Peroral Absorption of Octreotide in Pigs Formulated in Delivery Systems on the Basis of Superporous Hydrogel Polymers

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Purpose. The aim of this study was to investigate the enhancement of peroral octreotide absorption using delivery systems based on superporous hydrogel (SPH) and SPH composite (SPHC) polymers.

Methods. Six female pigs (BW of 23.5 kg) were used in this study. SPH-based delivery systems were made of two components: 1) a conveyor system made of SPH and SPHC; 2) a core that contained octreotide. The core was inserted into the conveyor system (core inside, c.i.) or attached to the surface of the conveyor system (core outside, c.o.). Four different peroral formulations were investigated: c.i., c.o., core outside including trimethyl chitosan chloride (c.o.t.), and octreotide only in the absence of any polymer (o.o.). All formulations were placed in enteric-coated gelatin capsules (size 000) and administered perorally. Intravenous administration was used to determine bioavailability (F) values. Blood samples taken from the cannulated jugular vein were analyzed by radioimmunoassay.

Results. Peroral administration of 15 mg o.o. resulted in low F values of 1.0 ± 0.6% (mean ± SEM) whereas c.i. and c.o. administrations resulted in remarkably higher F values of 12.7 ± 3.6% and 8.7 ± 2.4%, respectively. By the addition of trimethyl chitosan chloride as an extra absorption enhancer to c.o.t., the highest bioavailability (16.1 ± 3.3%) was achieved.

Conclusions. These novel delivery systems based on SPH and SPHC polymers are able to increase the peroral bioavailability of octreotide by mechanical fixation and increasing the retention of the dosage form at the absorption site.

KEY WORDS: peroral peptide drug delivery; superporous hydrogel (SPH); SPH composite (SPHC); octreotide; pigs.

INTRODUCTION

Developing controlled drug delivery technologies using biodegradable polymers as drug carriers is one of the most rapidly advancing areas in pharmaceutical science. Applying such polymers to peroral peptide drug delivery is an important issue because various types of these polymers have shown to enhance the oral bioavailability of peptide drugs and proteins (1,2). Beyond these biodegradable polymers (well-known as absorption enhancers), different peptide analogues have been developed by chemical modification of the peptide structure to prevent proteolytic degradation and increase the bioavailability of peptide drugs (3,4). Among the available analogues, oligopeptides with low molecular weight such as octreotide (a somatostatin analogue) were studied for peroral absorption (5,6). Octreotide is a synthetic octapeptide, which has retained the essential pharmacophoric part (Phe-Trp-Lys-Thr) of the native molecule somatostatin. Octreotide is more potent than somatostatin and clinically used for the treatment of acute variceal bleeding in cirrhotic patients (7), insulin-dependent diabetes (8), and inhibition of gall bladder contraction during continuous jejunal feeding in patients with pancreatic pseudocyst (9). The currently available delivery system for octreotide is an injectable dosage form, which is not ideal for long-term drug administration in cirrhotic and diabetic patients. Therefore, the intestinal application of octreotide was investigated using different delivery systems and absorption enhancers such as poly (alkyl cyanoacrylate) nanocapsules and N-trimethyl chitosan chloride (5,6,10).

Recently, novel delivery systems based on superporous hydrogels (SPHs) and SPH composites (SPHCs) have been developed in our laboratories for peroral peptide drug delivery. SPH and SPHC polymers are a group of hydrogels, which swell very quickly as a result of their highly porous structure. SPH is a soft polymer with lower mechanical stability and faster swelling properties than SPHC, which is mechanically much more stable (11,12). These delivery systems based on SPH and SPHC polymers are able to achieve mechanical fixation of the dosage form at desirable site(s) of absorption in the intestine by controlled rapid swelling (13). The aim of the present study was to formulate octreotide in SPH- and SPHC-based delivery systems and to investigate the potential of these systems for enhancement of intestinal octreotide absorption after peroral administration in healthy pigs.

MATERIALS AND METHODS

Materials

Octreotide acetate, I^{125}- radiolabeled Tyr-1-octreotide, and octreotide-antiserum were donated by Novartis Pharma (Basel, Switzerland). Narketan® (ketamine) was purchased from Chassot (Vught, The Netherlands). Sufenta® (sulfentanyl) was obtained from Jansen-Cilag (Tilburg, The Netherlands). Clamoxyl® (amoxicilline) was from Smith-Kline Beecham Farma (Rijswijk, The Netherlands). Rapinovet® (propofol) was purchased from Schering-Plough (Brussels, Belgium). Vials containing Li-Heparin (Monovette®) for blood sample collection were from Sarstedt (Etten-Leur, The Netherlands). SPH and SPHC were synthesized as described previously (12). Eudragit S100 was supplied by Röhm (Darmstadt, Germany). Gelatin capsules were kindly donated by Capsugel® (Colmar, France). All other compounds were of analytical grade.

Experimental Setup

The approval of experimental protocol was obtained from the Ethical Committee for Animal Experimentation (Veterinary Faculty of Utrecht University). Six female pigs (body weight 23.5 ± 4.0 kg) were used in this study. Two weeks before the experiments, the pigs were housed in the

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animal facilities of the Central Laboratory Animal Institute (Utrecht University, Utrecht, The Netherlands). One week before the start of experiments, all animals were anesthetized to insert a silicone cannula into their jugular vein for blood sampling. The animals received a mixture of ketamine and atropine as pre-anesthesia and during the surgery the anesthesia was sustained by infusion of sulfentanyl forte. A thin silicone and heparinized cannula was inserted into the jugular vein and the other edge of the cannula was placed with a metal bar at the dorsal part of the animal's neck. To avoid (post) operative infections, amoxicilline was administered before and after the surgery. One week after surgery, the pigs received different octreotide administrations according to a randomized cross-over setup. Animals were fasted overnight before each administration but had access to water *ad libitum*.

Preparation of SPH- and SPHC-Based Delivery Systems

The following SPH- and SPHC-based delivery systems were prepared (13): 1) core inside the conveyor system and 2) core attached to the surface of the conveyor system (Fig. 1). Both systems were made of two components: 1) a conveyor system made of SPH and SPH composite; and 2) a core that also contained the peptide drug octreotide. The core was either inserted into the conveyor system (core inside, c.i.) or attached to its surface (core outside, c.o.). To make the conveyor system, first SPH and SPH composite polymers were synthesized (12). For the c.i. system, a hole was made in the center of SPHC polymer as a body and a piece of SPH polymer was used as a cap of the conveyor system (Fig. 1A). For the c.o. system, two holes were made on the surface of SPHC polymer as the conveyor system (Fig. 1B). The core component of the c.i. delivery system consisted of octreotide microparticles. To make these microparticles, 85 mg of PEG 6000 was melted. Then 15 mg of octreotide was completely dispersed in melted PEG 6000 while the whole mass was cooling down. The cooled mass was crushed using a mortar and sieved through sieve with a mesh size of 400 μm. Microparticles smaller than 400 μm were used as a core formulation. These microparticles were filled in the hole inside the SPH composite and the hole was closed with a piece of SPH as a cap. The reason for using SPH as a cap is that the swelling ratio of SPH is higher than SPH composite, and that the cap is ejected for allowing a burst release of the peptide drug (13).

The core component for c.o. delivery system contained a mixture of 7.5 mg of octreotide and 92.5 mg of lactose, which were pressed as minitablets (4 mm in diameter). Two tablets were attached to the conveyor system using a biodegradable glue (Histoacryl ®). In an additional formulation for the core component of c.o. delivery system, 20 mg *N*-trimethyl chitosan chloride (TMC) was added as an additional absorption enhancer to the c.o. formulation (14); however, because TMC is a sticky powder, 20 mg Explotab® was also added to the tablet formulation as a disintegrant to achieve a burst release profile. All peroral formulations were placed in gelatin capsules (size 000; 9 × 12 mm) and the capsules were enteric coated with 6% Eudragit S100 solution. As a negative control only 15 mg of octreotide without any polymer (o.o.) was filled in a gelatin capsule and enteric-coated. For intravenous (i.v.) administration, octreotide acetate was prepared as a 2-mL stock solution in sterile water for injection and added in 98 mL of sterile, pyrogen-free physiological saline solution at a final concentration of 100 μg/mL.

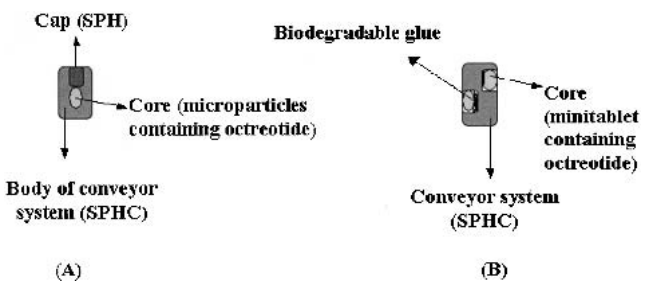

Fig. 1. Schematic figures of superporous hydrogel– and superporous hydrogel composite–based delivery systems. A, Core inside the delivery system; B, Core attached to surface of delivery system.

Administration of Octreotide Formulations

The formulations were administered to the animals every other day at 48-h intervals between administrations as a washout period. Before administration, the animals were sedated by i.v. injection of 0.6 mL/kg propofol (10 mg/mL) to facilitate peroral and i.v. administration. The duration of sedation was approximately 15 min, during which three blank blood samples of 4 mL were taken from the jugular vein. Thereafter, each capsule was administered via the mouth into the stomach using a plunger applicator during the sedation period. For i.v. administration, 5 mL of an octreotide solution (100 μg/mL) was given via the cannulated juglar vein with a 5-mL syringe, followed by 5 mL of sterile pyrogen-free physiological saline to ensure complete dosing. Four-milliliter blood samples were collected from the cannula inserted in the jugular vein and 4 mL of sterile pyrogen-free physiological saline were given back. Two milliliters of heparinized physiological saline (25 U/mL) were administered to fill the dead volume of the cannula to avoid blood clotting in the tube. In case of peroral administrations, blood samples of 4 mL were withdrawn at 1, 2, 3, 3.5, 4, 4.5, 5, 6, 8, and 10 h. After i.v. administration, blood sampling occurred at 2, 5, 10, 15, 30, 60, 120, 240, 360, and 600 min. Blood samples were kept on ice and then centrifuged for 15 min at 2000 g and 4°C. The obtained plasma was stored at −20°C up to the time of analysis. All animals were fed 6 h post-dosing. At the end of all experiments the pigs were euthanized by an overdose of pentobarbital, and the gastrointestinal tract was inspected macroscopically for possible damage.

Analysis of Octreotide

The plasma samples were analyzed for the octreotide concentrations by radioimmunoassay as previously described (15). To avoid inter-assay variations, all samples were analyzed in one assay using one batch of radiotracer and one batch of antiserum.

Pharmacokinetic Analysis of Data

Pharmacokinetic parameters, including total area under the plasma concentration-time curve (AUC), peak plasma concentration (C_{max}), and time to reach peak plasma concentration (t_{max}), for all peroral administrations were calculated directly from the plasma octreotide concentrations. The

AUCs for the individual plasma profiles were calculated with the linear trapezoidal rule (16).

The plasma profiles of octreotide after i.v. administration were fitted using WinNonlin program (Scientific Consulting Inc., Palo Alto, CA, USA). The plasma concentration-time profiles were fitted according to:

$$C_t = A_1 e^{-\alpha_1 t} + A_2 e^{-\alpha_2 t}$$

in which C_t represents the plasma concentration of octreotide at time t, and A_1, A_2, α_1, and α_2 are the coefficients and exponents of this equation. The pharmacokinetic parameters were calculated according to Gibaldi and Perrier (16). Absolute bioavailability values after peroral administrations of octreotide were calcualted according to the following:

$$F = \frac{AUC_{peroral} \times D_{i.v.}}{AUC_{i.v.} \times D_{peroral}} \times 100\%$$

in which F is the absolute bioavailability and D is the administered dose.

The obtained data were evaluated for statistically significant differences by one-way analysis of variance (ANOVA) at $P < 0.05$.

RESULTS AND DISCUSSION

The pharmacokinetic parameters after i.v. administration of 500 μg of octreotide in pigs are summarized in Table I. The octreotide plasma profiles were fitted to a two-compartment model, resulting in a short distribution half-life of about 7 min and a long elimination half-life of 52 min, quite similar to previously reported values in pigs (6).

The plasma octreotide concentration vs. time profiles obtained for each one of the peroral administrations, including c.o., c.i., o.o., and c.o.t., are shown for two of the six pigs (subject nos. 2 and 6) in Fig. 2 A and B. Because these drug delivery systems based on SPH and SPHC polymers were placed in enteric-coated gelatin capsules size 000, the passage of capsules from the stomach to the intestine varied in each subject and also after each administration. It has been well established that dosage forms up to about 3 mm in size pass through the contracted pylorus within 30 to 120 min; however, if the size of dosage forms is increased to more than 3 mm, they may stay in stomach from 1 to 10 h (17,18). Therefore, the gastric emptying time for enteric-coated capsules size 000 can vary from 2 to 6 h, as clearly observed in Fig. 2 A and B. As a result of this, the t_{max} for each administration is different, which can be the result of the natural contraction of the pylorus or anatomy and physiology of the animals with respect to gastric emptying time.

Table I. Pharamcokinetic Parameters after Intravenous Administration of Octreotide

Parameters	Mean ± SEM (n = 6)
t ½ dist. (min)	6.9 ± 1.5
t ½ elim. (min)	51.7 ± 8.1
V_d (mL/pig)	423 ± 54
Cl (mL/min/pig)	23.4 ± 2.4
AUC (ng/mL*min)	5621 ± 549

Note: t ½ dist. = distribution half-life; t ½ elim. = elimination half-life; V_d = volume of distribution; Cl = clearance; AUC = area under curve.

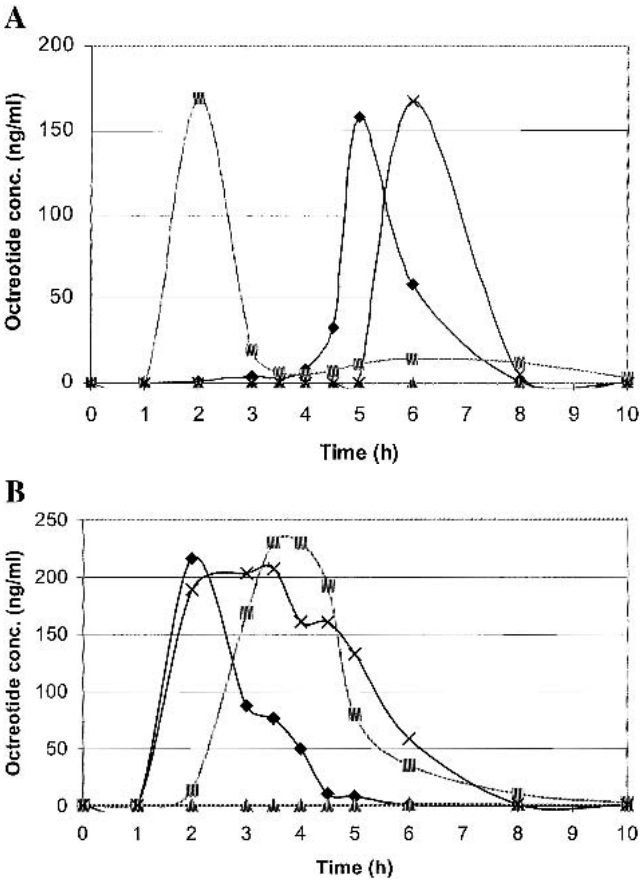

Fig. 2. Blood plasma profiles of octreotide after peroral administration of 15 mg/pig: A, Subject 2; B, Subject 6. Core outside (♦); core inside (■); octreotide without any polymer (▲); core outside with TMC (×).

As shown in Fig. 2, the plasma octreotide levels for the negative control (octreotide without SPH/SPHC polymers) was the lowest compared with the other peroral administrations. The enhanced octreotide absorption after peroral administration of the SPH/SPHC formulations is in accordance with our previous studies using porcine intestine *ex vivo* (19) and indicates that, after the enteric-coated capsules have reached the intestine and the enteric-coat and gelatin layers have been dissolved, the SPHC conveyor systems swelled rapidly and attached mechanically at the absorption site in the intestine. This mechanical fixation to the gut wall enhances the absorption of octreotide as a result of increased residence time of the delivery system in the intestine and to opening of tight junctions by mechanical pressure and water influx from the intestinal mucosa into the polymers (19,20). This water influx forced the intestinal epithelial cells to maintain their homeostatic pressure by compensating this water loss by opening of the tight junctions, to facilitate a rapid uptake of water together with octreotide molecules, and to maintain the homeostasis of the intestinal cells and thereby also enhancing plasma octreotide levels.

It is well known that, for the absorption of hydrophilic and macromolecular drugs such as peptides and proteins, initially the tight junctions should be opened and then a burst release of such drugs is necessary (13,20,21). This so-called "time-controlled burst release profile" can be achieved using

the present SPH- and SPHC-based delivery systems (22) because a rapid increase in plasma octreotide levels was observed after peroral administration of these systems (c.o., c.i., and c.o.t. formulations; Fig. 2 A and B). Moreover, the absorption and elimination of octreotide in some of the pigs (e.g., subject no. 2; Fig. 2A) were very fast, showing plasma octreotide profiles between two time point measurements with rather sharp peaks and probably resulting in underestimated bioavailability values. If the time points between measurements would have been shorter, it might have caused broader octreotide absorption peaks and higher bioavailability values.

The pharmacokinetics of octreotide in each pig after peroral administration of the different formulations are given in Table II. T_{max} appeared to vary between 2 to 6 h in most of the administrations in different subjects, except for c.i. administration in subject no. 3 in which the capsule did not pass the pylorus and showed no octreotide absorption; therefore, this administration was omitted from the bioavailability calculations. Mean C_{max} values (± SEM) for c.o., c.i., and c.o.t. administrations were 152.0 ± 14.7, 175.9 ± 15.9, and 157.9 ± 15.4 ng/mL, respectively, which are significantly higher than C_{max} for o.o. administrations (17.8 ± 11.5 ng/mL). This demonstrates that the present SPH- and SPHC-based delivery systems are able to increase substantially the intestinal absorption of octreotide, by mechanisms already discussed above. When the chitosan derivative TMC was added to the c.o. formulations (c.o.t.), the octreotide absorption profile was observed to be broader. This can be explained by the fact that TMC is a sticky powder, therefore prolonging the octreotide absorption time.

The average absolute bioavailabilities for octreotide after each administration are also given in Table II. Peroral administration of o.o. resulted in a bioavailability (F) of 1.0 ± 0.6%, whereas using SPH and SPHC polymers in c.i. and c.o. administrations resulted in F values of 12.7 ± 3.6% and 8.7 ± 2.4%, respectively. These absorption enhancements were significantly ($p < 0.05$) higher than o.o. administration. By the addition of TMC as an additional absorption enhancer to the c.o. formulation, an even higher bioavailability of octreotide (16.1 ± 3.3%) was achieved. It has already been reported that TMC increases substantially the intestinal absorption of octreotide *in vivo* in rats and pigs (6,14). Adding TMC to the present c.o.t. formulation is an extra factor to enhance the intestinal absorption of octreotide because TMC is well soluble at neutral pH values and interacts with the tight junctions by its positive charge, thereby facilitating the transport of hydrophilic molecules like octreotide across the paracellular pathway.

During the present experiments, no stress or discomfort was observed with the animals. At the end of the experiments, all pigs were sacrificed and the gut was macroscopically inspected for possible damages. No abnormalities or lesions were observed in the intestinal tract of these animals.

In conclusion, SPH and SPHC polymers showed the ability to enhance the peroral absorption of octreotide as a result of their swelling properties and mechanical attachment to the intestinal wall. This mechanical fixation increases the residence time of the delivery systems in the intestine and also opens the tight junctions by water influx from the intestinal cells. The increased peroral bioavailability of octreotide using SPH- and SPHC-based delivery systems indicates that effec-

Table II. Pharmacokinetic Parameters of Octreotide after Peroral Administration in Pigs

	c.o.				c.i.				o.o.				c.o.t.			
Pig no.	t_{max} (h)	C_{max} (ng/mL)	AUC (ng/mL*min)	F%	t_{max} (h)	C_{max} (ng/mL)	AUC (ng/mL*min)	F%[a]	t_{max} (h)	C_{max} (ng/mL)	AUC (ng/mL*min)	F%	t_{max} (h)	C_{max} (ng/mL)	AUC (ng/mL*min)	F%
1	3.5	138.8	5249	3.1	4.5	171.1	6277	3.7	5	70.5	4996	3.0	5	100.0	9442	5.6
2	5	157.9	13832	8.2	2	169.6	14817	8.8	4	0.1	3.0	0.01	6	167.5	15603	9.3
3	6	120.7	13022	7.7	0	0	0	–	3	29.8	4571	2.7	6	177.3	28539	16.9
4	4	160.8	30282	18.0	6	178.3	35789	21.2	3	0.2	14	0.01	3	129.4	36291	21.5
5	4.5	117.9	4566	2.7	5	130.8	14570	8.6	3.5	5.2	157	0.1	5	166.3	25336	15.0
6	2	215.8	21390	12.7	3.5	230.1	36003	21.4	6	1.3	211	0.1	3.5	206.8	47644	28.3
Mean[a] (SEM)				8.7 (2.4)[b]				12.7 (3.6)[b]				1.0 (0.6)				16.1 (3.3)[b]

[a] mean of 5 pigs

[b] c.o., c.i. and c.o.t. are significantly different from o.o. at $p < 0.05$.

c.o. = core outside delivery system; c.i. = core inside delivery system; o.o. = only octreotide (negative control); c.o.t. = core outside delivery system including TMC; t_{max} = time to reach plasma peak concentration; C_{max} = plasma peak concentraion; AUC = area under curve; F = absolute bioavailability.

tive peroral absorption of peptide drugs requires appropriate delivery systems containing different absorption enhancers and excipients for drug targeting and opening of the intestinal tight junctions.

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