Intestinal Absorption of Octreotide Using Trimethyl Chitosan Chloride: Studies in Pigs

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Purpose. To investigate the enhancing effect of trimethyl chitosan chloride (TMC) on the enteral absorption of octreotide and to delineate the required doses of both TMC and peptide *in vivo* in juvenile pigs.

Methods. Six female pigs (body weight, 25 kg) were operated to induce a stoma at the beginning of their jejunum and to insert an in-dwelling fistula for intrajejunal (IJ) administration of the formulations. A silicone cannula was inserted at the jugular vein for blood sampling. One week after surgery the pigs received IJ octreotide solution administrations with or without TMC at pH 7.4 or chitosan HCl at pH 5.5. For determining bioavailability (F) values, the pigs also received an octreotide solution intravenously (IV). Blood samples were taken from the cannulated jugular vein and subsequently analyzed by radioimmunoassay.

Results. Intrajejunal administration of 10 mg octreotide without any polymer (control solution) resulted in F values of 1.7 ± 1.1% (mean ± SE). Chitosan HCl 1.5% (w/v) at pH 5.5 led to a 3-fold increase in F compared to the control (non-polymer containing) formulations. Co-administration of octreotide with 5 and 10% (w/v) TMC at pH 7.4 resulted in 7.7- and 14.5-fold increase of octreotide absorption, respectively (F of 13.9 ± 1.3% and 24.8 ± 1.8%). IJ administration of 5 mg octreotide solutions resulted in low F values of 0.5 ± 0.6%, whereas co-administration with 5% (w/v) TMC increased the intestinal octreotide bioavailability to 8.2 ± 1.5%.

Conclusions. Cationic polymers of the chitosan type are able to enhance the intestinal absorption of the peptide drug octreotide in pigs. In this respect, TMC at neutral pH values of 7.4 appears to be more potent than chitosan HCl at a weak acidic pH of 5.5.

KEY WORDS: peroral delivery; chitosan; trimethyl chitosan (TMC); octreotide; pigs; intestinal absorption.

INTRODUCTION

The successful peroral delivery and absorption of macromolecular drugs appears to be one of the most challenging fields in drug delivery (1). Delivery of macromolecules such as peptide drugs across the intestinal tract has met limited success until recently. The main barriers a peptide encounters after oral administration are its susceptibility toward enzymatic degradation in the gastrointestinal tract and its poor permeation across the intestinal mucosa (2). The use of peptide analogs or protease inhibitors (free or bound to polymers) is a promising approach to avoid enzymatic degradation (3). The permeability barrier can be overcome by the use of permeation/absorption enhancers formulated with the drug of interest. These approaches can increase the clinical utility of peptide drugs, and also the patient compliance (4).

Recently, a class of amino acid derivatives (Emisphere Technologies, NY, USA) has been reported to successfully overcome the intestinal permeability barrier. When taken orally, these formulations show remarkably increased enteral absorption of heparin (unfractionated and low molecular weight), human growth hormone, salmon calcitonin, and interferon-α (5,6). The mechanism of absorption enhancement using this approach is not fully understood. It is assumed that the enhancement effect is achieved by condensing the macromolecular structure with such agents, resulting in facilitated membrane transport. The therapeutic and enhancing agents dissociate after permeation across the intestinal barrier to release the therapeutic into the lymph system. Another assumption is that the macromolecule's hydrophilicity is decreased, thereby facilitating the permeation across the cell membranes. Evidence for the involvement of P-glycoprotein efflux pump has also been found (7).

A different strategy to overcome the intestinal permeability barrier is the use of agents, which are able to increase the paracellular permeation by opening of the intercellular tight junctions. Structurally different polymers have been shown to elicit absorption-promoting effects when co-administered with hydrophilic macromolecular drugs (8). In our department a modified chitosan was synthesized, which appeared be a non-cytotoxic absorption enhancer (9). This trimethylated chitosan (TMC; degree of quaternization, 60%) was demonstrated to be a potent enhancer of the paracellular transport of hydrophilic markers and peptide drugs *in vitro* in Caco-2 cell monolayers, and also an effective intestinal absorption enhancer of peptides *in vivo* after enteral administration in rats (10,11). Compared with the absorption enhancing approach using the amino acid derivatives, the strategy of using polymers with absorption enhancing properties such as TMC has the following advantages: a) because of their polymeric nature (M_w > 100 kDa) and hydrophilic characteristics, the intestinal absorption of these polymers is expected to be negligible (12), and b) they allow for reversibly increased permeation only by the paracellular routes along the enterocytes (8). Additionally, such polymers may show mucoadhesive properties useful for the development of dosage forms.

It should be noted that when a novel excipient is developed the performance of *in vivo* studies with different animal species is required to validate the efficiency and to delineate the effective doses of both absorption enhancer and therapeutic agent. These species should be chosen by especial criteria such as similarity of the digestive system with that of humans and experimental feasibility. TMC was recently found to substantially increase the intestinal absorption and bioavailability of octreotide (an octapeptide drug) in rats (11). TMC is also expected to be a functional polymeric excipient for peroral solid dosage forms of octreotide.

Octreotide acetate was discovered in 1982 to display 7000 times the biological activity of somatostatin and introduced to the market in 1987 as Sandostatin® for the therapy of acromegaly and for the symptomatic treatment of gastroentero-

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pancreatic (GEP) endocrine tumors (13). Sandostatin® is administered by subcutaneous injections two or three times daily. Several approaches have been explored to diminish the inconvenience of the parenteral route, the most successful one being the development of LAR (long-acting release) preparations. Sandostatin®, LAR®, and OncoLar® are composed of polymeric microcapsules for intramuscularly injected depot systems (14). However, dosage forms for peroral administration would exhibit higher patient compliance and other advantages over parenteral formulations. The design of such formulations should be based on information regarding the dosing of both polymer and peptide. Therefore, it was decided to perform intestinal absorption studies in a different animal species. The juvenile pig was chosen because of the similarity of its gastrointestinal tract with that of humans (15).

MATERIALS AND METHODS

Materials

Chitosan (Seacure 244) and Chitosan HCl (Seacure CI 210) were generous gifts of Pronova AS (Drammen, Norway). Octreotide acetate, [I^{125}]-radiolabelled Tyr-1-octreotide and octreotide-antiserum were kindly donated by Novartis Pharma, (Basel, Switzerland). Methyliodide and N-methylpyrrolidinone were obtained from Acros (Geel, Belgium). Narketan® (ketamine) was purchased from Chassot (Vught, The Netherlands). Sufenta® (sufentanil) and Stresnil® (azaperon) were from Jansen-Cilag (Tilburg, The Netherlands). Dormicum® (midazolam) was from Hoffmann-La Roche (Mijdrecht, The Netherlands). Temgesic® (buprenorfine) was purchased from Schering-Plough (Maarsen, The Netherlands). Clamoxyl® (amoxicilline) was obtained from Smith-Kline Beecham Farma (Rijswijk, The Netherlands). Depomycin® (aqueous suspension containing procaine, penicilline-G and dihydrostreptomycin) was from Mycofarm (De Bilt, The Netherlands). Vials containing Li-Heparin (Monovette®) for blood sample collection were obtained from Sarstedt (Etten-Leur, The Netherlands). All other reagents were of analytical grade.

Preparation of TMC Polymer

N-trimethyl chitosan chloride (TMC) was synthesized as previously described (16). Briefly, sieved chitosan (93% deacetylated; viscosity 40 mPas · sec) with a particle size of 200–400 μm was mixed with methyliodide in an alkaline solution of N-methylpyrrolidinone at 60°C for 75 min. This obtained product underwent a second step of reductive methylation, to yield the final product TMC60 iodide (60% degree of trimethylation). The product was precipitated by addition of ethanol and isolated by centrifugation. The purification step of the final product included the exchange of the counterion iodide with chloride in a NaCl aqueous solution and extensive washing with ethanol and diethylether. The product was dried in vacuo and measured for the degree of quaternization by ^{1}H-NMR, using a 600 MHz spectrometer (Bruker, Switzerland). The degree of trimethylation was calculated to be 60%.

Animals

The experimental protocol was approved by the Ethical Committee for animal experimentation (Veterinary Faculty of Utrecht University). Female pigs were used. Before and during the experiments the pigs were housed in the animal facilities of the Central Laboratory Animal Institute (Utrecht University, Utrecht, The Netherlands). Before surgery and experimentation, the animals were fasted overnight, but having access to water *ad libitum.* The animals weighed approximately 25 kg before starting the experiments.

Surgery of the Piglets

Six female pigs were anesthetized to induce a stoma at the beginning of the jejunum and to insert a silicone cannula at the jugular vein. The animals received a mixture of ketamine, atropine, and thiopental as pre-anesthesia. During surgery the anesthesia was sustained by infusion of a mixture of sufentanyl forte. A vertical incision of 10 cm at the peritoneum was made to localize the beginning of the jejunum and the insertion of the fistula. Then a stoma of 1.5 cm diameter was opened through the skin, underlying muscles, and intestinal wall of the located point. A silicone fistula of T-shape (2 cm o.d. and 1.5 cm i.d.) was inserted into the stoma and fixed at position with the semicylindrical horizontal part fitting to the interior of the intestine and the cylindrical vertical part protruding through the intestinal wall, adipose tissue, abdominal muscles, and skin out of the body. The fistula was closed firmly with a silicone tap. The peritoneum of the animals was closed by stitching. Subsequently, the fistula was fixed on the skin, using a wide adhesive band which was changed every other day. A thin silicone and heparinized cannula was inserted into the jugular vein, and the other edge of the cannula was guided with a metal bar at the dorsal part of the animal's neck. To avoid postoperative pain, the animals received buprenorfine intravenously. Amoxicilline was administered before and after surgery for the prophylaxis of infection. To prevent infection of the opening created at the dorsal part during the experiments, the animals were receiving three to four times/day Depomycin® locally.

Preparation of Octreotide Formulations

Octreotide acetate for IV administration was prepared as a 2 ml stock solution in sterile water for injection and added in 98 ml sterile, pyrogen-free physiological saline solution at final concentration of 100 μg/ml. Control solutions of octreotide acetate alone were prepared at 500 μg/ml and 250 μg/ml in physiological saline (pH = 7.4; control solutions). Chitosan HCl was dissolved at 1.5% (w/v) in control solution of 500 μg/ml and the pH was adjusted at 5.5 with 0.1 M NaOH. TMC was dissolved at 10 and 5% (w/v) in control of 500 μg/ml and 5% (w/v) in control solution of 250 μg/ml. The pH of all TMC formulations was 7.4. The compositions of the aforementioned solutions are given in Table I.

Administration of Octreotide Formulations

The pigs received administrations according to a randomized cross-over setup. The drugs were administered to the animals every other day at 48 h interval between administrations to ensure complete wash-out of the peptide. Before administration, the animals were sedated by subcutaneous injection of an azaperon/ketamine mixture to facilitate intrajejunal (IJ) and IV administration. The duration of the sedation was approximately 15 min. During the sedation of the

Table I. Octreotide Formulations[a]

Administration	Concentration octreotide (μ/ml)	Route	Polymer	Polymer conc. (% w/v)	pH
OA10	500	IJ	—	—	7.4
OA5	250	IJ	—	—	7.4
CS1.5	500	IJ	Chitosan HCl	1.5	5.5
TMC10	500	IJ	TMC	10	7.4
TMC5	500	IJ	TMC	5	7.4
TMC5/5	250	IJ	TMC	5	7.4
OA	100	IV	—	—	7.4

[a] IJ and IV are the abbreviations of intrajejunal and intravenous administrations, respectively. OA, octreotide acetate; TMC, trimethyl chitosan chloride; CS, chitosan. Number codes after OA refer to octreotide dose per animal; number codes after polymer refer to polymer concentration (w/v) in 10 mg octreotide dosing solution; in case for TMC5/5 the second number refers to the 5 mg octreotide dose per animal.

animals, three blank blood samples of 4 ml were withdrawn from the jugular vein. An elastic tube of 15 cm connected to a syringe of 50 ml containing the IJ octreotide formulations, was inserted into the fistula and guided into the jejunum approximately 5 cm to ensure complete dosing. 20 ml of IJ formulations were administered at a rate of 10 ml/min. Thereafter, the fistula was tightly closed and inspected for possible leaking. At the end of this procedure the animals recovered to consciousness. For IV purposes, 5 ml of an octreotide solution (100 μg/ml) were given via the cannulated jugular vein with a 5 ml syringe, followed by 5 ml of sterile pyrogen-free physiological saline to ensure complete dosing. Blood samples of 4 ml were collected from the cannula inserted in the jugular vein, and 4 ml of sterile pyrogen-free physiological saline were given back. Two ml of heparinized physiological saline (50 U/ml) were administered to fill the dead volume of the cannula to avoid blood clotting. Blood samples of 4 ml were withdrawn at 2, 5, 10, 15, 30, 45, 60, 120, and 180 min after IV administration, and at 20, 40, 60, 90, 120, 150, and 180 min after IJ administration. Blood samples were placed directly on ice and then centrifuged for 15 min at 3000 rpm and 4°C. The plasma obtained was stored at −20°C until analysis. At the end of all experiments the animals were sacrificed by an overdose of pentobarbital and the intestines were inspected macroscopically for possible damage.

Analysis of Octreotide

The analysis of plasma samples on octreotide concentrations was performed by radioimmunoassay as previously described (17). 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

The plasma profiles of octreotide, after IV bolus injection, were fitted using WinNonlin program (Scientific Consulting Inc., Palo Alto, CA USA). The serum 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 equals the serum concentration of octreotide at time t, and A_1, A_2, α_1, α_2 are the coefficients and exponents of this equation. The pharmacokinetic parameters were calculated according to Gibaldi and Perrier (18). The areas under the individual concentration-time curves (AUC) were calculated with the linear trapezoidal rule. Absolute bioavailability values after IJ administration of octreotide were calculated according to:

$$F = \frac{AUC_{IJ} \times D_{IV}}{AUC_{IV} \times D_{IJ}} \times 100\%$$

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

Absorption enhancement ratios were calculated by the following formula:

$$ER = \frac{F(\text{octreotide + polymer})}{F(\text{octreotide alone})}$$

The data were evaluated for statistically significant differences by one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Table II summarizes the pharmacokinetic parameters after IV administration of 500 μg octreotide in pigs. The plasma levels were fitting to a 2-compartment model, resulting in a short distribution half-life of about 5 min and a relatively long elimination half-life of about 40 min, quite similar as previously reported in rats (11,19).

Two cationic polymers were tested for their ability to increase the intestinal absorption of octreotide in pigs: chitosan and its quaternized modification TMC. Chitosan HCl dissolved at concentrations of 1.5% (w/v) in physiological saline, yielded a gel at pH value of 5.5 (CS1.5; Table I). Increasing

Table II. IV Administered Octreotide in Pigs

Parameters	Mean ± SE (n = 6)
$t_{1/2}$ dist[a] (min)	5.1 ± 1.2
$t_{1/2}$ elim (min)	39.5 ± 4.2
V_d (ml/kg)	86.9 ± 17.7
Cl (ml/min · kg)	6.4 ± 0.5

[a] $t_{1/2}$ dist., distribution half-life; $t_{1/2}$ elim., elimination half-life; Cl, clearance; V_d, volume of distribution.

the concentration, this type of chitosan started to flocculate. The same phenomenon was observed by increasing the pH value of the solution above 6. On the contrary, the quaternized polymer TMC was well soluble and yielded a gel at concentrations of 10% (w/v) and neutral pH value (TMC10; Table I), and with similar viscosity as 1.5% (w/v) chitosan HCl at pH 5.5. This relatively high concentration of the TMC polymer was chosen to counteract the dilution of the 20 ml administration volume by the luminal fluids and mucus of the intestinal tract and to ensure that substantial amounts of both peptide and enhancer could reach the absorptive site of the intestinal mucosa. A second octreotide formulation with 5% (w/v) TMC and pH 7.4 (TMC5; Table I) was administered to examine the effect of the concentration of the absorption enhancer on the intestinal absorption of the same dose of octreotide (10 mg).

The effect of the polymer concentrations on the intestinal absorption of 10 mg octreotide acetate is depicted in Fig. 1. Chitosan HCl at concentrations of 1.5% (w/v) and pH 5.5 significantly increased the absorption of the peptide (Fig. 1; Table III). This effect is in agreement with previously presented data, demonstrating that protonated chitosan is a potent absorption enhancer (20–22). Nevertheless, TMC containing formulations exceeded the potency of chitosan HCl, and increased the intestinal absorption of a 10 mg octreotide dose even more (Fig. 1; Table III). This could be attributed to the absorption enhancing action of TMC at neutral pH values, which is related to its solubility properties and possibly to the high TMC concentrations of the formulation. As shown in Table III, TMC 10% (w/v) remarkably increased the bioavailability of the peptide up to 25% with an enhancement ratio ER of 14.5, whereas TMC 5% (w/v) resulted in an absolute bioavailability of 13.9% and an ER of 7.7.

It may be possible that smaller M_w chitosans would have been better soluble, and that higher concentrations of the polymer would have comparable absorption enhancing effects as TMC. However, it has been reported that soluble chitosans with low average M_w were not able to decrease the transepithelial electrical resistance (TEER, being an indicative measure for the opening of the paracellular pathways) of Caco-2 cells, and that a degree of polymerisation (DP) ≥50 was necessary to induce a reduction in TEER (23). Therefore, chitosan may encounter problems in its application as absorption enhancer in the small intestine. Soluble chitosans of low M_w would have little effect as absorption enhancers, whereas chitosans of high M_w would show poor aqueous solubility and minimal effect in the neutral environment of the small intestine. On the contrary, TMC polymers do not show these aqueous solubility problems and are therefore suitable for application as absorption enhancer in the intestinal tract.

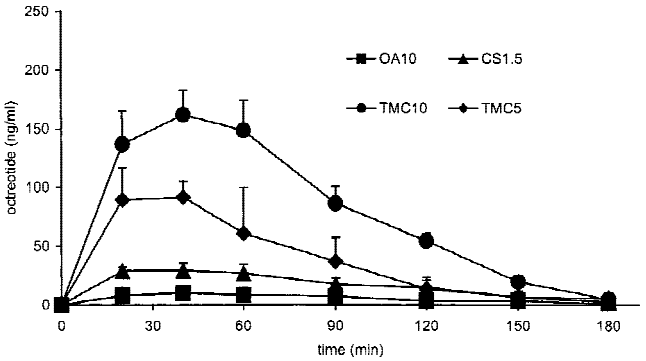

Fig. 1. Plasma octreotide concentration (mean ± SE) versus time curves after IJ administration of 10mg/20ml/pig with the polymers chitosan HCl [CS1.5: 1.5% (w/v); pH = 5.5; n = 6] and TMC [TMC10: 10%(w/v); pH = 7.4; n = 6 and TMC5: 5%(w/v); pH = 7.4; n = 3] or without any polymer [OA10: octreotide in 0.9% NaCl; pH = 7.4; n = 5].

Following IJ administration of octreotide without any polymer (OA 10; Table I), peptide bioavailability was observed to be low (1.7 ± 1.1%; Table III). These results are quite similar to previously reported data in rats (11,24). It has also been shown that orally administered octreotide-containing capsules in healthy male volunteers resulted in a bioavailability of 0.6% (25).

Table III. Intestinal Absorption of Octreotide in Pigs

Application (see Table I)	t_{max}[a] (min)	C_{max} (ng/ml) mean ± SE	AUC (ng/ml*min) mean ± SE	F(%) mean ± SE	ER	n
OA10	40	9.8 ± 5	1001 ± 625	1.7 ± 1.1	1	5
CS1.5	40	29.0 ± 6.4[b]	3016 ± 648	5.1 ± 1.1[c]	3.0	6
TMC10	40	161.8 ± 20.7[d,e]	14523 ± 1070	24.8 ± 1.8[d,e]	14.5	6
TMC5	40	91.5 ± 13.6[d,e,g]	7719 ± 786	13.9 ± 1.3[d,f,h]	7.7	3
OA	2	206.4 ± 26.0	2926 ± 497	100	—	6
OA5	40	1.7 ± 1.1	178 ± 159	0.5 ± 0.6	1	3
TMC5/5	40	43.9 ± 13.8[i,k]	3836 ± 795	8.2 ± 1.5[j,k]	16.2	3

[a] t_{max}, time to reach plasma peak concentration; C_{max}, plasma peak concentration; F, absolute bioavailability; ER, absorption enhancement ratio; n, number of animals.
[b] Significantly different from OA10, $P < 0.005$.
[c] Significantly different from OA10, $P < 0.05$.
[d] Significantly different from OA10, $P < 0.001$.
[e] Significantly different from CS1.5, $P < 0.001$.
[f] Significantly different from CS1.5, $P < 0.005$.
[g] Significantly different from TMC10, $P < 0.05$.
[h] Significantly different from TMC10, $P < 0.005$.
[i] Significantly different from OA5, $P < 0.001$.
[j] Significantly different from OA5, $P < 0.005$.
[k] Significantly different from TMC5, $P < 0.05$.

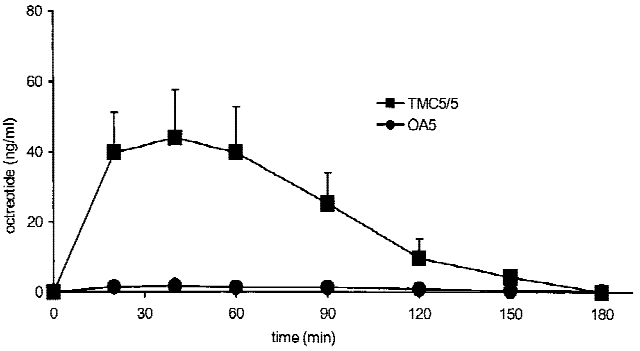

Fig. 2. Plasma octreotide concentration (mean ± SE) versus time curves after IJ administration of 5mg/20ml/pig with TMC [TMC5/5: 5% (w/v); pH = 7.4; n = 3] and without any polymer [OA5: octreotide in 0.9% NaCl; pH = 7.4; n = 3].

When 5% (w/v) TMC polymer was co-administered with .5 mg octreotide intrajejunally (TMC5/5; Table I) a significant increase in all absorption parameters was observed compared to the absorption of octreotide alone (see Fig. 2 and Table III). Mean octreotide bioavailability and enhancement ratio were 8.2% and 16.2, respectively. These values were significantly lower than those obtained with formulation TMC5 (10 mg octreotide with 5% TMC), indicating that the dose of peptide drug is also an important factor in studying the influence of TMC as intestinal absorption enhancer.

During experimentation the animals showed no stress or discomfort due to the presence of the fistula. At the end of all experiments the pigs were sacrificed and the gut was macroscopically inspected for possible lesions. All animals showed normal intestinal morphology and no bleeding or damage was observed either around the stoma or along the intestinal tract.

Several parameters have recently been studied that affect the intestinal absorption of the peptide drug salmon calcitonin. Using the beagle dog as an *in vivo* model, it was found that regional pH is an important factor that influences the absorption of this particular peptide (26). Additionally, using intestinal access ports it was observed that less dilution and spreading in the lower small intestine favored the calcitonin absorption, due to the induced high concentrations of pharmaceutical excipients and peptide at a restricted site in the intestine (26). Intestinal spreading and dilution of the present TMC/octreotide formulations may be the reason for the observed peptide absorption peaks at 40 min after IJ administration, thereafter followed by a decline (Fig. 1 and 2). This implies that the design of solid dosage forms of octreotide with TMC should entail controlled and/or delayed release characteristics of both peptide and polymeric absorption enhancer. Current research is now focused on the development and optimization of solid dosage forms containing TMC as absorption enhancing excipient. These oral formulations will also be evaluated *in vivo* in pigs.

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