ELSEVIER

International Journal of Pharmaceutics 137 (1996) 103–110

international journal of pharmaceutics

Cyclodextrins — Useful excipients for oral peptide administration?

Barbara Haeberlin, Thomas Gengenbacher, Armin Meinzer, Gert Fricker*

Sandoz Pharma Ltd., CH-4002 Basle, Switzerland

Received 12 December 1995; accepted 19 February 1996

Abstract

Cyclodextrins have been investigated for their potential use as excipients for the oral delivery of peptides. A modified calcitonin and the somatostatin analog octapeptide octreotide (Sandostatin®) were chosen as model drugs. Both the potential of cyclodextrins for metabolic and physicochemical stabilization, as well as their use as absorption enhancers were evaluated in vitro using the Caco-2 cell monolayer model and in in situ absorption experiments with rats. Physical mixtures of the peptides with α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, hydroxypropyl-β-cyclodextrin and dimethyl-β-cyclodextrin were used throughout the experiments. The use of β-cyclodextrin and hydroxypropyl-β-cyclodextrin resulted in an increased chemical and enzymatic stability of the peptides. β-Cyclodextrin, γ-cyclodextrin and hydroxypropyl-β-cyclodextrin also showed absorption enhancing properties in the in vitro system as well as in the in situ study. No beneficial effects were observed for α-cyclodextrin. An enhanced permeation of the paracellular marker PEG-4000 across the cell monolayers in the presence of distinct cyclodextrins indicated an impairment of the tight junctional integrity as one reason for improved peptide absorption. The results suggest that distinct cyclodextrins have protective and absorption enhancing effects on peptides by preparing simple physical mixtures of the two components. However, the extent of protection and absorption enhancement seems to depend strongly on the nature of the peptide used as well as the chosen cyclodextrin.

Keywords: Octreotide; Calcitonin; Cyclodextrins; Oral peptide delivery; Tight junctions

1. Introduction

Cyclodextrins (CyD) are cyclic oligosaccharides consisting of 6–8 glucopyranose units. The α-1,4-glycosidic linkage of the glucose units results in the formation of torus-like molecules with a polar outer surface and an apolar interior cavity (Szejtli, 1987). As a consequence of this apolar cavity, the CyDs are able to form inclusion complexes with a variety of guest molecules (Saenger, 1980; Szejtli

* Corresponding author, Institut für pharmazeutische Technologie und Biopharmazie, Im Neuenheimer Feld 366, D-69120 Heidelberg, Germany. Tel.: +49-6221-548336; fax: +49-6221-545971; e-mail: JW3@IX.URZ.UNI-HEIDELBERG.DE.

Abbreviations: CyD, cyclodextrin; HP-β-CyD, hydroxypropyl-β-cyclodextrin.

PII S0378-5173(96)04499-7

et al., 1982; Uekama et al., 1983). CyDs offer several advantages in drug delivery including improved drug solubilization and protection against physico-chemical and enzymatic degradation, as well as the potential for enhanced absorption afforded by direct interaction of the CyDs with membrane components such as cholesterol, which might induce changes in fluidity and permeability (Irie et al., 1982).

Though, extensive data concerning encapsulation of drug molecules, as well as requirements with respect to lipophilicity and the relative size, geometry and characterization of the resulting complexes have been published, only limited knowledge on the interaction of peptides and proteins with CyD-molecules is available (Brewster et al., 1991; Irie et al., 1992). Therefore, the goal of the present study was to investigate mixtures of various CyDs with selected peptides with respect to stabilization (enzymatic and physico-chemical) and absorption enhancement in the gastrointestinal tract. The absorption enhancing effects were evaluated in an intestinal cell culture model (Caco-2) and in in situ absorption studies in rats. To account for the variable physico-chemical properties of the different CyDs, the natural occurring α-, β- and γ-CyD and two CyD derivatives (hydroxy-propyl-β-CyD and dimethyl-β-CyD) were chosen for the investigations. As model peptides, a glycosylated calcitonin (modified calcitonin), which is sensitive against moisture (deglycosylation) and labile against enzymatic degradation, and the cyclic somatostatin analog octapeptide octreotide were chosen. Octreotide is preferably absorbed in the jejunal part of the small intestine. However, its overall bioavailability is rather low when given without absorption enhancer (ca. 0.3–0.5%) (Fricker et al., 1991, 1992; Drewe et al., 1993; Fricker and Drewe, 1995).

2. Materials and methods

2.1. Materials

Modified calcitonin, octreotide and ^{14}C-labelled octreotide with a specific activity of 40 μCi/mg were provided by Sandoz Pharma, Basle. β-CyD, γ-CyD, hydroxypropyl-β-CyD (HP-β-CyD) and dimethyl-β-CyD were purchased from Wacker (Munich, Germany); α-CyD was from Fluka (Buchs, Switzerland). Caco-2 cells, originally derived from a human colorectal carcinoma, were obtained from the American Type Culture Collection (Rockville, MD). All other chemicals were of reagent grade and purchased from commercial sources.

2.2. Methods

2.2.1. Preparation of CyD/peptide mixtures

Mixtures of modified calcitonin/CyD [ratio 1:15 (w/w)] and octreotide/CyD [ratio 1:50 (w/w)] were prepared by sieving (< 0.375 mm) and 10 min of mixing with a turbula-mixer (Bachhofen Maschinenfabrik, Basle, Switzerland). Different ratios in terms of weight were used, since equimolar ratios of peptide/cyclodextrin were applied in the cell culture experiments. The drug content of the resulting mixtures was assessed by HPLC.

2.2.2. Assessment of the physico-chemical stability

The modified calcitonin samples were stored in tightly closed glass vials for 0, 1 and 3 months at 5°C, 25°C, and 30°C, respectively. Drug content and degradation products (mainly deglycosylated peptide) were determined by HPLC. In addition, water content (Karl Fischer titration) and particle size of the samples were taken as quality parameters for the physical mixtures.

The content of active ingredient and degradation products of physical mixtures of octreotide and β-CyD and HP-β-CyD at -25°C, 25°C/60% relative humidity, and 40°C/75% relative humidity after storage for 1 and 3 months in open glass vials were used as quality characteristics to assess the effect of cyclodextrins on the stability of octreotide (determination by HPLC).

2.2.3. Assessment of the enzymatic stability

2.2.3.1. Pepsin. 1:15 (w/w) mixtures of modified calcitonin with the respective CyD or modified calcitonin alone (0.5 mg/ml) were incubated with

pepsin (0.4 mU/ml) at 37°C in 0.1 N HCl. At given time intervals the enzymatic reaction was stopped by mixing 0.2 ml of the incubation mixture with 0.8 ml of methanol.

2.2.3.2. α-Chymotrypsin. 1:15 (w/w) mixtures of modified calcitonin with the respective CyD or modified calcitonin alone (0.5 mg/ml) were incubated with α-chymotrypsin (7.3 U/ml) at 37°C in 0.15 M potassium phosphate pH 7.5. At predetermined time intervals, the reaction was stopped by mixing 0.5 ml of the incubation mixture with 0.5 ml of 10% H_3PO_4.

For both reactions, the remaining intact peptide was determined by HPLC. These enzymatic assays were only performed for the modified calcitonin since octreotide exhibits an outstanding high resistance against enzymatic degradation in the GI-tract (Fricker et al., 1991).

2.2.4. In vitro study with Caco-2 cell monolayers

The in vitro absorption studies with octreotide and CyDs were performed as previously described (Drewe et al., 1993). Briefly, Caco-2 cell monolayers were grown on polycarbonate membrane filters (Snapwell®, Costar) for 2 weeks. After that time confluency of the monolayers was reached, which was verified by the determination of the extent of permeation of the extracellular marker PEG 4000 and the transepithelial resistance (220–290 $\Omega \times cm^2$ at confluency). The filters were placed in side-by-side diffusion chambers filled with supplemented Dulbecco's modified Eagle medium containing 10% fetal calf serum. Then, the cells were incubated with 0.1 μM ^{14}C-labelled octreotide from the apical side. At time intervals of 15 min, aliquots of the medium were taken from the basolateral side of the monolayers and the peptide concentration was determined by liquid scintillation counting. After 1 h, CyD (1% w/v) was added to the apical side. The sequential addition of peptide and cyclodextrin was justified, because in separate experiments an immediate release of peptide out of all powder formulations was determined. The permeation of the peptide during the first hour was used as an internal control value, which was compared to the permeation rate of the peptides after the addition of the respective CyD. Reference experiments confirmed that in the absence of CyDs no change in the permeation rate occurred over 2 h. The absorption enhancing effect was determined by calculating the increase (in percent) of the relative permeation rate before and after adding the CyD.

2.2.5. In situ absorption study in rats

In situ absorption studies were performed as described previously (Fricker et al., 1991, 1992; Drewe et al., 1993). All animal studies were approved by the Committee of the Cantonal authorities according to the Swiss Animal Welfare Act. Male Wistar rats (BRL, Füllinsdorf, CH), with a body weight of approximately 300 g were kept without food, but with free access to water, for 1 day prior to the experiment. The animals were anaesthetised by intraperitoneal injection of urethane ($1 g \times kg^{-1}$). The peritoneum was opened by a midline incision and 5-cm segments of the desired intestinal area were ligated in order to prevent transit of the administered peptide down the gut while maintaining normal blood supply. The beginning of the jejunum was localized 5 cm distal to the ligamentum of Treitz. The mixtures of CyD and peptide (100 μg octreotide/5 mg CyD and 200 μg modified calcitonin/3 mg CyD, respectively) were dissolved in 0.5 ml physiological saline and injected into the ligated jejunal loop. Blood samples were taken up to 5 h after administration from a vena jugularis. The samples were analyzed by use of specific RIAs for octreotide and modified calcitonin (Sandoz Pharma AG, Basle, CH), respectively. C_{max} and T_{max} were determined and AUCs were calculated using the trapezoidal rule. The relative absorption efficiencies were calculated by setting the AUC of octreotide and modified calcitonin after administration of the peptide alone as 100%.

3. Results

3.1. Stabilizing effect of cyclodextrins in mixtures with modified calcitonin

All investigated mixtures of peptide and CyDs showed a stabilizing effect on the extent of degra-

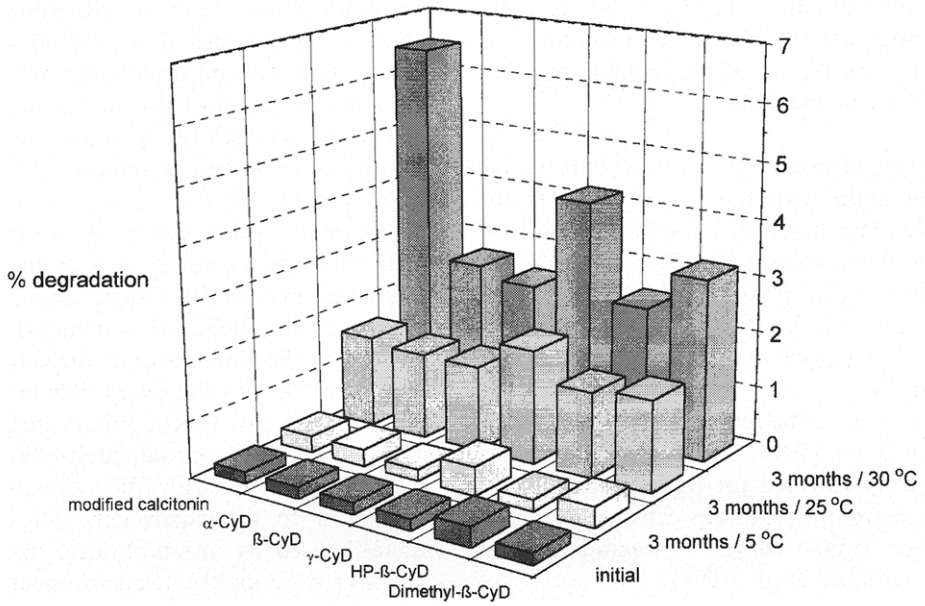

Fig. 1. Chemical stability of modified calcitonin in mixtures with cyclodextrins after 3 months (n = 4). The ratio of modified calcitonin to cyclodextrin was 1:15 (w/w). The bars represent the means of four determinations.

dation (deglycosylation) of modified calcitonin (Fig. 1; Table 1). The highest stabilizing effect was found for mixtures of modified calcitonin with β-CyD and HP-β-CyD, where only 2.5% degradation was observed as compared to 6.4% for modified calcitonin alone after storage at 30°C for 3 months. The water content and particle size of the different mixtures was not affected by storage time and conditions. However, the water content of the different mixtures was strongly dependent on the CyD used and varied between 1.8% (dimethyl-β-CyD) and 13.3% (β-CyD). But it has to be noted that mixtures with the highest stability were either low (i.e. HP-β-CyD, 2.8%) or high in water content (i.e. β-CyD, 13.3%). No direct correlation could be drawn between the stabilizing effect of the chosen CyD and the water content of the investigated mixture. The particle size of the mixtures varied dependent on the CyD used between 19 μm and 305 μm. However, for each mixture it was rather constant over the whole time interval, suggesting that no significant agglomeration occurred during the time of incubation.

Studies concerning the enzymatic degradation of the modified calcitonin also showed differences in the stabilizing effect of the various CyDs against the hydrolytic activity of pepsin and α-chymotrypsin (Fig. 2). α-CyD exhibited no stabilizing effect, whereas modified calcitonin in the mixture with dimethyl-β-CyD showed the highest resistance against proteolytic degradation. In the presence of α-CyD only 33% and 40% of intact peptide were found after 60 min incubation with pepsin and α-chymotrypsin, respectively. In the presence of dimethyl-β-CyD, 66% of the intact peptide after incubation with pepsin and 54% after incubation with α-chymotrypsin were found for the same time period. The reference values for the peptide alone were 33.8% after incubation with pepsin and 40.2% after incubation with α-chymotrypsin.

3.2. Stabilizing effect of CyD on octreotide

For octreotide, no stabilizing effect of the CyDs was observed after storage for 3 months at 25°C/60%. The degradation of octreotide alone was 0.3%, while degradation of octreotide

Table 1
Water content and particle size of modified calcitonin/CyD mixtures

Incubation time	% Degradation	Water content (%)	Particle size Q3 max
Calcitonin alone			
Initial	<0.1	—	—
3 months, 5°C	0.2	—	—
3 months, 25°C	1.9	—	—
3 months, 30°C	6.4	—	—
α-CyD/calcitonin			
Initial	<0.1	9.0	215
3 months, 5°C	0.2	9.0	215
3 months, 25°C	1.7	9.9	180
3 months, 30°C	2.7	9.7	180
ß-CyD/calcitonin			
Initial	<0.1	9.2	215
3 months, 5°C	0.1	12.7	305
3 months, 25°C	1.7	13.3	215
3 months, 30°C	2.5	12.0	215
Dimethyl-ß-CyD/calcitonin			
Initial	0.1	1.8	38
3 months, 5°C	0.2	2.7	38
3 months, 25°C	1.7	3.1	38
3 months, 30°C	3.1	5.1	38
γ-CyD/calcitonin			
Initial	0.1	5.0	38
3 months, 5°C	0.4	4.7	38
3 months, 25°C	2.2	4.9	38
3 months, 30°C	4.4	4.7	38
HP-ß-CyD calcitonin			
Initial	0.2	2.8	38
3 months, 5°C	0.1	4.0	19
3 months, 25°C	1.6	3.7	38
3 months, 30°C	2.5	3.7	19

All values are given as means of duplicate determination; the particle size determination was done by laser light diffraction.

in the presence of CyD varied between 2.3–3.6%. It can be concluded that, if CyDs exerted any effect upon octreotide stability, it was rather destabilizing than stabilizing.

3.3. In situ absorption studies

CyD/peptide mixtures were dissolved in saline and administered in situ by intrajejunal injection. The pharmacokinetic data after intrajejunal administration of octreotide and modified calcitonin with the various CyDs are summarized in Tables 2 and 3. The most pronounced absorption enhancing effects for the modified calcitonin were observed in the presence of γ-CyD (5.96-fold), HP-β-CyD (3.75-fold) and dimethyl-β-CyD (4.70-fold), whereas for octreotide an absorption enhancement was obtained with β-CyD (4.39-fold) and HP-β-CyD (3.52-fold). T_{max} for all administrations varied between 0.5 and 1.5 h, suggesting a very rapid absorption of both peptides.

3.4. In vitro permeation studies with mixtures peptide/CyD

Caco-2 cell experiments were performed to get an insight into the mechanism of absorption enhancement. For these studies, only octreotide was used since radioactive labelled peptide was available allowing immediate and sensitive detection of small amounts of peptide permeated through the cell monolayer. From previous experiments, it is known that the permeation of octreotide through Caco-2 cell monolayers is in the range between 1 and 3% of a given dose per hour (Drewe et al., 1993; Fricker and Drewe, 1995). Addition of CyDs to the apical side of the cell monolayers after 1 h permeation measurement in the absence of CyD resulted in the following rank order of a permeation enhancing effect: dimethyl-β-CyD (2.43-fold control) < HP-β-CyD (2.69-fold) < γ-CyD (2.86-fold) < β-CyD (2.94-fold) < α-CyD (3.51 fold) (Fig. 3). The numbers in parentheses indicate the increase in relative absorption rates.

In order to see whether the CyDs have an effect upon the tight junctional integrity of the cell monolayers, the same permeation experiments were performed with the ^{3}H-labelled extracellular

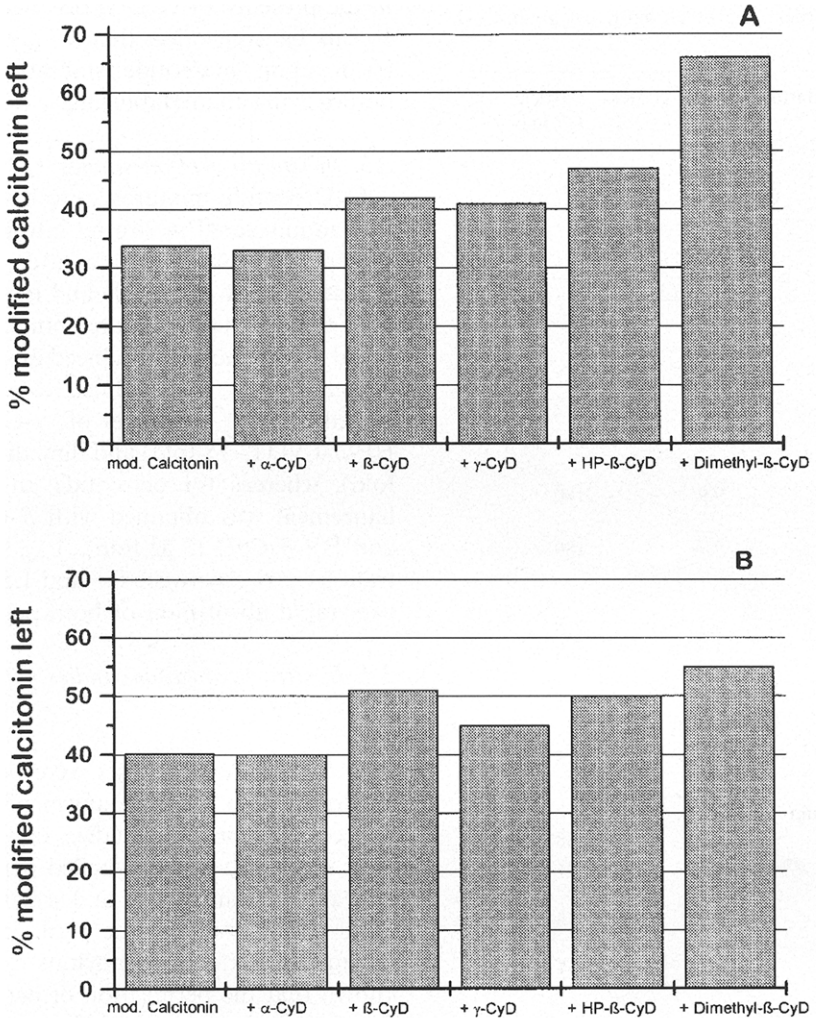

Fig. 2. Enzymatic stability of modified calcitonin in mixtures during incubation with pepsin and α-chymotrypsin ($n = 4$). The ratio of modified calcitonin to cyclodextrin was 1:15 (w/w). The bars represent the means of four determinations. A, Incubation with pepsin; B, Incubation with α-chymotrypsin.

marker PEG-4000. Its permeation across the monolayers was increased in the same rank order in the presence of the respective CyD than the permeation of octreotide, suggesting that addition of the CyDs affects paracellular pathways. The permeation of PEG-4000 varied between 0.1 and 0.4%/2 h of the given dose in the absence of CyD and increased up to 3% in the presence of α-CyD. Determination of lactate dehydrogenase activity in the apical incubation medium gave no indication of increased enzyme levels, suggesting that no significant cell impairment occurred.

The results indicate that the rank order for the absorption enhancing potential for peptides by different CyDs cannot generally be predicted, but depends strongly on the compounds tested. Comparison of the in vitro and the in situ studies with octreotide showed a similar rank order for the effects of β- and HP-β-CyD. However, α-CyD resulted in the best absorption enhancing properties in the Caco-2 studies, while its absorption enhancing potential in the in situ study was rather poor.

Table 2
Absorption enhancing effect of CyD/octreotide mixtures in situ in rats

	AUC (0–5 h)	C_{max} (ng/ml)	Rel. absorption efficiency
Octreotide	6.15 (130)	7.31 (99)	1.00
+ α-CyD	7.91 (179)	4.52 (147)	1.28
+ ß-CyD	27.00 (115)	17.86 (58)*	4.39
+ γ-CyD	10.14 (58)	6.58 (34)	1.69
+ hydroxypropyl-ß-CyD	21.67 (37)*	50.31 (40)*	3.52
+ dimethyl-ß-CyD	3.79 (34)	3.34 (29)	0.62

Means and coefficient of variation (%); $n = 6$.
*Significantly different with $\alpha = 0.05$.

Table 3
Absorption enhancing effect of CyD/modified calcitonin mixtures in situ in rats

	AUC (0–5 h)	C_{max} (ng/ml)	Rel. absorption efficiency
Mod. calcitonin alone	2.21 (31)	1.41 (53)	1.00
+ α-CyD	4.32 (46)*	3.54 (26)*	1.95
+ ß-CyD	4.52 (25)*	2.54 (35)*	2.05
+ γ-CyD	13.17 (92)*	6.10 (78)*	5.96
+ hydroxypropyl-ß-CyD	8.30 (47)*	7.38 (41)*	3.75
+ dimethyl-ß-CyD	10.40 (62)*	11.02 (67)*	4.70

Means and coefficient of variation (%); $n = 6$.
*Significantly different with $\alpha = 0.05$.

4. Discussion

Our studies showed that simple mixtures of CyDs with a modified calcitonin and octreotide resulted in stabilizing and absorption enhancing effects on the peptides tested. While applying CyDs to the investigated peptides, formation of inclusion complexes is not necessarily required for a beneficial effect. However, the observed results cannot directly be transferred from one peptide to the other. The stabilizing effects with modified calcitonin might be due either to a direct interaction of CyDs with the peptide or to physico-chemical phenomena like adsorption of moisture or complexation of other components, that may cause peptide degradation, by the CyDs. Since the calcitonin is moisture sensitive, it is likely that the stabilizing effects are caused by a protective action from humidity by the CyDs due to their different capacities to bind water, although in our experiments, no clear correlation was found between water content of the mixture and modified calcitonin stability.

Because CyDs have been described to form inclusion complexes, additional attempts to form such complexes with the peptides investigated

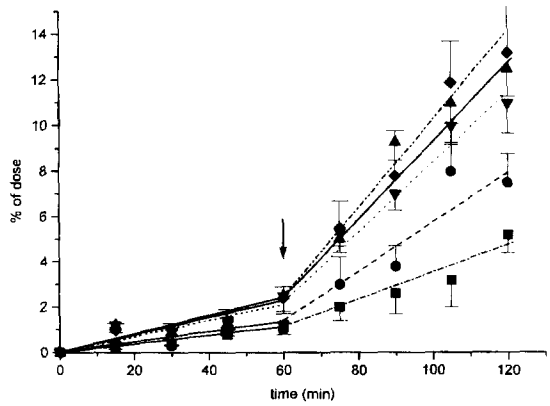

Fig. 3. Absorption enhancing effect of CyDs upon peptide permeation through Caco-2 cell monolayers ($n = 6$). Peptide concentration in the apical compartment was 0.1 μM. After 1 h (arrow) the CyDs were added to the apical side to give a final concentration of 1% (w/v). ■, dimethyl-β-CyD; ●, HP-β-CyD; ▲, β-CyD; ▼, γ-CyD; ◆, α-CyD.

have been performed by cosuspension and precipitation under reduced pressure. However, all attempts to obtain direct proof of 'inclusion complexes' by analytical methods like X-ray powder diffraction, IR-spectroscopy, thermogravimetrical analysis or H-NMR-analysis failed. Preliminary data from two-dimensional NMR-analysis (ROESY technique) suggest that at least a partial inclusion compound of octreotide and β-CyD in aqueous solution was formed. By this technique it could be shown that the aromatic phenylalanine and tryptophane residues of octreotide were partially incorporated in the CyD cavity. However, preliminary permeation studies showed that preparations manufactured by 'coprecipitation' did not result in higher permeabilities of the peptide across cell monolayers and in situ. Nevertheless, the results demonstrate that distinct CyDs possess the potential as effective excipients for the oral application of peptides. Complexation of such compounds seems to be unnecessary for a stabilizing effect or an increased transepithelial passage.

The absorption enhancing potential can be caused by a direct membrane fluidizing action of the CyDs as was recently shown for nasally administered CyDs (Shao et al., 1992). The present finding of an increased permeation of PEG-4000, which paralleled the absorption of octreotide, indicated that an opening of cellular tight junctions is also involved in the absorption enhancement without a significant impairment of cell membrane integrity as suggested by the lack of an increased LDH release in the presence of the CyDs.

Considering all data, we conclude that the beneficial effects of the CyDs are highly dependent on the chosen combination of peptides and CyDs. Therefore, the potential of CyDs as stabilizing and absorption enhancing excipients has to be investigated in each case on a compound-based approach.

Acknowledgements

The contributions of Dr S. Pfeffer, Dr J. France (Sandoz Pharma AG) and Professor Seelig (Biozentrum Basel) to the analytical characterization of the peptide/CyD interactions are highly appreciated.

References

Brewster, M.E., Hora, M.S., Simpkins, J.W. and Bodor, N., Use of 2-hydroxypropyl-β-cyclodextrin as a solubilizing and stabilizing excipient for protein drugs. *Pharm. Res.*, 8 (1991) 792–795.

Drewe, J., Fricker, G., Vonderscher, J. and Beglinger, C., Enteral absorption of octreotide: absorption enhancement by polyoxyethylene-24-cholesterol ether. *Br. J. Pharmacol.*, 108 (1993) 298–303.

Fricker, G. and Drewe, J., Enteral absorption of octreotide. Modulation of permeability by distinct carbohydrates. *J. Pharmacol. Exp. Ther.*, 274 (1995) 826–832.

Fricker, G., Bruns, C., Munzer, J., Briner, U., Albert, R., Kissel, T. and Vonderscher, J., Intestinal absorption of the octapeptide octreotide 201-995 visualized by fluorescence derivatization. *Gastroenterology*, 100 (1991) 1544–1552.

Fricker, G., Drewe, J., Vonderscher, J., Kissel, T. and Beglinger, C., Enteral absorption of octreotide. *Br. J. Pharmacol.*, 105 (1992) 783–786.

Irie, T., Otagiri, M., Sunada, M., Uekama, K., Ohtani, Y., Yamada, Y. and Sugiyama, Y., Cyclodextrin induced hemolysis and shape changes of human erythrocytes in vitro. *J. Pharm. Dyn.*, 5 (1982) 741–744.

Irie, T., Wakamatsu, K., Arima, H., Aritomi, H. and Uekama, K., Enhancing effects of cyclodextrins on nasal absorption of insulin in rats. *Int. J. Pharm.*, 84 (1992) 129–139.

Saenger, W., Cyclodextrin inclusion compounds in research and industry. *Angew. Chem. Int. Ed. Engl.*, 19 (1980) 344–362.

Shao, Z., Krishnamoorthy, R. and Mitra, A.Z., Cyclodextrins as nasal absorption promoters of insulin: mechanistic evaluation. *Pharm. Res.*, 9 (1992) 1157–1163.

Szejtli, J., Chemistry, characterization and properties of cyclodextrins. In Breimer, D.D. and Speiser, P. (Eds.), *Topics in Pharmaceutical Sciences*, 1987, 151 pp.

Szejtli, J., Bolla-Pusztei, E. and Kajatar, M., The β-cyclodextrin inclusion complex of menadione (vitamin K3). *Pharmazie*, 37 (1982) 725–728.

Uekama, K., Narisawa, S., Hirayama, F. and Otagiri, M., Improvement of dissolution and absorption characteristics of benzodiazepines by cyclodextrin complexation. *Int. J. Pharm.*, 16 (1983) 327–338.