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Intranasal delivery of recombinant human growth hormone (somatropin) in sheep using chitosan-based powder formulations

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Abstract

The effectiveness of chitosan in promoting the intranasal bioavailability of recombinant human growth hormone (hGH) has been evaluated. hGH was formulated with chitosan to produce a powder blend (Formulation A) and granules (Formulation B) for intranasal administration. The in vivo pharmacokinetic performance of the formulations was evaluated in a group of six sheep in a randomised crossover study. A subcutaneous injection of hGH solution was administered as a control. The intranasal and subcutaneous doses of hGH were 0.3 and 0.03 mg/kg, respectively. The intranasal formulations appeared to be well tolerated. Mean bioavailabilities of hGH from Formulations A and B were 14 and 15%, respectively relative to subcutaneous injection. It is concluded that chitosan-based intranasal powder formulations may provide a practical means for non-injectable delivery of hGH and, potentially, other therapeutic protein molecules.

Keywords: Nasal; Chitosan; Human growth hormone; Sheep

1. Introduction

The intranasal route offers a number of attractions as a non-invasive means of delivering therapeutic macromolecules. It is convenient to the patient, offers a relatively benign environment for macromolecule stability, is generally well tolerated and, by comparison to the pulmonary route, is less demanding in terms of formulation technology, such as particle size requirements, and complexity of delivery device (Arora et al., 2002; Illum, 2003; Illum and Fisher, 1997).

Synthetic human growth hormone (hGH, somatropin) is manufactured by recombinant DNA technology and is a 191 amino acid polypeptide (MW 22 kDa) with an amino acid sequence and two internal disulphide bridges identical to that of the major component of human pituitary growth hormone (Pearlman and Bewley, 1993). Therapeutically, hGH is used in children to treat growth retardation, for example short stature due to insufficient growth hormone secretion, Turner's syndrome or chronic renal insufficiency. In adults it is used as a treatment for growth hormone deficiency and for management of HIV-related wasting and cachexia (Sweetman, 2002)

There are a number of reports on the intranasal administration of hGH. The effect of sodium tauro-24,25-dihydrofusidate (STDHF) on the nasal absorption of hGH in the rat, rabbit and sheep has been evaluated (Baldwin et al., 1990). Compared to a simple aqueous solution of hGH, the addition of STDHF produced a 11-fold increase in intranasal hGH bioavailability (area under curve) in rats and rabbits and a 21-fold increase in sheep. STDHF-based hGH solutions were also administered to growth hormone-deficient patients (Hedin et al., 1993). The bioavailability relative to subcutaneous injection (s.c.) was in the range 1.6–3.0%.

In a similar study, formulations containing hGH and L-α-phosphatidylcholine (LPC) were administered to rats (up to 17.5% bioavailability achieved), rabbits (72.8%) and sheep (up to 16%) (Fisher et al., 1991). Bioavailabilities in the

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absence of LPC were 2.3, 1.4 and 0.2% in the rat, rabbit and sheep, respectively.

In sheep the intranasal absorption of hGH administered as an aqueous solution and as lyophilised powders containing starch microspheres and starch microspheres mixed with LPC has been reported (Illum et al., 1990). Relative to s.c. injection, the nasal formulations produced bioavailabilities of 0.1, 2.7 and 14.4%, respectively. The possibility of LPC causing damage to cell membranes was noted. An intranasal hGH formulation containing α-cyclodextrin and didecanoyl-L-α-phosphatidylcholine provided a bioavailability of 20% in rabbits, but was associated with severe damage to the nasal epithelial membrane (Agerholm et al., 1994). Formulations containing hGH and didecanoyl-L-α-phosphatidylcholine have been intranasally administered to growth hormone-deficient patients (Laursen et al., 1996). Depending on dose, the bioavailability ranged from 3.8 to 8.9%.

It is therefore clear that there may be opportunities to deliver efficacious amounts of hGH into the systemic circulation via the intranasal route, but this will require the use of additives to enhance bioavailability. For long term use in a human medicine, any such additive(s) will need to be demonstrated to be both effective and safe.

A number of studies have reported the use of chitosan as an excipient for improving intranasal absorption of polar small molecules and peptides (Dyer et al., 2002; Illum et al., 1999, 2000, 2002; Illum, 2003; Roon et al., 1999; Sinswat and Tengamnuay, 2003) and for enhancing the efficacy of intranasal vaccines (Illum et al., 2001; Mills et al., 2003). Chitosan is a polysaccharide comprising copolymers of glucosamine and *N*-acetylglucosamine and is derived by the partial deacetylation of chitin, a material found in abundance in shells of Crustacea such as lobsters, prawns and crabs. Being positively charged, chitosan can bind strongly to negatively charged materials such as epithelial cell surfaces and mucus. Chitosan has been shown to behave as a bioadhesive polymer, increasing significantly the half-time of nasal clearance (Soane et al., 1999). In addition to improved adhesion between the formulation and the nasal tissue, there are also reports that chitosan can increase the permeability of cell monolayers and may have transient effects, in vitro, on the integrity of tight junctions (Artursson et al., 1994; Dodane et al., 1999; Ranaldi et al., 2002; Smith et al., 2004), although it is not clear how relevant this phenomenon is to the ability of chitosan to improve drug absorption in vivo.

From a pharmaceutical regulatory viewpoint the use of chitosan as an additive to improve intranasal drug absorption is attractive since it has pharmacopoeial recognition (European Pharmacopoeia, 2002) and is available commercially as a high purity material made in compliance with Good Manufacturing Practice (Illum, 2002). A considerable body of human clinical data is also being generated on chitosan-based intranasal formulations: In clinical trials to date, in excess of 700 human subjects have received a total of more than 2000 intranasal doses (unpublished data).

However, there are no reports on whether chitosan has any utility in improving the intranasal absorption of high molecular weight (>10 kDa) therapeutic proteins. This paper presents work to explore the feasibility of delivering hGH intranasally using chitosan-based delivery systems. The sheep was used as the animal model. Both chitosan powder blend and granule formulations were prepared and characterised. The latter was developed with a view to exploring whether this particular presentation would provide for improved powder flow and/or blend homogeneity compared with a powder blend formulation.

2. Materials and methods

2.1. Materials

Recombinant human growth hormone (frozen bulk solution containing 8.8 mg/ml protein) was supplied by Biochemie, Kundl, Austria. Chitosan glutamate (Protasan UP G213) was purchased from NovaMatrix, Drammen, Norway and polyvinylpyrrolidone (PVP) (Kollidon® 30) from BASF, Ludwigshafen, Germany. Dichloromethane, disodium hydrogen orthophosphate dihydrate, sodium dihydrogen orthophosphate dihydrate, hydrochloric acid and sodium hydroxide were purchased from Fisher Scientific, Loughborough, UK. Deionised water was used throughout (Prima and Maxima water purification units, ELGA Lab Water, High Wycombe, UK).

2.2. Formulation preparation

Defrosted hGH solution (170 ml) was transferred into a glass beaker, frozen using liquid nitrogen and lyophilised for 48 h (ThermoSavant ModulyoD freeze dryer, Thermo Life Sciences, Basingstoke, UK). The resultant powder was passed through a sieve (0.85 mm aperture size) before use.

To prepare the powder blend (Formulation A), 648 mg of freeze-dried hGH and 408 mg of chitosan glutamate were gently mixed in a glass mortar using a pestle. This powder was transferred to a glass vial and mixed using a Turbula T2 mixer (Willy Bachofen, Bubendorf, Switzerland) for 30 min. The final powder was stored refrigerated in a glass vial.

To prepare granules (Formulation B), 15 mg of PVP was dissolved in 2–3 ml of dichloromethane in a glass beaker. To the PVP solution, were added 864 mg of freeze-dried hGH and 529 mg of chitosan glutamate and these were mixed using a spatula to form a homogeneous mass. The majority of the dichloromethane was allowed to evaporate in a fume hood and then the wet mixture was passed through a 0.25 mm aperture size sieve and oven dried at 40 °C to constant weight. The dried granules were gently milled in a mortar and passed through a 0.15 mm aperture size sieve. The sieved material was stored desiccated in a refrigerator in a glass vial.

Phosphate buffer solution was prepared by dissolving 56 mg of disodium hydrogen orthophosphate dihydrate and 29 mg of sodium dihydrogen orthophosphate dihydrate in