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Nasal administration of an angiotensin antagonist in the rat model: Effect of bioadhesive formulations on the distribution of drugs to the systemic and central nervous systems

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Abstract

The effect of bioadhesive formulations on the direct transport of an angiotensin antagonist drug (^{14}C-GR138950) from the nasal cavity to the central nervous system was evaluated in a rat model. Three different bioadhesive polymer formulations (3% pectin LM-5, 1.0% pectin LM-12 and 0.5% chitosan G210) containing the drug were administered nasally to rats by inserting a dosing cannula 7 mm into the nasal cavity after which the plasma and brain tissue levels were measured. It was found that the polymer formulations provided significantly higher plasma levels and significantly lower brain tissue levels of drug than a control, in the form of a simple drug solution. Changing the depth of insertion of the cannula from 7 to 15 mm, in order to reach the olfactory region in the nasal cavity significantly decreased plasma levels and significantly increased brain tissue levels of drug for the two formulations studied (1.0% pectin LM-12 and a simple drug solution). There was no significant difference between the drug availability for the bioadhesive formulation and the control in the brain when the longer cannula was used for administration. It is suggested that the conventional rat model is not suitable for evaluation of the effects of bioadhesive formulations in nose-to-brain delivery.

Keywords: Bioadhesive formulations; Olfactory region; Pectin; Chitosan; Nasal administration

1. Introduction

There is an increasing need to identify drug delivery systems that enable efficient delivery of drugs to the central nervous system for treatment of neurological diseases such a Alzheimer's and Parkinson's diseases, by direct transport from the nasal cavity via the olfactory pathway (Illum, 2004). It is known from numerous animal studies, that for most drugs, the amount of drug transported to the CNS via this route is only of the order of 1% or less. It is envisaged that the transport of drugs to the CNS in man would be even lower due to the difficulty in reaching the olfactory region with a normal spray device and the much smaller area of the olfactory region in man as compared to for example that in rat and dog. Hence, with such limited targeting of the olfactory region expected, only very potent drugs would likely result in a beneficial therapeutic effect for the patient.

Novel nasal spray and powder devices have been described in the literature (e.g. OptiMist™ and ViaNase ID™) that claim to enable targeting of formulations to the olfactory region (Djupesland et al., 2006; Reger et al., 2006). However, so far no proof of improved brain targeting of drugs from these devices as compared to normal nasal sprays has been reported. It is likely that enhanced delivery systems will be necessary for significant drug transport across the olfactory epithelium, especially for polar drugs (Illum, 2004). In this regard it is expected that the olfactory epithelial barrier to the transport of large peptides will be similar to that seen for the respiratory epithelium. As suggested in previous publications by our group (Illum, 2004; Charlton et al., 2007a,b) a strategy to achieving improved delivery to the brain via the olfactory route would be a combination of a vector that specifically targets receptors in the olfactory region, and a bioadhesive formulation that retains the drug at the absorption site (and if necessary an enhancer that improves

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transport across the epithelium) (Illum, 2004; Charlton et al., 2006, 2007a,b).

The literature indicates that very few bioadhesive formulations have been tested for the improvement of nose-to-brain delivery of drugs. Jansson et al. (2005) have shown increased residence time and drug transfer across the nasal epithelium in rats using a gellan gum formulation; and Vyas et al. (2006) have demonstrated increased brain uptake from a mucoadhesive microemulsion.

We have previously studied the use of the bioadhesive materials pectin and chitosan for nasal administration. Low methylated pectins were shown to gel rapidly after administration into the nasal cavity of sheep and were furthermore retained for an extended period of time resulting in an increased half-time of clearance of the formulation (Illum, 2000, 2003). Chitosan has similarly been shown to have bioadhesive characteristics and to be retained in the nasal cavity for prolonged periods (Illum, 2004; Soane et al., 1999). Chitosan has also been proven to be an efficient absorption enhancer that can transiently open tight junctions between epithelial cells and thereby significantly enhance the absorption of even large molecular weight polar drugs (Illum et al., 2000; Hinchcliffe and Illum, 1999; Smith et al., 2004).

In a previous publication, low molecular weight pectins LM-5 and LM-12, together with chitosan G210, each combined with a model zwitterionic drug (GR138950) showed significantly slower rates of drug release as compared to a control solution as determined in a Franz Diffusion Cell (FDC) study (Charlton et al., 2007a). It was concluded that these formulations could have potential as nasal controlled release vehicles. Studies in MDCK1 cell monolayers showed that chitosan significantly decreased transepithelial electrical resistance (TEER) values, and significantly increased the transport of mannitol across the membrane, whereas both pectins showed minimal effects on the TEER and minimal increases in mannitol transport. Both the chitosan and pectin formulations reduced the transport of the lipophilic (positively charged) drug propranolol (Charlton et al., 2007a). Another previous paper has reported that bioadhesive pectin and chitosan formulations were able to reach the olfactory region in the nasal cavity of man when delivered using a simple nasal drop device (Charlton et al., 2007b). Furthermore, the formulations displayed a significantly increased residence time on the epithelial surface. This was in contrast to a non-bioadhesive control delivered with the same device. It was further shown that a pectin formulation administered with a nasal spray system did not show an increase in residence time in the olfactory region.

The purpose of the present study was to evaluate the effect of bioadhesive pectin and chitosan solution formulations on the uptake of an angiotensin antagonist across the nasal mucosa into the blood and the CNS using the rat model. Two pectin formulations, 3% pectin LM-5 and 1% LM-12 and a 0.5% chitosan G210 formulation containing GR138950 were administered to rats after which the drug concentration in plasma and in different areas of the brain were monitored. Furthermore, the effect of two different lengths of the application cannula on plasma and CNS uptake was investigated in the same model.

2. Materials and methods

2.1. Materials and equipment

Genu pectin LM-5 CS (BN91837) and Genu pectin LM-12 CG (BN G1451) were kindly donated by Hercules (Hercules Inc., Salford, Lancashire, UK). Chitosan Seacure G210 (glutamate salt, molecular weight: ~150 kDa, degree of deacetylation: 82%) (BN604-583-08B1.1) (now known as Protosan UP G213) was donated by Pronova Biopolymer (now known as Novomatrix, FMC Biopolymer, Drammen, Norway). The model drug candidate ^{14}C-GR138950L (BN R2838/35/2, specific activity 3.01 MBq/mg), a zwitterionic angiotensin antagonist molecule (pKa_1 3.6, pKa_2 5.5), was kindly donated by GlaxoWellcome (Stevenage, Hertfordshire, UK) (Fig. 1).

OptiPhase 'HiSafe' 2 liquid scintillation cocktail and OptiSolv tissue solubiliser were purchased from Fisher Scientific UK (Loughborough, Leicestershire, UK). Heparin sodium salt (porcine, 174 USP units/mg) was purchased from Sigma–Aldrich, Poole, Dorset, UK. Sodium chloride injection/infusion BP 0.9% (w/v) and water for injection (WFI) were purchased from Medical School Stores, QMC Hospital, Nottingham, UK. Hypnorm (Janssen-Cilag Ltd., High Wycombe, Bucks, UK) and Hypnovel (Roche Ltd., Welwyn Garden City, Herts, UK) were obtained from Biomedical Science Unit, QMC Hospital, Nottingham, UK.

A microcentrifuge (MSE Micro Centaur, Sanyo, UK) was used for spinning down blood samples. BD Microtainer plasma separator tubes with lithium heparin (Becton Dickson and Company, Franklin Lake, New Jersey, USA; purchased from NHS Supplies, UK) were used for blood collection and plasma

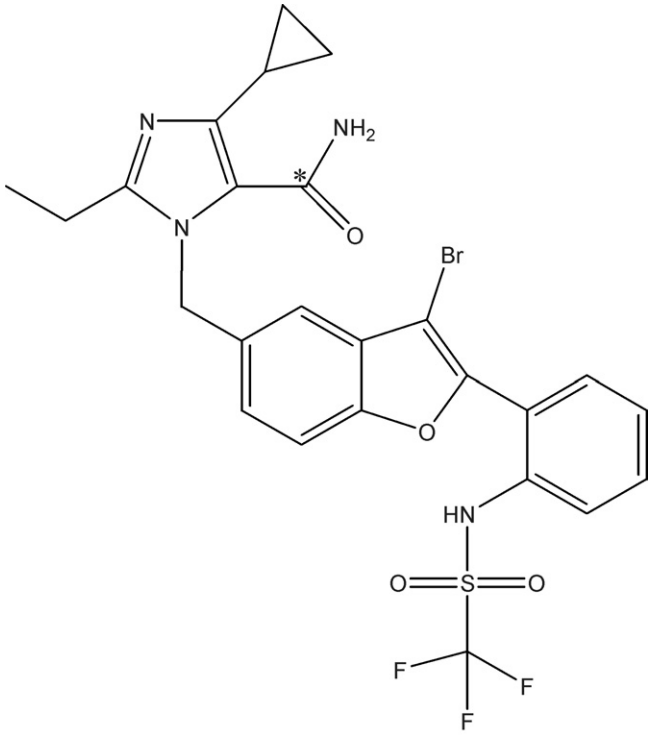

Fig. 1. Molecular structure of GR138950, (*) denotes site of radiolabelling.

Table 1
Formulation components and doses

Formulation	Polymer concentration (%)	Formulation viscosity (mPa.s)	GR138950 concentration (mg/ml)	Dose/250 g rat (g)	Volume dosed (μl/250 g)
Pectin LM-5	3.0	30	2.24	67	30
Pectin LM-12	1.0	18	1.36	41	30
Chitosan G210	0.5	23	1.60	48	30
Control	–	–	2.50	75	30

isolation. Brain sections were prepared using a brain slicer (1 mm slice, for 200–300 g rats; Zivic Miller, supplied by Harvard Apparatus Ltd., Edenbridge, Kent, UK).

2.2. Formulation preparation

For the rat absorption study three different ^{14}C-GR138950-polymer solution formulations were employed together with a ^{14}C-GR138950 solution control as detailed in Table 1.

The polymers were reconstituted in WFI and stirred (magnetic stirrer and bar) overnight. ^{14}C-GR138950 (in powder form) was added to each formulation in aliquots up to the final concentration. The solutions were thoroughly mixed between additions of aliquots. Drug concentrations were determined from radioactivity as measured by liquid scintillation using a 1219 Rackbeta 'Spectral' Liquid Scintillation Counter (EG&G Wallac, Milton Keynes, UK).

2.3. Animal absorption studies

Groups ($n = 3$) of male Wistar rats weighing approximately 250 g were anaesthetised intravenously with a solution of 2 ml Hypnorm (fentanyl/fluanisone) + 2 ml Hypnovel (midazolam) + 10 ml water for injection, via an indwelling needle implanted into the caudal (tail) vein. 0.2 ml of anaesthetic was administered for induction of anaesthesia, and 0.1 ml was given as a top-up dose, as required, in order to maintain anaesthesia throughout the study. The rats were tracheotomised to maintain a patent airway and to prohibit airflow through the nasal cavity. The oesophagus was occluded by ligation against the tracheal cannula to prevent drug absorption from the gastrointestinal tract. The carotid artery was cannulated to allow blood sampling. After cannulation and after blood sampling, heparinised saline (0.25 mg/ml heparin in 0.9% saline) was used to clear the cannula to prevent clot formation in the tube. This surgical methodology was taken from Hirai et al. (1981) with modifications by Fisher et al. (1985). This method used a 7 mm nasal insertion of the administration cannula.

Intranasal doses (0.12 ml/kg, divided evenly between both nostrils) were administered via a cannula (polyethylene, 0.28 mm I.D., 0.56 mm O.D.) inserted 7 mm into the nasal cavity.

Blood samples (150–200 μl) were taken via the carotid cannula pre-dose and at the following time points as appropriate; 1, 2, 3, 5, 7, 8, 9, 10, 11, 15, 20, 25 and 30 min. Blood samples were collected into Microtainers, which were pre-coated with heparin and contained a polymer plug that enabled plasma separation by density-gradient centrifugation (13,000 rpm for 5 min).

The rats were sacrificed at 1, 5, 10, 15 and 30 min after dosing, and the brain; olfactory lobes; nasal turbinates, lining and septum were removed. The brain was removed intact (excluding the olfactory lobes) and then divided into four sections, as described in Fig. 2, with the aid of a brain slicer. The first section was made across the transverse sinus, followed by two further cuts at 5 mm intervals towards the anterior of the cerebral hemispheres.

The animal study protocol was approved by the Ethics Committee at QMC, BMSU, Nottingham University and the study carried out under a project license approved by the Home Office, UK.

2.4. Determination of effect of cannula length on absorption of drug

Nasal administration in the rat model using the 7 mm cannula delivers the dose into the nasal passage at the anterior end of the nasoturbinate and maxilloturbinate. The nasal cavity is narrow at this point, therefore, resistance to polymer flow is high and mucoadhesion is likely to be extensive. At 15 mm from the nares in the rat, the nasal passage widens at the posterior of the maxilloturbinate and the anterior part of the ethmoid turbinates where the olfactory region is situated (Fig. 3). The

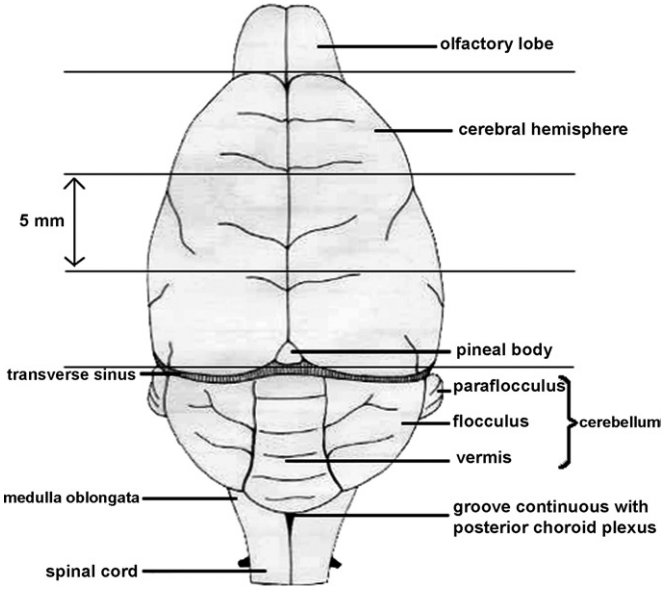

Fig. 2. Schematic of the brain, illustrating how the brain was sectioned for analysis. The brain was removed separate to the olfactory lobes and was divided into sections, with section 1 being the front of the cerebral hemisphere and section 4 being the cerebellum. The horizontal sectioning lines represent the division cuts (adapted from Rowett, 1979).

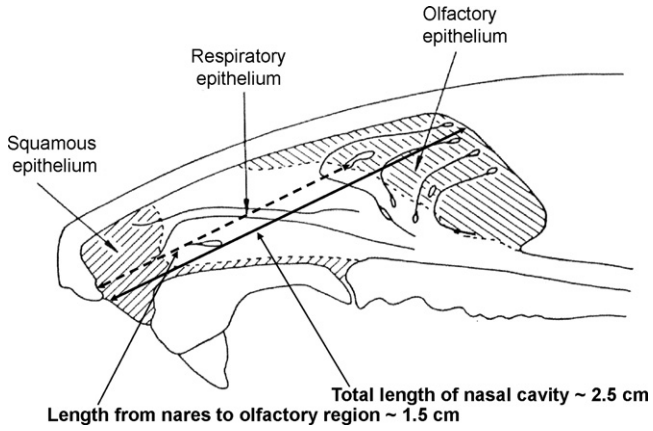

Fig. 3. Schematic representation of the rat nasal cavity.

use of a 15 mm cannula is feasible, and delivery of a polymer formulation behind the maxilloturbinate will minimise the loss of dose through adhesion to the anterior turbinates and should deliver the formulation to the edge of the olfactory region. This concept for potentially increasing the delivery of drug to the olfactory region was tested in the rat model.

To minimise the use of animals, only one polymer formulation was tested and only 5 and 10 min end-points were examined. The formulations tested were ^{14}C-GR138950 in a simple solution formulation and together with 1% pectin LM-12. Pectin LM-12 was chosen because it was found to produce the highest drug exposure in the olfactory lobes and blood (based on AUC) compared to the other two polymer formulations tested in the first absorption study. Furthermore, the two time-points nearest to the C_{max} in the blood and the brain were 5 and 10 min post-dose, which matches the end-point times for this experiment.

2.5. Blood and tissue sample analysis

The drug concentrations in the blood and tissue samples were determined by a liquid scintillation method. Plasma analysis was performed using 80 μl of plasma with 10 ml of OptiPhase scintillation cocktail in a glass scintillation vial. Each tissue sample was treated using Optisolv (1 ml/200 mg of tissue) and left to stand until fully solubilised (approximately 3 weeks). Optisolv is a toluene-based reagent that breaks down tissue and bone, resulting in a coloured, viscous liquid. The olfactory lobe and nasal lining samples were processed by analysing 200 μl of solubilised tissue/Optisolv with 10 ml of OptiPhase in a glass scintillation vial. For brain tissue samples, 1 ml of sample was added to 10 ml of OptiPhase in a glass scintillation vial.

Before measuring radioactive content, samples were stored in the dark at 3–5 °C for a minimum of 6 h to eliminate chemiluminescence from the scintillation cocktail. All samples were analysed with the RackBeta counter for 300 s which was repeated to give six readings for each sample. Samples of control plasma and brain tissue were spiked with known quantities of drug to produce standards for calibration. Clean solubilised tissue samples and spiked controls were used as background and control samples.

The ratios of drug concentration in the olfactory lobe over plasma concentration were calculated at specific time points. AUC of the concentration over time plot gives a cumulative measure of drug concentration in a sample. Multiplying the AUC by the total blood volume or weight of tissue gives the cumulative amount of drug delivered to the blood or brain tissue. This represents the 'availability' of drug. The total blood volume of a rat is 65 ml/kg (Laboratory Animal Science Association, 1998). The average weight of each brain section was determined from the weights of the experimental samples.

2.6. Statistical analysis

Statistical analysis was performed by GraphPad Prism version 4.03 for Windows (GraphPad Software, San Diego, California, USA) using two-way ANOVA, the two factors being formulation and time, followed by Bonferroni post-test analysis. The interaction component was used to determine a significant difference.

3. Results

3.1. Animal absorption studies

The chitosan and pectin GR138950 formulations were administered nasally to rats and the resultant plasma concentration compared to that obtained from a drug control solution. The plasma profiles are given in Fig. 4. Each of the formulations resulted in plasma drug concentration profiles that were statistically different to each other (LM-12 – $p < 0.0001$; LM-5 – $p = 0.0006$; G210 – $p = 0.0406$; simple solution – $p = 0.0406$). The use of 1% pectin LM-12 resulted in an increased rate of drug appearance in the blood, and the drug concentration was higher throughout the 30 min post-dose period compared to the other three formulations. 3% pectin LM-5, 0.5% chitosan G210 and the simple drug solution control all displayed the same rate of drug absorption over the first three minutes, after which the pro-

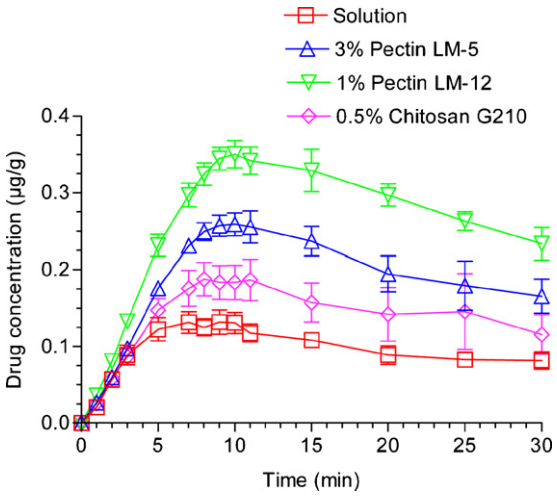

Fig. 4. Concentration profiles of GR138950 in plasma over 30 min following intranasal dosing of the drug in simple solution, and with 3% pectin LM-5, 1% pectin LM-12 or 0.5% chitosan G210. Error bars represent the S.E.M., $n = 3$.

Table 2
Drug distribution data following intranasal administration of the three polymer formulations

	Solution		Pectin LM-5		Pectin LM-12		Chitosan G210	
	Olf	Pl	Olf	Pl	Olf	Pl	Olf	Pl
t_{max} (min)	5	9	10	10	10	10	15	8
C_{max} (μg/g)	0.151 (0.022)	0.132 (0.016)	0.064 (0.020)	0.259 (0.015)	0.089 (0.036)	0.350 (0.018)	0.091 (0.048)	0.188 (0.022)
AUC (min μg/g)	2.93 (0.57)	2.88 (0.27)	1.49 (0.13)	5.68 (0.53)	1.63 (0.27)	7.96 (0.76)	1.48 (0.05)	4.23 (0.45)
Availability (μg/min)	0.23 (0.05)	46.80 (4.45)	0.12 (0.01)	92.30 (8.69)	0.13 (0.02)	129.38 (12.38)	0.12 (0.00)	68.66 (7.33)
Absorption (%)	23.4	45.3	63.4	34.0				

Olf: olfactory lobes, Pl: plasma. The S.E.M. is given in brackets.

files were different. All of the polymer formulations had higher C_{max} compared to that of the simple drug solution, whereas the t_{max} were not significantly different for the four formulations (~9 min.). (Table 2).

Analysis of the calculated drug availability showed that all of the polymer formulations provided increased delivery of drug into the blood compared to the simple drug solution (Fig. 5). The addition of 1% pectin LM-12 to the original formulation resulted in the largest (176%) increase in plasma drug availability, followed by 3% pectin LM-5 (97%), and 0.5% G210 (45%).

The time–drug concentration profiles for uptake of drug into the olfactory lobes after nasal application showed notable differences between the bioadhesive polymer formulations and the simple drug solution formulation, with the polymer formulations resulting in a later t_{max} and lower C_{max} (Table 2). Pectin LM-5 produced a significantly lower drug concentration profile in the olfactory lobes compared to the drug solution ($p = 0.0250$); the difference at the 5 min point being most prominent (0.04 mg/g for the LM-5 as compared to 0.15 mg/g for the solution). There were no other statistically significant differences between the formulations with respect to drug concentration in the olfactory lobes.

The ratios of drug concentration in the olfactory lobes to plasma concentration at 1 min post-dose varied between the different polymer formulations, with pectin LM-5 producing the highest value, followed by chitosan G210 (Fig. 6). At the 1 min sampling point, pectin LM-12 produced the highest blood concentration and lowest concentration in the olfactory lobes, and thus produced the lowest ratio, as expected. The simple drug solution produced the lowest blood concentration and highest concentration in the olfactory lobes at 1 min, and thus had the highest ratio value (more than double the value for 3% pectin LM-5 ($p = 0.0153$). The polymer formulations all produced similar ratio values at 5 min post-dose and later. This indicated surprisingly that the simple solution was most effective of the four formulations in delivering drug to the olfactory lobes.

Analysis of the cerebral hemispheres and cerebellum sections showed that there was no significant difference between drug concentration profiles over 30 min for the polymer formulations and the simple drug solution ($p > 0.05$) (Fig. 7, Table 3). Both pectin formulations produced similar drug concentration profiles in all of the brain sections, and displayed good reproducibility. However, chitosan G210 showed a high variability in drug level concentration (cerebral hemispheres 1; $C_{max} = 0.066$, S.E.M. = 0.052) at 10 min post-dose (Table 3).

Analysis of the relative drug availability supported the previous observations that the use of the polymers resulted in decreased delivery of GR138950 to the olfactory lobes (Fig. 7). Although there was no difference in drug delivery to the cerebral hemispheres, for the cerebellum the simple drug solution resulted in the highest concentration of drug in this tissue section.

3.2. Determination of effect of cannula length on absorption of drug

Following nasal administration in the rat model using a 15 mm dosing cannula, the drug concentration profiles for the

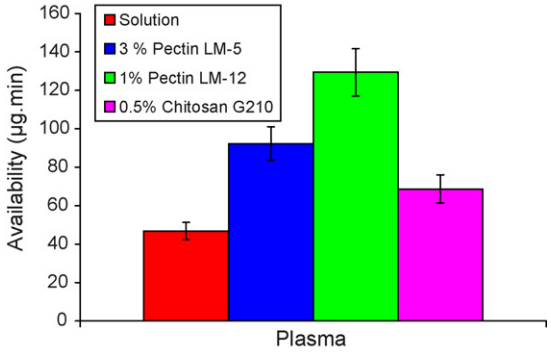

Fig. 5. Availability of GR138950 in plasma over 30 min after intranasal dosing of the drug in simple solution, and with 3% pectin LM-5, 1% pectin LM-12 or 0.5% chitosan G210. Error bars represent the S.E.M., $n = 3$.

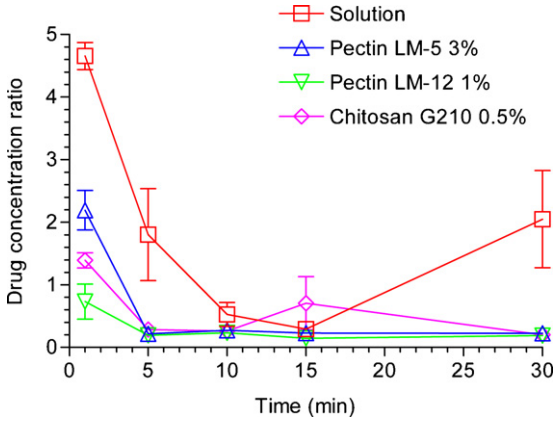

Fig. 6. Ratios of drug concentration in the olfactory lobes (μg/g) against drug concentration in plasma (μg/g) over 30 min after intranasal dosing of the drug in simple solution, and with 3% pectin LM-5, 1% pectin LM-12 or 0.5% chitosan G210. Error bars represent the S.E.M., $n = 3$.

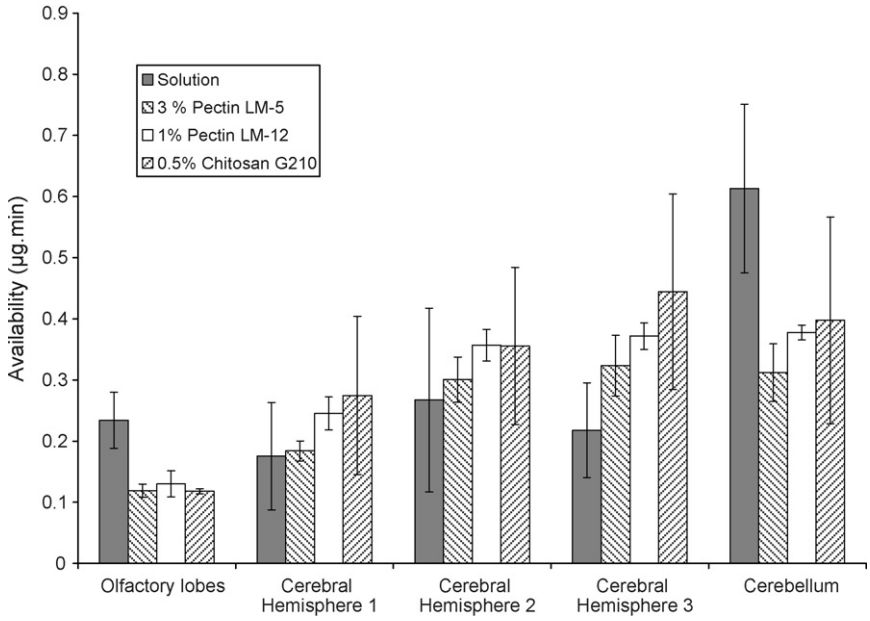

Fig. 7. Availability of GR138950 in brain regions over 30 min after intranasal dosing of the drug in solution, and with 3% pectin LM-5, 1% pectin LM-12 or 0.5% chitosan G210. Error bars represent the S.E.M., $n = 3$.

Table 3
Mean t_{max} and C_{max} values for each brain sample after intranasal dosing of GR138950 in solution, and with 3% pectin LM-5, 1% pectin LM-12 or 0.5% chitosan G210

Brain region	Solution		Pectin LM-5		Pectin LM-12		Chitosan G210	
	t_{max} (min)	C_{max} (μg/g)	t_{max} (min)	C_{max} (μg/g)	t_{max} (min)	C_{max} (μg/g)	t_{max} (min)	C_{max} (μg/g)
Olfactory lobes	5	0.151 (0.022)	10	0.064 (0.020)	10	0.089 (0.036)	15	0.091 (0.048)
Cerebral hemispheres 1	5	0.042 (0.036)	15	0.022 (0.001)	5	0.040 (0.017)	10	0.066 (0.052)
Cerebral hemispheres 2	5	0.049 (0.045)	10	0.022 (0.006)	5	0.030 (0.008)	15	0.019 (0.004)
Cerebral hemispheres 3	5	0.024 (0.018)	10	0.029 (0.007)	5	0.040 (0.011)	15	0.029 (0.002)
Cerebellum	10	0.087 (0.065)	10	0.035 (0.008)	5	0.041 (0.005)	15	0.034 (0.009)

The S.E.M. is given in brackets, $n = 3$.

pectin LM-12 and the drug control solution formulations were similar for both the olfactory lobes and for the plasma (Fig. 8, Table 4). Statistical analysis indicated that there was no significant difference between the two distribution profiles for GR138950 in the olfactory lobes nor the plasma concentration associated with the two formulations ($p = 0.7102$). The drug levels in the plasma after dosing either of the two formulations with the 15 mm cannula were significantly lower than the levels recorded after dosing with the 7 mm cannula ($p < 0.0001$) (Fig. 9 and Table 5). The change from 7 to 15 mm cannula resulted in the plasma C_{max} decreasing 43% for the simple solution and 71% for the pectin LM-12 formulation.

The availability data for the drug in plasma show that a similar amount of drug was absorbed into the blood over a 10 min period after dosing either formulation using a 15 mm cannula (Fig. 10). Drug availability in the plasma was greater for both formulations after dosing with the 7 mm cannula compared to the 15 mm cannula; the availability being 111% higher for the pectin formulation compared to the simple drug solution.

Using the 15 mm cannula for nasal administration resulted in higher drug levels in the olfactory lobes for both formulations compared to using the 7 mm cannula (Table 5). The statistical

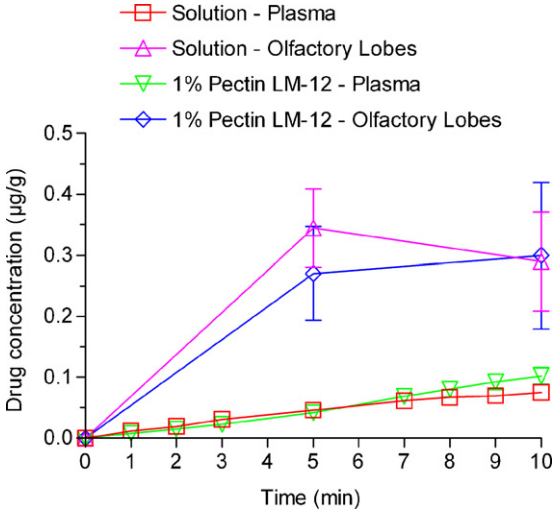

Fig. 8. Concentration profiles of GR138950 in the olfactory lobes and plasma over 10 min following intranasal dosing of the drug in solution and with 1% pectin LM-12 using a 15 mm dosing cannula. Error bars represent the S.E.M., $n = 6$.

Table 4
Drug distribution data for the olfactory lobes and plasma following intranasal dosing of GR138950 in solution and with 1% pectin LM-12 using a 15 mm dosing cannula

	Solution		Pectin LM-12	
	Olfactory lobes	Plasma	Olfactory lobes	Plasma
t_{max} (min)	5	10	10	10
C_{max} (μg/g)	0.290 (0.064)	0.075 (0.005)	0.300 (0.120)	0.102 (0.013)
AUC (min μg/g)	2.45 (0.42)	0.44 (0.05)	2.10 (0.34)	0.47 (0.07)
Availability (μg min)	0.20 (0.03)	7.08 (0.78)	0.17 (0.03)	7.61 (1.10)
Absorption (%)	5.9		6.3	

The S.E.M. is given in brackets, $n = 6$.

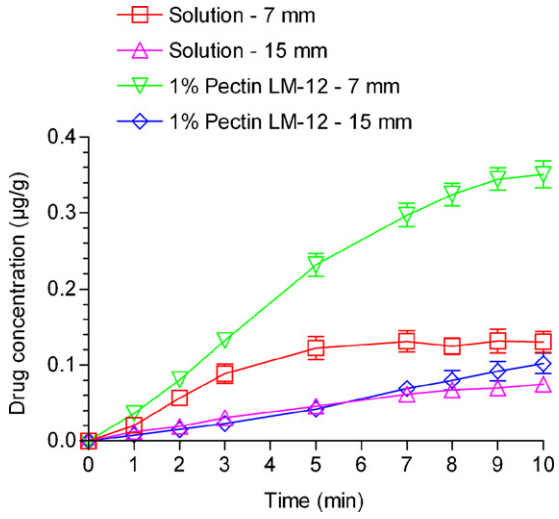

Fig. 9. Concentration profiles of GR138950 in plasma over 10 min following intranasal dosing of the drug in solution and with 1% pectin LM-12 using either a 7 mm or 15 mm dosing cannula. Error bars represent the S.E.M., $n = 3$ (7 mm cannula) or 6 (15 mm cannula).

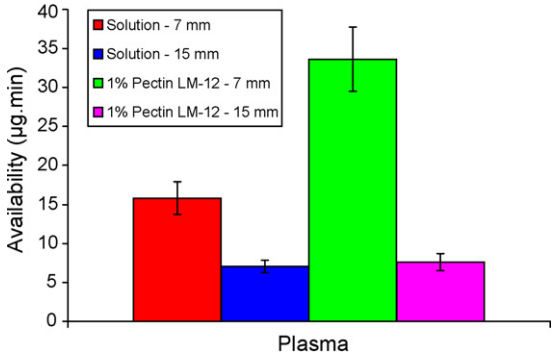

Fig. 10. Availability of GR138950 in plasma over 10 min following intranasal dosing of the drug in solution and with 1% pectin LM-12 using either a 7 mm or 15 mm dosing cannula. Error bars represent the S.E.M., $n = 3$ (7 mm cannula) or 6 (15 mm cannula).

analysis was inconclusive due to the low number of time points. However, the differences in C_{max}, t_{max} and availability are large, and are regarded as being noteworthy. Cannula length did not affect the t_{max} values. Changing to the 15 mm cannula produced a 92% and 237% increase in C_{max} for the simple drug solution and the pectin LM-12 formulation, respectively. Contrary to the trend observed in the olfactory lobes, the use of the 15 mm dosing cannula had little effect on drug levels recorded in the cerebral hemispheres and cerebellum.

Drug availability data confirmed that the use of the 15 mm dosing cannula resulted in a significant larger amount of drug being delivered to the olfactory lobes (Fig. 11). The increase in drug availability was 117% and 246% for the simple drug solution and pectin LM-12 formulation, respectively. Drug availability was lower in the cerebral hemispheres and cerebellum after administering either formulation with the longer cannula.

4. Discussion

It has been suggested that a promising approach to obtaining a higher CNS concentration of drug after nasal application using the direct nose-to-brain pathway, would be the use of bioadhesive formulations that after application to the olfactory region would provide increased residence time and thus potentially an increased transport of drugs across the membrane. The olfactory neuron cells end in a knob like protrusion at the surface of the epithelium which is exposed to the nasal cavity and which carry a number of long cilia. However, these cilia are non-mobile and do not have any mucociliary clearance function. It is however

Table 5
Average t_{max} and C_{max} values for GR138950 in the olfactory lobes and plasma following intranasal dosing of the drug in solution and with 1% pectin LM-12 using either a 7 mm or 15 mm dosing cannula

Formulation–cannula length	Olfactory lobes		Plasma	
	t_{max} (min)	C_{max} (μg/g)	t_{max} (min)	C_{max} (μg/g)
Solution – 7 mm	5	0.151 (0.022)	9	0.132 (0.016)
Solution – 15 mm	5	0.290 (0.064)	10	0.075 (0.005)
1% Pectin LM-12 – 7 mm	10	0.089 (0.036)	10	0.350 (0.018)
1% Pectin LM-12 – 15 mm	10	0.300 (0.120)	10	0.102 (0.013)

The S.E.M. is given in brackets, $n = 3$ (7 mm cannula) or 6 (15 mm cannula).

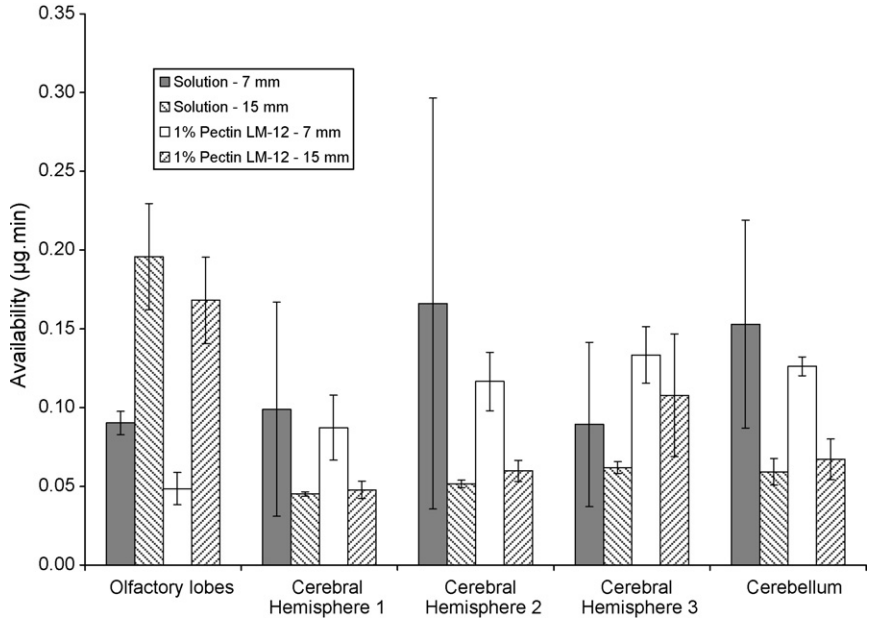

Fig. 11. Availability of GR138950 in brain regions over 10 min following intranasal dosing of the drug in solution and with 1% pectin LM-12 using either a 7 mm or 15 mm dosing cannula. Error bars represent the S.E.M., $n = 3$ (7 mm cannula) or 6 (15 mm cannula).

envisaged that, under normal conditions, mucus is cleared rapidly from the olfactory region in man by gravity. Hence, if formulations reach the olfactory region, they will normally be cleared rapidly from the region. However, pectin and chitosan formulations, administered as drops, have displayed significantly increased residence times at the apex of the olfactory cleft compared to a control solution (Charlton et al., 2007b).

In the present study, the amount of drug absorbed into the blood was considerably higher when the drug GR138950 was administered in a pectin or chitosan solution formulation as compared to a simple drug solution when using a 7 mm dosing cannula. The plasma C_{max} and AUC were significantly larger for all the polymer formulations than for the simple drug solution, with the C_{max} and AUC for the two pectin formulations significantly larger than for the chitosan formulation. t_{max} values for all formulations were similar.

The drug levels in the olfactory lobes were lower after dosing the polymer formulations compared to the simple solution, and the $AUC_{0–30\,min.}$ values were approximately half that of the simple drug solution. Also, the C_{max} were all lower for the polymer formulations, whereas, the t_{max} values were all larger than the corresponding t_{max} value for the simple drug solution, indicating that the polymers were acting in a controlled release fashion or that the formulations reached the olfactory epithelium at a later time.

The higher drug levels (expressed as C_{max} and AUC) seen in the blood when using the polymer formulations can be explained as follows. Gelation or adhesion forces associated with the polymers resulted in the dose being held at the point of delivery; thus resulting in increased residence time on the respiratory epithelium. The respiratory mucosa has a rich vasculature, therefore drug absorption into the blood is rapid from this part of the nasal cavity. This effect was prominent when the formulations were dosed with the 7 mm cannula to the respiratory region (anterior end of the nasoturbinates and maxilloturbinates) rather than to the posterior of the maxilloturbinates and the end of the end of the ethnoid turbinates where the olfactory epithelium is situated. It has previously been proposed that GR138950 is absorbed via the paracellular route due to its hydrophilic nature (Charlton et al., 2006). Drug transport studies using MDCK cells have shown that chitosan was able to enhance the uptake of GR138950 by opening cell tight junctions whereas this was not seen for the pectin formulations (Charlton et al., 2007a).

An increased residence time of the polymer formulations on the respiratory mucosa would account for increased drug absorption into the blood, with a smaller proportion of the dose reaching the olfactory epithelium. This would result in lower drug levels in the olfactory lobes and a later t_{max}, which is supported by the data. Due to the fact that the rat was on its back throughout the study delivery of the simple solution to the respiratory region would result in the majority of the solution quickly draining to the roof and the back of the nasal cavity where the olfactory epithelium is situated. Hence, the absorption into the blood for the simple drug solution would be lower and absorption into the olfactory lobes would be higher than for the bioadhesive polymer formulations, which is supported by the data.

It was shown previously that both the pectin and chitosan formulations were able to significantly change the rate of transport of drug through a model membrane in a Franz Diffusion Cell (FDC) study (Charlton et al., 2007a). Absorption of drug into the blood from the polymer formulations mirrored the results of the FDC studies, which demonstrated that the rate of GR138950 release (drug transport across the membrane) was fastest for the 1% pectin LM-12 formulation, slower for the 3% pectin LM-5, and slowest for the 1% chitosan G210 formulation. Furthermore, the FDC studies showed that the difference in rate of drug release between 1% LM-12 and 3% LM-5 was the same as between 3% LM-5 and 1% G210, which is similar to the variation in

drug concentration profiles, indicating that the superior ability of chitosan to enhance paracellular drug movement did not significantly affect GR138950 absorption in the current study. It is proposed that drug release, and not absorption, was the rate limiting factor.

Jansson et al. (2005) found that a gellan gum formulation (containing fluorescein dextran 3000) increased the residence time of the drug in the nasal cavity of rats and increased its transfer across the nasal epithelium. This was verified by fluorescent microscopy. The formulation was administered by a tube inserted to a depth of 5 mm in the nasal cavity. No blood samples were collected and hence it is not possible to evaluate whether the plasma concentration was increased as seen in the present studies with the similarly formulations of pectin and with chitosan. Vyas et al. (2006) evaluated the effect of mucoadhesive microemulsions containing clonazepam on the transport of the drug from nose to brain in rats. They found that the brain uptake was significantly increased (in terms of C_{max} and AUC) for the mucoadhesive microemulsion formulation as compared to a simple microemulsion with the calculated "direct nose to brain transport" value increasing from 0.23 to 0.44. There was no information provided concerning the depth of insertion of the administration tube. Hence, these results are difficult to explain in relation to those in the present study.

In the present work, when the cannulae used for nasal administration were changed from 7 to 15 mm length, a significant decrease in drug delivery to the blood was found as expected for both the simple drug solution and the polymer formulations. Simultaneously, a significant increase in drug delivery to the olfactory lobes was seen for both formulations. The difference in drug distribution between the two cannulae lengths was significantly greater for the selected pectin formulation than for the simple drug solution. The deeper dosing position in the nasal cavity reduced the contact between the formulation and the respiratory epithelium covering the nasoturbinates and maxilloturbinates, therefore, drug absorption into the blood stream was reduced. In addition, less of the dose was potentially trapped in the anterior turbinates, which is particularly significant for bioadhesive polymer formulations. The increased drug levels in the olfactory lobes indicate that a higher amount of the dose reached the olfactory region when the longer cannula was used. The drug concentration profiles for the olfactory lobes showed that GR138950 concentration was higher at 10 min compared to at 5 min post-dose with the polymer formulations, but the opposite was true for the simple solution, irrespective of dosing cannula length. This indicates that distribution of the polymer formulation to the olfactory epithelium was slower than the simple solution. This is particularly demonstrated by greater drug delivery to the blood and reduced delivery to the olfactory lobes by the polymer formulation compared to the simple solution when dosed using the shorter cannula.

These results raise the question of the appropriateness of an anaesthetized rat model for nose-to-brain delivery, especially when testing bioadhesive formulations, where the application of the formulation is performed by use of a short cannula or where the formulation is delivered by drops into the nares. Another notable problem with this model is the orientation of the animal on its back for the duration of the study. This does not mimic normal use of nasal products by humans. While it is feasible for a patient to administer nasal drops in a "nostril-up" position that facilitates pooling of the dose in the olfactory region; the patient would then return to being upright, and gravity would cause the dose to drain away from the olfactory region. This is particularly significant for a simple drug solution, which will drain away faster than mucoadhesive polymer formulations. A further problem is the decreased mucociliary clearance in an anaesthetized rat model resulting in a less pronounced (or missing) beneficial effect of bioadhesive formulations (Major and Illum, 1994). Investigation of drug absorption and distribution in a restrained, conscious animal model in a "normal position" would be of value for future studies.

5. Conclusion

In previous studies, it was found that materials, such as low methylated pectins, that gel in the nasal cavity after application, and chitosan, have characteristics that potentially can improve transport of drugs from the nasal cavity to the brain tissue. Both polymers are bioadhesive and furthermore, chitosan is an efficient absorption enhancer. In other previous studies, it was shown that in man such bioadhesive formulations were able to increase the residence time on the olfactory region as compared a non-bioadhesive formulation. The present studies showed that when such a bioadhesive formulation containing a model angiotensin antagonist drug was applied nasally in a conventional rat model the bioadhesive formulations (as compared to a control) improved the transmucosal delivery of the drug into the blood stream rather than improving the transport into the CNS. However, when the application cannula was inserted deeper into the nasal cavity and the formulation thereby placed near the olfactory region in the rat the absorption into the CNS was significantly improved for both pectin and a control formulation. However, there was no significant difference between the bioadhesive formulations and the control formulation. It can further be concluded that due to the decreased mucociliary clearance in an anaesthetized rat model which might result in a less pronounced (or missing) beneficial effect of bioadhesive formulations, and pooling in the olfactory region of the simple solution formulation due to the supine position of the rat, the anaesthetised rat model is not suitable for the evaluation of such formulations for improvement of nose-to-brain drug delivery.

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References

Charlton, S.T., Davis, S.S., Illum, L., 2007a. Evaluation of bioadhesive polymers as delivery systems for nose to brain delivery: in vitro characterization studies. J. Control Rel. 118, 225–234.

Charlton, S.T., Jones, N.S., Davis, S.S., Illum, L., 2007b. Distribution and clearance of bioadhesive formulations from the olfactory region in man: effect of polymer type and nasal delivery device. Eur. J. Pharm. Sci. 30, 295–302.

Charlton, S.T., Whetstone, J., Fayinka, S.T., Read, K.D., Davis, S.S., Illum, L., 2006. Evaluation of the mechanism of direct transport of an angiotensin antagonist from the nasal cavity to the central nervous system. Pharm. Res. (submitted for publication).

Djupesland, P.G., Skretting, A., Winderen, M., Holland, T., 2006. Breath actuated device improves delivery to target sites beyond the nasal valve. Laryngoscope 116, 466–472.

Fisher, A.N., Brown, K., Davis, S.S., Parr, G.D., Smith, D.A., 1985. The nasal absorption of sodium cromoglycate in the albino-rat. J. Pharm. Pharmacol. 37, 38–41.

Hinchcliffe, M., Illum, L., 1999. Intranasal insulin delivery and therapy. Adv. Drug Deliv. Rev. 35, 199–234.

Hirai, S., Yashiki, T., Matsusawa, T., Mima, H., 1981. Absorption of drugs from the nasal mucosa of rat. Int. J. Pharm. 7, 317–325.

Illum, L., 2000. Transport of drugs from the nasal cavity to the central nervous system. Eur. J. Pharm. Sci. 11, 1–18.

Illum, L., 2003. Nasal drug delivery—possibilities, problems and solutions. J. Control. Rel. 87, 187–198.

Illum, L., 2004. Is nose-to-brain transport of drugs in man a reality? J. Pharm. Pharmacol. 56, 3–17.

Illum, L., Watts, P., Fisher, A.N., Jabbal–Gill, I., Davis, S.S., 2000. Novel chitosan based delivery systems for nasal administration of a LHRH-analogue. STP Pharm. 10, 89–94.

Jansson, B., Hagerström, H., Fransen, N., Edsman, K., Björk, E., 2005. The influence of gellan gum on the transfer of fluorescein dextran across rat nasal epithelium in vivo. Eur. J. Pharm. Biopharm. 59, 557–564.

Major, S.H., Illum, L., 1994. Investigation of the effect of anaesthesia on nasal absorption of insulin in rats. Int. J. Pharm. 149, 123–129.

Reger, M., Baker, L.D., Watson, G.S., Fishel, M., Cholerton, B., Green, P., Breitner, J.C.S., DeGroodt, W., Frey, W.H., July 2006. Therapeutic effects of daily intranasal insulin administration in early Alzheimer's disease. In: Abstract from 10th International Conference on Alzheimer's Disease and Related Disorders, Madrid, Spain.

Rowett, H.G.O., 1979. Dissection guides-III. In: The Rat. John Murray Ltd., London.

Smith, J.M., Dornish, M., Wood, E.J., 2004. Involvement of protein kinase C in chitosan glutamate-mediated tight junction disruption. Biomaterials 26, 3269–3276.

Soane, R.J., Frier, M., Perkins, A.C., Jones, N.S., Davis, S.S., Illum, L., 1999. Evaluation of clearance characteristics of bioadhesive systems in humans. Int. J. Pharm. 78, 55–65.

Vyas, T.K., Babbar, A.K., Sharma, R.K., Singh, S., Misra, A., 2006. Intranasal mucoadhesive microemulsions of clonazepam: preliminary studies on brain targeting. J. Pharm. Sci. 95, 570–580.