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In vivo evaluation of nicotine lyophilised nasal insert in sheep

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Abstract

The nasal route offers an attractive means of delivering a drug directly to the systemic circulation and avoiding hepatic first-pass metabolism, although rapid mucociliary clearance can be detrimental to nasal absorption. The in vitro and in vivo characteristics of a nasal insert formulation prepared by lyophilisation of a viscous HPMC gel solution designed to overcome this problem were studied. In vitro release of nicotine from the lyophilised insert was compared with powder and spray formulations. Stability and characterisation studies were carried out using dynamic vapour sorption, scanning electron microscopy and HPLC analysis. Nicotine formulations were administered to eight wether sheep in a randomised four-way cross-over study, and plasma nicotine assessed comparing the nasal insert formulation with conventional nasal powder, nasal spray and IV doses. In vitro release studies demonstrated prolonged nicotine release from the nasal insert formulation compared to a powder and liquid. In vivo plasma profiles appeared to show prolonged plasma nicotine levels compared to the conventional formulations, although T_{max}, C_{max} and AUC parameters for the insert were not significantly different due to high variability in the pharmacokinetic data. In conclusion, the nasal insert displayed a promising prolonged plasma profile, which must be investigated further to provide statistical significance to prove the effect.
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1. Introduction

Intranasal (IN) delivery offers an attractive potential route for systemic absorption of drugs as an alternative to oral and parenteral administration, and the range of nasal preparations currently available in the market demonstrate patient acceptability of the route. The intranasal route bypasses first-pass hepatic metabolism, delivering drug directly into the sys-

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temic circulation via the high arterial blood perfusion (Mygind and Dahl, 1998) of the nasal mucosa.

Mechanisms of nasal absorption have been investigated for various drugs, with reports showing that absorption of small molecular weight hydrophilic compounds is thought to occur passively via aqueous pores in the nasal mucosa (Chien et al., 1989), with others suggesting that above 1000 Da such compounds show limited, or no absorption (McMartin et al., 1987). Some lipophilic compounds such as propranolol and progesterone have shown nasal bioavailability similar to that of intravenous (IV) injection (Donovan et al., 1990), and IN absorption of these drugs is thought to occur via a transcellular pathway linked to lipophilicity and partitioning into the nasal mucosa (Corbo et al., 1998). In common with other sites of absorption, factors including pH and drug ionisation are important to the overall behaviour and bioavailability of the dose (Behl et al., 1998; Dondeti et al., 1996).

The nose forms a part of the body's defence mechanism against foreign assault, and therefore, a major disadvantage of nasal administration is the rapid mucociliary clearance of substances from the nasal cavity, yielding typical half-lives of approximately 15 min (Mygind and Dahl, 1998). As a result, larger molecular weight compounds such as peptides and proteins, which may require extended mucosal contact in order to allow maximum uptake, can pass through the nasal cavity with little or no absorption and systemic effect. Even compounds which are easily absorbed nasally will only have a short window for absorption, particularly in the case of powder formulations, where rate of dissolution of the drug must also be taken into consideration, and it may be necessary to repeatedly administer the compound throughout the day in order to sustain appropriate therapeutic plasma levels. In attempts to overcome the absorption problems resulting from rapid nasal transit rates, extensive use of absorption enhancers such as bile salts (Shao and Mitra, 1992; Dondeti et al., 1995; Illum et al., 2001), chitosan (Fernandez-Urrusuno et al., 1999; Dyer et al., 2002) fusidate derivatives (Shao and Mitra, 1992; Longenecker et al., 1987), cyclodextrins (Yang et al., 2004), and various other compounds (Shao and Mitra, 1992; Longenecker et al., 1987; Dondeti et al., 1995; Illum et al., 1990; Natsume et al., 1999; Takenaga et al., 1998; Morimoto et al., 1985) have been employed in an attempt to increase nasal absorption during this relatively brief period of contact with the mucosa. However, there are concerns regarding the long-term effects of some absorption enhancers on the nasal mucosa, particularly where chronic therapy is necessary. In some cases, it has been reported that increased absorption of a particular compound may in part have been a consequence of mucosal damage (Shao and Mitra, 1992; Carreno-Gomez and Duncan, 1997; Witschi and Mrsny, 1999).

An alternative approach to increase nasal absorption of drugs is to increase nasal residence time using bioadhesive formulations that resist mucociliary clearance. Bioadhesion is the extent to which a substance adheres to tissue by means of mechanisms thought to include interaction of polymer chains with glycoproteins on the cell surface (Nakamura et al., 1999; Illum et al., 1987), a process aided by water uptake and swelling that increases the flexibility of the polymer chain (Alur et al., 1999). Other factors implicated in, and influencing bioadhesion are the number of hydrophilic groups contained in the polymer (Mortazavi, 1995), molecular weight (Dondeti et al., 1996; Smart et al., 1984), hydrogen bonding (Duchene et al., 1988), ionic interactions (Duchene et al., 1988) and viscosity (Dondeti et al., 1996; Dondeti et al., 1995), with the general consensus being that the over adhesion of a formulation will often occur as a result of a complex combination of a number of these factors. Additionally, water uptake by polymers has been suggested to have the extra benefit of enhancing drug absorption, by causing a transient osmotic opening of mucosal cell tight junctions allowing easier passage of drug molecules (Edman et al., 1992).

Hydroxypropylmethylcellulose (HPMC), a non-ionic polymer, has been shown to exhibit bioadhesivity (Henriksen et al., 1996; Dyvik and Graffner, 1992) and is a GRAS excipient (generally regarded as safe, Kibbe, 2000), making it an attractive option for application to a sensitive mucosal surface such as the nose. This polymer is used for many controlled release applications, and in vitro characterisation of a lyophilised HPMC nasal insert formulation has previously demonstrated that prolonged drug release can be achieved from such preparations (Thapa, 2000). In theory, therefore, incorporation of HPMC in a nasal formulation may allow both increased nasal residence time in combination with controlled release of drug, potentially

providing improved absorption over conventional nasal formulations.

The administration of highly viscous polymer solutions nasally would prove extremely difficult, presenting a limitation on the concentration of polymer used, so the use of a mechanically robust nasal insert is described, formed by the lyophilisation of a viscous HPMC gel solution. Such a lyophilised dosage form would then be expected to rapidly rehydrate on contact with the moist nasal mucosa and humid conditions of the nasal cavity, forming a more viscous gel than the original solution. The present study in sheep was carried out to investigate in vivo absorption of nicotine from such a lyophilised nasal insert and to determine how formulation factors influenced absorption. The sheep is considered to be an ideal animal model for investigation of nasal absorption as the ratio of surface area: kg body weight is of a similar order to that of humans, and the general anatomical structure is also very similar (Cheng et al., 2002).

Nicotine was selected for absorption studies as it is known to be readily absorbed from the nasal mucosa in sheep (Cheng et al., 2002), has been shown to be a relatively stable compound under a variety of storage conditions in liquid and freeze-dried form (Cornaz and Buri, 1998), and is of interest in nasal application with direct systemic absorption as it has a therapeutic application in humans as an aid to smoking cessation. Nicotine hydrogen tartrate (NHT) was used in the formulation as it is more stable than nicotine base and has been previously used to demonstrate mucosal absorption (Cheng et al., 2002; Ikinci et al., 2004). The lyophilised HPMC insert formulation, was compared with nasally administered nicotine powder and spray formulations.

2. Materials and methods

2.1. Materials

HPMC (grade K4MP) was donated by the Dow Chemical Company (MI, USA). D(−)Mannitol, and water (HPLC grade) were purchased from BDH (VWR, Poole, UK). Nicotine hydrogen tartrate, nicotine-methyl-d_3, methanol, ammonia, sodium phosphate buffer (pH 3) and phosphate buffered saline (PBS) pH 7.4, were purchased from Sigma (Gillingham, UK).

2.2. Manufacture of nasal insert

Inserts were prepared by lyophilisation of solutions of 2% (w/w) HPMC, 1% (w/w) mannitol and the required amount of NHT to give 4 mg nicotine base per insert. Mannitol, a commonly used bulking agent was added to provide mechanical strength to the lyophilised product when handled. The mannitol and NHT were first dissolved in about one third of the required amount of distilled water. The required weight of HPMC powder was then added slowly, with constant stirring to obtain uniform gels which were made up to volume with distilled water, stirred to homogeneity and stored at 4 °C overnight to allow removal of air bubbles. Gel containing 4 mg of nicotine base was transferred into 0.6 ml polythene centrifuge tubes (Life sciences, Basingstoke, UK) as required, and freeze dried for 26 h using a Virtis Advantage freeze drier (Virtis, NY, USA), with pre-set cycle stages; freezing (8 h, reducing temperature from −30 to −60 °C in 10 °C increments), primary drying (18 h increasing temperature from 10 to 22 °C in 5 °C increments, with chamber pressure decreasing from 100 to 40 mTorr) and post heat (10 min at 25 °C, 40 mTorr).

2.3. In vitro release of nicotine

The in vitro release profile of nicotine was studied using a diffusion chamber based on a design previously described by Cornaz et al. (1996). Briefly, the receptor compartment contained PBS, pH 7.4 at 37 °C, and the donor compartment contained air saturated with moisture generated by the temperature and the closed system nature of the experimental setup. The lyophilisate was placed on Whatman Grade 1, 42.5 mm diameter filter paper (VRW, Poole, UK) maintained just in contact with the liquid phase of the receptor compartment, which was constantly agitated with a magnetic stirrer. Samples of 0.5 ml were withdrawn at regular time intervals from the acceptor compartment and analysed spectrophotometrically (using a UV1 double beam spectrophotometer, Thermospectronic, Rochester, USA) at 260 nm. Each sample taken from the acceptor compartment was replaced immediately with 0.5 ml of fresh medium. The release profiles

of nicotine from a 2% HPMC nasal insert, NHT powder, and a solution of NHT in 5% (w/w) mannitol were plotted.

2.4. Vapour sorption studies

The dynamic vapour sorption (DVS) apparatus (DVS 1000, Surface Measurement Systems, Cheshire, UK) regulates the temperature and humidity of the environment surrounding a sample, allowing any weight changes in a sample due to sorption or desorption of water vapour to be accurately measured. Examining the behaviour of the samples under such conditions was considered of importance in characterising a formulation which would be subjected to a high humidity environment in the nasal cavity.

In order to minimise exposure to atmospheric humidity all samples were stored in a desiccator until required for study, when they were transferred immediately to the DVS sample pan. Samples were subjected to a controlled cycle of changing relative humidity (RH), beginning with an initial drying phase at 0% RH, to quantify and remove residual moisture from the freeze drying process and exposure to atmospheric humidity. The temperature was maintained at 25 °C, and RH was increased in 10 stepwise increments to 95% RH. RH was decreased through the same steps in reverse, and the entire cycle was then repeated. Progression to the next RH increment of a cycle was triggered when either the weight of the sample had remained constant (rate of weight change over 20 min <0.002 mg/min), or that the maximum individual step time of 999 min had been reached. In each case, a single sample was analysed, due to the extended period of analysis required for the samples (up to 5 days).

2.5. Scanning electron microscopy (SEM)

Lyophilised samples containing HPMC, NHT and mannitol were prepared for microscopy by using a scalpel blade to make a fine initial incision in the surface of the insert, allowing a section of the lyophilisate to be prised apart, exposing an internal area that had not been damaged by the slicing action of the scalpel. The sample was fixed to the sample stub by means of copper electrical tape and was gold coated using a Polaron SC515 SEM Coating System (Bio-Rad sputter coater, Bio-Rad Ltd., Hemel Hempstead, UK). SEM images were obtained using a Phillips SEM 500 (Bio-Rad Ltd., Hemel Hempstead, UK), with a spot size of 320 Å and 12 KV intensity.

2.6. Preparation of doses

2.6.1. Nicotine powder

For each dose, NHT powder equivalent to 4 mg of nicotine base was weighed out and packed into the last 2 cm cut from the narrow end of a 200 μl Gilson pipette tip. An equal weight of mannitol powder was packed into the pipette tip, on top of the layer of NHT powder as a bulking agent, and to allow greater comparability with the solid dosage form of the lyophilisate which also contained mannitol. In this way, the mannitol would contact the nasal mucosa first upon dosing, maximising any potential for an osmotic effect improving absorption. For dosing, the narrow end of the pipette tip was inserted into a length of silicone tubing (Portex, Kent, UK, internal diameter 1.95 mm, external diameter 5 mm), with a 50 ml syringe with the plunger fully withdrawn placed at the other end.

Depressing the plunger on the syringe created a rapid expulsion of air from the barrel, which propelled the powder from the pipette tip into the nasal cavity of the sheep. The powder was applied directly to the turbinate site of the nasal mucosa by inserting the pipette tip and tubing to the required depth into the nasal cavity via a Portex Z79 naso-pharyngeal tube (Portex, Kent, UK, internal diameter 7.0 mm, external diameter 10.00 mm) marked to ensure consistent 85 mm depth of administration when inserted to the nasal cavity.

2.6.2. Nicotine spray

NHT solution at a concentration of 4 mg/200 μl nicotine base was administered via silicone tubing attached to a 50 ml syringe with puncture holes made at the opposite end of the tube. A Gilson pipette was first used to draw 200 μl of nicotine solution into the tubing which was inserted into the nasal cavity via the naso-pharyngeal tube (marked to ensure consistent depth of administration as detailed in Section 2.6.1), following which the 50 ml syringe was attached and the plunger was rapidly depressed, causing the solution to spray out onto the turbinate site through the puncture holes in the silicone tubing.

2.6.3. Lyophilised insert

Lyophilised nasal inserts were prepared as described above from 2% HPMC, mannitol and NHT to give a dose of 4 mg nicotine base per insert. The insert was administered to the turbinate site in the nasal cavity of the sheep by inserting the lyophilisate into the naso-pharyngeal tube (marked to ensure consistent depth of administration as detailed in Section 2.6.1), and gently ejecting it into the nasal cavity by pushing a flexible nylon rod through the interior of the naso-pharyngeal tube.

2.6.4. Intravenous

NHT solution was filter sterilised at a concentration of 0.2 mg/ml nicotine base and 5 ml of this solution (1 mg nicotine base) administered by venipunture into the jugular vein which was not cannulated (see Section 2.7 below).

2.7. Study protocol

Eight wether sheep, 1–2 years old, weighing 50–70 kg, were obtained from Cochno Farm and Research Centre (University of Glasgow, UK). They were housed indoors and received food and water ad libitum for the duration of the study, which was approved by the University of Glasgow Ethical Review Committee and performed under a Home Office (UK Government) Licence. A randomised cross-over study design was adopted, with a wash out period of 7 days between treatments.

For serial blood sampling, an in-dwelling intravenous cannula (50 mm × 16 g, Dunwood, Aberdeen, UK) was placed in the jugular vein of each sheep, and retained in place for the duration of each experimental leg. The cannula was kept patent by flushing with heparinised normal saline when required, and was removed at the end of the blood sampling period. The sheep were not sedated to avoid impairing the mucociliary function, and required only minimal manual restraint during dosing and sampling. A pre-dose blood sample was taken prior to NHT administration, following which blood was sampled at 15, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min after dosing, except for IV administration when sampling was at 2, 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min. Blood samples were collected in heparinised vacuette tubes (Lithium Heparin 9 ml Monovette, Sarstedt, Leicester, UK), and plasma was separated by centrifugation at 3000 × *g* for 15 min and stored at −20 °C until analysis.

2.8. Analysis of plasma samples

Plasma samples (1 ml) spiked with 50 ng of internal standard (nicotine-methyl-d_3) and mixed with 0.5 ml of 0.1 M sodium phosphate buffer (pH 3) were filtered using Whatman 0.45 μm 22 mm Nylon GD/X syringe filters (BDH, VRW, Poole, UK). The filtrate was extracted on an Isolute® SCX column (Argonaut, Mid Glamorgan, UK), the analyte eluted with 1 ml of 3% (w/v) ammonia in methanol solution and then blown to near dryness under a stream of nitrogen. The remaining concentrated solution was transferred to sample vials for LC–MS analysis on an Automass GC/LC–MS system (TSQ 7000, ThermoFinnegan, San Jose, USA). Selected ion monitoring was carried out for the base peak of the electron mass spectra of nicotine (*m*/*z* 84) and nicotine-methyl-d_3 (*m*/*z* 87).

2.9. Pharmacokinetic data analysis

The data were analysed assuming first-order absorption and one compartment model kinetics with first-order elimination. Maximum plasma concentration (C_{max}), its time of occurrence (T_{max}) and the area under the curve (AUC) were obtained by calculating the mean of the plasma concentration versus time data for four NHT formulations in each individual sheep. The area under the curve from 0 to 8 h was calculated using the trapezoidal rule, and the data was analysed statistically using analysis of variance (Anova).

3. Results and discussion

3.1. In vitro release

The in vitro release data gathered showed that nicotine was released from the lyophilised formulation over an extended period of time (up to 4 h). The time to attain 90% release of NHT could be ranked 2% HPMC > NHT solution ≥ NHT powder, with the nasal insert significantly different from both the solution and powder ($p < 0.05$ using an unpaired *t*-test). The in vitro release profiles are shown in Fig. 1.

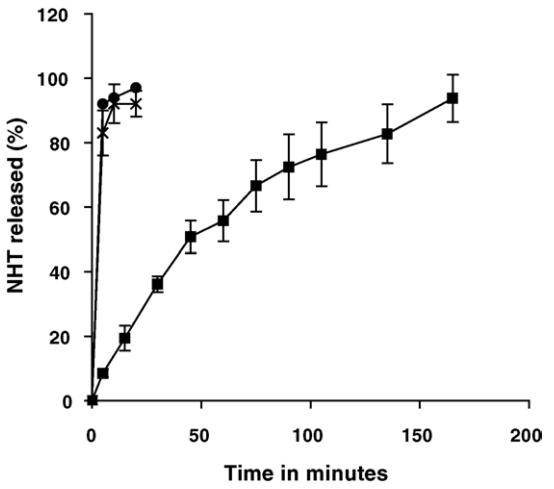

Fig. 1. In vitro release of NHT ($n = 4$) in PBS pH 7.4, at 37 °C. NHT solution (●), NHT powder (×), lyophilised insert (■).

Due to the complex geometry of the nasal insert, one end narrowing in relation to the other, and the processes of collapse, swelling and spreading that occur on hydration, the classical application of the Peppas equation for a flat cylinder does not necessarily have relevance. However, in order to describe the data in a manner which allowed comparison of formulations, a means of obtaining linear data was sought. When plotted as % nicotine released against exponential time (t^n), a best fit plot was obtained for $n = 0.7$. The value of the exponent suggests that NHT release was at least in part dependent on erosion or dissolution of the polymer gel. Similar results have been described for the release of a poorly soluble drug from tablets containing different ratios of HPMC K4MP and K100LVP (Eyolfsson, 1999) and for the release of alprazolam from sustained release HPMC tablets (Skoug et al., 1993).

Compared to the lyophilised insert, release of NHT from powder or solution was rapid, which was expected as in either formulation the NHT would be available for almost instantaneous dissolution, although release from the powder was very slightly delayed (not significant, $p > 0.05$) due to time taken for hydration and dissolution. In the case of the lyophilised formulations, hydration of the HPMC lyophilisate is initially required before NHT release through the gelled matrix, explaining the delayed and prolonged release profile.

3.2. Vapour sorption

The DVS analysis for the lyophilised formulation demonstrated a substantial increase in weight (approximately equivalent to the dry weight of the insert) as a result of sorption of water vapour by the lyophilised NHT formulation. Table 1 demonstrates that mannitol absorbs very little water vapour, either in powder or lyophilised form, while HPMC absorbs over 50% of its dry weight in both powder and lyophilised states. NHT, on the other hand, absorbs a low level of water vapour in its original powder state, while displaying a 10-fold increase in vapour sorption capacity following

Table 1
Vapour sorption data on lyophilised formulations and their individual components

Formulation	Initial moisture content (percentage of original weight)	Total H_2O sorbed per cycle (percentage of dry weight)	
		Cycle 1	Cycle 2
NHT nasal insert[a]	1.7	107.0	88.7
NHT powder	1.8	4.3	3.9
Lyophilised NHT[b]	0.4	45.1	44.5
HPMC powder	0.0	57.7	54.4
Lyophilised HPMC[c]	0.7	51.3	51.6
Mannitol powder	0.0	0.3	0.2
Lyophilised mannitol[d]	0.0	0.00	1.0
Placebo nasal insert[e]	1.5	46.1	36.7

[a] 2% HPMC/1% mannitol/NHT.
[b] Lyophilised NHT solution (4 mg nicotine base/ml).
[c] Lyophilised 5% HPMC solution.
[d] Lyophilised 10% mannitol solution.
[e] 2% HPMC/1% mannitol.

lyophilisation. This increase in vapour sorption capacity of NHT following lyophilisation may explain the fact that addition of NHT to the nasal insert more than doubles the percentage of water absorbed compared with a placebo nasal insert.

This data suggests that in the humid environment of the nasal cavity, the nasal insert will be capable of rapidly absorbing moisture. However, this data also has implications for the storage and packaging of such a formulation on a commercial basis.

Initial moisture content of the samples varied, as shown in Table 1, although with the lyophilised nasal inserts it is difficult to ascertain whether the moisture remained from the freeze drying process, or was absorbed from the environment during the brief period of transfer to the DVS.

3.3. SEM

A typical SEM image obtained for a lyophilised nasal insert containing HPMC, mannitol and NHT is shown in Fig. 2. It can be seen that the formulation forms a highly porous structure upon lyophilisation, potentially providing an ideal route for water ingress and subsequent rehydration of the polymer, an important step in gel formation within the nasal cavity.

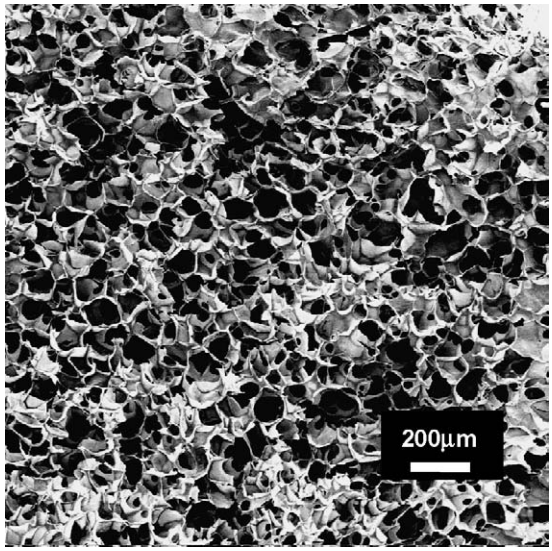

Fig. 2. SEM image showing the characteristic high internal porosity of the lyophilised nasal insert.

3.4. In vivo nicotine plasma concentrations and pharmacokinetic analysis

The observed maximum concentrations (C_{max}), times of maximum plasma concentration (T_{max}) and the area under the curve (AUC) are shown in Table 2. The mean plasma concentration time profiles for the four formulations in sheep (Fig. 3—error bars omitted for clarity, data presented in Table 2) indicated that the nasal powder and liquid doses showed a relatively rapid increase to the peak nicotine absorption, followed by a decrease in plasma levels, similar to results previously reported (Cheng et al., 2002) for a nasal NHT solution in sheep (T_{max} of 2.3 min), and in rats (Jung et al., 2000), when it was concluded that plasma profiles of nicotine were similar to those of IV administration. Sampling times for the nasal formulations in this investigation were chosen according to both the constraints of the experimental procedure, and the design of a suitable sampling regimen for the slow releasing nasal insert with which the relative behaviour of a powder and liquid could then be compared. It can be seen that in the case of the solution and powder formulations an ideal sampling regimen would have included more time points between 0 and 15 min such as those reported by Johansson et al., 1991, in order to give clearer information on T_{max}, C_{max} and AUC for these formulations. The information obtained, however, serves to confirm

Table 2
Pharmacokinetic parameters (mean values ± S.D.) following intranasal (4 mg) and intravenous (1 mg) administration of nicotine in sheep

Formulation	T_{max} (min)	C_{max} (ng/ml)	AUC (ng h/ml) (0–480 min)	Relative bioavailability[a] (%)
IV ($n = 8$)	2.80 ± 1.40	9.4 ± 5.8	16.3 ± 32.25	100
Lyophilised insert ($n = 7$)	39.4 ± 33.0[b]	27.6 ± 23.4	54.4 ± 69.4	83.4
Spray ($n = 6$)	21.0 ± 13.4	45.6 ± 24.0	33.6 ± 32.7	51.5
Powder ($n = 6$)	20.0 ± 7.7	36.4 ± 19.4	17.7 ± 6.40	27.2

[a] Relative to 1 mg IV dose.
[b] Significantly different from IV.

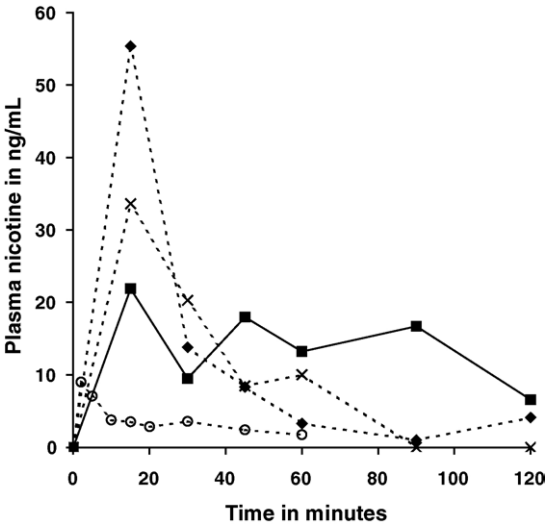

Fig. 3. Mean plasma concentration time profiles of different formulations following intranasal and intravenous administration of 4 and 1 mg, respectively, of nicotine in sheep ($n = 6$–8). IV (◯), nasal insert (■), nasal spray (♦), nasal powder (×).

the rapid absorption of this drug nasally. In this present study, 30 min after dosing, plasma nicotine values for nasal NHT powder and spray were approximately one half and one quarter of their peak values, respectively, and at 90 min the amount of nicotine detected in the plasma was negligible.

The IV dose gave rise to a rapid T_{max} of 2.8 ± 1.4 min and the conventional nasal powder and spray formulations yielded T_{max} values of 20.0 ± 7.7 and 21.0 ± 13.4 min, respectively. The nasal insert, on the other hand, yielded a mean T_{max} of 39.4 ± 33.0 min, although the delayed T_{max} of the insert differed significantly from only the IV formulation ($p < 0.05$) due to the variability of the data. The mean profile for the nasal insert shows a more gradual rise in plasma nicotine, with significant levels sustained over approximately 2 h, followed by a gradual decrease in plasma levels at a slower rate than for the other nasal preparations. Corresponding C_{max} values decrease in the order nasal spray > nasal powder > nasal insert > IV, although there were no significant differences observed ($p > 0.05$) between the C_{max} values of any of these formulations (when corrected to allow for the difference in IV and intranasal dose). The prolonged plasma nicotine levels for the nasal insert, with a low C_{max} in comparison with the other nasal formulation is consistent with results reported by Jung et al. (2000)), using proliposomes to achieve prolonged nasal delivery in rats.

The apparent contradiction between C_{max}/T_{max} values presented in Table 2, and those presented graphically in Fig. 3 are explained by the fact that the data in the table represents the average value calculated from the C_{max} or T_{max} for each individual sheep, irrespective of time of occurrence, while the plasma profiles show the average plasma nicotine level at the particular time point at which plasma samples were obtained.

The mean AUC of the insert was greater than that for the other nasal formulations, although again due to the high variability of the data, there were no statistically significant differences between the formulations ($p > 0.05$), and it is possible that the full AUC for the powder and spray formulations may be underestimated due to the sampling regimen used. Nevertheless, for comparative purposes the bioavailability of the nasal formulations relative to the IV preparation was calculated from the mean AUC, allowing for the difference in dose by using the formula:

$$F_{rel} = \left(\frac{AUC_{(IN)}/\text{IN dose}}{AUC_{(IV)}/\text{IV dose}} \right) \times 100\%$$

The nasal insert formulation gave a relative bioavailability of 83.4%, compared to 51.5 and 27.2% for the nasal spray and powder, respectively, a promising increase over the conventional nasal formulations, despite the high variability of the data meaning that this effect is not statistically significant. This data may also suggest that despite the low C_{max} of the nasal insert, the apparent prolonged plasma levels might result in a higher bioavailability.

As far as the lyophilised insert was concerned the prolonged plasma nicotine levels are likely to be a result of a combination of two factors. Firstly, the insert would require time for hydration of the polymer on the nasal mucosa before nicotine release would begin. This factor would not be present with the nasal spray or readily soluble powder formulations from which the NHT should be immediately available for absorption. This is reflected in the relatively rapid plasma nicotine peaks (around 20 min) for these formulations, and the T_{max} value of the nasal insert which while not statistically different, is higher than for the conventional preparations.

The second, probably more significant contribution to the extended plasma profile would be the viscous

nature of the hydrated gel layer, through which the nicotine has to diffuse to be released. This is consistent with the in vitro results, which showed a significantly extended NHT release compared with the powder and liquid formulations. Jung et al. (2000) similarly concluded that such a combination of "retarded hydration and sustained release" explained the extended half-life and mean residence time of nicotine from nasal proliposomes. Prolonged nicotine plasma levels suggest that the lyophilised insert remains in the nasal cavity releasing nicotine for approximately 2–3 h. In vitro observations show that a viscous adhesive gel is formed on hydration of the dosage form. If this gel is formed on administration of the insert to the sheep nasal cavity, then it would be expected to resist the rapid mucociliary clearance rate by adhering to the mucosa as a large cohesive mass, making the slowly diffusing drug available for absorption over an extended period.

A further mechanism of action on the nasal mucosa has been suggested for bioadhesive formulations, that of water uptake affording a transient opening of the tight junctions between the cells of the nasal mucosa, allowing an alternative path of transport of drug through aqueous pores. This opening of the tight cell junctions is suggested to be caused by a slight dehydration of mucosal cells as a result of water uptake by the bioadhesive formulation (Edman et al., 1992). If this effect does occur, then the insert formulation is likely to display this behaviour, due to its requirement for hydration by water from the nasal mucosa, and the demonstrated uptake of water and swelling of HPMC (Columbo et al., 1999).

A combination of these factors may explain the apparent "double peak" which can be seen in the plasma profile for the lyophilised insert. The preliminary plasma nicotine peak may be attributed to an initial release of nicotine from the surface of the insert, with the secondary peak being a result of absorption following diffusion of nicotine through the slowly hydrating formulation, assumed to be slowly spreading over the surface of the nasal mucosa. The lyophilised insert formulation may, therefore, have potential applications for any drug requiring sustained delivery, and also potentially where an initial burst release followed by a sustained level of drug absorption is required, for example with nicotine as a smoking cessation aid. Such a "double peak" effect would, however, have to be further investigated, as the lack of statistical significance in the data means that the existence of the effect is not proven.

Differences in metabolic rate may in part explain the variability between the data obtained from the different animals, although previous researchers investigating nasal nicotine formulations in sheep (Cheng et al., 2002) have also reported high inter-animal variability resulting in a lack of statistical significance attached to apparent observed differences in plasma profiles, while other researchers investigating nasal insulin in sheep present data with similar high levels of variability (Dyer et al., 2002; Illum et al., 2001). Another report into the correlation of nasal data in sheep, rabbits and man also demonstrates data showing a greater degree of variability in bioavailability of nasal buprenorphine in sheep than in the other two models (Lindhart et al., 2001). It is also possible that on administration of some of the nasal formulations, the nasal mucosa of the sheep may have become temporarily damaged in some way, resulting in higher than anticipated plasma levels. Conversely, if the nasal dose were not delivered effectively to the correct site on the nasal mucosa for some reason, the plasma levels obtained for the formulation in that particular sheep may have been lower than those observed for the same formulation in the other sheep. It is possible for example, that the use of an insufflation technique may have resulted in some of the powder or spray doses missing the intended deposition site, or passing through the nasal cavity altogether, despite the care taken to ensure consistent depth of administration. In common with the majority of nasal studies reported, it is impossible to be completely sure of site of deposition without employing the use of an imaging technique.

The nasal insert appears to show slightly higher variability of data than the other formulations studied, which is considered to be a result of the complex hydration and diffusion processes occurring in the insert, which is a fundamentally different formulation concept to the conventional powder and liquid dose, and may be more susceptible to the physiological differences of individual sheep.

In conclusion, the lyophilised insert displayed a significantly extended NHT release profile over the conventional formulations studied. The high variability of the pharmacokinetic data observed means that statistically the only parameter of the nasal insert which was proven to be different was the T_{max} when com-

pared with the IV dose. However, despite this, general observations on the higher relative bioavailability and prolonged plasma profile of the nasal insert is suggestive of an extended period of absorption from the nasal cavity, meriting further study to confirm this effect.

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