J. Pharm. Pharmacol. 1998, 50: 467–473
Received October 21, 1997
Accepted January 15, 1998

The Effect of a Drug-delivery System Consisting of Soybean Phosphatidyl Choline and Medium-chain Monoacylglycerol on the Intestinal Permeability of Hexarelin in the Rat

URBAN FAGERHOLM, BRITA SJÖSTRÖM* JANINA SROKA-MARKOVIC*†, ANNIKA WIJK*, MARIANNE SVENSSON*† AND HANS LENNERNÄS

*Department of Pharmacy, Division of Biopharmaceutics and Pharmacokinetics, University of Uppsala, PO Box 580, S-751 23 Uppsala and * Pharmacia & Upjohn, Pharmaceutical Development, S-112 87, Stockholm, Sweden*

Abstract

The aim of this study was to investigate if the effective in-situ permeability (P_{eff}) of a new growth hormone-releasing peptide, hexarelin, along rat intestine was enhanced by a lipid matrix drug-delivery system comprising a mixture of soybean phosphatidyl choline and medium-chain monoacylglycerol (PC-MG). The study was performed with and without a protease inhibitor, Pefabloc SC. To enable better understanding of the mechanism of action of this delivery system we also studied the uptake of a small hydrophilic molecule, atenolol.

PC-MG at a concentration of 15 mmol L^{-1} increased the jejunal P_{eff} of hexarelin approximately 20-fold, both in the presence and absence of Pefabloc SC, whereas P_{eff} was not increased in the ileum and colon. PC-MG had no effect on the jejunal, ileal and colonic P_{eff} of atenolol. Complete recovery of the non-absorbable molecule PEG 4000 showed that functional intestinal viability was maintained in all experiments.

Although the results obtained in this study are promising, pharmacokinetic and toxicological studies are required to investigate if this delivery system is a suitable and safe candidate for improving the oral bioavailability of hexarelin.

Although many peptides and proteins are potent drugs, when administered orally their bioavailability, and hence their pharmacological activity, is often very low (Lee & Yamamoto 1990; Drewe et al 1993; Taki et al 1995). This might be because of metabolism in the intestinal lumen and wall, poor membrane permeability or extensive liver extraction, or a combination of these (Lee 1995; Bai et al 1995; Taki et al 1995). Various attempts have been made to overcome these barriers, for example, synthesis of more stable and lipophilic analogues, and the use of absorption enhancers and peptidase and protease inhibitors (Tomita et al 1988; Aungst et al 1991; Drewe et al 1993; Muranishi & Yamamoto 1994; Yodoya et al 1994; Lee 1995; Taki et al 1995; Bouras et al 1996). Chemical modification might reduce the molecular size, increase the lipophilicity and also reduce the sensitivity to enzymatic degradation (Yodoya et al 1994; Stewart & Taylor 1995). Such strategies of drug design include identification of a minimum fragment with maintained pharmacological activity, substitution of L-amino acids with unnatural (i.e. D) amino acids, substitution of hydrogen-bonding groups, cyclization and targeting for carrier-mediated transport across the intestinal mucosa (Stewart & Taylor 1995).

Among additives reported to enhance the intestinal permeability and oral bioavailability of both hydrophilic and lipophilic compounds is biosomes, a mixture of soybean phosphatidyl choline and medium-chain monoacylglycerol (PC-MG), which in excess water spontaneously forms lipid particles (liposomes) (Bruce 1994; Bohlinder et al 1994; Lohikangas et al 1994a, b). On the basis of in-vitro data it has been suggested that the lipid components of PC-MG interact with intestinal cell membranes (Betageri et al 1993; Lohikangas et al 1994a, b; Aungst et al 1996). One hypothesis is that MG,

† Present address: Astra Pain Control AB, S-151 85 Södertälje, Sweden.

Correspondence: H. Lennernäs, Department of Pharmacy, Division of Biopharmaceutics and Pharmacokinetics, University of Uppsala, PO Box 580, S-751 23 Uppsala, Sweden.

which is believed to be responsible for the permeability enhancer effect, extracts cholesterol from cell membranes, with increased membrane permeability as a consequence (Lohikangas et al 1994a; Kararli 1995). The enhancing effect of PC-MG seems to be dose-dependent and, at concentrations ≥ 6 mmol L^{-1}, is also correlated with in-vitro epithelial morphological changes observed by microscopy in Caco-2 cell monolayers (Lohikangas et al 1994a). However, it is suggested that compared with many other products this delivery system promotes clinically safe absorption enhancement (Muranishi 1990; Aungst et al 1996).

The enzymatic activity profile of the gastrointestinal tract suggests that delivery of peptide and protein drugs to the colon and rectum would be attractive (Bai 1993; Krishnamoorthy & Mitra 1995; Bai et al 1995; Rubinstein 1995). These regions have also been shown to be most promising with regard to enhancement of absorption (Muranishi 1990). However, it must be taken into account that the colonic microflora, which has a broad spectrum of metabolic reactions, might extensively degrade peptides and proteins (Bai et al 1995). Furthermore, the colonic and rectal permeabilities of several compounds have been reported to be lower than in the small intestine (Chadwick et al 1977; Schultz & Winne 1987; Lennernäs et al 1995; Fagerholm et al 1997).

Hexarelin (His-D-2-methyl-Trp-Ala-Trp-D-Phe-Lys-NH_2) is a synthetic peptide that substantially increases plasma levels of growth hormone after intravenous administration in man (Ghigo et al 1994; Imbimbo et al 1994). However, when delivered orally the growth hormone-releasing activity is only $0{\cdot}3 \pm 0{\cdot}1\%$ of that measured after intravenous administration (Ghigo et al 1994). Plausible reasons for this low bioavailability might be low intestinal permeability, metabolism in the gastrointestinal tract and/or extensive extraction in the liver.

The main aim of this study was to investigate whether a soybean phosphatidyl choline-medium-chain monoacylglycerol matrix (PC-MG) formulated with hexarelin in two different ways is an effective means of enhancing the effective in-situ permeability (P_{eff}) of hexarelin along the rat intestine. The study was performed with and without a protease inhibitor, Pefabloc SC. To enable better understanding of the mechanism of action of PC-MG, the uptake of a small hydrophilic compound, atenolol, was also studied. Local effects of these additives on the intestinal mucosa were assessed by monitoring the uptake of a non-absorbable marker, PEG 4000, and the release of lactate dehydrogenase into the lumen.

Materials and Methods

Drugs

Hexarelin is a hexapeptide, MW 887 Da, with three pK_a values, 5·2, 7·2 and 10·1 at ionic strength 0·15 mmol L^{-1}. The log D (octanol–water) at pH 6·5 and 7·4 were estimated to be −2·3 and −0·9, respectively (Sokolowski, personal communication). In its un-ionized form hexarelin has a partition coefficient (log P) of 2·2. The log D and log P values were assessed by use of a PCA 101 automatic titrator (Sirius Analytical Instruments, Forest Row, UK). Atenolol is a small (MW 266) hydrophilic drug (log D −1·8 octanol–water at pH 7·4) with a pK_a value of 9·6 (Fagerholm et al 1996).

Study design and composition of perfusion solutions

We used a previously validated intestinal in-situ rat single-pass perfusion approach to determine the effective intestinal permeability (P_{eff}) of hexarelin and atenolol in the absence and presence of 15 mmol L^{-1} PC-MG (30 : 70, %w/w) (Fagerholm et al 1996, 1997). The study was divided into three parts. In part I the P_{eff} of hexarelin (0·15 mmol L^{-1} hexarelin acetate) and atenolol (0·83 mmol L^{-1}) in the rat jejunum ($n = 6$), ileum ($n = 5$) and proximal colon ($n = 5$) were estimated in the absence (Ia) and presence (Ib) of PC-MG. Hexarelin was mixed with the lipid matrix before addition of excess perfusion solution containing atenolol (a detailed description of the preparation of the lipid hexarelin mixture is given below). Part II was identical to part I (IIa, without PC-MG; IIb, with PC-MG) except that the protease inhibitor Pefabloc SC (0·3 g L^{-1}) was added to both perfusion solutions (IIa and IIb) and jejunal experiments were performed with five animals.

Pefabloc SC (Pentapharm, Basel, Switzerland) has been shown to inhibit enzymatic degradation of hexarelin in luminal fluids (data not shown) and was chosen because it has been shown to be safer and more effective than other serine protease inhibitors. In part III, atenolol (0·028 mmol L^{-1}) was mixed with the lipid matrix before addition of excess perfusion solution containing hexarelin. These experiments were performed in the jejunum of five rats and without Pefabloc SC. Basal estimates of the P_{eff} of atenolol have previously been determined in the rat (Fagerholm et al 1996, 1997). The perfusion solution (pH 6·5, 290 mOsm L^{-1}) contained 48 mmol L^{-1} NaCl, 5·4 mmol L^{-1} KCl, 28 mmol L^{-1} Na_2HPO_4, 43 mmol L^{-1} NaH_2PO_4, 35 mmol L^{-1} mannitol, 1 g L^{-1} polyethylene glycol 4000 (PEG 4000), 2·5 μCi L^{-1} [^{14}C] PEG 4000 (a marker for fluid flux and intestinal functional via-

bility) (Amersham, Buckinghamshire, UK) and 10 mmol L^{-1} D-glucose.

Preparation of the hexarelin formulations
In parts I and II, hexarelin acetate was dissolved in sterile water at a concentration of 26% (w/w) (pure peptide 22·1% w/w). The solution was subsequently blended with a mixture of soybean phosphatidyl choline and medium-chain monoacylglycerol (30 : 70, % w/w) (Scotia LipidTeknik AB, Stockholm, Sweden). The amount of hexarelin solution in the lipid mixture was 11·8% (w/w). The tube containing the mixture was filled with nitrogen, sealed, and then shaken moderately until the mixture appeared to be homogenous and clear (1·5 h at 37°C). The hexarelin-lipid mixture was then stored at −20°C until the perfusion study. In part III, the same procedure was followed for atenolol. The amount of atenolol solution in the lipid mixture was 11·9% (w/w). On starting the perfusion experiments isotonic saline solution was added to allow for swelling of the lipid matrix for 10 min at room temperature. Before the start of the perfusion experiment the perfusion solution was added to the lipid mixture.

Intestinal single-pass perfusion experiments
Fasted male Sprague-Dawley rats, 200–280 g, were anaesthetized by intraperitoneal injection of inactin-byk (thiobutabarbital sodium; 120 mg kg^{-1}) before surgery, which is described elsewhere (Fagerholm et al 1996). This class of anaesthetic agents has been shown to have little or no influence on P_{eff} in rats (Yuasa et al 1993). After surgery the experiment was immediately started with administration of a bolus dose of 4 mL via the plastic inlet tube. Each regional segment (jejunum, ileum, colon) was single-pass perfused at 0·2 mL min^{-1} (Harvard Apparatus 22) for 180 (parts I and II) or 90 min (part III). Perfusions in parts I and II were divided into two separate experimental periods of 90 min each: 0–90 min without PC-MG (Ia and IIa) and 90–180 min with PC-MG (Ib and IIb). Period b was also initiated with a bolus of 4 mL so that equilibrium in the perfusion system would be reached more rapidly. During each period perfusate was quantitatively collected from 0–45, 45–60, 60–75 and 75–90 min into test tubes kept on ice. To recover the amounts of substances remaining in the perfusion system, at the end of perfusion the segments of intestine were rinsed for 2–5 min with approximately 15 mL saline. All perfusion syringes and perfusate samples were weighed and the samples were frozen immediately and stored at −70°C until analysis.

Approval for these animal studies was given by the Uppsala Animal Research Ethics Committee (application number C246/95).

Functional viability tests
The effects of PC-MG and Pefabloc SC on intestinal tissue was assessed by monitoring the recovery of a non-absorbable marker molecule, PEG 4000, and the release of lactate dehydrogenase into the intestinal lumen (Swenson et al 1994; Aungst et al 1996; Fagerholm et al 1996). Lactate dehydrogenase analyses were not performed in the initial jejunal perfusion experiments in parts I and II.

Stability
The stability of hexarelin (0·15 mmol L^{-1}) was investigated in perfusion solution and in different gastrointestinal fluids from the rat (jejunum, ileum, caecum, colon and rectum) for 60 min at 37°C. The contents of these sections were taken and placed in individual homogenization tubes. The contents were diluted with perfusion buffer (pH 6·5, 1 : 5 w/w) and homogenized using an Ultra-Turrax homogenizer (model T 25); they were then centrifuged (Sorvall RC 58) at 35 000 *g* and 4°C for 15 min. The stability of hexarelin in rat ileal content was also determined in the presence of Pefabloc SC (0·025 and 0·05 g L^{-1}). The samples were immediately frozen at −70°C until analysis. The stability of atenolol has previously been investigated, and there was no sign of degradation of this compound after 180 min at 37°C (Lennernäs et al 1994).

Analytical methods
All chemicals used were of analytical grade. Levels of hexarelin in perfusate were assessed by a validated high-performance liquid chromatographic (HPLC) method at Pharmaceutical Development, Pharmacia & Upjohn, Sweden. Separations were performed on a 300 mm × 3·9 mm i.d. column packed with 10-μm μBondapak C_{18} (Waters, Milford, MA) connected to a 25 mm × 4 mm i.d. guard column packed with 5-μm Zorbax SB C_{18} (Rockland Technologies, Nuenen, The Netherlands). The column temperature was kept at 30°C. The mobile phase, 72·2/27·7/0·1 (v/v) water–acetonitrile–trifluoroacetic acid, was filtered through a 0·45-μm type FH membrane filter (Millipore, Bedford, MA) before use. The flow rate was 1·0 mL min^{-1}. Fluorescence detection was performed at 350 nm after excitation at 280 nm.

Perfusion/perfusate solution (100 μL) was diluted with aqueous trifluoroacetic acid solution (0·5%, 500 μL) and 10 μL was injected directly on to the chromatographic column. The precision of

the method was 5·2% and the limit of quantification (LOQ) 1·2 $\mu g\,mL^{-1}$. The concentrations of atenolol were analysed by a previously validated HPLC method (Lennernäs et al 1994).

Levels of [^{14}C]PEG 4000 were determined by liquid scintillation counting for 10 min (Beckman model 244 instrument) after addition of 8 mL Beckman Ready Safe; the concentrations of lactate dehydrogenase in perfusate were assessed by use of a colorimetric analytical method (LDH kit, Sigma Diagnostics). Partition coefficients of hexarelin were determined using a PCA 101 automatic titrator (Sirius Analytical Instruments, Forest Row, UK).

Calculations and predictions

The recovery of [^{14}C]PEG 4000 (PEG_{rec}) was estimated from equation 1:

$$\sum PEG_{rec} = \sum PEG_{out} / \sum PEG_{in} \quad (1)$$

where $\sum PEG_{in}$ and $\sum PEG_{out}$ are, respectively, the accumulated amounts of [^{14}C]PEG 4000 entering and leaving the intestinal segment during equilibrium. The parallel-tube model was used to estimate the effective intestinal permeability coefficients (P_{eff}, $cm\,s^{-1}$) of hexarelin and atenolol (Komiya et al 1980; Fagerholm et al 1996):

$$P_{eff} = (-Q_{in}\ln(C_{out}/C_{in}))/A \quad (2)$$

where C_{in} and C_{out} are the inlet and fluid-transport-corrected outlet solute concentrations, respectively. $[PEG]_{in}$ and $[PEG]_{out}$ are the inlet and outlet concentrations of the water flux marker [^{14}C] PEG 4000, respectively. Q_{in} is the perfusion flow rate, and A is the mass transfer surface area within the intestinal segment and is assumed to be the area of a cylinder of length (L) 7–12 cm in the jejunum and ileum and 1·5–6 cm in the colon, and of radius (r) 0·18, 0·18 and 0·25 cm in the jejunum, ileum and proximal colon, respectively (Komiya et al 1980; Kararli 1995).

Student's paired *t*-test was used to determine the significance of differences between P_{eff} values measured in different experimental periods. P_{eff} coefficients are presented as means ± standard deviation (s.d.).

Results and Discussion

Hexarelin was stable for 60 min in perfusion solution and in fluids from the rat caecum, colon and rectum. In fluids from the small intestine, however, some degradation occurred. After 60 min approximately 80 and 60% of intact hexarelin was found in jejunal and ileal fluids, respectively. Hexarelin was stable in the incubation studies when Pefabloc SC was added. Furthermore, degradation by intraluminal and brush-border peptidases and proteases during the perfusion studies seemed to be negligible, as Pefabloc SC had no effect on measured P_{eff}. In addition, the low basal P_{eff} on hexarelin throughout the rat intestine, $\approx 0{\cdot}1 \times 10^{-4}\,cm\,s^{-1}$, also suggests less pronounced pre-systemic metabolism in the lumen (Table 1, Figure 1).

Irrespective of the method used to mix the lipid matrix and hexarelin, and whether Pefabloc SC was present, the jejunal P_{eff} of hexarelin increased approximately 20-fold in the presence of 15 $mmol\,L^{-1}$ PC-MG (Table 1, Figure 1). According to our previous classification, the measured P_{eff} values in the absence and presence of PC-MG, $0{\cdot}14 \times 10^{-4}$ (pooled jejunal data) and $2{\cdot}7 \times 10^{-4}\,cm\,s^{-1}$, correspond to intermediate and high intestinal permeability, respectively (Fagerholm et al 1996). In contrast, PC-MG had no effect on the P_{eff} of hexarelin in the ileum or colon.

Table 1. Steady-state effective intestinal permeability ($P_{eff} \times 10^{-4}\,cm\,s^{-1}$) of hexarelin and atenolol during in-situ perfusion of the rat jejunum, ileum and colon.

	Hexarelin		Atenolol	
	Without PC-MG	With PC-MG	Without PC-MG	With PC-MG
Part I (without Pefabloc SC)				
Jejunum	0·08 ± 0·08**	2·80 ± 0·52**	0 ± 0	0·07 ± 0·08
Ileum	0·10 ± 0·16	0·83 ± 0·93	0·09 ± 0·14	0·12 ± 0·14
Colon	0·13 ± 0·12	0·27 ± 0·42	0·17 ± 0·20	0·02 ± 0·04
Part II (with Pefabloc SC)				
Jejunum	0·21 ± 0·06*	2·70 ± 0·94*	0·13 ± 0·09	0·08 ± 0·09
Ileum	0·09 ± 0·09	0·51 ± 0·36	0·08 ± 0·07	0·08 ± 0·12
Colon	0·07 ± 0·16	0·07 ± 0·13	0·04 ± 0·08	0·06 ± 0·14
Part III (without Pefabloc SC)				
Jejunum	–	2·70 ± 0·84	–	0·03 ± 0·03

Values are means ± s.d. * $P < 0{\cdot}01$, ** $P < 0{\cdot}0001$, significant difference between results obtained in the presence and absence of PC-MG.

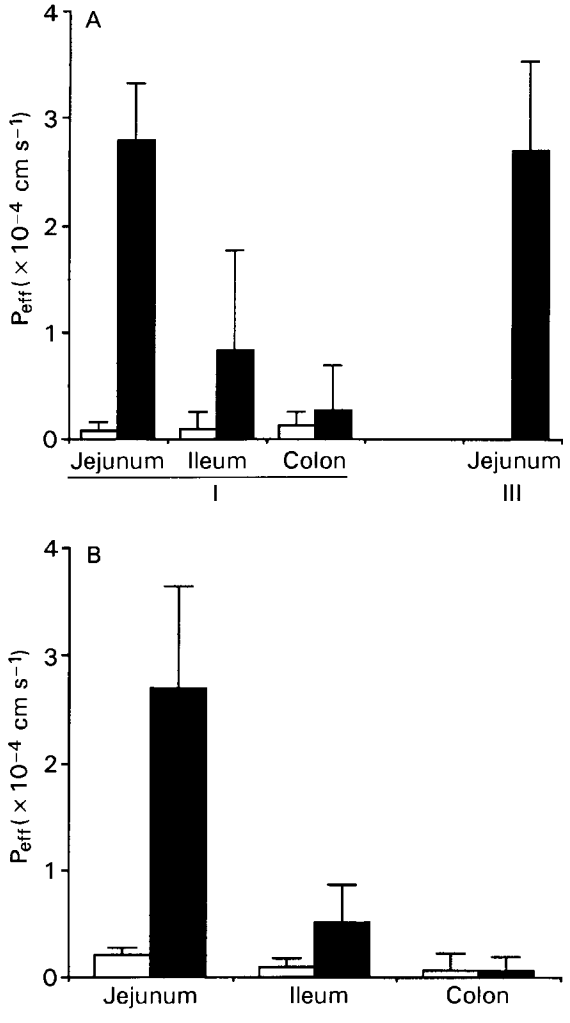

Figure 1. Effective permeability coefficients (P_{eff}) of hexarelin in the absence (□) and presence (■) of 15 mmol L^{-1} PC-MG, without (A; $P < 0.0001$ in the jejenum) and with (B; $P < 0.01$ in the jejenum) the protease inhibitor Pefabloc SC, in the single-pass in-situ perfused rat jejunum, ileum and colon. Values are means ± s.d.

The P_{eff} for atenolol was low and unaffected by PC-MG in all investigated regions of the rat intestine (Table 1). Because the jejunal P_{eff} of hexarelin was enhanced, but not that of atenolol, it seems unlikely that PC-MG has affected the paracellular permeability by opening up the tight junctions (Lennernäs et al 1994). The mechanism of action might instead involve interaction with the intestinal membrane, which was suggested by previous in-vitro studies with PC-MG and monoglycerides (Betageri et al 1993; Lohikangas et al 1994a; Aungst et al 1996). It has been proposed that monoglycerides cause disordering of the interior of cell membranes, and thereby increase membrane fluidity and permeability (Muranishi 1990; Muranishi & Yamamoto 1994). Increased permeability might also be because of extraction of cholesterol from the cell membranes (Lohikangas et al 1994a; Kararli 1995). The lack of an absorption-enhancing effect of PC-MG in the lower regions of the intestine might therefore be explained by reduced depletion of cholesterol from ileal and colonic cell membranes. This is in accord with the more rigid cell membranes in these regions, because of higher cholesterol and saturated fat content (Kararli 1995). Another hypothesis for the enhanced jejunal P_{eff} of hexarelin is that the P-glycoprotein efflux pump (which has been shown to limit the absorption of peptides across intestinal epithelia) becomes inactivated when the membrane characteristics are altered (Karlsson et al 1993; Bai et al 1995; Saitoh & Aungst 1995; Fricker et al 1996; Nerurkar et al 1996). However, the quantitative importance of this efflux system for oral drug absorption is not yet clear.

The effects of PC-MG have previously been investigated in Caco-2 cell monolayers and in-vivo in the rabbit rectum and duodenum (Bohlinder et al 1994; Lohikangas et al 1994a, b). In contrast with the lack of effect of PC-MG on the uptake of atenolol and PEG 4000 (data not shown) in the current study, Lohikangas et al (1994a,b) measured increased permeabilities for two other compounds of similar size and hydrophilicity, mannitol (MW 182) and fragmin (MW 5000), in the Caco-2 model. The basal permeabilities of mannitol and fragmin in Caco-2 cell monolayers were estimated to be 0·6 and $0{\cdot}8 \times 10^{-7}$ cm s^{-1}, respectively, and during exposure of 8 mmol L^{-1} PC-MG these P_{eff} values increased approximately 20- and 8-fold, respectively (Lohikangas et al 1994a). Although these increases seem large, the permeabilities still lead to prediction of low oral absorption (Lennernäs et al 1996). The discrepancy between the two models might, therefore, be the inability of the rat in-situ perfusion model to detect such small quantitative changes in intestinal absorption. In all, the PC-MG experiments suggest that this delivery system might be most effective in the upper small intestine for larger molecules with peptide structure and physicochemical properties similar to those of hexarelin. Hexarelin is a very flexible molecule that can assume many different conformations with various physicochemical properties. This dynamic molecular structure—and therefore properties—might explain why this compound was affected by PC-MG whereas the smaller and more rigid molecule atenolol was not (Palm et al 1996). The in-vivo potential of PC-MG and other enhancers is, however, difficult to predict. The concentration of the enhancer might be too low in-vivo, as a consequence of intestinal motility and secretion, and degradation by lipases (Swenson & Curatolo 1992; Ungell 1993). In addition, luminal bile salts and

dietary fats might form mixed micelles with the component(s) of the enhancer.

As indicated by the complete recovery of PEG (PEG_{rec}) in all perfusion experiments (data not shown), 15 mmol L^{-1} PC-MG and Pefabloc SC seemed not to affect functional intestinal viability. The implication of the perfusate lactate dehydrogenase levels measured is, however, difficult to interpret, firstly because no values of lactate dehydrogenase activity are available from the jejunal perfusions in study parts I and II, where an enhancer effect was observed, and secondly because a significant ($P < 0.05$) increase in lactate dehydrogenase perfusate concentrations were observed in the presence of PC-MG in ileal perfusions in part I of the study (a, 895 ± 854 units mL^{-1}; b, 1731 ± 641 units mL^{-1}) but not in part II. In the colonic experiments lactate dehydrogenase levels remained unchanged when PC-MG was added.

In summary, these results demonstrate that PC-MG might enhance the effective in-situ permeability in rats of peptide analogues such as hexarelin. However, although promising results were obtained, pharmacokinetic and toxicological studies are required to investigate whether PC-MG drug-delivery systems are suitable candidates for optimizing the oral bioavailability of hexarelin. Our data also suggest that the apparently low systemic bioavailability of hexarelin is partly because of low membrane permeability in the intestine.

Acknowledgements

Soybean phosphatidyl choline and monoacylglycerol were kindly supplied by Scotia LipidTeknik AB, Stockholm, Sweden. We thank Mrs Britt Jansson for analysis of atenolol, Håkan Johansson for skilful technical assistance during the perfusion experiments, and Dr Anders Sokolowski for the determination of pK_a and partitioning coefficients of hexarelin.

References

Aungst, B. J., Blake, J. A., Hussain, M. A. (1991) An in-vitro evaluation of metabolism and poor membrane permeation impeding intestinal absorption of leucine enkephalin, and methods to increase absorption. J. Pharmacol. Exp. Ther. 259: 139–145

Aungst, B. J., Saitoh, H., Burcham, D. L., Huang, S. -M., Mousa, S. A., Hussain, M. A. (1996) Enhancement of the intestinal absorption of peptides and non-peptides. J. Contr. Rel. 41: 19–31

Bai, J. P. F. (1993) Distribution of brush-border membrane peptidases along the intestine of rabbits and rats: implication for site-specific delivery of peptide drugs. J. Drug Targ. 1: 231–236

Bai, J. P. F., Chang, L. -L., Guo, J. -H. (1995) Targeting of peptide and protein drugs to specific sites in the oral route. Crit. Rev. Ther. Drug Carrier Syst. 12: 339–371

Betageri, G. V., Jenkins, S. A., Parsons, D. L. (1993) In: Betageri, G. V., Jenkins, S. A., Parsons, D. L. (eds) Liposome Drug-Delivery Systems. Technomic Publishing, Lancaster

Bohlinder, K., Olsson, B., Ragnarsson, G., Singh, S. K., Stjernström Öhman, G., Wadsten, T., Carlsson, A., Herlsöf, B. (1994) Use and characteristics of a novel lipid particle-forming matrix as a drug-carrier system. Eur. J. Pharm. Sci. 2: 271–279

Bouras, M., Huneau, J. -F., Tomé, D. (1996) The inhibition of intestinal dipeptidylaminopeptidase-IV promotes the absorption of enterostatin and des-arginine-enterostatin across rat jejunum in-vitro. Life Sci. 59: 2147–2155

Bruce, L. (1994) Biosomes: A New Approach to Drug Delivery. LipidTeknik and Pharmacia

Chadwick, V. S., Phillips, S. F., Hofmann, A. F. (1977) Measurements of intestinal permeability using low molecular-weight polyethylene glycols (PEG 400). II. Application to normal and abnormal permeability states in man and animals. Gastroenterology 73: 247–251

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

Fagerholm, U., Johansson, M., Lennernäs, H. (1996) Comparison between permeability coefficients in rat and human jejunum. Pharm. Res. 13: 1335–1341

Fagerholm, U., Lindahl, A., Lennernäs, H. (1997) Regional intestinal permeability in rats of compounds with different physicochemical properties and transport mechanisms. J. Pharm. Pharmacol. 49: 687–690

Fricker, G., Drewe, J., Huwyle, J., Gutman, H., Beglinger, C. (1996) Relevance of P-glycoprotein for the enteral absorption of cyclosporin A: in-vitro and in-vivo correlation. Br. J. Pharmacol. 118: 1841–1847

Ghigo, E., Arvat, E., Gianotti, L., Imbimbo, B. P., Lenaerts, V., Deghenghi, R., Camanni, F. (1994) Growth-hormone-releasing activity of hexarelin, a new synthetic hexapeptide, after intravenous, subcutaneous, intranasal, and oral administration in man. J. Clin. Endocrin. Metab. 78: 693–698

Imbimbo, B. P., Mant, T., Edwards, M., Amin, D., Dalton, N., Boutignon, F., Lenaerts, V., Wuthrich, P., Dengenghi, R. (1994) Growth hormone-releasing activity of hexarelin in humans. A dose-response study. Eur. J. Clin. Pharmacol. 46: 421–425

Kararli, T. T. (1995) Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. Biopharm. Drug Dispos. 16: 351–380

Karlsson, J., Kuo, S. -M., Ziemniak, J. A., Artursson, P. (1993) Transport of celiprolol across human intestinal epithelial (Caco-2) cells: mediation of secretion by multiple transporters including P-glycoprotein. Br. J. Pharmacol. 110: 1009–1016

Komiya, I., Park, J. Y., Kamani, A., Ho, N. F. H., Higuchi, W. I. (1980) Quantitative mechanistic studies in simultaneous fluid flow and intestinal absorption using steroids as model solutes. Int. J. Pharm. 4: 249–262

Krishnamoorthy, R., Mitra, A. K. (1995) Peptide metabolism by gastric, pancreatic, and lysosomal proteinases. In: Taylor, M. D., Amidon, G. L. (eds) Peptide-Based Drug Design. Chapter 3. American Chemical Society, Washington

Lee, H. J. (1995) Biopharmaceutical properties and pharmacokinetics of peptide and protein drugs. In: Taylor, M. D.,

Amidon, G. L. (eds) Peptide-Based Drug Design. Chapter 4. American Chemical Society, Washington

Lee, V. H. L., Yamamoto, A. (1990) Penetration and enzymatic barriers to peptide and protein absorption. Adv. Drug Del. Rev. 4: 171–207

Lennernäs, H., Ahrenstedt, Ö, Ungell, A. -L. (1994) Intestinal drug absorption during induced net water absorption in man: a mechanistic study using antipyrine, atenolol and enalaprilat. Br. J. Clin. Pharmacol. 37: 589–596

Lennernäs, H., Fagerholm, U., Raab, Y., Gerdin, B., Hällgren, R. (1995) Regional rectal perfusion: a new in-vivo approach to study rectal drug absorption in man. Pharm. Res. 12: 426–432

Lennernäs, H., Palm, K., Fagerholm, U., Artursson, P. (1996) Comparison between active and passive drug transport in human intestinal epithelial (Caco-2) cells in vitro and human jejunum in-vivo. Int. J. Pharm. 127: 103–107

Lohikangas, L., Wilen, M., Einarsson, M., Artursson, P. (1994a) Effects of a new lipid-based drug-delivery system on the absorption of low molecular weight heparin (Fragmin) through monolayers of human intestinal epithelial Caco-2 cells and after rectal administration to rabbits. Eur. J. Pharm. Sci. 1: 297–305

Lohikangas, L., Wilen, M., Einarsson, M., Artursson, P. (1994b) Relative contribution of phosphatidylcholine and monoglyceride to absorption enhancement of low molecular weight heparin (Fragmin) by a new lipid-based drug-delivery system in monolayers of human intestinal epithelial Caco-2 cells and after rectal administration to rabbits. Eur. J. Pharm. Sci. 1: 307–312

Muranishi, S. (1990) Absorption enhancers. Crit. Rev. Ther. Drug Carrier Syst. 7: 1–34

Muranishi, S., Yamamoto, A. (1994) Mechanisms of absorption enhancement through the intestinal epithelium. In: de Boer, A. (Bert) G. (ed.) Drug Absorption Enhancement. Harwood Academic Publishers

Nerurkar, M. M., Burton, P. S., Borchardt, R. T. (1996) The use of surfactants to enhance the permeability of peptides through Caco-2 cells by inhibition of an apically polarized efflux system. Pharm. Res. 13: 528–534

Palm, K., Luthman, K., Ungell, A. -L., Strandlund, G., Artursson, P. (1996) Correlation of drug absorption with molecular surface properties. J. Pharm. Sci. 85: 32–39

Rubinstein, A. (1995) Approaches and opportunities in colon-specific drug delivery. Crit. Rev. Ther. Drug Carrier Syst. 12: 101–149

Saitoh, H., Aungst, B. J. (1995) Possible involvement of multiple P-glycoprotein-mediated efflux systems in the transport of verapamil and other organic cations across rat intestine. Pharm. Res. 12: 1304–1310

Schultz, R., Winne, D. (1987) Relationship between antipyrine absorption and blood flow rate in the rat jejunum, ileum and colon. Naunyn Schmiedebergs Arch. Pharmacol. 335: 97–102

Stewart, B. H., Taylor, M. D. (1995) Prodrug approaches for improving peptidomimetic drug absorption. In: Taylor, M. D., Amidon, G. L., (eds) Peptide-Based Drug Design. Chapter 9. American Chemical Society, Washington

Swenson, E. S., Curatolo, W. J. (1992) Means to enhance penetration: intestinal permeability enhancement for proteins, peptides and other polar drugs: mechanisms and potential toxicity. Adv. Drug Del. Rev. 8: 39–92

Swenson, E. S., Milisen, W. B., Curatolo, W. (1994) Intestinal permeability enhancement: structure-activity and structure-toxicity relationships for nonylphenoxypolyoxyethylene surfactant permeability enhancers. Pharm. Res. 11: 1501–1504

Taki, Y., Sakane, T., Nadai, T., Sezaki, H., Amidon, G. L., Langguth, P., Yamashita, S. (1995) Gastrointestinal absorption of peptide drug: quantitative evaluation of the degradation and the permeation of metkephamid in rat small intestine. J. Pharmacol. Exp. Ther. 274: 373–377

Tomita, M., Hayashi, M., Horie, T., Ishizawa, T., Awazu, S. (1988) Enhancement of colonic drug absorption by the transcellular permeation route. Pharm. Res. 5: 786–789

Ungell, A. -L. (1993) Absorption from the gastrointestinal tract. In: Current Status on Targeted Drug Delivery to the Gastrointestinal Tract. Capsugel Library

Yodoya, E., Uemura, K., Tenema, T., Fujita, T., Murakami, M., Yamamoto, A., Muranishi, S. (1994) Enhanced permeability of tetragastrin across the rat intestinal membrane and its reduced degradation by acylation with various fatty acids. J. Pharmacol. Exp. Ther. 271: 1509–1513

Yuasa, H., Matsuda, K., Watanabe, J. (1993) Influence of anaesthetic regimens on intestinal absorption in rats. Pharm. Res. 10: 884–888