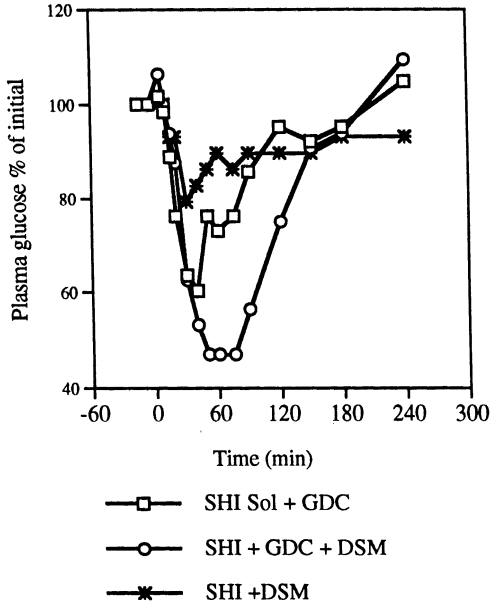

Fig. 1. The plasma glucose levels in sheep following nasal administration of insulin in combination with glycodeoxycholate as a solution formulation, with starch microspheres as a freeze-dried powder or with glycodeoxycholate and starch microspheres as a freeze dried powder. SHI Sol, Sodium insulin solution; SHI, Sodium insulin; DSM, Starch microspheres; GDC, Glycodeoxycholate.

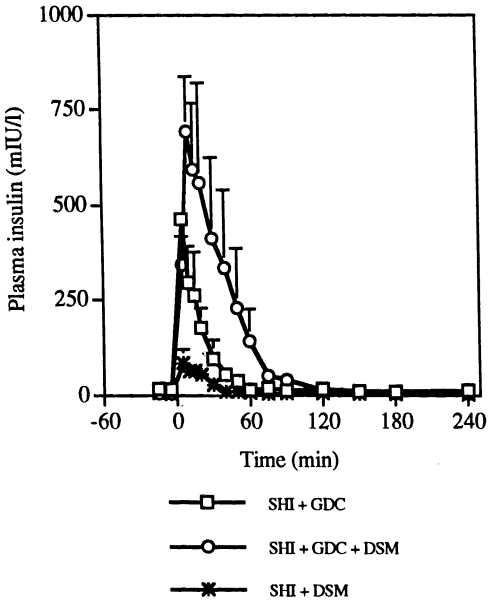

Fig. 2. The plasma insulin levels in sheep following nasal administration of insulin in combination with glycodeoxycholate as a solution formulation, with starch microspheres as a freeze-dried powder or with glycodeoxycholate and starch microspheres as a freeze dried powder. SHI, Sodium insulin; DSM, Starch microspheres; GDC, Glycodeoxycholate.

nounced for the bioadhesive nasal powder formulation than for the solution. It can also be seen that both of these GDC formulations had a more pronounced effect on the plasma glucose level than insulin administered nasally with starch microspheres alone.

The pharmacodynamic effect was mirrored in the pharmacokinetic characteristics for the three formulations (Fig. 2, Table 2). A higher plasma insulin peak level (C_{max}) of 776 mIU/l and a 2.3 fold larger bioavailability for the GDC starch microsphere formulation (31.9%) was obtained as compared to the GDC solution formulation (C_{max} of 463 mIU/l and bioavailability of 10.0%). The control starch microsphere formulation without the GDC gave a bioavailability of just 3.6%. The tmax was reached within 5 min for all formulations.

A similar effect was seen for the enhancing agent of STDHF given in a dose of 0.08 mg/kg (Fig. 3) with a maximum fall in blood glucose levels of 20% for the STDHF solution formulation and 43.5% for the STDHF starch microsphere formulation. The minimum plasma glucose levels were reached at 40 min and 60 min after the administration of the STDHF solution formulation and powder formulation, respectively.

As for the case of the GDC enhancer system, the plasma insulin levels (*C*max) were increased from 243 mIU/l to 409 mIU/l and the bioavailability increased 2.5 fold, when insulin

Table 2
Intranasal administration of different enhancer formulations in combination with insulin to sheep

Formulation	Area under curve (AUC)+/−SEM (mIU·min/l)	Mean C_{max} [a]+/−SEM (mIU/l)	Relative [b] bioavailability (%)	Ratio: AUC DSM AUC sol
SHI Sol (2 IU/kg)	1546 (+/−417)	52 (+/−13)	1.8	–
SHI Sol (2 IU/kg)+GDC (0.08 mg/kg)	12136 (+/−2621)	463 (+/−21)	14.1	–
SHI (2 IU/kg)+DSM (2.5 mg/kg)+GDC (0.08 mg/kg)	27484 (+/−11830)	776 (+/−155)	31.9	2.3
SHI Sol (2 IU/kg)+STDHF (0.08 mg/kg)	5812 (+/−555)	243 (+/−17)	6.7	–
SHI (2IU/kg)+DSM (2.5 mg/kg)+STDHF (0.08 mg/kg)	14207 (+/−2057)	409 (+/−59)	16.5	2.4
SHI (2IU/kg)+DSM (2.5 mg/kg)	3078 (+/−1140)	97 (+/−31)	3.6	–
SC SHI Sol (0.2 IU/kg)	8615 (+/−1297)	95 (+/−14)	100.0	–

Effect of formulation on pharmacokinetic parameters
[a] Calculated from individual sheep insulin profiles.
[b] To s.c. administration.

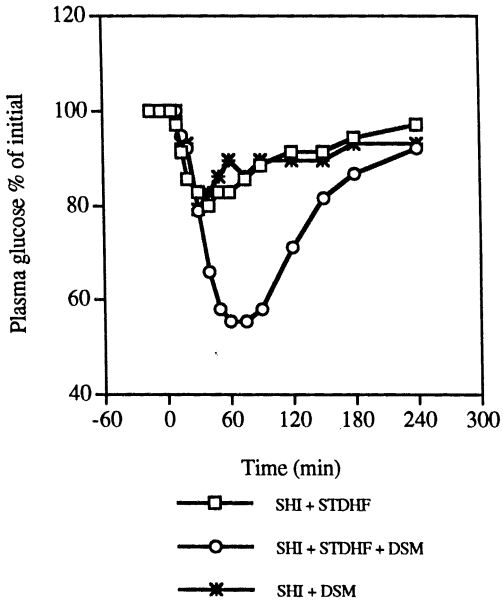

Fig. 3. The plasma glucose levels in sheep following nasal administration of insulin in combination with sodium tauro-24,25-dihydrofusidate as a solution formulation and with starch microspheres with or without sodium tauro-24,25-dihydrofusidate as a freeze-dried powder. SHI, Sodium insulin; DSM, Starch microspheres; STDHF, Sodium tauro-24,25-dihydrofusidate.

was administered as a freeze-dried formulation in combination with STDHF and starch microspheres as compared to the STDHF solution formulation (Fig. 4, Table 2). When compared to the control starch microsphere formulation without enhancer the bioavailability was significantly increased, by 4.6 times. For both STDHF formulations tmax was reached within 5 min after nasal administration.

When insulin was administered nasally in solution formulations containing various concentrations of the lysophospholipid LPC the decrease in plasma glucose levels were directly related to the amount of LPC administered in the formulation. The formulations, split equally between the two nostrils, were administered on the basis of a total volume of 0.01 ml/kg with LPC concentrations of 0.5, 2.0, 5.0 and 10.0 mg/ml and identical concentrations of insulin. Within 50 min, the plasma glucose levels fell to 96.9, 87.1, 76.2 and 71.4% of base levels, respectively for LPC doses of 0.005, 0.02, 0.05 and 0.1 mg/kg (Fig. 5). The plasma insulin levels reached their tmax values within 3–5 min. The *C*max was lowest for the 0.005 mg/kg dose and the highest for the three higher doses given (Fig. 6, Table 3). There was no significant difference between the plasma insulin levels reached for the three highest doses. The highest plasma insulin level obtained was 142 mIU/l compared to 463 mIU/l for the GDC solution formulation and 243 mIU/l for the STDHF solution formulation. Thus, it was evident from the pharmacodynamic and the pharmacokinetic results that, compared to the GDC and the STDHF in the concentrations and the doses used, LPC was not such an effective nasal absorption enhancer.

Interestingly, combining LPC with starch microspheres and formulating it into a freeze-dried powder had a dramatic effect on the effectiveness of LPC as an enhancer system. (Only the three highest doses of LPC were combined with the starch microspheres since the effect of the lowest

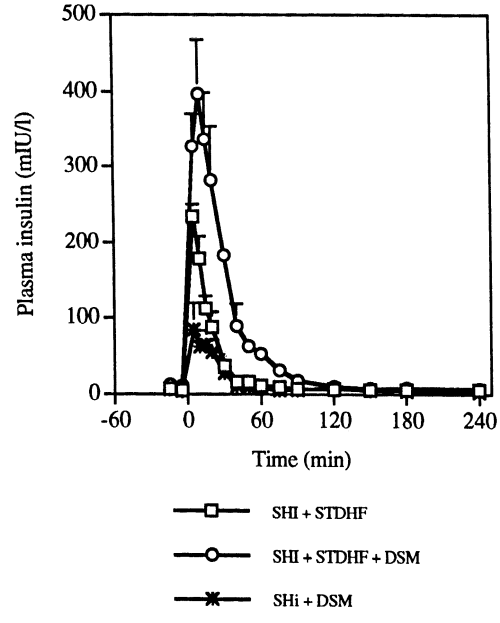

Fig. 4. The plasma insulin levels in sheep following nasal administration of insulin in combination with sodium tauro-24,25-dihydrofusidate as a solution formulation or with starch microspheres with or without sodium tauro-24,25-dihydrofusidate as a freeze-dried powder. SHI, Sodium insulin; DSM, Starch microspheres; STDHF, Sodium tauro-24,25-dihydrofusidate.

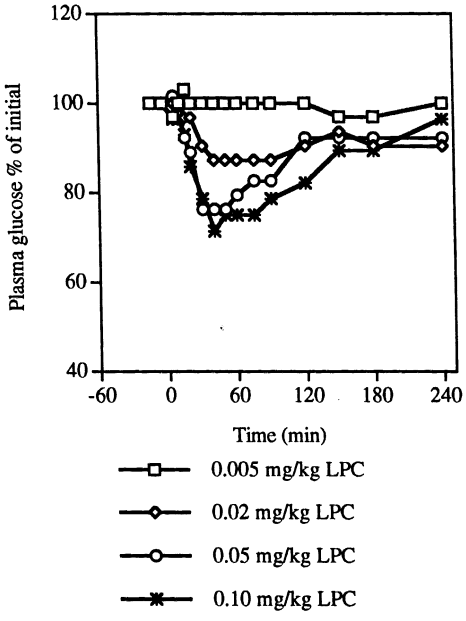

Fig. 5. The plasma glucose levels in sheep following nasal administration of insulin in combination with L-α-lysophosphatidylcholine at various concentrations as a solution formulation. LPC, L-α-lysophosphatidylcholine.

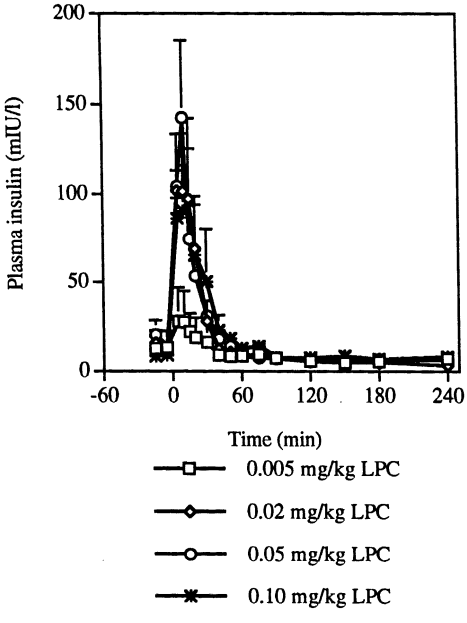

Fig. 6. The plasma insulin levels in sheep following nasal administration of insulin in combination with L-α-lysophosphatidylcholine at various concentrations as a solution formulation. LPC, L-α-lysophosphatidylcholine.

dose was negligible when given as a solution formulation). The minimum plasma glucose levels fell to 89.6, 46.4 and 41.6% of base levels, respectively, for LPC doses of 0.02, 0.05 and 0.1 mg/kg as shown in Fig. 7. Similarly, the maximum plasma insulin levels obtained increased to 380 mU/l. The tmax was reached within 20–30 min after administration (Fig. 8). For the three doses of LPC used, the bioavailability increased by 1.4, 5.0 and 3.6 times respectively, when given as a starch microsphere powder formulation as compared to the equivalent simple solution formulations. These increases were significant for the two highest doses of LPC. There was no significant difference between the plasma insulin levels or the AUCs obtained for the two highest doses of LPC used in combination with the starch microspheres.

The use of starch microspheres as a freeze dried nasal formulation for insulin, without the employment of absorption enhancing agents, showed that the starch microspheres themselves were able to enhance the nasal absorption of insulin with a Cmax plasma insulin level of 97 mIU/l and a relative bioavailability of 3.6% as compared to a simple insulin solution (Table 2). However, it is evident, that the combination of the starch microspheres with an absorption enhancing agent (as a freeze dried powder formulation) is able to increase significantly the efficiency of the different types of enhancing agents used here and to provide an effect greater than would be gained by the use of starch microspheres alone.

It is interesting to note that the ratio of the AUCs for the enhancer plus powder formulation and the enhancer solution formulation are similar for the two potent surfactant enhancers GDC and STDHF, with values of 2.3 and 2.4. This suggests that, as discussed above, the mechanism by which these two enhancers achieve their effect is similar for the two materials. STDHF is structurally similar to bile salts and is thought to increase peptide absorption across the nasal mucosa by similar mechanisms. It has been suggested that insulin may be solubilised by STDHF to form reverse micelles and hence pores within the cell membrane thereby allowing diffusion of insulin across the cell layer (Longenecker et al., 1987; Eppstein and Longenecker, 1988; Duchateau et al., 1986, 1987;

Gordon et al., 1985; Ennis et al., 1990) but no real evidence for this has been provided. It has also been suggested that STDHF (and bile salts) may act on the tight junctions (Eppstein and Longenecker, 1988; Shao and Mitra, 1992). It is likely, that modification of the cell membrane is the major contributor to the enhanced transport of peptides across the nasal membrane since considerable cell loss and cell separation has been demonstrated after the application of STDHF and bile salt to mucosal surfaces (Richardson et al., 1992; Ennis et al., 1990; Marttin et al., 1995). The starch microspheres have been shown by transmission electron microscopy to transiently dilate the tight junctions in the cell membrane (Bjork et al., 1995). This effect could also enhance peptide absorption across mucosal membranes. It was suggested that after application to the membrane the starch microspheres absorbed water from the mucosa and hydrated, which caused a hydrostatic pressure within the paracellular space that resulted in the structural separation of the tight junctions (Bjork et al., 1995). Importantly, morphological examinations of rabbit nasal mucosa after application of starch microspheres for 8 weeks showed no toxic effect on the membrane apart from a mild hyperplasia (Bjork et al., 1991). Therefore, it is considered likely that the reason for the synergistic effect seen for the combination of the surfactant enhancers, GDC and STDHF with starch microspheres on the enhancement of insulin transport across the nasal membrane in sheep is due to a combination of the mainly different mechanisms of actions exerted by the starch microspheres and the enhancer on the membrane.

It has been shown that insulin, when freeze-dried with starch microspheres is both absorbed into the matrix of the microspheres and also adsorbed to the microsphere surface. The rate of release of insulin from the microsphers is rapid in vitro, with 90% released within 10 min. (Edman et al., 1992). It would be expected that the smaller molecular weight enhancer materials would also be released rapidly from the microspheres upon application on the nasal mucosal surface and hence the absorption promoting effects of enhancer and microspheres would be initiated simultaneously.

Table 3
Intranasal administration to sheep of LPC formulations in combination with insulin

Formulation	Area under curve (AUC)+/−SEM (mIU min/l)	Mean C_{max}[a] +/−SEM (mIU/l)	Relative[b] bioavailability (%)	Ratio: AUC DSM AUC sol
SHI Sol (2 IU/kg)	1546 (+/−417)	52 (+/−13)	1.8	–
SHI Sol (2 IU/kg)+LPC (0.005 mg/kg)	2229 (+/−1223)	28 (+/−17)	2.6	–
SHI Sol (2 IU/kg)+LPC (0.02 mg/kg)	3949 (+/−792)	122 (+/−22)	4.6	–
SHI (2IU/kg)+DSM (2.5 mg/kg)+LPC (0.02 mg/kg)	5517 (+/−532)	94 (+/−7)	6.4	1.4
SHI Sol (2 IU/kg)+LPC (0.05 mg/kg)	4300 (+/−580)	142 (+/−43)	5.0	–
SHI (2IU/kg)+DSM (2.5 mg/kg)+LPC (0.05 mg/kg)	21765 (+/−5674)	380 (+/−58)	25.3	5.1
SHI Sol (2 IU/kg)+LPC (0.10 mg/kg)	4612 (+/−880)	125 (+/−33)	5.4	–
SHI (2IU/kg)+DSM (2.5 mg/kg)+LPC (0.10 mg/kg)	16891 (+/−2121)	335 (+/−48)	19.6	3.7
SC SHI Sol (0.2 IU/kg)	8615 (+/−1297)	95 (+/−14)	100.0	–

Effect of formulation on pharmacokinetic parameters
[a] Calculated from individual sheep insulin-time profiles.
[b] To s.c. administration.

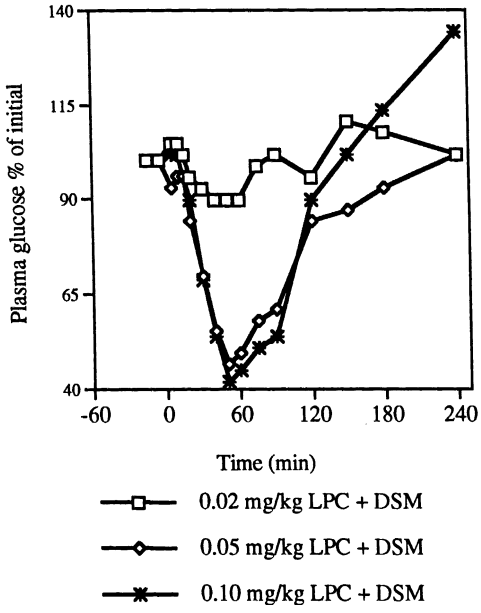

Fig. 7. The plasma glucose levels in sheep following nasal administration of insulin in combination with L-α-lysophosphatidylcholine and starch microspheres as a freeze-dried powder. LPC, L-α-lysophosphatidylcholine; DSM, Starch microspheres.

For the LPC systems, the ratio between the AUCs for the microsphere formulations containing enhancer and the equivalent solution formulations is 1.4 for the lowest dose of LPC used in combination with the starch microspheres. This ratio is different to the ratios of 5.1 and 3.7 found for the two highest doses of LPC used in the formulations. The reason for this difference is that the dose of 0.02 mg/kg LPC is equivalent to the administration of a 0.2% LPC solution. This concentration is at the lower end of the concentration range needed for a maximum enhancing effect. When combined with starch microspheres some of the LPC could be retained in the microspheres and hence the 'real' LPC concentration on the nasal membrane is lower, which would affect the absorption enhancing capacity. The higher enhancement ratios for the two highest concentrations of LPC formulated with the starch microspheres, reflect the fact that the LPC is a relatively weak enhancer. In a similar fashion to GDC and STDHF, LPC is a surfactant-type enhancer, with its main absorption enhancing effect being due to an interaction with cell membranes (El-Hariri et al., 1991; Ropke et al., 1996; Marttin et al., 1995, 1996).

It can be concluded that bioadhesive starch microspheres are able to increase synergistically the effect of absorption enhancers (that predominantly work by interacting with the lipid cell bilayer) on the transport of a model polypeptide, insulin across the nasal membrane in sheep. Dependent on the potency of the absorption enhancer the increment in absorption enhancement was shown to be from 1.4 times to 5 times that obtained for the absorption enhancer in solution.

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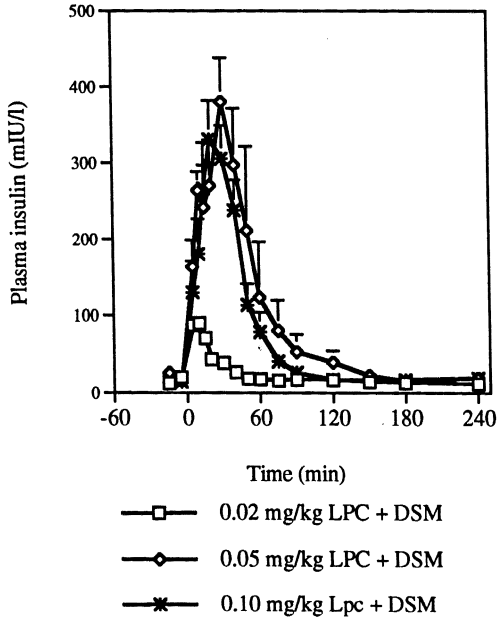

Fig. 8. The plasma insulin levels in sheep following nasal administration of insulin in combination with L-α-lysophosphatidylcholine and starch microspheres as a freeze-dried powder. LPC, L-α-lysophosphatidylcholine; DSM, Starch microspheres.

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