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Nasal delivery of octreotide: Absorption enhancement by particulate carrier systems

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Abstract

The potential of various powder formulations to enhance the nasal absorption of the somatostatin analogue peptide octreotide (Sandostatin®) was studied by a combination of in vitro and in vivo experiments. The particulate carriers under investigation were microcrystalline cellulose (Avicel PH101), semicrystalline cellulose (Elcema P050), hydroxyethyl starch, cross-linked dextran (Sephadex G25), microcrystalline chitosan, pectin and alginic acid. Determination of the Ca^{2+}-binding capacity of these carriers demonstrated large differences for excipients of the same chemical composition, depending on the physical appearance. Whereas Avicel PH101 bound 0.22 μg Ca^{2+}/mg carrier, no Ca^{2+} binding could be detected for Elcema P050. The following rank order was obtained: swollen Sephadex G25 > alginic acid > microcrystalline cellulose = hydroxyethyl starch ≫ chitosan = pectin = semicrystalline cellulose = 0. For Sephadex G25 a pre-swelling time of at least 30 min was necessary to observe calcium binding (0.55 μg /mg). Determination of water uptake by the different excipients showed a very rapid water uptake of more than 200% (wt/wt) by microcrystalline chitosan and Avicel PH101. The rate and extent of water uptake can be ranked for the nasal particulate carriers as follows: chitosan > microcrystalline cellulose > semicrystalline cellulose ≫ pectin = hydroxyethyl starch = alginic acid = Sephadex G25. When the absorption of octreotide was determined in vivo in rats after nasal administration together with the respective carrier, the highest bioavailability was seen after coadministration of alginic acid and Sephadex G25 (4,1% and 5.56%). T_{max} of plasma concentration was between 0.08 and 0.34 min. It was delayed after coadministration of Sephadex G25 and pectin (1 and 2 h), which might be explained by swelling time and gel formation of the excipients. The data suggest a correlation between calcium-binding properties of nasal carriers and their potential as nasal absorption enhancers for peptides under in vivo conditions.

Keywords: Nasal absorption; Particulate carrier; Peptide transport; Ca^{2+}-adsorption; Tight junctions

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1. Introduction

The mechanism by which particulate carriers enhance the nasal bioavailability of drugs are incompletely understood. Different hypotheses (Edman and Björk, 1992) have been put forward for specific nasal delivery systems, such as an increase in nasal contact time by bioadhesion of starch microspheres (Illum et al., 1987). Microcrystalline cellulose was suggested to provide prolonged residence time, due to its insolubility (Nagai et al., 1984; Nagai, 1986) combined with an increase in local drug concentration. Degradable starch microspheres were found to induce a transient opening of tight junctions by dehydration of mucosal cells (Edman et al., 1991; Edman et al., 1992). Also, uptake of particles via macrophages was suggested for nasal absorption of drugs (Almeida et al., 1993). All these hypotheses are related to very specific physicochemical properties of the respective carrier system. A more general mechanism explaining absorption-enhancing effects of all those different excipients has not yet been described. The mechanisms of action of surface active agents or complexing agents used as absorption enhancers are better understood, but a relationship to the absorption-enhancing mechanisms of particulate carriers has not been established. It is well known that sodium-EDTA and other Ca^{2+} chelators such as citric acid may act as absorption enhancers by complexing Ca^{2+} ions, which are important for the functional integrity of the intercellular spaces (Tomita et al., 1988; Artursson and Magnusson, 1989,). By that mechanism, Ca^{2+} chelators influence the paracellular transport of molecules, which are normally excluded from this pathway, e.g. peptides and proteins (Lee, 1986). However, the use of calcium chelators is limited due to their local intolerability, which might be related to their membrane damaging activity. Enhancers may also penetrate into the cells where they interact with intracellular Ca^{2+}, thus causing severe damage or even cell death. Water uptake or swelling of dry particles is also considered to be an important factor for the enhancement of nasal absorption (Björk et al., 1995; Pereswetoff-Morath and Edman, 1995). Nevertheless, only few quantitative data are available (Illum, 1987) addressing this issue. In addition, a correlation of water uptake of nasal powders and their corresponding penetration-enhancing effect has not yet been published.

In the present work, we investigated the Ca^{2+}-binding capacity of different nasal carriers. Considering the effects of starch microspheres on tight junctions (Edman and Björk, 1992) and the mode of action of Ca^{2+} chelators, we expected that also dry powders may exhibit a potential for Ca^{2+} binding resulting in an increased absorption of drugs. In addition, we quantified the water uptake of these excipients and compared it to the improvement of drug absorption.

2. Materials and methods

2.1. Chemicals

Microcrystalline cellulose Avicel PH101 (FMC, Hamburg, Germany), semicrystalline cellulose powder Elcema P050 (Degussa, Frankfurt, Germany), hydroxyethyl starch (HES) and pectin type FPA (Sigma, St. Louis, USA), cross-linked dextran Sephadex G25 (Pharmacia, Uppsala, Sweden) and alginic acid (Fluka, Buchs, Switzerland) were used as nasal particulate carriers. [^{14}C]-octreotide (Sandostatin®) with a specific activity of 40.4 Ci mg^{-1} and unlabelled peptide were kindly provided by Sandoz Pharma Ltd, Basel, Switzerland. All other chemicals were purchased in reagent grade quality. Microcrystalline chitosan was prepared as described previously (Struszczyk, 1987). Microcrystallinity of the obtained product was shown by X-ray diffractometry.

2.2. In vitro-Ca^{2+}-binding capacity

Ca^{2+} adsorption from a solution was determined for the dry excipients. An absorption time of 20 min was chosen, since in a normal healthy human nose the residence time of an inspired particle does not markedly exceed 20 min. Therefore, only effects occurring within this time interval may be of relevance for nasal transport. The Ca^{2+} concentration in the test solution was chosen lower than it occurs under physiological con-

ditions, where a concentration of about 4 mM Ca^{2+} has been found for human nasal mucus (Widdicombe and Wells, 1982). In order to further increase the discriminative power of this method, the tested amount of powder was 5–25-fold higher than the amount which may be applied in humans. Defined quantities (100 mg, 300 mg and 500 mg) of dry, particulate carrier were suspended in 5.0 ml of 1.25 μM aqueous $CaCl_2$ solution. After 20 min of stirring, the supernatant was filtered through a 0.45 μm membrane filter (Gelman, Switzerland) to remove the suspended particles. The Ca^{2+} content of the resulting clear solution was determined by atomic absorption spectroscopy (atom absorption spectrometer model 5000, Perkin Elmer, Beaconsfield, UK) versus a calibration standard (Baker, Phillipsburg, USA). The quantification limit of the method used was below 1 ppm. In order to take into account the individual Ca^{2+} content of the excipients, control samples of each carrier were suspended in demineralized water and the Ca^{2+} content of the supernatant was determined after 20 min of stirring. The Ca^{2+}-binding capacity of each carrier was calculated in μg Ca^{2+} per mg carrier from the decrease of Ca^{2+} concentration in solution.

2.3. Water uptake of nasal carriers

According to the theory of nasal permeation enhancement by local dehydration of cells, the rate and the extent of water uptake (wicking effect) of a particle is estimated to be more important than concomitant 'swelling' (i.e. increase in volume). Therefore, particle size determination by laser diffractometry before and after contact with water did not seem to be an adequate method. Thus, a new method was applied for the kinetic measurement of water uptake similar to that reported by Enslin (1933). Pasteur pipettes with similar tip dimensions were sealed at their tip ends with small pieces of dialysis membrane (Spectra pore, MWE 8.000, Spectrum, LA). The prepared pipettes (n = 6) were filled with 0.5 g of each vacuum dried excipient. Tapping and thereby changes in density of the powder bed was avoided. After determination of the starting weight, the pipettes were placed vertically in a 3-mm high water reservoir. The increase in weight was measured after 15, 30, 45 min, 1 h, 2 h, 4 h, 6 h, 8 h and 24 h. Water uptake was calculated from the weight increase in mg water per mg carrier.

2.4. Drug release studies

Twenty milligrams of powder, containing 500 μg octreotide, placed in a flow through glass perfusion chamber in closed loop configuration were perfused with 30 mM phosphate buffer, pH 6.5 at a rate of 2 mL/min. The concentration of octreotide in the circulating perfusate was determined continously by UV-detection.

2.5. Animal experiments

All animal studies were performed according to the Guidelines of the Committee of the cantonal authorities according to the Swiss Animal Welfare Act. Fasted male Wistar rats (BRL, Füllinsdorf, Switzerland) with a mean body weight of 300 g were anaesthetised by intraperitoneal injection of urethane (1 g kg^{-1}). Powder mixtures of different carriers with octreotide were prepared by dry blending and sieving through a 250-mesh sieve. For each animal, a nominal dose of 50 μg octreotide in 500 μg carrier (microcrystalline cellulose, powder cellulose or hydroxyethyl starch) was filled into an Eppendorff 100 μl-pipette tip. The powder was administered into the left nostril of each animal, by pushing a small rubber ball on the pipette tip. Control animals received an i.v. injection of 50 μg octreotide in 0.1 ml physiological saline. Another series of animals received 12 μg octreotide dissolved in 0.9% saline into the nostril (n = 6 animals for all formulations). Blood samples (100 μl) were taken before and after drug administration at 0 min, 10 min, 1 h, 2 h, 3 h and 5 h and immediately centrifuged at 10 000 g for 5 min at 4°C. The plasma was kept frozen until the concentration of octreotide was determined by a radioimmunoassay (Marbach et al., 1985). The rabbit antiserum was found to recognize only intact peptide with a very low cross-reactivity to peptide fragments, somato-

statin-14 or somatostatin-28. The area under the plasma-time curve (AUC) was estimated by the trapezoidal rule.

3. Results

3.1. Ca^{2+}-binding capacity

The Ca^{2+}-binding studies showed that particulate carriers, such as microcrystalline cellulose (Avicel PH101) or hydroxyethyl starch, are capable of removing significant amounts of Ca^{2+} ions from an aqueous vehicle (Fig. 1). The reproducibility of the test was good. Whereas Avicel PH101 did not release measurable amounts of Ca^{2+} into an aqueous medium, it was able to bind about 0.22 μg $\pm$0.03 μg Ca^{2+} per mg. Additionally, it could be shown that Avicel PH101, saturated with demineralized water, or even Avicel PH101 suspended in water still absorbed the same amount of Ca^{2+} from a Ca^{2+} containing solution. Unexpectedly, large differences were observed in Ca^{2+}-binding capacity of excipients of the same chemical composition, but with differences in their physical appearance. In contrast to the microcrystalline cellulose, the powder cellulose Elcema P050 did not absorb measurable amounts of Ca^{2+}. Microcrystalline chitosan and pectin also exhibited no Ca^{2+}-binding capacity. Some of the carriers, such as Sephadex G25, developed a Ca^{2+}-binding capability only after a pre-swelling time of at least 30 min. Consequently, the method of preconditioning of Sephadex G25-particles prior to nasal administration may be crucial for a potential absorption-enhancing effect of such particles. As anticipated, alginic acid showed high Ca^{2+} absorption due to formation of insoluble Ca^{2+}-salts. No differences in Ca^{2+} binding could be observed when increasing amounts of powder (100–300 mg) were used in the assay.

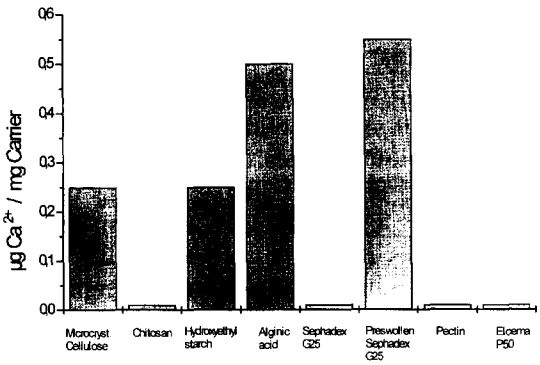

Fig. 1. Ca^{2+}-binding capacity of particulate carriers for nasal peptide absorption: 300 mg carrier were suspended in an aqueous solutiuon containing 1.25 μM Ca^{2+}. After 20 min, the remaining Ca^{2+}-concentration in the supernatant was determined by atomic absorption spectroscopy.

3.2. Water uptake of the carrier particles

Comparing the maximum water uptake and the rate of water uptake of nasal dry powders, major differences could be observed (Fig. 2). Microcrystalline cellulose (Avicel PH101) and microcrystalline chitosan showed a very rapid uptake of more than 210% and 290% [wt/wt] water, respectively. For both excipients, the equilibrium was reached after 2 h. In contrast, the rate and the extent of water absorption were lower for powder cellulose Elcema P050 (170%), and the pharmaceutically-used starches. Water uptake was hardly detectable by this method for hydroxyethyl starch, pectin, alginic acid and Sephadex G25. Gel formation of these excipients within the pipette tip may have delayed further diffusion of water into the powder bed.

3.3. Drug release studies

In order to assure that all powders have comparable release characteristics in the in vivo studies, we determined the time-dependent release of octreotide out of the powder mixtures into phosphate buffer in a perfusion chamber system. Complete release occurred within 10 min from all powder formulations. Avicel PH101, Chitosan and Sephadex G25 released the drug within 2 min; Elcema P050 and alginic acid released it within 4 min; hydroxyethyl starch released it within 6 min and pectin in 10 min.

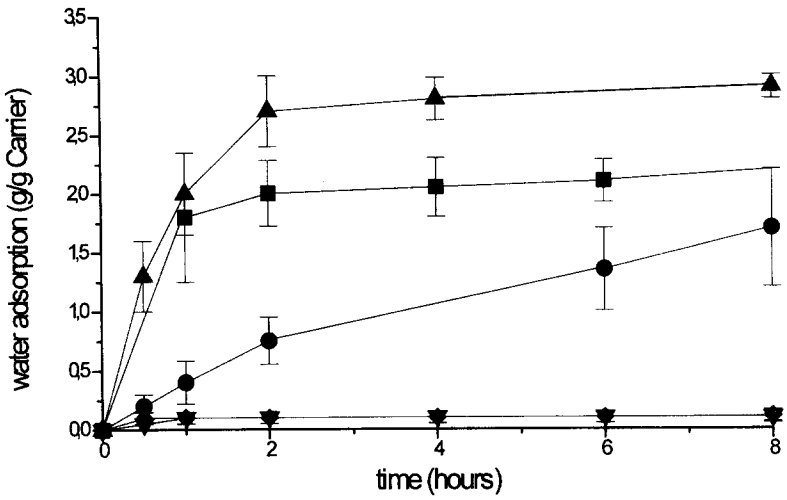

Fig. 2. Water uptake of particulate carriers: Time-dependent adsorption of water by the carriers: ▲ = Chitosan; ■ = Avicel PH101; ● = Elcema P050; ▼ = Sephadex™ G25; X = pectin; ◆ = Hydroxyethyl starch; □ = alginic acid. (Mean ± stand. dev.; n = 3).

3.4. Animal experiments

In contrast to a perfusion model described earlier (Hirai et al., 1981), our model is based on the nasal application of a dry powder and the determination of the resulting plasma-concentration-time profiles. The application of dry powder mixtures of octreotide as model drug and different nasal carriers into the nostrils of anaesthetised rats resulted in large differences in drug absorption, depending on the carrier system. The absolute bioavailability of octreotide when combined with Sephadex G25 or alginic acid was found to be rather high (4.10% and 5.56%). Considerably lower bioavailabilities were achieved when octreotide was mixed with pectin or microcrystalline cellulose (1.84% and 1.67%). Mixtures with microcrystalline chitosan or hydroxyethyl starch resulted only in a low availability of the peptide (0.35% and 0.67%, respectively). All powders yielded a T_{max} around or below 0.5 h, indicating a very rapid and transient absorption of the peptide except for Sephadex G25 and pectin (T_{max} of 1.0 and 2.0 h). When a series of control animals received 12 μg octreotide dissolved in 50 μl 0.9% saline, a bioavailability of 0.59% was obtained. All results of these rat studies are summarized in Table 1.

4. Discussion and conclusions

A variety of particulate materials, such as starch, dextran and microcrystalline cellulose, have been evaluated as drug carriers enhancing the nasal bioavailability of peptides. Several hypotheses have been put forward explaining the mode of action of these excipients, yet a generally-applicable mechanism is still lacking. In the present study, we investigated whether water uptake and the ability to bind Ca^{2+} play a major role for the extent of peptide absorption using the somatostatin analogue octreotide as model peptide.

Comparing the water absorption of the tested particulate carriers and their influence on nasal bioavailability of octreotide in rats, the experimental results do not suggest any correlation of these two parameters (Fig. 3). However, the capability of the particulate carriers to bind Ca^{2+} ions seems to be of major importance for their potential to enhance absorption of the coadministered peptide (Fig. 4). We, therefore, conclude that the absorption-enhancing effect of particulate carriers correlates directly with their Ca^{2+}-binding capacity. Changes in the function of tight junctions and permeability of Caco-2 monolayers after application of starch microspheres was recently at-

Table 1
Absolute bioavailability of octreotide in rats after nasal administration of various powder formulations

Excipient	Mean dose per animal in μg	Absolute bioavailability in %	C_{max} in ng l^{-1}	T_{max} in h
i.v. Solution	50,0	100	377.6 (63.9)	0.08 (0.01)
Avicel PH101	22.9 (4.6)	1.67 (0.6)	4.9 (2.6)	0.25 (0.3)
Chitosan	48.4 (4.5)	0.35 (0.2)	2.44 (1.9)	0.25 (0.1)
Hydroxy-ethyl starch	34.7 (8.4)	0.67 (0.4)	13.04 (12.4)	0.08 (0.8)
Alginic acid	47.3 (9.5)	4.10 (1.8)	39.6 (18.8)	0.25 (0.1)
Sephadex G25	51.3 (10.2)	5.56 (4.3)	18.5 (13.5)	1.00 (0.1)
Pectin	29.1 (4.7)	1.84 (1.3)	2.05 (1.3)	2.00 (0.8)
Elcema P050	7.8 (1.1)	1.27 (0.7)	1.03 (0.52)	0.34 (0.1)
Saline (control)	12 (0.0)	0.59 (0.1)	0.9 (0.3)	1.00 (0.1)

Numbers in parentheses: S.E.M.

tributed (Edman et al., 1992) to a 'shrinkage' of the cells by dehydration caused by water absorption of the powder applied. Nevertheless, the opening of the tight junction may also be explained by a local decrease in Ca^{2+} concentration. Our bioavailability data in rats show the clear advantage of Ca^{2+}-absorbing systems as compared to powders without an ability for Ca^{2+} binding. The Ca^{2+}-binding potential of any carrier may depend on special conformational conditions of the molecule regarding position of hydroxyl groups, thereby facilitating formation of Ca^{2+} complexes but not of drug complexes. This may explain the large differences in Ca^{2+}-binding properties of substances with the similar chemical composition, but differences in physicochemical properties, such as starches. It may also explain the low absorption promoting effect of materials like Sephadex and DEAE-Sephadex, which — as for their original use — tend to form complexes with drugs.

Our hypothesis explains many in vivo findings on nasal drug delivery systems, including 'bioadhesive' polyanionic gel systems such as alginic or hyaluronic, which enhance the nasal bioavailability of drugs (Ryden and Edman, 1991). The rheology of respiratory mucus (Forstner et al., 1977) and the ciliary beat frequency of the nasal epithelium may also be affected by Ca^{2+} ions. The nasal ciliary beat frequency is also Ca^{2+}-dependent: Increased ciliary beat frequencies were reported for increased intracellular Ca^{2+} concentrations (Lansley and Sanderson, 1993).

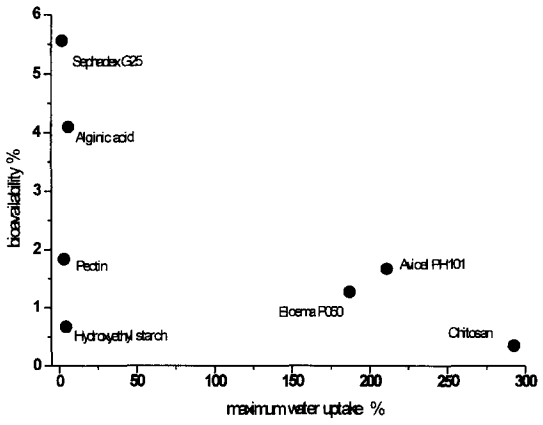

Fig. 3. Correlation between water uptake of excipients and their effect on nasal absorption of octreotide in rats.

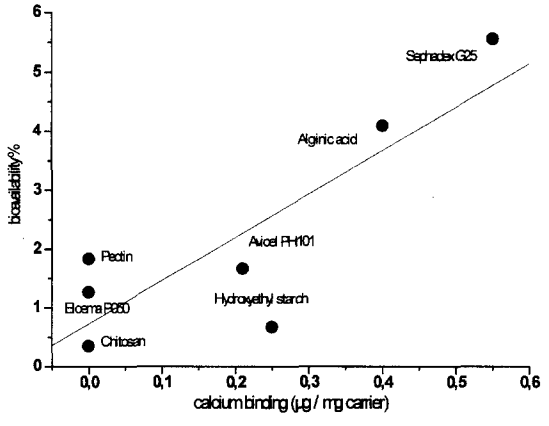

Fig. 4. Correlation between Ca^{2+}-binding capacity of excipients and their effect on nasal absorption of octreotide in rats.

This may explain the prolonged nasal residence time of microcrystalline cellulose (Nagai et al., 1984) or the increased residence time of degradable starch microspheres (Illum et al., 1987).

Although a clear advantage of the powder formulations as compared to the liquid formulation could be seen (bioavailabilities up to 5.56% versus 0.59%), the nasal bioavailabilities of octreotide formulations were rather low. Earlier studies with octreotide administered with microcrystalline cellulose described relative bioavailabilities of 20–30% in humans using s.c. administration as reference (Harris et al., 1992). Taking into consideration that nasal administration in rats is rather difficult, it might well be that species differences contribute to the observed discrepancies. Similar species differences have been observed by other groups for insulin which was nasally administered to rabbits and rats (Schipper et al., 1993).

The time of maximum plasma concentrations of octreotide was quite different for the investigated carriers. T_{max} was below 20 min except for Sephadex™ G25 and pectin with T_{max}s of 1 and 2 h, respectively. Since all carriers showed a rapid drug release in vitro, it may be speculated that the prolonged increase in plasma concentrations may be related to the swelling behaviour of the powders. This assumption is supported by the observation that only pre-swollen Sephadex G25 showed a capacity for calcium binding. Therefore, this pre-swelling time should be taken into account if a delayed plasma profile is desired.

In summary, the calcium-binding capability of particulate nasal carrier systems transiently trapping Ca^{2+} from the mucosal environment seems to be a plausible explanation for their absorption-enhancing properties. Whereas absorption-enhancing mechanisms were so far correlated to specific physical properties of the individual particulate systems, a more general mechanism for the enhancement of nasal peptide absorption could now be identified applying to various particulate carriers as well as to gel forming agents. We cannot rule out entirely that other mechanisms, such as water uptake, contribute to the enhancer effect, but the dominant feature seems to be the calcium-binding capacity. The low local toxicity reported so far of particulate nasal carrier systems compared to other nasal enhancer principles, such as surfactants and calcium chelators, is an additional feature requiring further explorations.

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