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Investigation of the nasal absorption of biosynthetic human growth hormone in sheep—use of a bioadhesive microsphere delivery system

L. Illum [1], N.F. Farraj [1], S.S. Davis [1], B.R. Johansen [2] and D.T. O'Hagan [1]

[1] *Department of Pharmaceutical Sciences, University of Nottingham, Nottingham NG7 2RD (U.K.) and* [2] *Novo-Nordisk A/S, 1, Niels Steensensvej, 2820 Gentofte (Denmark)*

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Summary

This paper describes an assessment of the potential of using bioadhesive microspheres as a nasal delivery system for biosynthetic human growth hormone (hGH) in sheep. The microsphere system was used alone and in combination with a biological surfactant, lysophosphatidylcholine (LPC). For comparison, hGH was also administered nasally as a solution and subcutaneously as an injection. The levels of hGH in the blood samples obtained were determined by an ELISA technique. The hGH was absorbed to only a very low extent when administered as a nasal solution. However, the microsphere delivery system without added enhancer was capable of considerably enhancing the nasal absorption of hGH. A delay in absorption was observed with the microspheres alone, which may be partially due to low aqueous solubility of hGH. Rapid and much higher absorption was observed when hGH was administered in combination with the microspheres and LPC as an enhancer.

Introduction

Ever since developments in recombinant DNA technology first allowed the production of biosynthetic human growth hormone (hGH) (Martial et al., 1979), there has been increasing interest in the use of hGH for alternative therapeutic purposes other than as replacement therapy in short stature (Williams and Frohman, 1986). Currently, hGH is administered parenterally requiring, often painful, thrice weekly injections to GH-deficient children. Consequently, if hGH is to be optimally exploited as a therapeutic agent, particularly for chronic conditions, then the development of alternative delivery systems employing non-parenteral routes of administration is necessary.

Recently, the use of degradable starch microspheres (DSM) for the nasal delivery of gentamicin (Illum et al., 1988) and insulin (Bjork and Edman, 1988; Farraj et al., 1990) has been reported. In these studies, the DSM were shown to significantly enhance the absorption of the co-administered drug. However, higher levels of circulating drug were detected when the DSM were used in combination with a biological absorption enhancer, lysophosphatidylcholine (LPC) (Illum et

Correspondence: L. Illum, Department of Pharmaceutical Sciences, University of Nottingham, Nottingham NG7 2RD, U.K.

al., 1988; Farraj, et al., 1990). Furthermore, LPC has been shown to be an effective absorption enhancer for hGH (Fisher et al., 1990; O'Hagan et al., 1990) and insulin (Illum et al., 1990) following nasal administration to rats. The advantages of LPC as an absorption enhancer have been discussed previously (Illum et al., 1990; O'Hagan and Illum, 1990).

The aim of this study was to make an assessment of the potential of using DSM, both alone and in combination with LPC, as a nasal delivery system for hGH. Further to its potential therapeutic uses, hGH is also an excellent high molecular mass (22 kDa) model polypeptide for nasal absorption studies. These studies were performed in the conscious sheep model which has some important advantages over alternative animal models, including the maintenance of ciliary function during the study, the large size of the nasal cavity and the accessibility of the jugular vein for cannulation.

Materials and Methods

Materials

Biosynthetic human growth hormone (Batch 5022) was obtained from Novo-Nordisk, Denmark. Degradable starch microspheres (batch 29323) were a gift from Pharmacia A/B, Uppsala, Sweden. The fraction of particles used in this study had a mean volume diameter of 48 μm (swelled size) and a mean number diameter of 40 μm (swelled size). L-α-Lysophosphatidylcholine was purchased from Sigma, Poole, U.K. All other reagents were of reagent grade.

Experimental methods

Preparation of lyophilised formulations

The required quantity of DSM was dispersed in a solution of hGH, prepared at the appropriate concentration by reconstitution of the freeze-dried vials of hGH with distilled water, and any LPC required was added. The solution was stirred for 1 h at room temperature and then freeze-dried to obtain a powder formulation.

TABLE 1

Formulations of hGH administered to sheep

Formulation	Route of administration	No. of animals
hGH solution (0.1 IU/kg)	subcutaneous	3
hGH solution (0.9 IU/kg)	intranasal	2
hGH (0.9 IU/kg) and DSM (2.5 mg/kg) powder	intranasal	3
hGH (0.9 IU/kg), LPC (0.2 mg/kg) and DSM (2.5 mg/kg) powder	intranasal	3

Animal experiments

Cross-bred sheep (Suffolk and Texel) were housed indoors as experimental animals on straw beds as a flock and were fed on nut concentrate with ad libitum hay and water. The methods of intranasal administration of liquid and powder formulations were described previously (Farraj et al., 1990).

Eleven sheep were divided into three groups of three and one group of two animals. The mean weight ($\pm$S.D.) of the sheep was 35.8 ($\pm$3.0) kg. The hGH formulations were administered at 0.9 IU/kg (0.31 mg/kg) (51.4 IU/ml) intranasally and at 0.1 IU/kg (4 IU/ml) subcutaneously. Two animals received hGH as a nasal solution and three animals received hGH as a subcutaneous injection. The lyophilised formulations of hGH were administered intranasally in combination with 2.5 mg/kg DSM and in combination with 2.5 mg/kg DSM and 0.2 mg/kg LPC to two groups of three animals. The various formulations administered to the sheep are listed in Table 1.

Blood samples were collected in heparinised tubes (Li heparin) onto crushed ice prior to the hGH administration and at 10, 20, 30, 40, 60, 75, 90, 120, 150, 180, 240 and 300 min post-administration. The plasma was separated by centrifugation at 4°C and stored at -20°C until assayed by an enzyme-linked immunosorbent assay (ELISA).

Determination of plasma levels of hGH

The plasma levels of hGH were determined by an ELISA technique which has been described

TABLE 2

Mean plasma levels of hGH (ng/ml) ± S.E. achieved for the different formulations administered to sheep

Time (min)	s.c. hGH solution	Nasal hGH solution	Nasal DSM + hGH powder	Nasal DSM, LPC + hGH powder
0	< 4.4	< 0.55	< 2.2	< 4.4
10	5.5 (1.1)	0.9 (0.4)	< 2.2	6.7 (1.8)
20	7.9 (2.6)	0.7 (0.2)	< 2.2	25.7 (14.0)
30	12.1 (3.2)	0.8 (0.3)	< 2.2	41.7 (19.6)
40	13.0 (1.9)	1.0 (0.4)	< 2.2	54.3 (26.0)
60	22.6 (4.9)	0.9 (0.3)	3.3 (1.1)	55.4 (21.8)
75	23.2 (3.5)	0.7 (0.2)	3.9 (1.7)	49.0 (15.8)
90	21.8 (5.3)	0.8 (0.3)	8.0 (1.8)	40.9 (11.4)
120	18.0 (4.6)	< 0.55	9.0 (4.5)	18.7 (4.5)
150	17.5 (4.1)	< 0.55	8.1 (4.0)	13.0 (3.9)
180	15.2 (1.6)	0.6 (0.03)	7.4 (3.4)	10.1 (0.8)
240	17.2 (4.2)	< 0.55	3.9 (1.6)	5.1 (0.7)
300	16.2 (6.1)	< 0.55	< 2.2	< 4.4

previously (O'Hagan et al., 1990). The lowest level of sensitivity of the assay was 0.11 ng/ml hGH and the highest concentration of the prepared standard solutions of hGH was 7.0 ng/ml. All the plasma samples were diluted so that the concentration of hGH therein fell within this range. Hence, the lowest level of plasma hGH detectable for each formulation group (Table 2) is in accordance with the dilution factor used for the plasma prior to assay. The relative bioavailabilities obtained for the two hGH delivery systems, DSM with and without LPC, were calculated relative to the data obtained from subcutaneous injection of hGH after dose adjustment (Table 3).

TABLE 3

Relative effectiveness of the different formulations for intranasal delivery of hGH

Formulation	Peak concentration (ng/ml) (S.E.)	AUC (ng min ml^{-1})	Relative bioavailability [a] (ng min ml^{-1})
Subcutaneous hGH	23.2 (3.5)	3653	100%
Nasal hGH solution	1.0 (0.4)	28.3	0.1%
DSM + hGH powder	9.0 (4.5)	892.3	2.7%
DSM, LPC + hGH powder	55.4 (21.8)	4725	14.4%

[a] Bioavailability calculated from subcutaneous data ($t = 0$ to $t = 300$) after dose adjustment.

Results and Discussion

The mean plasma levels of hGH (ng/ml) and the standard errors of the means (S.E.) resulting from nasal administration of hGH solution, hGH in combination with DSM alone, and hGH with DSM and LPC are shown in Table 2 and represented graphically in Figure 1. The areas under the absorption curves (AUCs) from time $t = 0$ to $t = 300$ min and the calculated relative bioavailabilities are shown in Table 3.

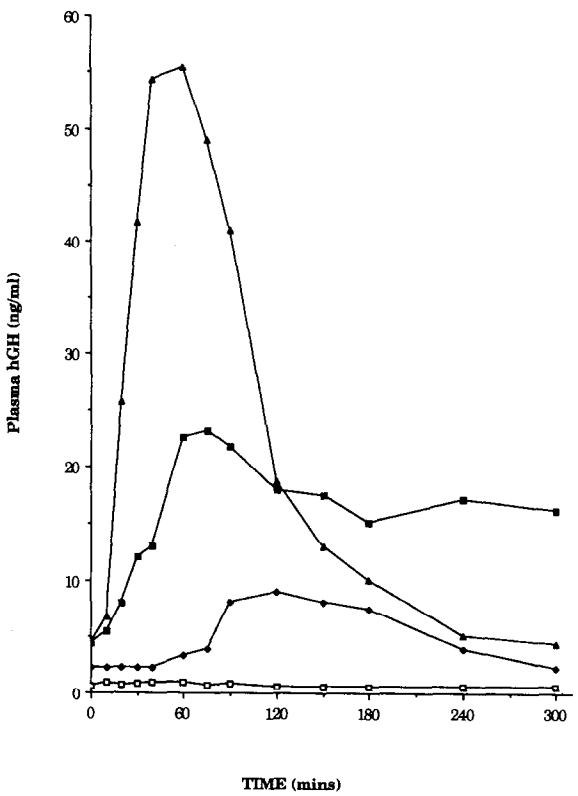

Fig. 1. Mean plasma levels of hGH following intranasal administration of hGH (0.9 IU/kg) as a solution (□), in combination with DSM (2.5 mg/kg) as a powder (◆), in combination with DSM (2.5 mg/kg) and LPC (0.2 mg/kg) as a powder (▲) and following subcutaneous injection of hGH (0.1 IU/kg) (■).

As expected, intranasal administration of the high molecular mass hormone hGH in solution resulted in only low absorption, with a plasma peak of 1.0 ng/ml 40 min after administration. This is in agreement with previous data from another research group who investigated the effect of molecular weight on nasal absorption in rats and found that hydrophilic molecules with a molecular mass > 1000 Da were poorly absorbed (Fisher et al., 1987). Previously, higher plasma levels of hGH were detected when an hGH solution was administered nasally to rats at the same dose as above (O'Hagan et al., 1990). However, while this result may be partly explained by differences in the sites of deposition and distribution of the administered formulation in the nasal cavities of the two animal models, it is also a further indication that the conscious sheep is a more appropriate model for the assessment of intranasal drug delivery systems than the rat, where the mucociliary clearance mechanism is impaired.

The use of DSM as a nasal delivery system for hGH resulted in an increase in plasma peak height of hGH from 1.0 ng/ml to 9.0 ng/ml, an increase in AUC from 28.3 to 892.3 and a relative bioavailability of 2.7% ($t = 0$ to $t = 300$). The time for the plasma peak of hGH was delayed to 120 min, which is probably an indication of the extended contact period of the DSM with the nasal mucosa (Illum et al., 1987), but may also indicate a slow dissolution of hGH. Drugs must be in solution before they are absorbed across membranes and hGH has only limited solubility (~ 18 mg/ml) relative to other macromolecules. Consequently, if most of the water present in the mucus layer is absorbed by the DSM during their swelling process, then initially, there may be little water remaining to dissolve the hGH.

The combination of DSM and LPC proved to be a potent nasal delivery system for hGH, with an increase in plasma peak height from 1.0 ng/ml to 55.4 ng/ml, an increase in AUC from 28.3 to 4725 and a relative bioavailability of 14.4% as calculated from the 300 min study period. It should be noted that at this time, the plasma concentrations for the nasal delivery systems were back to the initial values, whereas the plasma levels for the subcutaneously administered group were still relatively high. Hence, doses of hGH administered nasally appeared to be cleared more rapidly from the body than those administered by subcutaneous injection. Furthermore, with this delivery system, hGH was well absorbed initially and the plasma peak was only delayed slightly. LPC is a biological surfactant (Stafford and Dennis, 1988) and consequently, will promote the solubilisation of hGH and allow a more rapid absorption.

The results described here indicate that the DSM and LPC delivery system has considerable potential as a nasal delivery system for hGH. However, these results need to be confirmed in a larger study group and the potential membrane damaging effects of LPC need to be further assessed. Preliminary evidence indicates that LPC causes only limited morphological changes in the vaginal epithelium at low concentrations (Richardson et al., 1989). The use of delivery systems such as the bioadhesive microsphere system may encourage the application or exploitation of hGH for alternative therapeutic purposes.

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