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Permeation enhancement of octreotide by specific bile salts in rats and human subjects: *in vitro, in vivo* correlations

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1 The potential of bile salts to improve the enteral absorption of octreotide, an orally active somatostatin analogue, was investigated by a combination of *in vitro, in situ* and *in vivo* experiments.

2 Incorporation of octreotide into lipid monolayers (as measured by area increase of the monolayer at constant surface pressure using a Langmuir-Blodgett trough set-up) depended on the type of bile salt used for monolayer pre-treatment. Addition of 20 μM octreotide to the subphase containing 20 μM of the dihydroxylated bile salt ursodeoxycholate (UDCA) causes a 9% increase in area, whereas addition of octreotide to the subphase containing the 7α-enantiomer of UDCA, chenodeoxycholate (CDCA), resulted in an area increase of the lipid monolayer of 20%. Area increase by octreotide alone was not significantly different from the increase of octreotide and UDCA in combination.

3 CDCA and UDCA in combination with octreotide increased the permeability of liposomal membranes for rubidium ions, whereas octreotide alone did not significantly change the permeability. This indicates membrane distortion as a possible cause for the enhanced absorption of octreotide by bile salts.

4 In polarized Caco-2 cell monolayers octreotide exhibited a permeation coefficient of 0.008 ± 0.004 cm h^{-1}. Addition of 0.2-1% of UDCA to the apical incubation medium had no significant effect upon the permeation coefficient. In contrast, 0.2-1% CDCA in the incubation medium resulted in a significant increase ($P<0.05$) of the monolayer permeability of octreotide (0.015-0.037 cm h^{-1}).

5 Octreotide was absorbed as the intact peptide from the gastrointestinal tract in rats with an absorption efficiency of 0.26%. Coadministration of bile salt resulted in a dose-dependent increase in absorption efficiency of the peptide up to 20.2%. The observed effect was more pronounced for CDCA than for UDCA.

6 The effect of CDCA and UDCA on octreotide absorption *in vivo* was assessed in a pharmacokinetic study with healthy volunteers. After oral administration of 4 mg octreotide in the presence of 100 mg bile salt, an average bioavailability of the peptide of 1.26% was achieved in the presence of CDCA, whereas in the presence of UDCA a bioavailability of only 0.13% was reached. This difference was statistically significant ($P<0.01$).

7 In conclusion, the co-administration of CDCA is able to enhance the enteral absorption of octreotide. The *in vitro* and *in situ* experiments were predictive for the observed effect in human subjects.

Keywords: Octreotide; chenodeoxycholic acid; ursodeoxycholic acid; intestinal absorption

Introduction

The synthetic somatostatin analogue octapeptide, octreotide, is used in the therapy of acromegaly and diverse endocrine disorders. It has been stabilized against proteolytic degradation in gastrointestinal fluids (Pless *et al.*, 1986), thus providing intestinal bioavailability. Various studies have shown its activity after oral administration (Williams *et al.*, 1986; Köhler *et al.*, 1987; Fuessl *et al.*, 1987), with plasma levels that are sufficient to suppress growth hormone secretion. However, the peptide exhibits a very low overall bioavailability of less than 1%. Therefore, the use of absorption enhancing excipients is indicated in order to achieve therapeutically relevant effects after oral administration with lower amounts of peptide. Several studies have shown that bile salts or bile salt analogues are able to improve the bioavailability of poorly absorbed drugs at various sites of administration, such as the nasal cavity, upper intestinal tract or rectum (Gasco *et al.*, 1984; van Hoogdalem *et al.*, 1989; 1990; Tengamnuay & Mitra, 1990; Kissel *et al.*, 1992). It is generally accepted, that bile salts improve the enteral bioavailability of poorly soluble drugs by increasing the solubility due to mixed micelle formation. However, the mechanisms involved in the permeation of a drug/bile salt complex through the gastrointestinal (GI)-wall are as yet unclear. The influence of bile salts has been explained as an alteration of the intestinal barrier function as the result of the detergent like effect of the bile salt; alternatively, changes in the thermodynamic activity of the drugs might be the reason for an altered permeation (Poelma *et al.*, 1989). From many studies, it has become evident that the effects of bile salts on absorption are dependent both on the structure of the drug under investigation and the structure of the bile salt used. Thus, bile salts have also been shown to reduce the permeation of drugs through the gastrointenstinal wall (Yamaguchi *et al.*, 1986; Barnwell *et al.*, 1993).

The aim of the present study was to evaluate the effects of two clinically used bile salts upon interaction of octreotide with lipid membranes simulating a cellular membrane and then, to investigate the enteral absorption of octreotide and its systemic disposition in a comparative study, using Caco-2 cells as an *in vitro* model of the intestinal barrier, and rats and

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human subjects as *in vivo* models in the absence and in the presence of bile salts. These biophysical and biological approaches were combined in order to assess the predictability of the experimental models for the situation in man.

Methods

Lipid membrane interactions

A circular Langmuir-Blodgett trough with a Wilhelmy-balance for surface pressure measurement was used in the 'constant pressure' mode (Fromherz, 1975). The subphase consisted of 10 mM HEPES buffer, pH 7.5, the lipid monolayer was formed with a chloroformic L-α-dioleoyl lecithin (DOPC) solution over an area of approximately 40 cm^2. Bile salts were added to the subphase to a final concentration of 20 μM and the area increase of the lipid monolayer was monitored at a constant pressure of 25 $mN \times m^{-1}$. When no further increase was observed, octreotide was added to the subphase to achieve a final concentration of 20 μM.

Flux experiments were performed as described recently (Fahr *et al.*, 1994). Briefly, liposomes with a diameter of 120 nm were made by extrusion in rubidium chloride buffer (135 mM RbCl, 10 mM HEPES, pH 7.4). The bile salts and/or octreotide were added to the liposomes (2.7 mM). At time 0, tracer amounts of $^{86}RbCl$ were added to the liposomes, thoroughly mixed and kept at 25°C. At different time points 6 μl of the liposome suspension was added to 60μl of cold (0–2°C) iso-osmolar sucrose and mixed. The liposomes in this mixture were separated from external rubidium by applying on an ion-exchange Bio-Rex 70 microcolumn with a total bed volume of 0.5 ml. The liposomes from the sample were directly eluted with 1 ml cold (0–4°C) iso-osmolar sucrose into a scintillation counting vial and radioactivity was measured. The initial isotope influx could be described by a simple diffusion formula:

$$I_i(t) = I_{i\infty} \times (1 - e^{-t/\tau})$$

I denotes the influx of the tracer into the liposomes and was determined by scintillation counting of the liposomes at the given time points. The characteristic time τ is related to the permeability coefficient P by $r_{liposome} \times (3 \times P)^{-1}$, hence the quotient of $\tau_0 \times \tau^{-1}$ is a measure for permeability increase, r denotes the radius of the liposome.

In vitro *permeation studies with Caco-2 cells*

Transport studies with Caco-2 cells grown on polycarbonate filters (Pore size 0.45 μm; Nucleopore, Cambridge, MA, U.S.A.) were performed as recently described (Drewe *et al.*, 1993). Briefly, the cells were cultured for 12–14 days on the filters until formation of confluent monolayers. They were maintained in Dulbecco's modified Eagle medium (DMEM), containing 10% foetal calf serum, 1% nonessential amino acids and 2% glutamine. The cell medium was changed every third day. For the transport experiments only cells between passage 70 and 90 were used. Filters were placed in side-by-side diffusion chambers and the integrity of the monolayer was determined by measurement of the transepithelial electrical resistance (TEER) and the transport of radiolabelled PEG-4000 as macromolecular marker for paracellular permeation. For the determination of the permeation of radiolabelled octreotide, the cell monolayers were incubated from the apical side with 10 μM ^{14}C labelled peptide in the absence or in the presence of bile salts at concentrations indicated in the figure and table legends. Aliquots of the incubation medium were taken from the basolateral side of the monolayer at 15 min intervals up to 120 min. The radioactivity in the sample was determined by liquid scintillation counting and peptide permeability was calculated following the equation:

$$P_{app} = \frac{dQ}{dt} \times \frac{1}{A \times C_0} (cm \times h^{-1})$$

C_o is the concentration of the administered peptide at the apical cell monolayer side, A is the total surface area, and dQ/dt is the permeability rate ($\mu g \times h^{-1}$).

The effect of the bile salts upon cell monolayer integrity was determined by microscopic control of the cell monolayers and determination of the permeability of PEG-4000 in the presence of the bile salts as well as by the measurement of release of lactate dehydrogenase and trypan blue exclusion.

In situ *absorption studies in rats*

All animal studies were performed according to the guidelines of the Committee of the Swiss Cantonal Agency for Animal Protection. Male Wistar rats, weighing approximately 300 g (BRL, Fühlinsdorf, Switzerland) were kept without food, but with free access to water for one day prior to the experiment. The animals were anaesthetized with urethane (i.p. injection of 2×0.7 ml, 20% in 0.9% saline). The peritoneum was opened by a midline incision and 50 μg octreotide dissolved in 0.5 ml 0.9% saline was injected into the jejunum, about 5–6 cm distal to the ligamentum of Treitz, in the absence or in the presence of bile salts at various concentrations. For the determination of the absorption efficiency of octreotide, a second group of animals received a bolus of 2 μg of the peptide dissolved in 100 ml 0.9% saline by injection into a mesenteric vein.

Blood samples (0.5 ml) were taken by puncture of the vena cava 10 min before and 20, 60, 120 and 180 min after drug administration and immediately centrifuged at 10 000 *g* for 5 min at 4°C. The plasma was kept frozen until the concentration of octreotide was determined by means of a radioimmunoassay (Bauer *et al.*, 1982). The rabbit antiserum recognised only the intact peptide with a very low cross-reactivity to peptide fragments, somatostatin-14 or somatostatin-28. The area under the plasma curve (AUC) was estimated using the trapezoidal rule. The absorption efficiencies were determined by the ratio of areas under the curve (AUC) values after intra-jejunal (i.j.) and intra-mesenteric (i.v.) administration, respectively, following the equation:

$$((AUC_{i.j.} \times dose_{i.v.})/(AUC_{i.v.} \times dose_{i.j.})) \times 100 = \%[\text{absorption efficiency}]$$

In vivo *absorption study in human subjects*

The peptide absorption study was carried out according to the guidelines of the declaration of Helsinki as revised in Tokyo (1975) and in Venice (1983). It was approved by the Ethics Committee of the University of Basel/Kantonsspital. The study was performed with a randomised open-labelled Latin-square design in 10 healthy male volunteers having a mean age of 25 years. Octreotide (4 mg) was given to the volunteers as a mixture together with the sodium salts of either UDCA or CDCA in hard gelatine capsules. All volunteers also received an oral placebo capsule and an intravenous infusion of 100 μg peptide over a period of 30 min in order to assess pharmacodynamic effects and the absolute bioavailability of the peptide, respectively. The wash out period between administrations was at least 3 days. No food was allowed for at least 10 h prior to receiving the dose. An intake of maximum of 250 ml of water was permitted throughout the night. For the first 4 h after each administration the subjects did not consume more than 50 ml of water per hour. A standardized liquid lunch (500 ml Ensure, Abbott Lab.) was taken 4 h after drug administration. This lunch contained a caloric content of 2090 kJ (500 kcal). It was swallowed by the subjects within 2 min. During the 12 h after drug administration, no intake of xanthine or alcohol-containing beverages was allowed. The subjects refrained from smoking during the study.

Blood samples for post-prandial insulin and octreotide de-

terminations were taken before and up to 6 and 12 h after administration, respectively, and collected in EDTA tubes. Plasma was separated from the blood by centrifugation. Octreotide plasma concentrations were determined by means of a specific radioimmunoassay (Bauer *et al.*, 1982) using polyclonal rabbit antibodies and ^{125}I-labelled octreotide as a tracer. The detection limit was 0.01 ng ml^{-1}. Insulin plasma concentrations were measured with a commercially available radioimmunoassay kit (INS-RIA-100 Insulin kit [IRE-Medgenix]). The detection limit was 2 μiu ml^{-1}.

For the intravenous administration, individual plasma concentration/time curves of octreotide were evaluated by non-linear regression analysis using the Topfit software (Heinzel *et al.*, 1993). The kinetic profiles of all plasma curves could be well described by a linear two or three compartment disposition model. Choice of the respective models was made by the Akaike criterion (Yamaoka *et al.*, 1978). By means of this analysis, the area under the plasma concentration/time curve extrapolated to infinite times (AUC(0-inf.)), the terminal linear hybrid transfer constants for elimination (λ_z), and the respective half-life for elimination ($t_{1/2,z}$) were estimated. Values of AUC for oral administrations were calculated by the linear trapezoidal rule and extrapolated to infinity using the last octreotide values above detection limit and λ_z as described in Gibaldi & Perrier (1982).

For insulin concentrations, AUC was calculated over a period of 6 h from the time of administration. In addition, the maximum plasma concentrations (C_{max}) and the time to maximum plasma concentration (T_{max}) were determined.

For comparison of pharmacokinetic parameters between the oral treatment groups, data were first tested for normal distribution by the Wilk-Shapiro test. If normal distribution of the data could not be rejected, samples were tested by analysis of variance. Otherwise, Kruskal-Wallis test was applied. If analysis of variance or Kruskal-Wallis test revealed significant differences, pairwise comparisons were performed by Student-Newman-Keuls test or Wilcoxon matched-pairs signed-ranks test with Bonferoni correction, respectively. All tests were performed by SPSS for Windows Software (1994). The level of significance was $P=0.05$.

Chemicals

[^{14}C]-octreotide with a specific activity of 40.99 μCi mg^{-1} and unlabelled peptide were provided by the Preclinical Research Department, Sandoz Pharma Ltd., Basle, Switzerland. The radiochemical purity of the labelled octreotide was greater than 98% as verified by high-performance thin-layer chromatography prior to the experiments. Caco-2 cells, originally derived from a human colorectal carcinoma, were obtained from the American Type Culture Collection, Rockville, Maryland, U.S.A., at passage 20–30. For transport studies they were used at passages 70–90. The sodium salts of 3α,7α-dihydroxy-5β-cholan-24-oic acid (chenodeoxycholate) and 3α,7β-dihydroxy-5β-cholan-24-oic acid (ursodeoxycholate) were obtained from Diamalt, Munich, Germany. All other chemicals were purchased in the highest purity from commercial sources.

Results

In vitro *experiments with lipid membranes*

The area increase of the DOPC lipid monolayer caused by octreotide was dependent on the type of bile salt, which was added to the subphase of the lipid monolayer. Addition of 20 μM UDCA to the subphase caused a small reduction of the lipid monolayer area (−1.5% at 25 mN × m^{-1} surface pressure, see Figure 1). Addition of 20 μM octreotide to this subphase resulted in an area increase of 9%. The same procedure with CDCA caused an area increase of 19% and after subsequent addition of octreotide an area increase of 20% was observed. Addition of octreotide alone caused an area increase of 12%, comparable to the area increase with the UDCA doped subphase alone (no significant differences for five individual experiments each).

Tracer flux measurements of ^{86}Rb across liposomal membranes showed a moderate initial permeability increase after addition of UDCA (see Figure 2, concentration for bile salts 2.7 mM, concentration ratio of bile salts to octreotide = 2415:1) in comparison to the control (increase 2.5 fold), whereas the complex UDCA/octreotide showed an increase of membrane permeability for ^{86}Rb (approximately 80 fold). CDCA showed *per se* a higher increase (230 fold compared to control) and the CDCA/octreotide complex showed the highest increase in permeability (610 fold). Octreotide alone did not significantly increase liposomal membrane permeability.

Transport experiments in Caco-2 cell monolayers

All transport experiments were performed with cells that had formed confluent monolayers after 12–14 days growth on polycarbonate filters with a pore size of 0.45 μm. By this time, transepithelial resistances of 300 ± 30 $\Omega \times cm^2$ were achieved indicating confluency of the cell monolayers. Control experiments were performed as recently described (Drewe *et al.*,

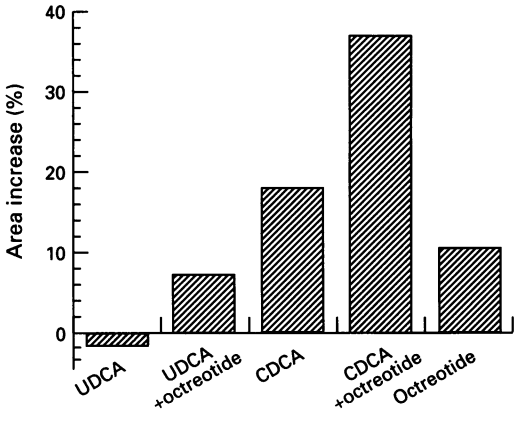

Figure 1 Area increase of a DOPC lipid monolayer at the air-water interface caused by addition of substances to the aqueous subphase. The subphase consisted of HEPES buffer pH 7.2 and was thermostatically controlled to 25°C. The apparatus was operated in the constant pressure mode (25 mN m^{-1}). Addition of substances to the subphase was by injection into the stirred subphase. Final concentration of each substance in the subphase was 20 μM (means of 3 experiments).

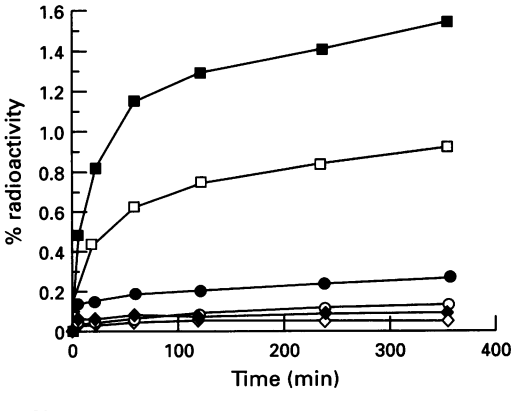

Figure 2 $^{86}Rb^+$-flux measurements into DOPC-liposomes in the presence of different test substances. Concentration of bile salts in the liposomal suspension: 2.7 mM, ratio of bile salts to octreotide = 2415:1. (●) UDCA/octreotide; (■) CDCA/octreotide; (□) CDCA; (○) UDCA; (◆) octreotide; (◇) control (liposomes). Data represent means of 3 experiments. Other conditions see Methods.

1993) in order to assess the functional integrity of the cells, such as the low permeation of [^{14}C]-PEG-4000 as extracellular marker compound (0.15% of a tracer dose per hour), or the directed transport of phenylalanine or taurocholate. All control experiments showed the formation of cell monolayers being suitable for the planned transport experiments.

When the cells were incubated from their apical side with 10 μM octreotide, the amount of peptide permeating the cell monolayer increased in a linear manner with time over a period of 2 h, resulting in a permeation coefficient of 0.008 ± 0.004 cm $\times$ h^{-1}. Addition of bile salts to the apical medium resulted in an enhanced permeation of the peptide dependent on the bile salt added and its concentration. In the presence of 0.5% (w/v) CDCA a 2.7 fold enhancement of octreotide permeation was observed. The presence of 0.5% (w/v) UDCA had no effect on octreotide permeation. When the amount of bile salt in the apical incubation medium was increased to 1% (w/v) a further enhancement of octreotide permeation was observed. In the presence of UDCA again no enhancement could be observed (Figure 3). In control experiments, the effect of the bile salts on the permeation of PEG-4000 was investigated. Both bile salts enhanced the permeation of the marker for paracellular transport. Whereas CDCA caused this effect at a concentration of 0.5% (w/v) in the apical medium, a concentration of at least 1% UDCA was necessary to enhance PEG-4000 permeation through the monolayer. In the presence of 1% CDCA the permeation rate of PEG was about three times higher than under control conditions, in the presence of 1% UDCA it was about 1.5 times higher. These observations indicate, that the integrity of the intercellular tight junctions is influenced by CDCA, thereby opening paracellular pathways. This was confirmed by microscopic observation of the cell monolayers demonstrating the detergent like effect of CDCA. After 60 min incubation, cell plaques were solubilized from the supporting filters with CDCA at concentrations greater than 1% (w/v), but not in the presence of UDCA. In order to assess the potential effects of both bile salts upon cell viability Trypan blue exclusion and release of lactate dehydrogenase were determined after incubation of the monolayers with the respective bile salts. Whereas no significant differences in dye exclusion could be seen between control monolayers and cells incubated with the bile salts, a significant increase of LDH release was observed predominantly after incubation with CDCA, indicating a direct interaction of the bile salt with the lipid membrane of the cells and giving an indication of increased membrane permeability (Figure 4).

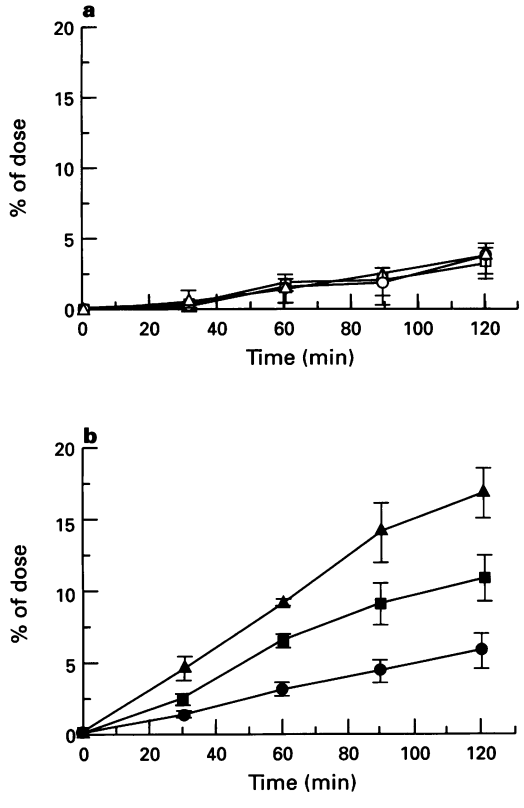

Figure 3 Permeation of octreotide through Caco-2 cell monolayers (a) alone (○) and in the presence of 0.5 (□) and 1 (△)% (w/v) UDCA; (b) alone (●) and in the presence of 0.5 (■) and 1 (▲)% (w/v) CDCA. Data represent means ± s.e.mean of 6 experiments.

Absorption experiments in rats

Recent experiments have shown that the upper jejunum is the site of highest octreotide absorption in rats (Fricker *et al.*, 1992). Therefore, the peptide was injected into a jejunal loop 5–6 cm distal to the ligamentum of Treitz in the absence and in the presence of the bile salts. The concentration of the bile salts in the administered solution was 1 and 2% (w/v), respectively.

In the absence of the bile salts a mean jejunal absorption

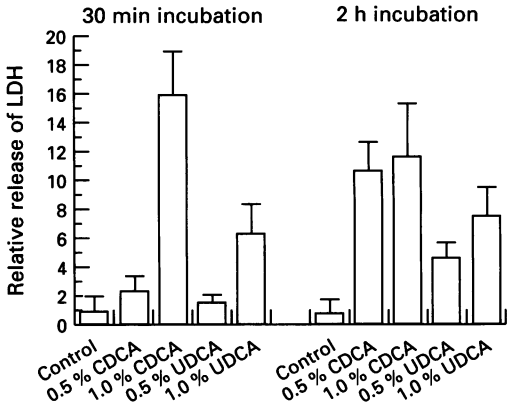

Figure 4 Relative release of lactate dehydrogenase by Caco-2 cells after incubation with increasing doses of bile salts. Data represent means ± s.e.mean of 6 experiments.

Table 1 Absorption of octreotide in rats

Substance/ composition	*AUC* 0–5 h (ng h ml^{-1})	C_{max} (ng ml^{-1})	*Absorption efficiency* (0–5 h) %
Octreotide i.v.	6.25 ± 1.25	5.84	100
Octreotide i.j.	0.82 ± 0.08	0.34	0.26
i.j. + 1% ursodeoxycholate	14.73 ± 7.21	9.78	4.71
i.j. + 1% chenodeoxycholate	63.19 ± 5.93	32.14	20.22
i.j. + 2% ursodeoxycholate	15.39 ± 1.63	9.56	4.92
i.j. + 2% chenodeoxycholate	56.72 ± 9.95	30.41	18.15

Data represent means ± s.e.mean; $n = 5$; i.v. = intramesenteric administration; i.j. = intrajejunal administration.

Table 2 Absorption of octreotide in human subjects

Composition	*Octreotide* (4 mg) + *NA^+-UDCA* (100 mg) (p.o.)	*Octreotide* (4 mg) + *Na^+-CDCA* (100 mg) (p.o.)	*Octreotide* (100 μg) (i.v.)
AUC (ng h ml^{-1})	0.63 ± 0.20	6.10 ± 2.69**	10.78 ± 0.92
F (%)	0.13 ± 0.03	1.26 ± 0.41	100 (reference)
C_{max} (ng ml^{-1})	0.13 ± 0.03	1.45 ± 0.64**	7.92 ± 0.59
T_{max} (h)	3.0 ± 0.47	2.7 ± 0.53	0.5 ± 0.00
$t_{\frac{1}{2},z}$ (h)			1.9 ± 0.16

Data represent mean ± s.e.mean; $n = 10$; p.o. = by mouth; i.v. = intravenous; AUC = area under the plasma concentration-time curve extrapolated to infinity; F = absolute bioavailability in percentage of the dose defined as: $F = (AUC_{p.o.} \times Dose_{i.v.})/(AUC_{i.v.} \times Dose_{p.o.}) \times 100$; C_{max} = maximum plasma concentration, T_{max} = time of C_{max}; $t_{\frac{1}{2},z}$ = terminal half-life for elimination; Na^+-UDCA = sodium ursodeoxycholate; Na^+-CDCA = sodium chenodeoxycholate. Significantly different from Na^+-UDCA-group, $^{**}P < 0.01$, Wilcoxon matched-pairs signed-ranks test.

efficiency of octreotide of 0.26% was determined (Table 1). Both bile salts had a significant enhancing effect upon the absorption of the peptide in the rank order CDCA > UDCA (20.2% absorption efficiency of the peptide as compared to 4.71% in the presence of UDCA). An increase of the bile salt concentration from 1% to 2% in the administered solution did not result in a further enhanced peptide absorption.

Absorption study with human volunteers

In addition to the *in situ* animal study, the efficacy of the two bile salts CDCA and UDCA in enhancing octreotide absorption was also investigated in a pharmacokinetic study involving 10 healthy male human volunteers. The participants of the study received an oral dose of either 4 mg octreotide together with 100 mg of the respective bile salt or an i.v. infusion of 0.1 mg octreotide over 30 min. The mean pharmacokinetic data of octreotide are given in Table 2 and Figure 5. Compared with the intravenous administration, the AUC values after the oral administration with CDCA and UDCA correspond to bioavailabilities of 1.26 ± 0.41% and 0.13 ± 0.03%, respectively. This difference was statistically significant ($P < 0.01$). The maximum plasma concentration after co-administration with CDCA (1.45 ± 0.64 ng ml^{-1}) was also significantly higher ($P < 0.01$) than after co-administration with UDCA (0.13 ± 0.03 ng ml^{-1})). Preliminary assessment of the pharmacological effect of the orally administered octreotide showed that there was a significant ($P < 0.05$) decrease of postprandial insulin secretion after co-administration with CDCA when compared with UDCA and placebo. This decrease was similar to that after the i.v. infusion, whereas the co-administration of UDCA had no effect (Table 3).

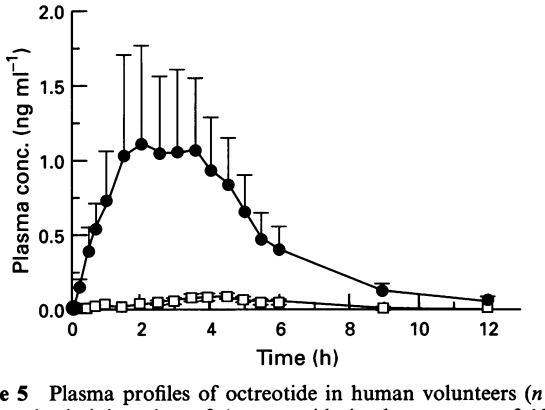

Figure 5 Plasma profiles of octreotide in human volunteers ($n = 10$) after oral administration of 4 mg peptide in the presence of 100 mg CDCA (●) or 100 mg UDCA (□). Data represent means ± s.e.mean.

Discussion

A drug administered *per os* has to overcome several barriers in the GI tract to become systemically available. The unstirred water layer forms the first barrier for a drug to pass, when it permeates the intestinal wall. It is known, that micellar complexation of drugs may decrease their absorption rate by decreasing their diffusion coefficient through the aqueous layer (Yamaguchi *et al.*, 1986). However, this effect can be compensated for by an increased overall mass transfer of drug in micelles as compared to un-associated drug in solution, where it is less soluble. In addition to the unstirred water layer, most epithelial cells are covered by a mucous layer consisting of a glycoprotein network with gel like structure. Bile salts and bile salt containing micelles decrease the viscosity and the thickness of this layer, thus increasing the permeability of co-administered drug molecules. Bile salts also directly alter the properties of the epithelial cell surface. The detergent like character of distinct bile salts affects the mucosal integrity and an opening of intercellular tight junctions may occur due to the bile salt-induced calcium complexation, thereby enhancing paracellular drug absorption (Murakami *et al.*, 1984).

The enhancing effects of bile salts or bile salts analogues upon mucosal peptide permeation through various epithelial tissues have been described for several peptides, such as insulin (Behl & Unowsky, 1987; Kissel *et al.*, 1992) and LHRH or

Table 3 Effect of octreotide on postprandial insulin

Composition	*Octreotide* (4 mg) + *Na^+-UDCA* (100 mg) (p.o.)	*Octreotide* (4 mg) + *Na^+-CDCA* (100 mg) (p.o.)	*Octreotide* (100 μg, i.v.)	*Placebo* (p.o.)
AUC insulin (ng h ml^{-1})	81.3 ± 15.3	44.4 ± 10.8	49.4 ± 7.1	85.2 ± 9.2
C_{max} insulin (ng ml^{-1})	74.0 ± 16.4	42.5 ± 13.4	47.2 ± 8.9	71.3 ± 8.1
T_{max} insulin (h)	5.3 ± 0.1	5.4 ± 0.1	5.5 ± 0.1	4.8 ± 0.2

Data represent mean ± s.e.mean; $n = 10$; p.o. = by mouth; i.v. = intravenous; AUC insulin = area under the plasma concentration time curve of insulin over a period of 6 h; C_{max} insulin = maximum insulin plasma concentration, T_{max} insulin = time of C_{max} insulin; Na^+-UDCA = sodium ursodeoxycholate; Na^+-CDCA = sodium chenodeoxycholate. Significantly different from Na^+-UDCA- and placebo group, $^{*}P < 0.05$, Wilcoxon matched-pairs signed-ranks test with Bonferoni correction.

growth hormone (Longenecker *et al.*, 1990). In the present study we have tested the effects of ursodeoxycholate and chenodeoxycholate upon interaction of octreotide, a somatostatin analogue octapeptide, with lipid membranes and the enhancement of the enteral absorption of octreotide.

The experiments show that bile salts exhibit a different effect upon the interaction of octreotide with lipid membranes in the rank order CDCA > UDCA. The monolayer experiments as well as the tracer flux experiments with ^{86}Rb-loaded liposomes indicate a significantly higher increase of membrane fluidity and membrane permeability for the peptide induced by CDCA than by UDCA. Effects on monolayer integrity could be observed at concentrations of 20 μM, which is far below the critical micellar concentration of the bile salts which is in the mmolar range. In this experiment the low concentration was used in order to prevent any abnormality in the monolayer structure. CDCA caused a 20% increase of the area and therefore it is likely that at higher concentrations in the subphase the monolayer would not be representative of an intact membrane. In contrast, the ^{86}Rb flux-experiments could be performed at bile salt concentrations in the same range as biological experiments. Both experiments support our recent *in vivo* studies with rats demonstrating a transcellular component in octroetide absorption (Fricker *et al.*, 1991; 1992; Drewe *et al.*, 1993).

As was to be expected from the biophysical *in vitro* models, a similar effect could be observed in the cell experiments and in the *in vivo* studies. The increase of the monolayer permeability correlated with the capability of both bile salts to increase the extent of permeation in the biological models: CDCA increased octreotide permeation more than UDCA. The observed effect can be explained, at least in part, by the chemical structure of the bile salts and their physicochemical properties. CDCA has both hydroxyl groups in the α-position, giving the molecule a hydrophobic and a hydrophilic side, whereas in UDCA the hydroxyl group in the 7-position is oriented so that there is no clearly hydrophobic side. This finds its expression in a lower critical micellar concentration and higher enthalpy and entropy values of CDCA for the aqueous-organic transfer (Vadnere & Lindenbaum, 1982; Roda *et al.*, 1990). Therefore, it can be assumed, that CDCA has a higher potency than UDCA for forming mixed micelles with amphipathic peptides such as octreotide.

The *in vivo* studies showed that the absorption enhancing properties of CDCA were superior to those of UDCA. Both, the Caco-2 experiments and the animal study indicated that a liquid composition containing 1% (w/v) of the respective bile salt should be sufficient for an optimal absorption enhancement. Further increase of bile salt concentration did not result in a significantly higher peptide permeation but started to damage the cell monolayer integrity.

There are several possible explanations for our observations. An inhibition of mucosal proteases and thus an inhibition of peptide degradation, as was recently described for insulin and the synthetic bile salt analogue, sodium taurodihydrofusidate (Lee & Longenecker, 1988) can be excluded as a possible reason for the increased absorption of octreotide, because previous studies have demonstrated proteolytic stability of the peptide in the GI-tract (Fricker *et al.*, 1991). It is more likely that the bile salts exerted their effects by the formation of mixed micelles and by their detergent-like properties as indicated by increased permeation of the paracellular marker PEG-4000 and by the increased release of LDH. The finding, that the release of cytosolic LDH is increased in the presence of increasing doses of CDCA supports previous results showing that mitochondrial dehydrogenase activity is inhibited by increasing bile salt concentrations in the medium (Anderberg *et al.*, 1992). Both results indicate an increased permeability of the cellular membrane. Interestingly, the relative potency of the two bile salts in enhancing the absorption of the peptide resembles their relative affinity with which they bind non-specifically to the mucosal surface of the intestine (Wilson & Treanor, 1977). The bile acid carrier in the apical cell membrane (Hidalgo & Borchardt, 1990) is presumably not involved in the observed effects, since it is rather specific for conjugated trihydroxylated bile salts, whereas unconjugated CDCA and UDCA permeate the cellular membrane by diffusion.

The bile salts enhance the peptide permeation both by the transcellular and by the paracellular route as indicated by the increased permeation of the paracellular marker PEG-4000 in the presence of CDCA. This finding is in agreement with other studies showing a decrease of transepithelial resistance and an increased mannitol or PEG-flux in the presence of di- and trihydroxylated bile salts (Anderberg *et al.*, 1992). It might also be speculated that insertion of the CDCA into the lipid membrane results in an altered membrane fluidity, thereby influencing the functional integrity of the intercellular tight junctions (Lee *et al.*, 1991).

In conclusion, our study has demonstrated the potential use of CDCA and UDCA as absorption enhancers for an otherwise poorly absorbed peptide drug. The *in vitro* models used correlated with the findings in the human study and proved to be predictable tools for the estimation of the situation in human subjects. However, the clinical relevance of these findings has to be investigated in trials with a larger number of patients.

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