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Enhanced vaginal absorption of insulin in sheep using lysophosphatidylcholine and a bioadhesive microsphere delivery system

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Summary

Insulin was administered vaginally to sheep as an aqueous solution and as a lyophilised powder with bioadhesive starch microspheres. The effect of lysophosphatidylcholine (LPC) on the vaginal absorption of insulin from both formulations was studied. While the vaginal absorption of insulin from insulin solution was minimal, the addition of LPC resulted in a rapid rise in plasma insulin and a pronounced fall in plasma glucose levels. The absolute bioavailability of the peptide from the latter solution was 13%. The hypoglycaemic response to vaginally administered insulin was also improved using the microsphere delivery system, compared to insulin solution alone, and was further enhanced by LPC. Vaginal absorption of insulin from each formulation appeared to be influenced by the oestrous cycle and was thought to correlate with changes in vaginal histology.

Introduction

Research into non-parenteral routes of administration for the systemic delivery of therapeutic peptides and proteins has largely focused on mucosal sites which are not gender-specific. However, the vaginal route may be particularly useful for the administration of peptide and protein drugs for the treatment of female-related conditions. Indeed, the vagina is permeable to a wide range of compounds, including to a certain degree peptides and proteins (Benzinger and Edelson, 1983). In common with other mucosal sites, absorption enhancers may be necessary to improve the vaginal bioavailability of the latter drugs, due to their high molecular weights and hydrophilic character (Lee, 1986). The vaginal absorption of peptides and proteins and the factors influencing drug delivery in experimental models and in woman have recently been reviewed by Richardson and Illum (1992).

The effect of a range of enhancers on the vaginal absorption of a model peptide, insulin, has been investigated in rats (Richardson et al., 1992). Amphiphilic compounds, including sodium

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taurodihydrofusidate (STDHF), laureth-9 and lysophosphatidylcholine (LPC), significantly increased the hypoglycaemic response to vaginally administered insulin but all of these enhancer systems affected mucosal histology. LPC emerged as one of the more promising enhancers, causing limited epithelial damage which was mainly confined to the surface cell layers.

LPC is a lysophospholipid which is surface-active in low concentrations and is found as a normal component of cell membranes as an intermediate in phospholipid metabolism (Weltzien, 1979). LPC proved to be an effective enhancer of the nasal absorption of gentamicin (Illum et al., 1988) and insulin (Illum et al., 1989) in rats and in sheep. The possible mechanisms by which absorption enhancers, including LPC, function have been reviewed by O'Hagan and Illum (1990). They reported that these may act by one or more of the following mechanisms: they may reduce mucus viscosity; increase membrane fluidity by the solubilisation of membrane components; open up intercellular junctions and inhibit peptide degradation by mucosal enzymes.

Our previous studies of the vaginal absorption of drugs have been performed in anaesthetised rats in which prolonged contact of the drug and enhancer solutions with the vaginal mucosa could be easily achieved. However, for further studies, conscious sheep were chosen as a more suitable model as these were thought to better represent the situation likely to be encountered when drug formulations are administered vaginally to women. The vaginal cavity of the sheep is comparable in size to that of women and the docility of the animal allows drug administration and blood sampling to be performed without sedation or anaesthesia.

Insulin has been extensively investigated for administration by non-parenteral routes (see (Illum et al., 1989, for references) and was again selected as a model peptide. The use of degradable starch microspheres (DSM) for the vaginal delivery of insulin was compared with administration of the peptide as a solution. This bioadhesive microsphere system has previously been used for the nasal delivery of drugs in sheep and was thought to enhance absorption by prolonging the residence of the drug in the nasal cavity (Illum et al., 1988; Farraj et al., 1990).

The effect of LPC on the vaginal absorption of insulin from both the aqueous solution and the microsphere formulation was investigated in sheep during the breeding season. From late summer to late winter, sheep exhibit regular ovarian cycles characterised by fluctuating plasma oestrogen and progesterone concentrations and lasting for 17 days (Legan and Karsch, 1979). While corresponding cyclic changes in vaginal histology have been reported by Robinson (1959), their influence on the vaginal absorption of drugs in sheep has not been evaluated. In the present study, plasma progesterone levels were measured to estimate the stage of the oestrous cycle of each animal on the day of the experiment and hence to gain information on the effect of the oestrous cycle on vaginal absorption.

Materials and Methods

Materials

Semi-synthetic human sodium-insulin (SHI) was obtained as a gift from Novo-Nordisk. L-α-Lysophosphatidylcholine was purchased from Sigma Chemical Company Ltd (Poole, Dorset, U.K.). Degradable starch microspheres 45/25, batch no. 62004, were a gift from Pharmacia A/B (Uppsala, Sweden). These were manufactured by emulsion polymerisation of hydrolysed potato starch and were fractioned into defined size distributions by wind sieving of the microspheres in the dry form. The fraction used in these studies had a mean volume diameter of 48 μm and a mean number diameter of 40 μm, when swelled in 0.9% saline solution.

Preparation of insulin solutions

Insulin was dissolved in phosphate buffer, pH 7.3, at a concentration of 200 IU/ml and the solutions were filtered through 0.22 μm sterile filters (Corning 21052-25). The water and salt content of the insulin sample was determined by spectrophotometric analysis of the prepared solutions at 276 nm. Thus, the powder was found to

contain 86% pure insulin and the weights used were adjusted accordingly.

LPC, where required, was dissolved in the insulin solution at a concentration of 10 mg/ml. All solutions were prepared on the day of the study.

Preparation of the lyophilised powder formulations

The required quantity of DSM was dispersed in sterile, distilled water in which insulin, and where required, LPC, were dissolved. The formulation was freeze-dried to obtain a light free flowing powder.

Vaginal administration of insulin formulations to sheep

16 cross-bred ewes (Suffolk and Texel) were housed indoors on straw beds as a flock and were fed on a nut concentrate with ad libitum hay and water. The animals were not fasted prior to insulin administration. On the day before the study, an in-dwelling cannula with a flow switch was placed in one of the external jugular veins of each animal. The cannula was kept patent by flushing with heparinised saline (25 IU/ml) when necessary and was removed upon the completion of the experiment.

The animals were used in two absorption experiments, separated from each other by 2 months. The mean weights (±S.E.) of the ewes were 31.7 (±1.7) and 34.7 (±1.0 kg) in the first ($n = 10$) and second ($n = 12$) studies, respectively. In each study, the sheep were divided into groups of three or four animals and dosed as follows. Insulin solution (2 IU/10 μl per kg) was administered vaginally alone and with LPC (0.1 mg/kg). The lyophilised powder formulations were administered at a dose of 2 IU/kg of insulin, in combination with 2.5 mg/kg of DSM and, where necessary, 0.2 mg/kg of LPC.

The sheep were not sedated prior to drug administration but were restrained in an upright position during dosing. The aqueous formulations were administered vaginally using disposable syringes, whereas the lyophilised powder formulations were administered using oral tubes.

Blood samples (6 ml) were collected from the venous cannula 15 and 5 min prior to insulin administration and at suitable intervals over 4–6 h. Each sample was divided into two parts: for

TABLE 1

Mean plasma glucose levels following vaginal administration of a number of insulin formulations in sheep

Time (min)	SHI soln. ($n = 4$)	SHI soln. + LPC ($n = 7$)	SHI + SMS ($n = 4$)	SHI + SMS + LPC ($n = 7$)
−15	3.70 (0.08)	3 63 (0.14)	3.63 (0 18)	3.69 (0.16)
−5	3.75 (0.05)	3.63 (0.14)	3 60 (0.14)	3.74 (0.17)
5	3.73 (0.11)	3.67 (0.13)	3.60 (0.18)	3.81 (0.20)
10	3 85 (0 13)	3.56 (0.12)	3.68 (0.17)	3.83 (0.19)
15	3.90 (0 14)	3.26 (0.11)	3.65 (0.17)	3.83 (0.21)
20	3.90 (0.14)	2.94 (0.10)	3 65 (0 20)	3.76 (0.20)
30	3.93 (0.11)	2.31 (0.12)	3.63 (0.26)	3 71 (0.21)
40	3.83 (0.09)	1.89 (0.10)	3.53 (0 26)	3.54 (0.23)
50	3.78 (0.14)	1.66 (0.10)	3.45 (0.30)	3.33 (0 25)
60	3 70 (0 14)	1.56 (0.10)	3.35 (0.26)	3.21 (0.28)
75	3.68 (0.08)	1.63 (0.15)	3 28 (0.27)	3.04 (0.31)
90	3.60 (0 07)	1.70 (0.17)	3.23 (0.35)	2.86 (0.32)
120	3.50 (0.12)	2.13 (0.26)	3.00 (0.45)	2.61 (0.32)
150	3 40 (0.15)	2.76 (0.24)	2.65 (0.43)	2 44 (0.28)
180	3 38 (0.19)	3.16 (0.18)	2 43 (0.38)	2.40 (0.24)
240	3.38 (0.16)	3.63 (0.19)	2.58 (0.39)	2.41 (0.25)
300	–	3 98 (0.14)	2.75 (0.33)	2.58 (0.48)
360	–	4.05 (0.23)	2.95 (0.27)	2.68 (0.52)

The unit of measurement is mmol/l; figures in parentheses indicate S.E values; soln., solution; other abbreviations as in text.

insulin analysis 4 ml of the blood sample was mixed in lithium heparin tubes; the remainder was placed in sodium fluoride tubes for glucose analysis. The tubes were stored on crushed ice until centrifugation at 4°C and 3000 rpm for 10 min. The plasma was then separated and stored at −20°C awaiting insulin, glucose and progesterone analysis.

Analysis of plasma

Plasma glucose levels were determined by the glucose oxidase method using a Yellow Springs instrument 23AM analyser, calibrated for glucose measurement in the range 0–10 mmol/l. Plasma glucose levels were expressed as a percentage (%) of the initial concentration, calculated as the mean concentration of the two samples collected prior to drug administration.

Plasma insulin levels were determined by radioimmunoassay (RIA) using a double-antibody technique. The assay measured human insulin using a first antibody raised in guinea pigs and a second anti-guinea pig antibody which precipitated the insulin-insulin antibody complex. In control experiments, the mean recovery of human insulin from spiked sheep plasma was calculated to be 94% (S.E. ± 2.2, $n = 40$). Plasma insulin levels were expressed in mIU/l.

Plasma progesterone levels were determined by RIA using a Gamma-B progesterone assay kit (Immunodiagnostic Services Ltd). The assay was a double antibody technique designed to measure human progesterone using a sheep primary antibody and a donkey anti-sheep second antibody. Plasma progesterone levels were expressed in nmol/l.

Results and Discussion

The effects of the vaginal administration of insulin formulations on the plasma glucose concentrations in sheep are reported in Table 1 and the mean plasma concentrations are illustrated in Figs 1 and 2. In addition, Table 2 gives a summary of the main pharmacokinetic parameters of the glucose and insulin plasma profiles.

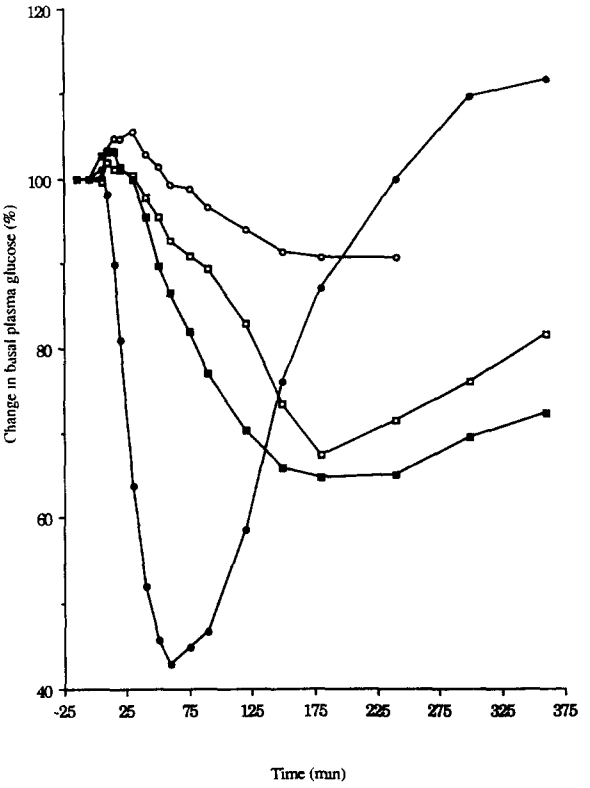

Fig. 1 Mean changes in basal plasma glucose concentrations (%) after vaginal administration of insulin solutions (sol, $n = 4$) and powders (pw, $n = 7$) to sheep; (○) SHI sol, (●) SHI + LPC sol, (□) SHI + DSM pw, (■) SHI + LPC pw

After vaginal administration of insulin (2 IU/kg) solution, the plasma glucose levels decreased slightly to 91% of basal levels after 4 h (Fig. 1) with little or no effect on the plasma insulin concentrations (C_{max} 10.4 mIU/l) (Fig. 2).

The addition of LPC (0.1 mg/kg) to the insulin solution greatly enhanced both insulin absorption and hypoglycaemia (Figs 1 and 2). In this group, insulin was rapidly absorbed with a mean peak concentration of 797 mIU/l (C_{max}) at 16 min (T_{max}) and a maximal hypoglycaemic response observed approx. 60 min after administration. Both plasma glucose and insulin concentrations had returned to near basal levels by the end of the experiment.

In terms of the extent of absorption of insulin, represented by the area under the plasma insulin time profile (AUC) (Table 1), the vaginal admin-

istration of insulin in solution with LPC resulted in significantly greater absorption than that achieved with insulin solution alone. The intravenous (i.v.) and subcutaneous (s.c.) administration of insulin to sheep was performed earlier by our research group and reported in detail by Farraj et al. (1990). The values for AUCs of the i.v. and s.c. profiles were used to calculate the bioavailability of insulin from each formulation (Table 2). The absolute bioavailability of insulin after vaginal administration of insulin in solution with LPC was 13.5% while the relative bioavailability (to s.c. injection) was 32.5%. Although inter-sheep variations in peak plasma insulin levels and AUCs were marked, these results were highly encouraging.

In similar studies of the nasal absorption of insulin in sheep, the absolute bioavailability of the peptide after administration as an aqueous solution with LPC (0.02 mg/kg) was reported to be 2.4% (Farraj et al., 1990). Rapid ciliary clearance of the solution from the nasal cavity, which reduces the contact with the nasal mucosa, was thought to account for the low bioavailability of the peptide even when administered in combination with an enhancer system. The results from the present study emphasise one of the advantages of the vaginal route in that prolonged contact time of a drug delivery system with the vaginal mucosa may be more readily achieved than for other administration sites such as the buccal and nasal cavities.

After vaginal administration of the lyophilised insulin powder formulations, slower but more sustained decreases in plasma glucose concentrations were observed (Fig. 1 and Table 1) although plasma insulin levels remained low (Fig. 2 and Table 2). A mean peak plasma insulin concentration of 56 mIU/l (C_{max}) was attained 70 min (T_{max}) after the administration of insulin (2 IU/kg) with DSM (2.5 mg/kg). Surprisingly, the addition of LPC (0.2 mg/kg) to this formulation did not result in more pronounced changes in plasma insulin levels although the hypoglycaemic response was increased. Thus, the biological effect of the insulin and LPC microsphere formulation was found to be greater than what should have been expected from the plasma insulin curve, a phenomenon which has been reported previously for both nasal (Pontiroli et al., 1982) and oral (Michel et al., 1991) administration of insulin and other peptide hormones (Fink et al., 1974; Overgaard et al., 1991).

Despite the more pronounced hypoglycaemic activity of the insulin and microsphere formulation containing LPC, the absolute bioavailability

TABLE 2

Main pharmacokinetic parameters of the plasma insulin-time profiles after vaginal administration of insulin formulations to sheep (mean ± S E)

Formulations	Mean C_{min} [a] (mmol/l)	Mean T_{min} [a] (min)	Mean C_{max} [a] (mIU/l)	Mean T_{max} [a] 0 (min)	Mean AUC_{0-240} [a] (mIU min l^{-1}) $\times 10^{-3}$	Absolute bioavailability [e] (%)	Relative bioavailability [b,e] (%)
SHI sol.	3 3 ± 0 15	120 ± 25	10.4 ± 2.0	20 ± 6.8	1.5 ± 0 2	0.7 ± 0.1	1.7 ± 0.2
SHI + LPC sol.	1.5 ± 0 10	59 ± 3	797 ± 210 [d]	16 ± 1.4	28 0 ± 8.6 [c]	13 5 ± 4 1	32 5 ± 9.9
SHI + DSM pw.	2.4 ± 0.37	195 ± 15	56 ± 22	70 ± 38	8.1 ± 2 8	3.9 ± 1 3	9 4 ± 3.2
SHI + DSM + LPC pw.	2.1 ± 0 29	236 ± 34	28 ± 7 [c]	85 ± 35	5.0 ± 1.0 [d]	2.4 ± 0.5	5 8 ± 1 2

[a] Calculated from individual sheep insulin-time profiles

[b] Relative to sc administration.

[c] Significantly different from control group (insulin solution) $p < 0.05$.

[d] Significantly different from control group (insulin solution) $p < 0.01$.

[e] Calculated using previously obtained data (Illum et al., 1957).

Sol, solution; pw., powder.

of the peptide was only 2.4% compared with 3.9% after administration with the DSM microspheres alone.

In similar studies by Farraj et al. (1990), the bioavailability of insulin after nasal administration of the peptide with DSM and LPC was 13%. In the present experiments, the low absorption of insulin from the powder formulations could reflect a slow diffusion of the drug through the gel layer in the vaginal tract prior to absorption.

In each of the treatment groups, inter-sheep variations in insulin absorption were marked and showed a weak correlation with the differences in plasma progesterone levels (Table 3). In particular, in the group treated with the insulin, LPC and microsphere formulation, the vaginal absorption of insulin was highest (C_{max} 66.4 mIU/l) in an animal in which plasma progesterone levels were double those seen in the remaining sheep.

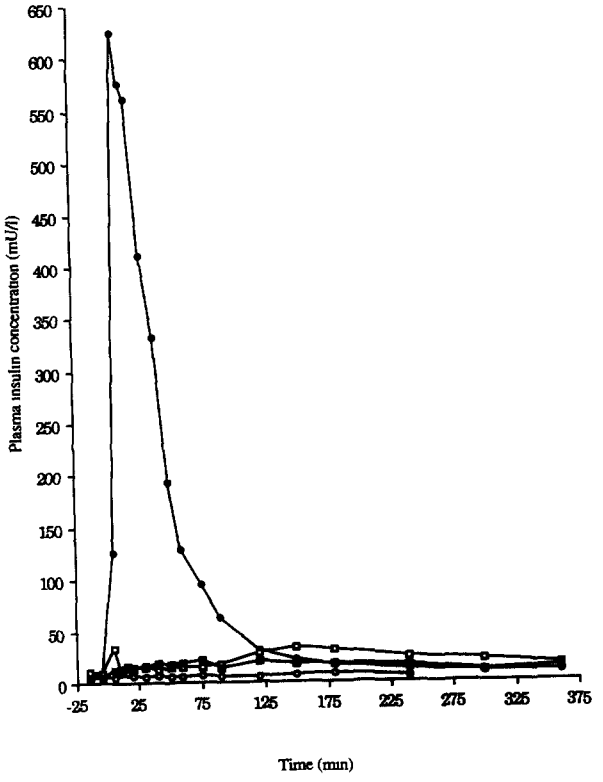

Fig 2. Mean plasma insulin concentrations (mIU/l) after vaginal administration of insulin solutions (sol, $n = 4$) and powders (pw, $n = 7$) to sheep. (○) SHI sol, (●) SHI + LPC sol, (□) SHI + DSM pw, (■) SHI + DSM + LPC pw.

TABLE 3

Plasma progesterone levels and the vaginal absorption of insulin in individual sheep

Sheep (A-Q) and study, 1 or 2	Treatment	AUC_{0-240} (mIU min l^{-1} $\times 10^{-3}$)	Plasma progesterone (nmol/l)
I-1	SHI sol.	1 03	99
J-1		1 35	151
K-1		1 79	226
L-1		1 83	231
B-2	SHI + LPC	9 79	230
N-1	sol	12 24	226
M-1		17.29	383
D-2		18 68	360
A-2		19 90	300
P-1		41 17	220
C-2		72 28	260
F-2	SHI + DSM	2 78	310
E-2	pw	2.98	480
H-2		5 74	300
G-2		11.03	> 1000
E-1	SHI + DSM	1.01	309
I-2	+ LPC pw	2 10	500
K-2		3.27	300
L-2		3.92	300
F-1		4.41	134
H-1		6.02	137
J-2		7 94	960

Several different values have been reported for plasma progesterone concentrations in sheep during the oestrous cycle. Progesterone levels in the follicular and luteal phases of the cycle were found to be 0.4 ng/ml (1.4 nmol/l) and 4 ng/ml (12.7 nmol/l), respectively (Pontiroli et al., 1982). However, other reports vary considerably according to the assay method used (Robinson, 1959). As the progesterone levels measured in the present study were between 8- and 80-fold higher than those reported by Chien (1982), it is difficult to predict the exact stage of the oestrous cycle from these results. During the follicular phase, under the influence of oestrogen, the vaginal epithelium will become thicker, while in the luteal phase, characterised by high progesterone levels, several cell layers may be shed (Robinson, 1959). These cyclic changes in epithelial thickness may certainly affect the vaginal absorption of insulin,

as indicated by the results of the present study. In contrast, the vaginal absorption of the lipophilic drug progesterone in sheep was reported to be similar in both follicular and luteal phases of the cycle (Chien, 1982). This reflects the findings of Okada et al. (1983), who suggested that cyclic variations in the vaginal absorption of drugs in rats were more pronounced for hydrophilic compounds.

Burgos and Roig de Vargas-Linares (1978) have described the changes which occur in the human vaginal epithelium during the menstrual cycle. The effect of these cyclic changes on drug absorption is not clear as conflicting reports have appeared (see Lee, 1986, for references). The results of the present study highlight one of the disadvantages of the vaginal route. In order to achieve predictable and consistent drug delivery, variations in vaginal absorption throughout the ovarian cycle must be characterised. Finally, although the vaginal absorption of insulin in sheep was greatest from the insulin administered as a solution in combination with LPC, formulations which optimise peptide stability, drug release kinetics and ease of administration need to be considered for routine vaginal drug delivery.

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