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Effects of the Mucoadhesive Polymer Polycarbophil on the Intestinal Absorption of a Peptide Drug in the Rat

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Abstract—The absorption across rat intestinal tissue of the model peptide drug 9-desglycinamide, 8-arginine vasopressin from bioadhesive formulations was studied in-vitro, in a chronically isolated internal loop in-situ and after intraduodenal administration in-vivo. A controlled-release bioadhesive drug delivery system was tested, consisting of microspheres of poly(2-hydroxyethyl methacrylate) with a mucoadhesive Polycarbophil-coating, as well as a fast-release formulation consisting of an aqueous solution of the peptide in a suspension of Polycarbophil particles. Using the controlled-release system, a slight improvement of peptide absorption was found in-vitro in comparison with a non-adhesive control system, but not in-situ or in-vivo. In contrast, bioavailability was significantly increased in all three models from the Polycarbophil suspension in comparison with a solution of the drug in saline. The effect appeared to be dose-dependent, indicative of intrinsic penetration-enhancing properties of the mucoadhesive polymer. A prolongation of the absorption phase in-vitro and in the chronically isolated loop in-situ suggested that the polymer was able to protect the peptide from proteolytic degradation. This could be confirmed by degradation studies in-vitro. The duration of the penetration enhancing/enzyme inhibiting effect was diminished with increasing complexity of the test model, in the same way as was previously found for the bioadhesive effect. This interrelationship suggests that the observed improvement in peptide absorption and the mucoadhesive properties of this polymer are associated. The development of a fast-release oral dosage form for peptide drugs on the basis of Polycarbophil appears to be possible.

Due to the rapid advances in biotechnology, an increasing number of peptides and proteins is becoming relevant for therapy, e.g. vasopressin, insulin, growth hormone, interferons, and calcitonin. Up to now, this class of drugs in general has to be administered parenterally. Although the so-called alternative (e.g. buccal, nasal) routes look promising, the oral route remains the greatest challenge. For several years now, the feasibility of bioadhesive drug delivery systems (BDDSs) has been extensively studied. This new concept might also offer possibilities to circumvent the difficulties related to oral delivery of peptides.

A BDDS is designed to adhere to the mucous linings of the gastrointestinal tract. It should then be able to exert a positive influence on drug absorption by different mechanisms, such as increasing the residence time of the delivery system at the site of drug absorption and intensifying its contact with the absorbing biological membrane. In particular the latter may help to overcome both the diffusional and enzymatic barriers which normally restrict the absorption of peptide drugs (Lee 1988).

As previously reported (Junginger et al 1990) the transport of the model peptide, 9-desglycinamide, 8-arginine vasopressin (DGAVP), across rat intestinal tissue in-vitro was significantly increased under the influence of a mucoadhesive polymer. In these experiments, the drug was slowly released from a BDDS based on microspheres of poly(2-hydroxyethyl methacrylate) which were loaded with the drug and coated with a mucoadhesive polymer of the poly(acrylic acid) type, such as Polycarbophil. As drug release was controlled only by the inner hydrogel core, the same microspheres without mucoadhesive coating could be used as a reference. The purpose of the study described here was to further investigate the mechanisms behind those findings and to determine their validity under in-vivo conditions.

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Materials and Methods

Drug formulations

Controlled release formulations. Mucoadhesive microspheres of poly(2-hydroxyethyl methacrylate) were prepared by suspension polymerization as previously described (Lehr et al 1990). Cross-linker content (ethyleneglycol-dimethacrylate) in the monomer feed was 5% (w/w). The sieved fractions in the 315–400 μm range from several polymerization runs were pooled. To remove possible residual monomers, the beads were extracted with ethanol in a Soxhlet for 24 h and thereafter with demineralized water at room temperature (21°C) for several days. Ten g of the dried beads were loaded with DGAVP by 48 h swelling in 20 mL water, containing 2 mg mL^{-1} of DGAVP (DGAVP-dicitrate was a kind gift from Organon, Oss, The Netherlands) under permanent magnetic stirring. The beads were rapidly frozen in liquid nitrogen and freeze-dried. One-half of the batch was coated with Polycarbophil (Carbopol EX-55, BF Goodrich, Cleveland, OH, USA) in an air suspension process as previously described (Lehr et al 1990); non-coated beads were used as control.

Drug release was studied in 0·9% NaCl (saline) (contain-

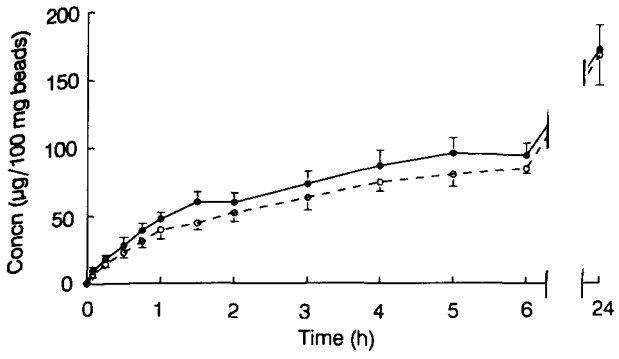

FIG. 1. Release of DGAVP from 100 mg PHEMA-microspheres in saline (USP paddle apparatus, 37°C). Shown is the mean ± s.d. Coated, ○, ns = 6; non-coated, ●, n = 5.

ing 0·01% Tween 20, Atlas, Essen, Germany) at 37°C (USP paddle method). After appropriate dilution of the samples, DGAVP was measured by radioimmunoassay (see below). As shown in Fig. 1, 100 mg of both coated and non-coated beads released about 80 µg DGAVP over 5 h. No correction was made for the slight difference in the total amount of drug released which was due to the weight contribution of the coating polymer (Lehr et al 1992a).

Fast release formulations. Polycarbophil was dispersed in isotonic saline under vigorous magnetic stirring and homogenized by further stirring for several hours. Up to concentrations of about 5% (w/v), the suspensions had a relatively low viscosity, permitting easy pipetting and injecting. After storage overnight at 5°C, the polymeric material formed a sediment which, however, was easy to redisperse by simply shaking, even after several weeks. One day before use, DGAVP was dissolved in saline and added to the polymer dispersions to yield the desired drug concentrations. As a control, the same amount of drug was dissolved in saline without further additives.

Peptide absorption studies

Intact DGAVP could be measured in saline, Krebs-Ringer buffer or rat plasma without further extraction, by a radioimmunoassay developed in this laboratory by van Bree et al (1988). Cross-reactivity of the antiserum used (J477C) with the peptide fragments, arginine-vasopressin, AVP-(1-7), AVP-(1-6) and [Cyt6]AVP-(2-8) was reported to be 1% or less (van Bree et al 1990). Blood was collected in heparinized 300 µL capillary sampling cups (Microvette CB300, Sarstedt BV, Etten-Leur, The Netherlands) from a small incision close to the tail tip. Samples were immediately placed on ice and spun down at 16000 *g* for 15 min at 4°C. Two 50 µL portions of the supernatant plasma were pipetted into assay tubes and kept at −20°C until analysis. Bound activity was separated using a second, anti-rabbit antibody coated on cellulose (Sac-Cel, Innogenetics, Abcoude, The Netherlands). DGAVP standards for calibration curves, ranging from 0·05 to 10 ng mL^{-1}, were prepared in the same matrix as the samples. Blank plasma was obtained from other rats.

In-vitro model. Rat intestinal loops (about 8 cm) were vertically perfused in-vitro as previously described (Junginger et al 1990). The luminal donor compartment was filled with 5 mL saline. The serosal acceptor compartment consisted of 40 mL Krebs-Ringer buffer. Both compartments were perfused with Carbogen (5% CO_2–95% O_2). The apparatus was placed in a water bath at 37°C.

Chronically isolated intestinal loop. An intestinal segment was isolated from rat ileum, about 10–15 cm proximal to the ileo-caecal junction. The loop remained in the peritoneal cavity with an intact blood supply, whilst the remaining intestine was restored by end-to-end anastomosis (Poelma & Tukker 1987). Rats were free to move in plastic cages (about 30 × 40 cm) and had access to food. Loops were rinsed daily with saline.

For these studies, 12 male Wistar rats were used between 1 and 4 weeks after surgery. If possible, rats were used repeatedly, up to five times. If blood was noted in the loop or body weight had decreased, the animal was not used. Between experiments, there were at least 3 days of rest. Average body weight was 264 ± 21 g (mean ± s.d., range, 227–298 g) which increased to 288 ± 36 g (range, 249–346 g). Average loop length was 10·3 ± 2·8 cm (range, 6–15 cm).

For the experiments, rats were placed in restraining cages; food was withdrawn but water was freely available. After carefully rinsing the loop with saline, drug formulations were injected with 1 mL saline or Polycarbophil suspension, respectively. The loops were not perfused. Short flexible tubes were connected to the ends of the loop and bent upwards to buffer volume changes of the loop when the animal moved.

Intraduodenal administration. The experimental technique is described elsewhere (Lehr et al 1992b). Briefly, a 4 mm teflon tube was inserted in rats under ether anaesthesia, passing through abdominal and stomach wall and ending in the duodenum. After a 2 h recuperation period, drug formulations were injected immediately into the small intestines via a smaller, flexible catheter (i.d. 1·6 mm, o.d. 2·1 mm). After the last blood sample, the animals were killed with an overdose of ether. In total, 24 male Wistar rats were used; average body weight was 217 ± 10 g (range, 190–230 g).

Peptide degradation studies

Luminal fluid. After 18 h fasting, one male Wistar rat, 256 g, was killed by suffocation with CO_2. The abdomen was opened and the small intestines removed. After resection of mesenteric tissue, the lumen of the gut was connected to a small funnel and filled with ice-cold phosphate buffered saline (PBS, pH = 7·4). The luminal contents (approx. 8 mL) were removed by careful rubbing and collected. The fluid was vortexed and centrifuged at 300 *g* for 10 min. The supernatant was stored in portions of 0·5 mL at −80°C. Protein content (Coomassie protein assay, Pierce, Oud Beijerland, The Netherlands) was 6·3 ± 0·1 mg mL^{-1}.

Mucosal homogenate. After removal of the luminal contents, the small intestine was opened longitudinally and rinsed with PBS. The mucosa was stretched over a microscope slide and scraped with another slide. The collected cellular material was diluted with PBS and homogenized in a 15 mL Potter-

Elvehjem tissue grinder chilled on ice. After centrifugation (10 min, 3000 *g*), three phases formed (fat, aqueous fluid and pellet). The aqueous fluid phase used for the further studies was stored in portions of 0·5 mL at −80°C. Protein content was 15 ± 1 mg mL^{-1}.

Incubation studies. Twenty five mL of saline or of a 1% (w/v) Polycarbophil suspension in saline were filled into glass tubes, kept at 37°C and mixed by perfusion with Carbogen. DGAVP (0·25 μg) was added to yield a theoretical concentration of 10 ng mL^{-1}. At t = 0, 250 μL luminal homogenate or 100 μL mucosal homogenate, respectively, were added to yield comparable concentrations of total protein. At given intervals, samples were taken and kept on ice. After spinning down the Polycarbophil particles, samples were analysed for DGAVP by radioimmunoassay. Control samples taken before the addition of DGAVP and before the addition of enzyme preparations, respectively, indicated that Polycarbophil interfered slightly with the assay which, however, could be compensated by 2-fold dilution with assay buffer.

Results

The results of analogous experiments with the two bioadhesive formulations in three different absorption models are shown synoptically in Figs 3 and 4. Statistical analysis was performed by nonparametric Wilcoxon U-test (Statgrafics, V. 2.6., Statistical Graphics Corp., Rockville, MD, USA) with the drug concentration data for each time point.

Drug absorption in-vitro

Administration of 100 mg drug-loaded microspheres into the lumen of rat small intestinal segments in-vitro led to a higher average drug concentration (although, statistically not significant) in the serosal acceptor compartment in the case of the coated (mucoadhesive) system in comparison with the non-coated (non-adhesive) system (Fig. 3a). A similar effect was observed when 10 μg DGAVP were added to the donor compartment in which previously 100 mg of coated or non-coated, but drug-free microspheres had been added (Fig. 2).

Administration of 1·0 mL of a 1% (w/v) Polycarbophil suspension containing 10 μg DGAVP led to significantly

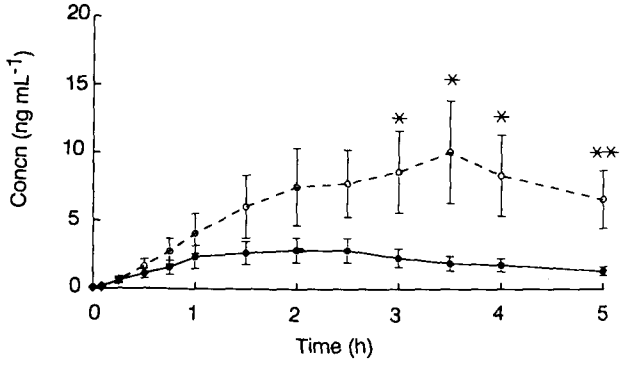

FIG. 2. Absorption of 10 μg DGAVP in-vitro in presence of coated (○) vs non-coated (●) drug-free microspheres. (Mean ± s.e.m. n = 7; $*P \leq 0{\cdot}1$, $**P \leq 0{\cdot}05$.)

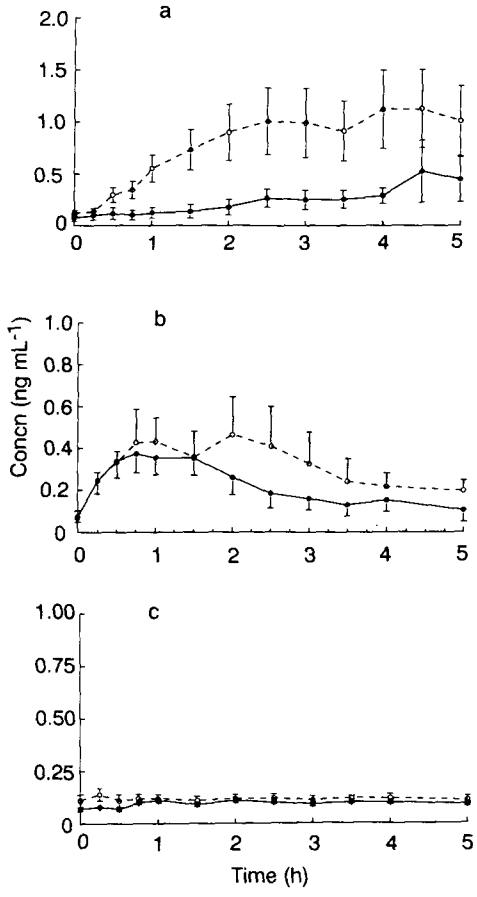

FIG. 3. (a) Absorption of DGAVP from 100 mg PHEMA-microspheres in-vitro (mean ± s.e.m.). Coated, ○, n = 6; non-coated, ●, n = 5. (b) Absorption of DGAVP from 100 mg PHEMA microspheres in the chronically isolated loop (mean ± s.e.m.). Coated, ○, n = 8; non-coated, ●, n = 7. (c) Absorption of DGAVP from 100 mg PHEMA-microspheres after intraduodenal administration (mean ± s.e.m.). Coated, ○, n = 6; non-coated, ●, n = 6.

higher acceptor concentrations and a shift in t_{max} in comparison with the same dose without Polycarbophil (Fig. 4a). Increasing the amount of Polycarbophil from 1 to 5% (w/v) further increased both the extent and duration of drug absorption. The area under the mean curves (AUC) was determined using the linear trapezoidal rule up to 300 min (Table 1). Because data points in the elimination phase were very limited, pharmacokinetic curve fitting could not be performed reliably. Nonetheless, the curves suggest that addition of increasing amounts of polycarbophil increased not only AUC but also the duration of the absorption phase.

Absorption in the chronically isolated loop model

In the chronically isolated loop model, there was no statistically significant difference between coated and non-coated microspheres, although the plasma concentrations in the terminal phase of the mean curves were slightly higher in the case of the coated system (Fig. 3b).

Administration of 100 μg DGAVP from a 4·5% Polycarbophil suspension led to a significantly different plasma concentration-time course in comparison with the control formulation (Fig. 4b). Pharmacokinetic analysis of the mean

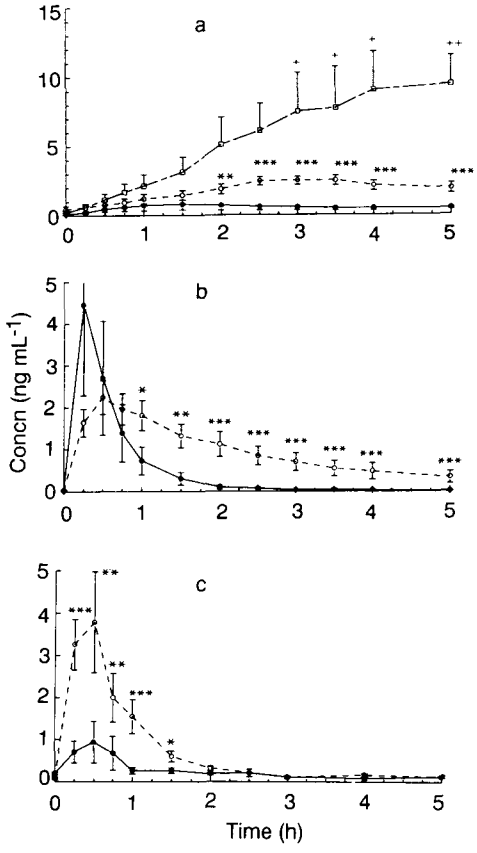

FIG. 4. (a) Absorption of 10 μg DGAVP from Polycarbophil suspensions in-vitro (mean ± s.e.m.). 5% PCP, □, n = 3; 1% PCP, ○, n = 10; saline, ●, n = 6. (b) Absorption of 100 μg DGAVP from a 4·5% Polycarbophil suspension (○, n = 8) vs saline (●, n = 7) in the chronically isolated loop (mean ± s.e.m.). (c) Absorption of 500 μg DGAVP from a 5% Polycarbophil suspension (○, n = 6) vs saline (●, n = 6) after intraduodenal administration (mean ± s.e.m.) $*P < 0.01$, $**P < 0.05$, $***P < 0.001$ compared with saline; $\dagger P < 0.1$, $\dagger\dagger P < 0.05$ compared with 1% Polycarbophil.

curves was performed by fitting the data to a two-exponential model (extravascular bolus without lag time, peeling or weighted least squares algorithm, SIPHAR, Rel. 3.3, Simed, Creteil, France). The obtained parameters are shown in Table 1.

Absorption after intraduodenal administration in-vivo

No immunoreactive DGAVP was detectable in plasma after administration of 100 mg coated or non-coated microspheres (Fig. 3c). After bolus administration of 500 μg DGAVP, however, the drug was detected in plasma, proving the intestinal absorption of the intact, immunoreactive peptide in-vivo. Also in this model, the use of a 4·5% Polycarbophil suspension led to significantly higher plasma concentrations in comparison with the control experiment with saline (Fig. 4c). Pharmacokinetic parameters are given in Table 1.

Pharmacokinetics of DGAVP in rats have previously been studied by van Hoogdaalem et al (1989). For a 10 μg intravenous infusion in rats of comparable body weight, a mean systemic clearance value of 3·9 ± 0·8 mL min^{-1} (mean ± s.d., n = 6) was reported, which allows the calculation of the AUC (2600 ± 500 min ng mL^{-1}). On the basis of this value, an estimate of the oral bioavailability (F) in the two in-vivo models was possible. Pharmacokinetics are assumed to be linear. For the in-vitro model, an imaginary bioavailability was calculated on the basis of an AUC which would have been found in the absence of any diffusion barrier or drug metabolism (i.e. 300 min × 10 μg/45 mL = 66·7 min μg mL^{-1}).

Peptide degradation in-vitro

In 100-fold diluted intestinal juice, DGAVP was as stable as in saline. This finding corroborates the results reported earlier by Matuszewska et al (1988). In the mucosal homogenate, the drug was degraded very rapidly (Fig. 5). The kinetics of this process, however, should be interpreted with

Table 1. Pharmacokinetic parameters of DGAVP in the presence or absence of suspended polycarbophil particles in three different models.

		Control (saline)	Polycarbophil suspension		Ratio (PCP/saline)
In-vitro model (dose = 10 μg)	C_{max} (ng mL^{-1})	0·8 ± 0·4	2·5 ± 0·4	(1%PCP)	3·1
			> 9	(5%PCP)	> 10·0
	t_{max} (min)	90	210		2·3
			> 300		> 3·3
	AUC (min ng mL^{-1})	166	540		3·3
			1756		> 10·6
	MRT (min)	—	—	—	
	F[a]	0·25%	0·8%		
			2·6%		
Chronically isolated loop (dose = 100 μg)	C_{max} (ng mL^{-1})	4·5 ± 2·2	2·3 ± 0·4		n.s.
	t_{max} (min)	< 15	30		> 2
	AUC (min ng mL^{-1})	155	296		1·9
	MRT (min)	35	154		4·4
	F[b]	0·6%	1·1%		
Intraduodenal administration (dose = 500 μg)	C_{max} (ng mL^{-1})	0·9 ± 0·5	3·8 ± 1·9		4·2
	t_{max} (min)	30	30		n.s.
	AUC (min ng mL^{-1})	57	210		3·7
	MRT (min)	57	50		n.s.
	F[b]	0·04%	0·16%		

[a] Based on imaginary AUC = t × D/$V_{distr.}$ with $V_{distr.}$ = 45 mL and t = 300 min. [b] Based on $AUC_{10\mu g\ i.v.}$ (van Hoogdaalem et al 1989), corrected for actual doses by linear extrapolation. n.s. = not significant.

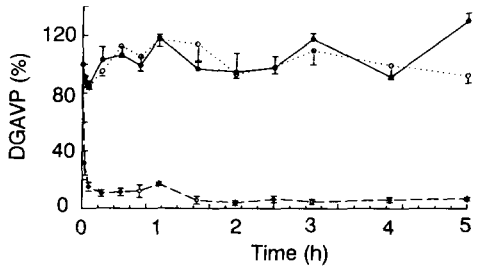

FIG. 5. Degradation of DGAVP in various media at 37°C in-vitro (mean ± s.d., n = 4). Saline, ●; mucosal homogenate, ◇; luminal homogenate, ○.

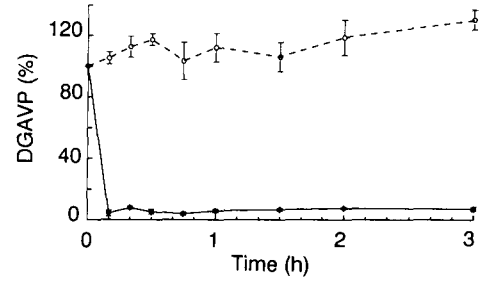

FIG. 6. Degradation of DGAVP in mucosal homogenate in presence or absence of 1% (w/v) Polycarbophil (○) and in saline (●). (Mean ± s.d., n = 4.)

care, as further degradation may have occurred in the withdrawn samples in spite of rapid cooling or freezing.

As shown in Fig. 6, the presence of 1% (m/v) Polycarbophil completely inhibited the degradation of DGAVP by the enzymes of the mucosal homogenate. As the antiserum used is specific for intact DGAVP (van Bree et al 1990), the formation of metabolites could not be measured. Interference of antibody binding with Polycarbophil after dilution of the samples with assay buffer could be excluded by control measurements.

Discussion

The previously observed effect of the mucoadhesive coating on DGAVP absorption from drug-loaded microspheres appeared to be reproducible in-vitro. Acceptor concentrations were lower than in the previously reported pilot experiments (Junginger et al 1990) which probably was a consequence of an improved protocol and experimental routine, leading to less tissue damage and leakiness. Similar effects, however, could be observed when the mucoadhesive polymer Polycarbophil was present in the donor compartment, either in the form of a coating on dummy-beads or as fine dispersed particles, but not directly associated with a drug delivery system. Furthermore, the effect was dependent on the amount of Polycarbophil. This indicated that the observed improvement in drug absorption was intrinsic to the polymer itself. Nonetheless, this might be interrelated with the fact that this polymer binds to mucosal tissue and mucus.

Viscosity effects exerted by the mucoadhesive polymer are not likely to play a role in case of the controlled release formulations. From previous studies (Lehr et al 1990) it was known that the Polycarbophil coating on the microspheres was very stable and did not dissolve during 24 h. Different viscosities between dispersions of coated and non-coated microspheres have not been reported. In contrast, for the fast release formulations, viscosity effects could be very important. In combination with the known mucoadhesive properties of the dispersed Polycarbophil particles, their interaction with soluble mucins, different pH and ionic strength might result in the spontaneous formation of a mucoadhesive hydrogel in-situ which coats the mucosa at the site of administration. This possible mechanism deserves to be further investigated.

With increasing physiological complexity of the test model (in-vitro to in-situ to in-vivo), the effectiveness of absorption barriers also increased. As a consequence, the same dose of DGAVP from microspheres was absorbed to a gradually decreased extent. Simultaneously, the effect of the mucoadhesive polymer disappeared. To compensate for the increased absorption barrier of the three test models, the amount of drug dissolved in the aqueous Polycarbophil suspensions was gradually increased. From this formulation the drug can be assumed to be released rapidly, as a coating of the mucoadhesive polymer did not affect peptide release from poly(2-hydroxyethylmethacrylate)-microspheres. Using this fast-release formulation, the presence of Polycarbophil particles induced a significantly increased bioavailability of the peptide in all three models.

An increased absorption of the peptide drugs insulin and calcitonin and the hydrophilic compound phenol red from an aqueous gel base of poly(acrylic acid) has been reported by Morimoto et al (1987) after rectal, vaginal and nasal administration. An enhanced influx of water in rat rectum and reversible structural changes of the mucosal tissue indicated that poly(acrylic acid) acted as a penetration enhancer, i.e. changing the permeability of epithelial tissue. As those authors noted, the penetration-enhancing effect of the poly(acrylic acid) gel was not suppressed by calcium ions and therefore not related to its chelating activity. The latter has been discussed as a mechanism for penetration-enhancing activities of EDTA or enamines, but is not generally accepted (Muranishi 1990).

The change in pharmacokinetic parameters for the in-vitro model and the chronically isolated loop under the influence of Polycarbophil indicated a prolongation of the absorption phase rather than an increased permeability of the tissue. In particular, in the in-situ experiment (Fig. 4b), the average C_{max} in the presence of Polycarbophil was even smaller than after administration in saline, which would not be expected if this polymer affected only tissue permeability. As the degradation studies demonstrated, Polycarbophil is able to inhibit proteolytic degradation of DGAVP by mucosal peptidases, at least in a non-buffered saline medium. The mechanism of enzyme inhibition is not yet known. The pH-dependence of this enzyme inhibition and the complexation of calcium and other metal ions as cofactors of proteolytic enzymes should be investigated.

After intraduodenal administration in-vivo, the duration of the absorption enhancement was decreased. In this model, the mean residence time (MRT) was not changed under the influence of Polycarbophil which means that the absorption phase was no longer prolonged. This may also be a

consequence of the presence of soluble mucin or mucus clots in the lumen of this model and a high mucus turnover rate, which was found to be crucial for the concept of mucoadhesion in controlling gastrointestinal transit (Lehr et al 1992b). This parallelism indicates that mucoadhesive and penetration enhancing properties of this polymer—whether by improving drug stability or tissue permeability—are associated. However, mucus turnover seems to limit this approach to fast-release formulations as there was no improvement in peptide bioavailability for the controlled-release formulations.

Conclusion

Polycarbophil enhanced the intestinal absorption of a peptide drug in-vitro and in-vivo. Besides being mucoadhesive, this polymer is likely to act as a penetration enhancer and was found to inhibit proteolytic degradation in-vitro. The mechanism of action should be further investigated. There are indications that penetration-enhancing or enzyme-inhibiting properties of this polymer are associated with its mucoadhesive properties. The development of oral dosage forms for peptides on the basis of Polycarbophil appears possible.

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