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Nasal residence of insulin containing lyophilised nasal insert formulations, using gamma scintigraphy

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ABSTRACT

Bioadhesive dosage forms are a potential method for overcoming rapid mucociliary transport in the nose. A lyophilised nasal insert formulation previously investigated in sheep demonstrated prolonged absorption of nicotine hydrogen tartrate suggestive of extended nasal residence, and increased bioavailability. The current study was performed to quantify nasal residence of the formulations using gamma scintigraphy, and to investigate the absorption of a larger molecule, namely insulin. A four-way crossover study was conducted in six healthy male volunteers, comparing a conventional nasal spray solution with three lyophilised nasal insert formulations (1–3% hydroxypropylmethylcellulose (HPMC)). The conventional nasal spray deposited in the posterior nasal cavity in only one instance, with a rapid clearance half-life of 9.2 min. The nasal insert formulations did not enhance nasal absorption of insulin, however an extended nasal residence time of 4–5 h was observed for the 2% HPMC formulation. The 1% HPMC insert initially showed good spreading behaviour; however, clearance was faster than for the 2% formulation. The 3% HPMC nasal insert showed no spreading, and was usually cleared intact from the nasal cavity within 90 min. In conclusion, the 2% HPMC lyophilised insert formulation achieved extended nasal residence, demonstrating an optimum combination of rapid adhesion without over hydration.

1. Introduction

The nasal route of administration offers an attractive alternative to the oral route for drug delivery, as the relatively large surface area and rich vasculature of the nasal mucosa provide the opportunity for direct absorption into the bloodstream (Mygind and Dahl, 1998; Newman et al., 2004). This makes nasal dosing a potential alternative route for drugs, such as proteins and peptides, which show poor oral bioavailability and are currently administered via injection.

Proteins and peptides often present a challenge for nasal delivery, both in terms of the large size of the molecule, and the rapid mucociliary clearance rate of the nasal cavity, with time to 50% clearance of approximately 12–15 min (Marttin et al., 1998). In an attempt to overcome these difficulties, many researchers use absorption enhancers, such as bile salts (Natsume et al., 1999), cyclodextrins (Yang et al., 2004), fusidate derivatives (Longenecker et al., 1987), and phosphatidylcholines (Illum et al., 1990). However, the use of absorption promoters has often been found to result in some damage to the nasal mucosa or the function of the cilia (Merkus et al., 1996; Gizurarson et al., 1990), and may not be considered suitable for long term use.

Another formulation strategy for nasal administration is the use of bioadhesive delivery systems. The aim is to promote adhesion of the formulation to the nasal mucosa, allowing an

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extended period of contact for drug absorption to occur. Nasal administration of bioadhesive polymer gels can be technically challenging and may require a specialised device, and there will also be a limit to the viscosity of gel that can be formulated for convenient nasal administration.

The preparation of a lyophilised hydroxypropylmethylcellulose (HPMC) nasal insert has been reported previously (McInnes et al., 2005), describing a dosage form robust enough to be handled and administered to the nasal cavity manually. On contact with a moist surface, such as the nasal mucosa, the lyophilisate hydrates, forming a gel of a higher concentration of HPMC than originally prepared prior to lyophilisation. It is proposed that this re-hydrated, concentrated HPMC gel could result in increased bioadhesion and therefore residence time in the nasal cavity, in combination with enhancement of absorption due to a transient dehydrating effect on the nasal mucosa.

The non-invasive imaging technique gamma scintigraphy has proved valuable and versatile in the assessment of nasal formulations in vivo, and has been used to elucidate transit times of nasal sprays and drops (Bryant et al., 1999; Guida et al., 2000), deposition patterns of nasal sprays (Suman et al., 1999; Eyles et al., 2001; Harris et al., 1988), and bioadhesive behaviour (Illum et al., 1987; Soane et al., 1999).

A previous in vivo study in sheep with a novel nasal insert formulation demonstrated prolonged absorption and increased bioavailability of nicotine hydrogen tartrate (NHT) in comparison with conventional nasal spray and powder formulations, suggestive of extended nasal residence (McInnes et al., 2005). Therefore, in the current study, it is proposed to investigate the nasal distribution and residence of the insert formulation using scintigraphy, and the effect of varying polymer concentration, in comparison with a conventional nasal spray. The incorporation of insulin will allow assessment of the performance in enhancing absorption of a more challenging molecule.

2. Materials and methods

2.1. Materials

HPMC (grade K4MP) was obtained from Dow Chemicals (Michigan, USA). Mannitol, insulin powder (human USP), phosphate buffered saline (PBS) pH 7.4 tablets, hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Sigma (Dorset, UK). Water for Injection was obtained from Baxter Healthcare (Glasgow, UK). BioRad Protein Assay was purchased from BioRad Ltd. (Hemel Hempstead, UK). Technetium-99m-diethylenetriaminepentaacetic acid (^{99m}Tc-DTPA) and Indium-111-diethylenetriaminepentaacetic acid (^{111}In-DTPA) were obtained from the West of Scotland Radionuclide Dispensary, Glasgow, UK.

2.2. Methods

2.2.1. Manufacture of nasal solution

The required quantity of insulin to produce 49IU of insulin activity per 100 μl was dissolved in 0.05 M HCl, and adjusted to pH 7.4 as required with dropwise addition of 0.05 M NaOH. The appropriate amount of ^{99m}Tc-DTPA was then added to give an activity of 4 MBq per dose.

2.2.2. Manufacture of nasal inserts

HPMC gels containing insulin were prepared by dissolving the required quantity of insulin to produce 49IU of insulin activity per dose in 0.05 M HCl, and adjusting to pH 7.4 as required with dropwise addition of 0.05 M NaOH. The required amount of mannitol to produce a 1% (w/w) concentration was dissolved in the insulin solution, followed by the appropriate amount of HPMC to make gels of 1, 2 or 3% (w/w) HPMC. ^{111}In-DTPA was added to produce an activity of 0.25 MBq per dose at the time of dosing, and the mixture was carefully stirred until a uniform solution was obtained. The resultant gel was allowed to settle to remove air.

HPMC gels were filled into polypropylene microcentrifuge tubes, and lyophilised using conditions described previously (McInnes et al., 2005). ^{111}In was used as a radiolabel for the nasal insert formulations as the short half-life of ^{99m}Tc (6.03 h) was unsuitable for the required length of freeze–drying cycle.

2.2.3. In vitro release

The in vitro release of insulin from lyophilised nasal inserts was studied using a diffusion chamber intended to mimic conditions within the nasal cavity, as previously described (McInnes et al., 2005). Insulin content in the receptor compartment was determined using the Bio-Rad assay, which is based on the binding of the agent Coomassie Brilliant Blue G-250 to protein, and undergoing a colour change from red to blue. This results in a change of the absorption maximum of the dye from 465 to 595 nm, allowing the quantity of protein present to be assessed using visible range spectrophotometry (Bradford, 1976). The assay was performed by vortexing 0.2 ml of the dye reagent with 0.8 ml of the insulin containing sample, allowing the colour change reaction to take place, and measuring the absorbance at 595 nm. Insulin content was calculated from a standard curve previously prepared.

2.2.4. Clinical study design

The study was a single centre, open label, four-way crossover trial. The study was performed in accordance with the relevant articles of the Declaration of Helsinki, and was approved by the North Glasgow Universities NHS Trust Ethics Committee and the Administration of Radioactive Substances Advisory Committee.

2.2.5. Study population

Six healthy male volunteers (aged 20–29) were entered into the study. Written informed consent was obtained from all volunteers, who underwent pre-study medical examinations to ensure compliance with study criteria. In particular, a normal medical history relating to the nasal cavity was required, as was satisfactory nasal mucociliary clearance as determined by the saccharin test. Exclusion criteria for the study included smokers, diabetic patients, recent respiratory tract infection, allergic rhinitis, regular medication, or recent participation in a clinical trial.