Intestinal Absorption of Octreotide: *N*-Trimethyl Chitosan Chloride (TMC) Ameliorates the Permeability and Absorption Properties of the Somatostatin Analogue *In Vitro* and *In Vivo*

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ABSTRACT: Octreotide acetate is a somatostatin analogue used for the control of endocrine tumors of the gastrointestinal (GI) tract and the treatment of acromegaly. The oral absorption of octreotide is limited because of the limited permeation across the intestinal epithelium. Both chitosan hydrochloride and *N*-trimethyl chitosan chloride (TMC), a quaternized chitosan derivative, are nonabsorbable and nontoxic polymers that have been proven to effectively increase the permeation of hydrophilic macromolecules across mucosal epithelia by opening the tight junctions. This study investigates the intestinal absorption of octreotide when it is coadministered with the polycationic absorption enhancer TMC. Caco-2 cell monolayers were used as an *in vitro* intestinal epithelium model, and male Wistar rats were used for *in vivo* studies. Octreotide with or without polymers (TMC; chitosan hydrochloride) was administered intrajejunally in rats, and serum peptide levels were measured by radioimmunoassay. All applications and administrations were performed at neutral pH values (i.e., pH = 7.4). In vitro transport studies with Caco-2 cells revealed an increased permeation of octreotide in the presence of TMC. Enhancement ratios ranged from 34 to 121 with increasing concentrations of the polymer (0.25–1.5%, w/v). In rats, 1.0% (w/v) TMC solution significantly increased the absorption of the peptide analogue, resulting in a 5-fold increase of octreotide bioavailability compared with the controls (octreotide alone). Coadministration of 1.0% (w/v) chitosan hydrochloride did not enhance octreotide bioavailability. These results in combination with the nontoxic character of TMC suggest that this polymer is a promising excipient in the development of solid dosage forms for the peroral delivery and intestinal absorption of octreotide. J Pharm Sci 89:951–957, 2000

Keywords: octreotide; TMC (trimethyl chitosan); tight junctions; permeation; absorption; rats

INTRODUCTION

The use of peptide analogues has been the therapeutic strategy for several diseases for the last two decades. Compared with native peptides, these analogues are resistant to metabolic degradation in the living organisms. Their unfavorable physicochemical properties, such as molecular weight and polarity, have excluded these agents from being formulated as solid dosage forms for transmucosal delivery and absorption (e.g., intestinal, rectal, or nasal). An example of these therapeutic peptides is the somatostatin analogue octreotide. In 1982,[1] octreotide acetate was shown to display 7000 times the biological activity of somatostatin and, in 1987, it was introduced to the

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market as Sandostatin® for the therapy of acromegaly and for the symptomatic treatment of gastroentero-pancreatic (GEP) endocrine tumors. Later on, it was registered for the control of refractory diarrhoea associated with AIDS[2] and for the prevention of complications following pancreatic surgery. 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 formation of the LAR (long-acting release) preparations. Sandostatin® LAR® and OncoLar® are both composed of polymeric microcapsules for intramuscularly injected depot systems.[3] Another approach that reached clinical studies was the employment of the nasal route.[4]

The peroral route was one of the early approaches for administration of octreotide.[5,6] Fricker and Drewe studied the enteral absorption of octreotide after coadministration with several structurally different absorption enhancers, both *in vitro* and *in vivo*.[7,8] Bile salts, like ursodeoxycholate (UDCA) and its 7-α enantiomer chenodeoxycholate (CDCA), substantially increased the oral bioavailability of octreotide in rats and healthy volunteers. From studies with liposomal membranes, it was suggested that membrane distortion was a possible cause of the enhanced absorption of octreotide in the presence of bile salts.[9] However, none of these approaches led to a solid dosage form for peroral administration because of harmful effects of the absorption enhancers.

The use of mucoadhesive polymers, which are able to open the intercellular tight junctions of the epithelia and promote the paracellular permeation of normally nonabsorbable and hydrophilic macromolecules, is another approach for improving peroral peptide delivery.[10] One of these polymers that display a significant absorption-enhancing effect, mucoadhesion, and nontoxic characteristics is chitosan. Chitosan has been proven to increase the absorption of peptides and their analogues *in vivo* when administered in a slightly acidic environment. At neutral pH values, however, chitosan is not soluble and is therefore ineffective as a penetration enhancer. To overcome this disadvantage, the quaternized chitosan derivative TMC (trimethyl chitosan chloride) was evaluated as a potential absorption enhancer of peptides and other hydrophilic macromolecular drugs.[11,12] TMC was nontoxic to Caco-2 cell monolayers, and no disruption of the cell membranes could be detected.[13] The present study is part of a series of *in vivo* studies with TMC polymers as potential enhancers for transmucosal absorption of peptide drugs.[14,15] Another purpose of this study is to prove the potency of TMC as an intestinal absorption enhancer for the peptide drug octreotide in vitro and *in vivo*. The permeation properties of octreotide across Caco-2 cell monolayers in the presence of TMC are investigated, and the enteral absorption of octreotide coadministration with TMC is evaluated *in vivo* in rats.

EXPERIMENTAL SECTION

Synthesis of TMC

N-Trimethyl chitosan chloride (TMC) was synthesized as previously described.[16] Briefly, sieved chitosan (Seacure 244, Pronova AS, Drammen, Norway; 93% deacetylated; viscosity 40 mPas · s) with a particle size 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 solution and extensive washing with ethanol and diethylether. The product was dried in vacuo and measured for the degree of quaternization by proton nuclear magnetic resonance spectroscopy (^{1}H NMR) with a 600 MHz spectrometer (Bruker, Switzerland). The degree of trimethylation was calculated to be 60%. This product is defined as TMC60.

Octreotide Acetate

Octreotide acetate (SMS -201-995), I^{125}-radiolabeled Tyr-1-octreotide, and SMS 201-995 antiserum were kindly donated by Novartis (Novartis Pharma AG, Basel Switzerland).

Caco-2 Cells

Caco-2 cell cultures of passage number 78 were used for the experiments. The cells were seeded on tissue culture polycarbonate membrane filters (pore size, 0.4 μm; area, 4.7 cm^2; noncoated) in Costar Transwell 6-well plates at a seeding density of 10^4 cells/cm^2. Dulbecco's Modified Eagle

Medium (DMEM), supplemented with 1% nonessential amino acids, 10% fetal calf serum, benzylpenicillin G (160 U/mL), and streptomycin sulfate (100 μg/ml) was used as culture medium, and added to both the donor and the acceptor compartments. The medium was changed every second day. The cell cultures were kept at a temperature of 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. For all experiments, cells were used 23–25 days after seeding. The transepithelial electrical resistance (TEER) was checked prior to the experiment with a Millicell® ERS meter (Millipore Corp., Bedford, MA) connected to a pair of thin, side-by-side electrodes, and the values ranged from 1000 to 1200 $\Omega \cdot cm^2$. Two hours before the experiments, the medium was changed to HBSS (Hanks' Balanced Salt Saline) buffered to pH 7.4 with 40 mM *n*-(2-hydroxyethyl) piperazine-*N*-(2-ethanosulfonic acid) (HEPES).

In Vitro Transport Studies

TMC60 at different concentrations was dissolved in HBSS–HEPES containing 200 μg/mL octreotide acetate. The pH of application was adjusted at 7.4. Apical aliquots of 2.5 mL containing the polymers and the peptide were applied on the cells, and the transport of the peptide was monitored by basolateral sampling over 4 h. Samples of 200 μL were added to 200 μL of 0.1 M ammonium acetate buffer solution (pH = 8.2) and analyzed by HPLC-UV_{218nm} spectroscopy for their content of octreotide. Isocratic elution was performed with 0.1M ammonium acetate buffer (pH = 8.2) containing 27% acetonitril at a flow rate of 1 mL/min. A 100 × 3 mm Chromspher 5 C18 column equipped with a Cromspher 5 10 × 46 mm precolumn (Chrompack, Middelburg, The Netherlands) was used. In this system, the retention time of octreotide was ~4 min. The apparent permeability coefficient was calculated according to the equation:

$$P_{app} = \frac{dQ}{dt(A \cdot 60 \cdot C_0)} \quad (1)$$

where P_{app} is the apparent permeability coefficient (cm/s), dQ/dt is the permeability rate (amount permeated per min), A is the diffusion area of the monolayer (cm^2), and C_0 is the initial concentration of the peptide. Transport enhancement ratios (ER) were calculated from P_{app} values according the formula:

$$ER = \frac{P_{app\ polymer}}{P_{app\ control}} \quad (2)$$

At the end of all experiments the viability of the monolayers was routinely checked by the trypan blue exclusion technique.[12] Cells that excluded trypan blue were considered to be viable.

In Vivo Studies in Rats

The protocol for the animal studies was approved by the Ethical Committee of Leiden University. The experimental procedure was slightly different from previously reported studies. In brief, male Wistar rats SPF (average body weight, 250 g) were obtained from Harlan (Zeist, The Netherlands). The animals were fasted for 18 h prior the experiment, with free access to water. The animals were anesthetized with Hypnorm® (1.5 mL/kg body weight) and Dormicum® (500 μg midazolam/kg body weight). Body temperature was monitored rectally and kept at 36.5–37 °C. After octreotide administration (intrajejunally or intravenously), blood sampling was performed through a cannula that was previously inserted into the right carotic artery. Samples of 200 μL were withdrawn at predetermined time points, and 200 μL of heparinized physiological saline (25 anti-Xa U/mL) were subsequently administered to the rat through the same cannula to prevent blood clotting and to compensate for blood loss during sampling. Blood samples were centrifuged (13,000 rpm for 15 min) and serum samples were collected and stored at –20 °C until analysis.

To determine the pharmacokinetic parameters of octreotide, a group of 6 animals received octreotide intravenously (iv). The femoral vein was cannulated and a bolus of 20 μg of octreotide acetate dissolved in 100 μL of sterile physiological saline was injected into the cannula. To ensure complete dosing, the injection cannula was flushed afterwards with 200 μL of physiological saline. Blood samples were taken from the same cannulated vein and treated as already described.

Formulations and Intrajejunal Administration

TMC60 solutions and chitosan hydrochloride dispersions were prepared at concentrations of 1% (w/v) in physiological saline. An amount of 100 μg octreotide acetate was dissolved per milliliter of the control (physiological saline) and the different polymer preparations. The pH of the formulations

was readjusted with 0.1 M NaOH or 0.1 M HCl to values of 7.4.

The peritoneum of the animals was opened and the beginning of the jejunum was localized 5 cm distally to the ligament of Treitz. To administer the octreotide formulations intrajejunally, a Teflon flexible tube connected to a syringe was inserted by a small incision (2 mm) into the jejunum in such a way that the end of the tube was located at least 5 cm distally from the incision. Then, 2 mL of the octreotide formulations were administered slowly. Afterwards, the tube was removed and the incision at the jejunum was closed and rinsed thoroughly with physiological saline before closing the abdomen of the animal.

Octreotide Analysis in Serum

The analysis of serum samples for octreotide concentrations was performed by radioimmunoassay as previously described.[18] To avoid interassay variations, all samples were analyzed in one assay.

Pharmacokinetic Analysis of Data

The serum profiles of octreotide after iv bolus injection were fitted using the WiNnonlin program (Scientific Consulting Inc., Palo Alto, CA). The serum concentration–time profiles were fitted according to:

$$C_t = A_1 e^{-\alpha 1 t} + A_2 e^{-\alpha 2 t} \quad (3)$$

where C equals the serum 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.[19] The areas under the individual concentration–time curves (AUC) were calculated with the linear trapezoidal rule. Absolute bioavailability values after intraduodenal administration of octreotide were calculated according to:

$$F = \frac{\mathrm{AUC}_{\mathrm{id}} \times D_{\mathrm{iv}}}{\mathrm{AUC}_{\mathrm{iv}} \times D_{\mathrm{id}}} \times 100\% \quad (4)$$

in which F is the absolute bioavailability and D is the administered dose. The data were evaluated for statistically significant differences by one way analysis of variance (ANOVA).

RESULTS

In Vitro Transport Studies Across Caco-2 Cell Monolayers

Caco-2 cell monolayers were used as an intestinal *in vitro* model to evaluate the potency of TMC60 to increase the paracellular permeation of octreotide. Figure 1 shows the P_{app} and ER values as a function of the polymer concentration. Octreotide permeation across the Caco-2 monolayer was very limited, resulting in P_{app} of $5.5 \cdot 10^{-9}$ cm/s. In the presence of TMC60 the P_{app} value was increased from $1.9 \cdot 10^{-7}$ cm/s for 0.25% (w/v) polymer up to $6.7 \cdot 10^{-7}$ cm/s for 1.5% (w/v) polymer. The respective enhancement ratios ER increased from 34 for 0.25%(w/v) to 121 for 1.5% (w/v) polymer as obvious from Figure 1. When the P_{app} values were plotted versus the concentration of the polymeric absorption enhancer, a linear correlation was observed ($R = 0.99$), indicating a specific interaction of the cationic polymer with components of the tight junctions, nonsaturated within the used concentration range. At the end of all experiments, no cellular uptake of trypan blue could be detected, which is indicative of the viability of the monolayers and the integrity of the cell membranes.

Enteral Absorption of Octreotide in Rats

Figure 2 shows the serum peptide concentrations versus time after intrajejunal administration of

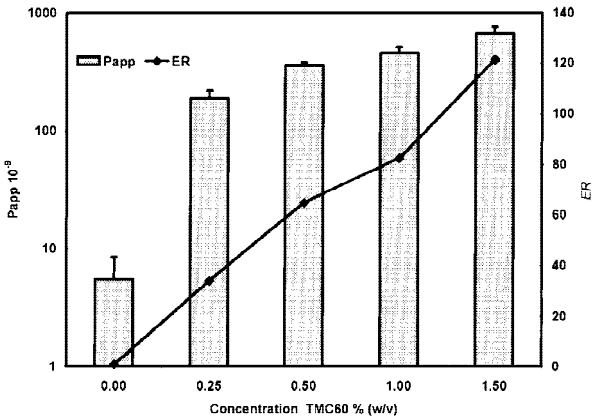

Figure 1. Transport of octreotide acetate across Caco-2 cell monolayers in the absence and the presence of increasing concentrations of TMC60 at pH = 7.4 (mean ± SE; $n = 3$). Bars represent P_{app} values, and line represents the enhancement ratio ($R = P_{app}$ polymer/$P_{app\ control}$). Linear correlation between P_{app} and concentration of the polymer; $R = 0.99$).

octreotide in rats. When octreotide was administered alone (i.e., without polymers), limited absorption was observed. This low level of absorption is in agreement with previously reported animal studies.[20] Chitosan hydrochloride (1%) did not manage to increase the absorption of octreotide compared with the control cases. This result can be explained by the poor solubility of chitosan at neutral pH values. However, when octreotide was coadministered with 1% TMC60, substantial increases in serum peptide concentrations were found. TMC60 increased the absolute bioavailability of the peptide up to 16%.

Table 1 summarizes the pharmacokinetic parameters of the intrajejunally administered octreotide. As obvious from this table, TMC60 provoked a 5-fold increase in C_{max}, AUC, and bioavailability (F) compared with control values. In contrast, chitosan hydrochloride did not enhance the intestinal octreotide absorption.

In Figure 2 the serum octreotide concentrations after iv administration of octreotide are also presented. These levels fit a two-compartment model with a short distribution half-life and a relatively longer elimination half-life, as previously described.[21] Table 2 summarizes the pharmacokinetic parameters after iv administration of 20 μg of octreotide per rat.

DISCUSSION

The present study demonstrates the efficacy of the cationic polymer TMC to increase the absorption of the peptide drug octreotide across intestinal epithelia. In this case, Caco-2 cell cultures were used to investigate the effect of increasing concentration of the polymer on the permeation properties of octreotide. Previous studies[12] showed that chitosan HCl was not able to increase the paracellular permeation of the radiolabelled marker [^{14}C] mannitol across Caco-2 epithelia at neutral pH values. Chitosan appeared to aggregate at these pH values and to loose its ability to open the tight junctions.[12] Therefore, chitosan itself was excluded from the present investigations on octreotide transport across Caco-2 epithelia. Octreotide alone as a hydrophilic molecule poorly permeated the Caco-2 intestinal epithelia, whereas TMC60 managed to increase the permeation of the peptide in a concentration-dependent way. Increasing concentrations of the polymer resulted in a linear increase of the transport of octreotide across the Caco-2 monolayers, which indicates a specific nonsaturated interaction of the soluble quaternized chitosan derivative with the tight junctions. *In vivo*, octreotide without coadministration of TMC60 showed an absolute bioavailability of 3% from the jejunum of rats, indicating that the Caco-2 intestinal epithelia represent a rather "tight" model concerning paracellular permeation because octreotide per se poorly permeated the Caco-2 cell monolayers as shown in the present study. TMC60 (1.0% w/v) turned out to increase the absolute bioavailability of the peptide up to 16%. The high absorption enhancement ratio caused by 1.0% TMC60 in the *in vitro* study with the Caco-2 cell monolayers just mentioned (ER = 80; Figure 1) was not found *in vivo* (5-fold increase in the bioavailability; Table 2). An explanation for this result may be the better permeation properties of octreotide per se across the jejunum compared with other sites of the small intestine.[20] In addition, *in vivo* phenomena such as distribution and clearance are also likely to diminish enhancement ratios compared with those observed *in vitro*.

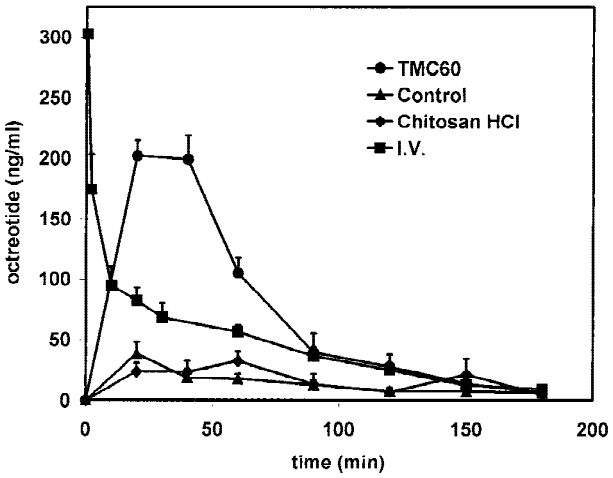

Figure 2. Serum octreotide levels after administration in rats (iv administration of 20μg per animal). Intrajejunal administration of 200μg in physiological saline with or without (control) 1.0% polymer. The pH of all applications was 7.4 (mean ± SE; $n = 6$).

Similar bioavailability values to the ones presented in this study were observed when octreotide was coadministered with bile salts. Chenodeoxycholate (CDCA) showed an increase on the absorption efficiency of the peptide of 20% when coadministered with 1% CDCA in rats. When healthy volunteers received 4 mg of octreotide with 100 mg of CDCA orally, an average bioavailability of the peptide of 1.26% was achieved.[9] Whereas the mechanism of the enhancing effect by bile salts could be attributed to increase of the

Table I. Intrajejunally Administered Octreotide Acetate[a]

Polymer	T_{max} (min)[b]	C_{max} (ng/mL)[c]	AUC (ng/mL · min)	F(%)[d]
Control	20	38.4 ± 9.4	2537 ± 485	3.1 ± 0.6
Chitosan HCl	60	32.5 ± 6.4	3095 ± 638	3.8 ± 0.8
TMC60	20	201.8 ± 12.5*[e]	12948 ± 735*	15.9 ± 0.9*

[a] Octreotide was administered at a dose of 200 μg/rat. Data are presented as mean ± SE of 6 animals.
[b] Time to reach serum peak concentration.
[c] Serum peak concentration.
[d] Absolute bioavailability.
[e] Asterisk indicates significant difference from control and chitosan HCl ($p < 0.005$).

permeation through both the paracellular and transcellular route (due to cell membrane disruption), TMC60 has not been found to affect the integrity of intestinal cell membranes but only allows for increased paracellular transport of hydrophilic marker compounds.[13]

The mechanism by which TMC60 opens the epithelial tight junction is not yet elucidated. Preliminary results from our laboratory indicate that TMC60 is able to open the tight junctions through a Ca^{++}-independent mechanism. TMC60 provokes a redistribution of cytoskeletal F-actin, as demonstrated by confocal laser scanning microscopy visualization studies, but does not influence the intracellular Ca^{++} concentrations of Caco-2 cell monolayers (to be published).

In the last two years the knowledge on the physiology and regulation of the tight junctions has increased remarkably.[22] New transmembrane proteins involved in the tight junction complex have been identified. Claudin-1 and -2 appear to be co-localized with occludin, and another transmembrane protein (the junctional adhesion molecule) has also shown to be localized to the tight junctions. It may be possible that TMC60 is able to interact with these tight junctional proteins by means of physicochemical interactions, leading to a transient disruption of the integrity of the tight junctions. Nevertheless, more investigations are necessary to elucidate the mechanism by which TMC60 regulates the opening of tight junctions.

Table II. Intravenously Administered Octreotide Acetate[a]

Parameter	Mean ± SE
body weight (g)	249 ± 15
$t_{1/2}$ dist (min)[b]	1.0 ± 0.3
$t_{1/2}$ elim (min)[c]	51.6 ± 2.5
V_d (mL/kg)[d]	51.6 ± 9.4
Cl (mL/min · kg)[e]	2.3 ± 0.1

[a] Octreotide was administered at a dose of 20 μg/rat. Data are presented as mean ± SE of 6 rats.
[b] Distribution half-life.
[c] Elimination half-life.
[d] Volume of distribution
[e] Clearance.

The cationic character of TMC60 appeared to be compatible with the basic properties of octreotide. No aggregation or precipitation phenomena were observed during the preparation of these formulations at neutral pH values. On the contrary, chitosan precipitated at this pH value, leading to limited intestinal absorption of octreotide after coadministration with 1.0% (w/v) chitosan hydrochloride dispersion and not significantly different from that found after intrajejunal administration of octreotide without any polymer.

In conclusion, at concentrations of 0.25–1.5% (w/v), TMC60 was found to increase the permeation of octreotide across Caco-2 cell monolayers. *In vivo* 1.0%(w/v) TMC60 was also able to substantially increase the absorption of octreotide after intrajejunal administration in rats. As shown in previous studies, TMC60 does not elicit deleterious effects on intestinal cell membranes.[13] Therefore, TMC60 is suggested to be a promising enhancer for the enteral absorption of octreotide.

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