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

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ARTICLE INFO

Article history:
Received 7 December 2006
Received in revised form
26 January 2007
Accepted 5 February 2007
Published on line 11 February 2007

Keywords:
Nasal residence
Gamma scintigraphy
Bioadhesion
Nasal administration
Lyophilisation
HPMC

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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doi:10.1016/j.ejps.2007.02.002

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.

2.2.6. *Study day procedure*

Subjects fasted for 10 h prior to administration of the test formulations. On each study day, external markers were attached to the forehead, on the same side as the nostril to which the formulation was administered. Immediately following dosing, subjects were seated in a lateral position in front of a Siemens E-Cam gamma camera, fitted with a low energy, high resolution collimator, with the nostril that received the dose closest to the camera. For the nasal spray, a dynamic series of 5 s images were acquired up to 3 min, followed by 15 s images up to 10 min, and static 60 s images at appropriate intervals thereafter. For the nasal insert formulations, static 60 s images were acquired at 10 min intervals until the activity had cleared from the nasal cavity. Images were stored electronically for subsequent analysis. Blood samples for glucose testing (1 ml) were taken at −10 and −5 min prior to dosing to establish a baseline value for each subject, following which samples were obtained at regular intervals post-administration. Whole blood samples were analysed immediately for glucose content using a Yellow Springs automated glucose sampler. At the completion of each study day, volunteers were fed a carbohydrate rich meal.

2.2.7. *Scintigraphic data analysis*

Scintigraphic images were analysed using WebLink® image analysis software. Using the images acquired, regions of interest were defined for the initial deposition site and posterior nasal cavity, and the counts in each area were determined. The posterior nasal cavity region of interest encompassed the entire posterior nasal area, including site of deposition where the formulation was deposited in this area. All data were corrected for background counts and radioactive decay. Parameters assessed for the nasal formulations were the time to 50% ($T_{50\%}$) and the time to 80% ($T_{80\%}$) clearance of counts from each region of interest. In instances where the counts in the region of interest had not fallen below 80% by the end of the imaging period, the value for $T_{80\%}$ was taken as the time of the last recorded image.

3. Results and discussion

3.1. *In vitro release*

Release profiles from the insulin spray and insulin loaded lyophilised inserts are shown in Fig. 1. The rate of insulin release from the lyophilised preparations decreased in the order 1% (w/w) HPMC > 2% (w/w) HPMC > 3% (w/w) HPMC, and it was observed from the profiles that for all of the lyophilised formulations, little or no insulin release occurred until 30 min. The maximum insulin release measured for the simple insulin liquid was approximately 80%, and this effect was considered to be due to a proportion of insulin becoming 'trapped' in the filter paper membrane. This was confirmed by soaking the filter paper membrane in a large volume of fresh distilled water with gentle agitation for a few hours and measuring the insulin content of the distilled water (data not shown). Despite this, it could clearly be seen that increasing the HPMC content of the nasal insert resulted in a decrease in in vitro release rate and overall maximum release, with the 3% (w/w) HPMC insert releasing less than 15% insulin in a 5 h period.

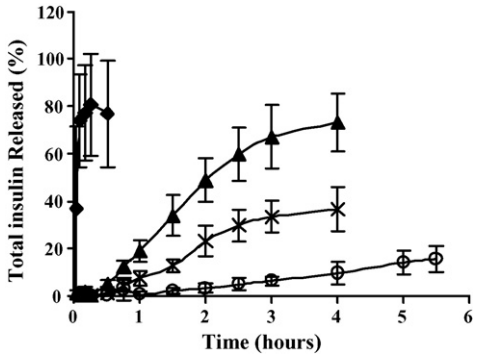

Fig. 1 – In vitro insulin release from nasal formulations in PBS pH 7.4: (♦) insulin solution, (▲) 1% (w/w) HPMC lyophilised insert, (×) 2% (w/w) HPMC lyophilised insert and (○) 3% (w/w) HPMC lyophilised insert.

It was noted during the in vitro release study that the 3% (w/w) HPMC formulation remained only partially hydrated for an extended period of time, while the 1% (w/w) HPMC formulation was reasonably rapidly hydrated on the membrane surface, which may also partially explain the corresponding decrease in total insulin release with increasing polymer concentration. Other researchers have made similar observations, concluding that polymer hydration was an important factor in the mobility of large molecular weight proteins (Simon et al., 1999). It has also been reported that for HPMC systems there are a combination of factors involved in drug release, including hydration, swelling, water solubility of drug and chain length and degree of substitution of the HPMC polymer used (Siepmann and Peppas, 2001), and with any matrix system, the possibility of some drug remaining entrapped in the matrix must be considered.

The profiles obtained may be evidence of the large molecular weight of the insulin molecule hindering its diffusion through the hydrated HPMC matrix, resulting in a release profile where this is the rate limiting step, as previous studies of the release of NHT from lyophilised HPMC nasal inserts demonstrated a more rapid release profile, with 50% NHT release at 52.5 min, and close to 100% release at 3 h for a 2% (w/w) HPMC insert (Thapa, 2000).

A drawback of the current in vitro release method is the use of filter paper as a membrane, which will not be entirely representative of the behaviour of tissue, and does not produce a more relevant mucosal surface pH while maintaining physiological pH in the reservoir. However, the use of excised tissue introduces variability as no two mucosal surfaces will be identical, there is potential for damaged tissue increasing apparent release rate, and the process can be time consuming and inconvenient. Therefore, the method used in the current study was considered to offer a more practical and rapid means of comparing different nasal formulations, while bearing in mind the limitations.

3.2. *Clinical study*

Three subjects completed all four study arms and three subjects completed three study arms only, due to unforeseen

Table 1 – Clearance behaviour of nasal insert formulations determined using gamma scintigraphy

Formulation	Initial deposition site		Posterior nasal cavity	
	$T_{50\%} \pm$ S.D. (h)	$T_{80\%} \pm$ S.D. (h)	$T_{50\%} \pm$ S.D. (h)	$T_{80\%} \pm$ S.D. (h)
1% (w/w) HPMC ($n=5$)	2.19 ± 1.43	2.77 ± 1.49	2.72 ± 1.43	2.99 ± 1.39
2% (w/w) HPMC ($n=4$)	1.19 ± 0.803	4.12 ± 0.16	3.13 ± 1.08	4.80 ± 0.43
3% (w/w) HPMC ($n=6$)	0.35 ± 0.45	0.80 ± 0.70	1.12 ± 1.11	1.28 ± 1.17

circumstances unrelated to the study. Data from all subjects were included in the analysis, and mean times to 50% and 80% clearance of activity ($T_{50\%}$ and $T_{80\%}$) from the initial deposition site and posterior nasal cavity are shown in Table 1.

It was not possible to calculate the mean $T_{50\%}$ or $T_{80\%}$ for the nasal spray, as the spray was deposited in the posterior nasal cavity in only one subject. In this instance, clearance from the posterior nasal cavity was rapid, with $T_{80\%}$ reached at 9.2 min. In all other subjects, the spray was deposited at the top of the anterior nasal cavity, due to poor administration technique. Consequently, the spray was retained within the anterior cavity, eventually draining forward via the nares. No drainage to the posterior cavity was noted in these subjects. This observation is similar to previous findings by Harris et al. (1988), where administration of a nasal pump spray resulted in deposition in the anterior region of the nasal cavity, and Soane et al. (1999) who reported that nasal formulations were deposited in either the anterior or turbinate region, depending on the administration technique of the volunteer. This effect of individual administration technique raises a separate question on the usefulness of nasal spray doses, aside from the formulation and residence time aspects discussed here.

Scintigraphic images revealed that the 1% (w/w) HPMC nasal insert formulation formed a gel which showed initial spreading between 10–20 min, and then gradually spread over the posterior nasal cavity. The gel was subsequently cleared to the nasopharynx area at the back of the nose. Nasal residence time was variable, demonstrated by the relatively large standard deviation of the $T_{50\%}$ and $T_{80\%}$ values for the posterior nasal cavity in Table 1.

The 2% (w/w) HPMC formulation demonstrated the longest residence time in the posterior nasal cavity, taking longer overall to move from initial deposition site ($T_{80\%}$), despite an initial clearance from this area ($T_{50\%}$) which was faster than for the 1% (w/w) HPMC formulation. Evidence of the gel formed from the insert was present for 4–5 h in all cases ($n=4$). Scintigraphic images also showed the spreading of the HPMC gel in the posterior nasal cavity, although it was observed that the gel formed was not as disperse as for the 1% (w/w) HPMC formulation. This was followed by the slow clearance of the gel by drainage from the nasopharynx. The standard deviations in Table 1 demonstrate that this formulation showed slightly less variability in its behaviour than the 1 or 3% (w/w) HPMC inserts.

The 3% (w/w) HPMC formulation displayed a shorter residence time than the other nasal inserts in the posterior nasal cavity ($n=6$), which was observed to be a result of a lack of spreading of the formulation in the nasal cavity and rapid movement from the site of deposition. In most instances, the dose was observed remaining as a more or less single point of activity, rather than the spreading observed for the other formulations, being expelled intact from the nose in all but one case. The dose either passed through the nasal cavity and out of the nasopharynx intact, or was expelled accidentally by the subjects, as the continued presence of an undissolved foreign object in the nasal cavity elicited subconscious sniffing, despite instructions to avoid doing so. In all but one case, the dose was expelled from the nasal cavity as a result of sniffing or rapid transit in 1.5 h or less. In one subject, however, the nasal insert remained in the nasal cavity for over 3.5 h. The performance of this formulation was considered to be unreliable and variable, and unlikely to be suitable for patient use due to the involuntary response it induced.

The observations regarding movement from initial deposition site and subsequent clearance reflect findings presented by Tafaghodi et al. (2004), who noted that the clearance from the deposition area was the main rate limiting step for overall nasal clearance of bioadhesive microspheres, and that following displacement from the initial deposition area, transit in the remainder of the nasal cavity was relatively rapid.

In vivo performance of the individual nasal insert formulations is shown in Fig. 2, and the profiles clearly show that the 2% (w/w) HPMC formulation was cleared from the posterior nasal cavity in a relatively controlled manner, with less inter subject variability than the other formulations. The 1% (w/w) HPMC inserts initially showed good nasal residence, reflected by the spreading observed in the images, however it was noted that when clearance from the posterior nasal cavity began it was reasonably rapid, and the overall variability in the behaviour of this formulation is obvious from the profiles. The rapid clearance of the generally intact 3% (w/w) HPMC formulation is evident in Fig. 2, with the profiles falling to 0% in a short space of time. For the individuals who accidentally expelled this formulation by sniffing, the percentage of initial counts remaining in the area did not immediately fall to zero, as a certain amount of hydration of the formulation would have occurred by this point, resulting in a residual amount of activity being detected in the nasal cavity. There were no volunteer specific effects observed.

Representative scintigraphic images are shown in Fig. 3, and demonstrate the rapid movement and spreading of the 1% (w/w) HPMC formulation, the relatively stationary and less disperse 2% (w/w) HPMC formulation, and the 3% (w/w) HPMC formulation which was almost cleared from the nasal cavity by 90 min. One-way ANOVA revealed that the $T_{80\%}$ values of the 2 and 3% (w/w) HPMC formulations at initial deposition site were significantly different ($p<0.001$), which reflect both the

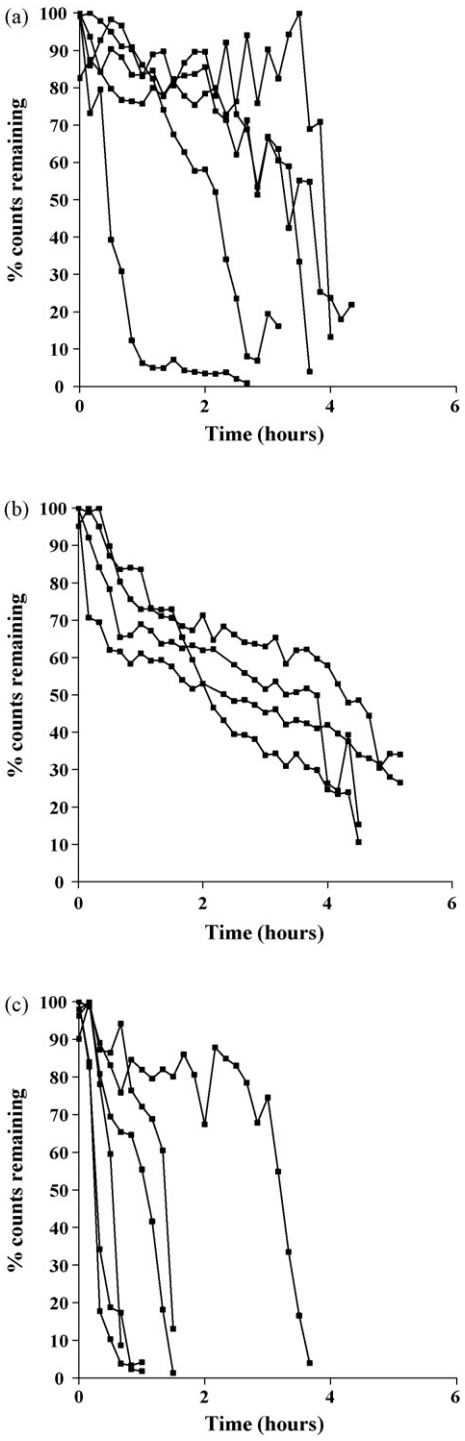

Fig. 2 – Individual clearance profiles from the posterior nasal cavity for (a) 1% (w/w) HPMC, (b) 2% (w/w) HPMC and (c) 3% (w/w) HPMC nasal inserts.

rapid clearance of the 3% (w/w) HPMC formulation along with the significantly reduced clearance from the initial deposition site of the 2% (w/w) HPMC insert. Due to the higher variability of the clearance behaviour of the 1% (w/w) HPMC formulation, clearance values here were not significantly different from the other two formulations. The $T_{80\%}$ clearance values of the 2 and 3% (w/w) HPMC formulations in the posterior nasal cavity were significantly different ($p < 0.001$), further emphasising the difference in behaviour between the two formulations. Variability in the data again meant that there was no significant difference detected for the 1% (w/w) HPMC formulation in comparison to the 2 and 3% (w/w) HPMC formulations.

Overall, the scintigraphic images obtained demonstrated that the 2% (w/w) HPMC nasal insert displayed the longest residence time on the nasal mucosa. As a previous study of the 2% (w/w) HPMC insert in sheep had demonstrated prolonged nasal absorption of NHT, it was expected that extended nasal residence may be observed, and the polymer concentration of this formulation would be likely to increase viscosity and polymer-mucous entanglement over that of the 1% (w/w) HPMC formulation. It has also been suggested that such viscous formulations may allow increased resistance to ciliary movements (Soane et al., 1999). The rapid spreading of the 1% (w/w) HPMC insert is considered to be a result of a more rapid hydration of this formulation, as observed during in vitro dissolution tests.

Blood glucose levels in the volunteers were not significantly altered by the administration of insulin in any study arm (data not shown), suggesting that while the current nasal insert formulation previously significantly enhanced nasal absorption of NHT in sheep (McInnes et al., 2005), it does not appear to be suitable for the enhancement of nasal insulin absorption in man. It is possible that no effect on blood glucose levels was observed as a result of the healthy physiological response to insulin compensating to ensure constant blood glucose levels, however previous studies have shown a reduction in blood glucose in healthy volunteers following nasal insulin administration (Newman et al., 1994).

The 3% (w/w) HPMC formulation did not disperse well in the nasal cavity, but was observed to move rapidly as a single unit, and this unexpectedly rapid clearance was considered to be a result of the higher concentration of polymer slowing down hydration of the dose, in this case to such an extent that the mucociliary clearance overcame any adhesion produced by the small amount of hydrated polymer. When the in vivo nasal clearance data are compared with in vitro release profiles it is interesting to note that at the time the 2% (w/w) HPMC insert clearance has reached $T_{80\%}$ (3.94 h), in vitro release had reached only 35% of the total insulin content. At this point, however, the release of insulin seems to have reached a plateau, and so it may be necessary to adjust the dose loaded into the formulation in accordance with the release profile of the compound determined by in vitro dissolution. The 1% (w/w) HPMC insert had reached $T_{80\%}$ clearance in 2.99 h, and at this time the in vitro dissolution had reached 65% drug release. These observations demonstrate the requirement for compromise in such polymer formulations, and the importance of considering which behavioural parameter is of most importance. Drug release rates must be balanced against the requirement for extended nasal residence, and likewise, the requirement for prolonged blood levels must be balanced against the target of increased overall bioavailability. Ultimately, it must also be remembered that there is a finite period of time in which hydration of the formulation can take place, to avoid producing a dose which passes through the nasal cavity with little effect.

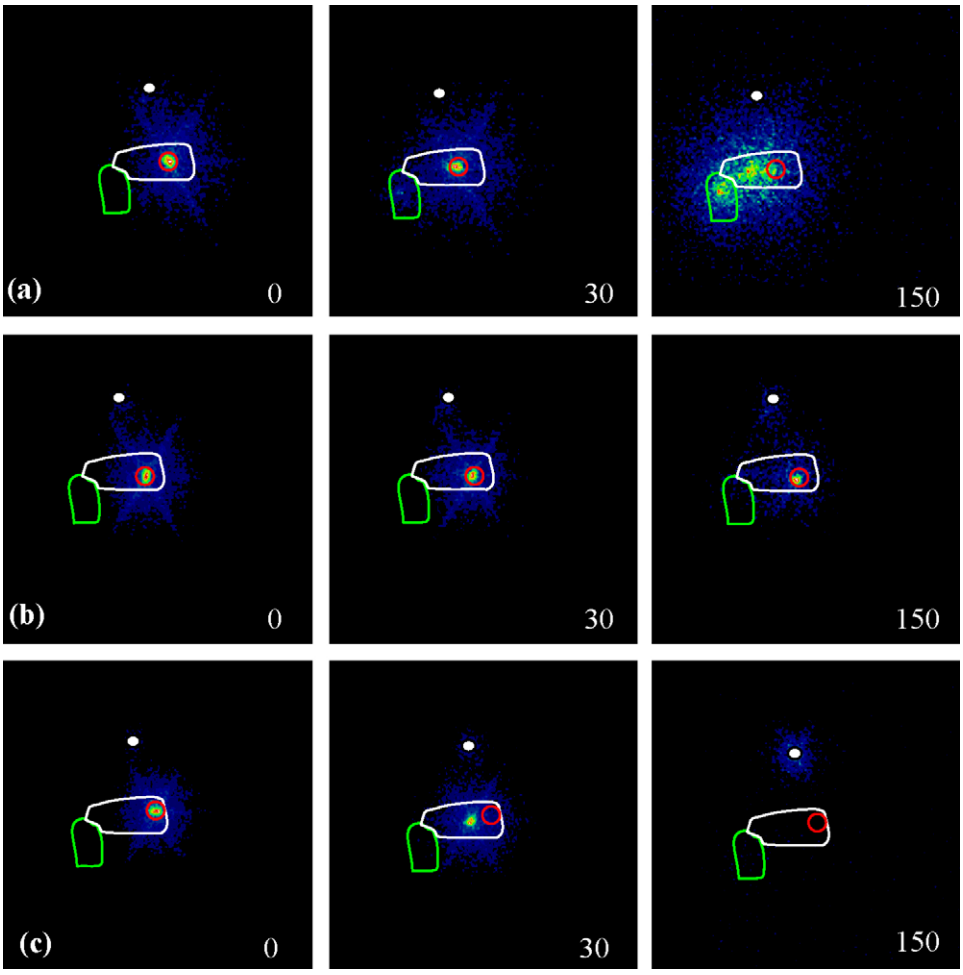

Fig. 3 – Representative scintigraphic images, showing (a) 1% (w/w) HPMC (subject 3), (b) 2% (w/w) HPMC (subject 4), and (c) 3% (w/w) HPMC (subject 4) nasal inserts in the nasal cavity at 0, 30 and 150 min. Initial deposition site is outlined in red, posterior nasal cavity in white, and the nasopharyngeal area in green (white dot shows location of a positional marker).

4. Conclusions

The in vivo spreading behaviour of the inserts was observed using gamma scintigraphy, and an extended nasal residence time of 4–5 h was demonstrated for the 2% (w/w) HPMC formulation. It was also noted for the conventional spray formulation that individual administration technique resulted in the majority of doses being deposited in the anterior rather than the posterior nasal cavity, with the dose then being cleared via the nares rather than the nasopharynx. The lyophilised nasal insert formulations did not however enhance nasal insulin absorption in healthy subjects. Increasing HPMC concentration did not necessarily lead to increased nasal residence time, which was hypothesised to be a result of inadequate hydration of the formulation leading to little or no spreading of gel. The optimal formulation will depend on the particular intended use of the system, and the properties of the compound being delivered, however caution should be exercised to avoid a situation where nasal residence time is reduced as a consequence of insufficient hydration of the formulation.

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