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Permeability and absorption of leuprolide from various intestinal regions in rabbits and rats

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Abstract

The in vitro permeability and in vivo absorption of leuprolide in different intestinal regions were measured to investigate the feasibility for site-specific delivery of leuprolide in the gastrointestinal (GI) tract. In vitro permeability of leuprolide in the rabbit GI tract was performed using a side-by-side diffusion apparatus and the permeability coefficients in the jejunum, ileum and colon were 0.27×10^{-7}, 2.96×10^{-7} and 7.85×10^{-7}cm/s, respectively. Varying the donor drug concentrations from 2 to 10 mg/ml, the permeability coefficients were independent of the donor concentration, suggesting the transport mechanism of passive diffusion. Using an intestine loop model in anesthetized rats, bioavailabilities of leuprolide in the jejunum, ileum and colon were 1.28, 5.62 and 9.59%, respectively. Drug recovery from the loop 5 h after dosing was 10.7% in jejunum, 24.5% in ileum and 40.7% in colon. Additional in vivo studies using conscious rats showed that the bioavailability of leuprolide was less than 1% for both ileal and colonic administration. In vivo absorption of leuprolide from ileum was not significantly different from colon in conscious rats. Sodium salicylate, a permeation enhancer, was co-administered with leuprolide to the rat ascending colon, and results showed a 4-fold increase in the bioavailability in conscious rats. Thus, in vivo studies indicate that both absorption and enzymatic degradation of leuprolide in the GI tract is site-dependent and the lower intestine may be an advantageous region for oral delivery of leuprolide.

Keywords: Abbott-43818; Leuprolide acetate; Peptide drug; Intestinal permeability; Oral absorption; Site-specific drug delivery

1. Introduction

Delivery through the gastrointestinal (GI) tract is the traditional method by which drugs are presented for systemic therapeutic effects in the body. Although the entire GI tract is capable of drug absorption, the small intestine (which can be

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subdivided into duodenum, jejunum and ileum) is the major site of absorption for most nutrients and drugs. However, most therapeutic peptide and protein drugs are poorly absorbed when given orally due to their high hydrophilicity, large molecular size and extreme instability in the GI tract (Wilding et al., 1994). Typically, the oral bioavailabilities of proteins and peptides are less than 2% (Banga and Chien, 1988). Several recent reviews on oral absorption of proteins and peptides have given insight on their low oral bioavailabilities (Lee et al., 1995). An increasing amount of evidence indicates that an advantageous region for the absorption of a specific peptide or protein drug may exist in the digestive tract. For instance, insulin and salmon calcitonin showed better absorption from the ileum than from the jejunum and the colon in rats (Morishita et al., 1993; Smith et al., 1994) and the apparent insulin permeability also varies with different intestinal regions (Schiling and Mitra, 1990). When 1-deamino-8-D-arginine vasopressin was given to the stomach, the duodenum, ileum, ileo-coecal junction or colon in rabbits, the highest plasma concentration of the drug was observed after ileo-coecal administration (Lundin and Vilhardt, 1986). Therefore, more attention has now been paid to the site-specific oral delivery of peptide and protein drugs in the GI tract.

Leuprolide acetate (Abbott-43818) is a potent agonist of the luteinizing hormone-releasing hormone (LH-RH) receptor. It is a nonapeptide chemically defined as 5-Oxo–Pro–His–Trp–Ser–Tyr–D-Leu–Leu–Arg–ProNHEt acetate with a molecular weight of 1209. Like most other peptide or protein drugs, leuprolide has very low oral bioavailability (Adjei et al., 1993), and is currently administered by subcutaneous or intramuscular injection (Okada et al., 1988). It has been found that leuprolide is rapidly degraded by chymotrypsin and rat intestinal mucosa homogenates (Haviv et al., 1992; Zheng et al., 1998). However, it does not exhibit notable hepatic first-pass clearance (Hoffman, 1991). Some proteases and peptidases appear to act as barriers limiting the intestinal absorption of leuprolide. Thus, lower small intestine or colon, where the enzymatic activity is relatively low, may be a preferred absorption site for leuprolide. The poor permeability of macromolecules in the intestine may further reduce the oral bioavailability of leuprolide. By gaining an understanding of the permeability of leuprolide in different intestinal regions, and further by using a site-specific delivery technique, the intestinal absorption of the drug may be improved. Although many efforts have been made to improve oral delivery of leuprolide, the site-specific permeability and absorption of leuprolide in the GI tract has not been investigated. The present study was undertaken to evaluate the feasibility of site-specific delivery of leuprolide in the GI tract. The work included in vitro permeability in various regions of rabbit intestine and in vivo site-specific absorption of leuprolide in rats.

2. Materials and methods

2.1. Materials and equipment

Abbott-43818 (leuprolide acetate) was obtained from Abbott Laboratories. HPLC grade water, acetonitrile and methanol were obtained from EM Industries (Gibbstown, NJ). Tetramethylammonium perchlorate and trifluoroacetic acid were from Sigma Chemical Co. (St. Louis, MO). All other reagents and materials were of the highest purity commercially available.

The HPLC used was a Spectra system model P-4000 equipped with an AS3000 autosampler, a UV/VIS detector and an PC 1000 data processing computer station (Thermo Separation Products, Fremont, CA). A vertical diffusion chamber system (Costar®) used in the permeability study was from Corning Inc. (Miami, FL).

Male New Zealand white rabbits, weighing 2.5–3.0 kg, were purchased from Hazleton Research Products (Kalamazoo, MI). Sprague–Dawley male rats (250–300 g) were obtained from Charles River (Portage, MI). The animals were maintained in a controlled temperature (21 ± 1°C) and light (12 h light/24 h) environment.

2.2. In vitro permeability studies

Animal intestinal tissues for the permeability study were obtained from the rabbits using the experimental method described previously (Day et al., 1994). Briefly, the rabbits were fasted for 16 h before each experiment and sacrificed by injection of Euthanasia T-61 through the marginal ear vein. Following a midline incision, the various segments of the rabbit intestine were removed, including jejunum (the proximal part of small intestine), ileum (the distal part of small intestine) and ascending colon (proximal to cecal-colonic junction), and placed into oxygenated Kreb's solution (1.1 mM $MgCl_2$, 1.25 mM $CaCl_2$, 114 mM NaCl, 5 mM KCl, 25 mM $NaHCO_3$, 1.65 mM Na_2HPO_4 and 0.3 mM NaH_2PO_4, pH 7.4). Each segment was opened along the mesenteric line, rinsed with Kreb's solution, and affixed between the opposing faces of the half-chambers of the diffusion cell using a series of pins surrounding the opening (Day et al., 1994). Serosal buffer (7 ml, 40 mM of D-glucose in Kreb's solution) was added to the receptor side of the chamber. An equal volume of mucosal buffer (40 mM of mannitol in Kreb's solution) containing leuprolide was simultaneously added to the donor side of the chamber. Mixing was achieved by bubbling a mixture of 95% O_2 and 5% CO_2 in a steady stream. The diffusion system was maintained at 37°C using a water circulator. Aliquots (500 μl) were periodically taken from the receptor (serosal) side for analysis of leuprolide up to 210 min. The sample volume was replaced immediately by an equal volume of blank serosal buffer. Immediately before HPLC analyses, the sample solution was subjected to centrifugation at approximately $2000 \times g$ for 5 min, and 100 μl of the resulting supernatant fraction was used for HPLC analysis. For each test, leuprolide in the donor solution was also assayed by an HPLC method at the beginning and end of the experiment to evaluate potential enzymatic and chemical degradation during the experiment.

A permeability coefficient (P) was calculated according to the relationship $P = dQ/dt/[A \times C_d]$, where Q is the amount of a compound traversing the tissue in time t (min), A is the area of exposed tissue (2.06 cm^2) and C_d represents drug concentration on the donor side.

2.3. Site-directed delivery of leuprolide in the rat GI tract

2.3.1. Anesthetized rat

Sprague–Dawley rats, anesthetized with nembutal/ketamine, were surgically prepared with portal vein and aortic artery sampling cannulas. Intestinal (10-cm) segments were tied-off at either the jejunum (just distal to the ligament of Treitz), the ileum (immediately proximal to the cecum) or the ascending colon. Leuprolide dose solution at 5 mg/ml containing 2.5 mM theophylline (absorption probe) was injected directly into the intestinal segment at a dose of 10 μmol/kg using an 18-gauge needle (Hoffman et al., 1995). Heparinized portal and aortic blood samples (0.3 ml) were simultaneously removed at 0, 3, 8 min, and 0.25, 0.5, 1, 1.5, 2, 3, and 5 h. After removing an aliquot for the theophylline assay, the blood was immediately processed for plasma by centrifugation. The plasma concentration of leuprolide was determined with a radioimmunoassay (Adjei et al., 1993). Blood theophylline was assayed by an HPLC method.

The fraction of the leuprolide dose absorbed by the intestine was estimated from the portal-systemic concentration gradients relative to the portal-systemic gradient and dose of the absorption probe, theophylline. Absorption rate constants were calculated from the initial slope of the fraction absorbed vs. time (Hoffman et al., 1995).

2.3.2. Conscious rat

Sprague–Dawley rats were surgically implanted with chronic portal and inferior vena cava vein and aortic arterial cannulas, and dosing cannulas at various intestinal sites according to the procedure described previously (Hoffman et al., 1995). Animals were individually caged and allowed to recover for at least 4 days following surgery. The dosing, sampling and analysis for the conscious animals was the same as described for anesthetized animals.

2.4. HPLC analysis of leuprolide acetate and theophylline

2.4.1. Method A

Leuprolide in the receptor or donor solution from permeability studies was analyzed by reverse phase HPLC on a Vydac™ protein and peptide C18 column (5 μm, 4.6 × 250 mm) at ambient temperature according to a previously described method with modification (Sutherland and Menon, 1987). The mobile phase consisted of water:acetonitrile:methanol (65:30:5) containing 6.68 mM tetramethylammonium perchlorate, and 0.066% trifluoroacetic acid (TFA). Elution of the HPLC column was conducted at a flow rate of 1.0 ml/min with the mobile phase, and the column eluate was monitored by absorbance at 220 nm. The concentration of leuprolide was determined from the HPLC peak area using a linear standard curve relating peak area to the concentration of leuprolide. The limitation of determination for leuprolide was 0.02 μg.

2.4.2. Method B

This method was used to determine the amount of leuprolide recovered from rat intestinal loops. Immediately following the absorption study in rats, the ligated intestinal segment (10 cm) was removed from the animal, cut open and rinsed with 50 ml of water into a beaker. The epithelium was scraped off with a glass slide and added to the wash. The remaining tissue was cut into small pieces (about 1 cm^2) and also added to the wash mixture. The entire mixture was transferred to a 100-ml volumetric flask and adjusted to volume with CH_3CN/water (1:1) and mixed. Prior to HPLC analyses, the samples were cleaned-up as follows using solid phase extraction (SPE): Alltech 1-ml ODS SPE columns were sequentially rinsed with two volumes of methanol, one volume of 0.1% TFA and one volume of water. A volume of sample (2 ml) was diluted with 2 ml of water and the entire solution (4 ml) passed through the SPE column. The SPE column was rinsed sequentially with three volumes of water, two volumes of methanol/water (1:1) and 0.5 ml of methanol and then dried by vacuum aspiration for 1 min. Leuprolide was eluted from the SPE column with 0.5 ml of 0.1% TFA in methanol. Eluents were diluted with 2 ml water and injected (25 μl) onto a Regis Little Champ® ODS HPLC column (5 × 0.46 cm). Leuprolide was eluted at 5.3 min using a mobile phase of CH_3CN/methanol/10 mM tetraethylammonium perchlorate in 0.1% TFA at 1 ml/min, and detected by UV absorbance at 205 nm.

2.4.3. Method C

Blood theophylline was assayed by adding 50 μl of blood sample to a 1-ml plastic bullet containing 100 μl of CH_3CN with rapid mixing. After centrifugation for 5 min on a clinical centrifuge, 75 μl of supernatant was added to a sample vial containing 1 ml water, capped and mixed. A 50 μl aliquot was injected onto the HPLC column. The HPLC assay for theophylline was performed on a Regis Little Champ® ODS column with a mobile phase of CH_3CN/10 mM phosphate (pH 3) (4:96) at 1 ml/min, and the elution was monitored at 273 nm.

3. Results and discussion

3.1. Permeability of leuprolide in various intestinal regions of rabbits

A variety of in vitro approaches have been used to assess the transport characteristics of peptides and proteins across the intestinal epithelium and model this barrier function of the gut (Ungell, 1993). One of the most common methods determines the intestinal permeability of a compound using isolated segments of intestinal tissue. Intestinal permeability directly reflects the interactions of the molecule with the tissue, and is affected by the physiological properties of the tissue as well as the physicochemical properties of the transported compound. Thus, permeability measurements for a compound should be more useful to predict absorption than physicochemical data alone.

The intestinal permeability of leuprolide was investigated in vitro using isolated rabbit intestinal tissues, including jejunum, ileum and ascending colon. It was observed that the amount of leuprolide in the receptor side increased with time.

The permeability coefficients of leuprolide in the jejunum, ileum and colon of rabbits, which were calculated from the slopes of the linear portion of the diffusion profiles, are listed in Table 1. When the donor concentration was 6 mg/ml, the mean permeability coefficients of leuprolide in jejunum, ileum and colon were 0.27×10^{-7}, 2.96×10^{-7} and 3.58×10^{-7} cm/s, respectively. Thus, the permeability of leuprolide in ileum and colon was about ten times higher than that in jejunum, indicating a significant difference ($p < 0.01$). The results indicate that the permeability of leuprolide may vary in the different regions of the intestine and the ileum and colon are more permeable to leuprolide than jejunum. No significant difference in leuprolide permeability was obtained between ileum and colon at donor concentrations of 6 and 10 mg ($p > 0.05$). However, at the donor concentrations of 4 mg/ml, the mean permeability coefficients of leuprolide were 1.38×10^{-7} cm/s in ileum and 2.83×10^{-7} cm/s in colon showing a statistical difference ($p < 0.05$). The data indicates that the order of the permeability in rabbit intestines is colon > ileum ≫ jejunum. Generally, paracellular transport of a molecule has a tendency to decrease from the small intestine to the colon due to the decreases in the surface area, cell density, and tight junction between cells. However, similar observation of the increase in in vitro permeability in ileum and colon compared with jejunum was also reported by Jezyk et al. for different compounds (Jezyk et al., 1992). Although the mechanisms behind this observation have not been understood, the data suggest that leuprolide may be absorbed not only via paracellular route but also by transcellular route.

Table 1
In vitro permeability of leuprolide in different regions of the rabbit gastrointestinal tract (mean ± SD)

Region of the gut	Leuprolide in donor side (mg/ml)	Permeability coefficients (P, $\times 10^{-7}$cm/s)
Jejunum	6	0.27 ± 0.17 ($n = 4$)
	10	0.37 ± 0.27 ($n = 3$)
Ileum[d]	4	1.38 ± 0.90 ($n = 5$)
	6	2.96 ± 3.41 ($n = 6$)[a]
	10	3.52 ± 1.03 ($n = 3$)[a]
Colon[d]	2	2.04 ± 0.41 ($n = 4$)
	4	2.83 ± 0.55 ($n = 6$)[b]
	6	3.58 ± 0.81 ($n = 6$)[a,c]
	10	2.99 ± 1.23 ($n = 3$)[a,c]

[a] t-Test; $p < 0.01$ compared with jejunum at same donor concentration indicates very significant differences at the 5% level.

[b] t-Test; $p < 0.05$ compared with ileum at same donor concentration indicates significant differences at the 5% level.

[c] t-Test; $p > 0.05$ compared with ileum at same donor concentration indicates no significant differences at the 5% level.

[d] ANOVA test; $p > 0.05$ among different donor concentrations indicates no significant differences at the 5% level.

Molecule transport through the membrane can often occur either as a result of passive diffusion or active transport. Unlike the active process, the passive diffusion of a compound through the cell membrane is dependent on the concentration gradient with a constant permeability coefficient. In this study, varying the donor concentration of leuprolide did not lead to a significant change in the permeability coefficient of the drug in the ileum and colon ($p > 0.05$, see Table 1). This suggests that a passive diffusion process may not be excluded for transport of leuprolide through the intestinal membrane.

In this study, the intestinal segments were not stripped of the serosal muscle layer (muscularis externa). This may result in a longer lag time. The lag time in ileum and colon was 63 ± 20 min ($n = 4$) and 75 ± 10 min ($n = 6$), respectively. Impact of the serosal muscle layer on leuprolide permeability in the intestinal segments was not evaluated since significant degradation of leuprolide in the receptor (serosol) side was observed using the stripped rabbit ileum. It is possible that the enzymes for degradation of leuprolide become accessible after the serosal muscle is stripped off.

3.2. Degradation of leuprolide

The enzymatic barrier is one of the most important factors limiting the absorption of peptide and protein drugs from the gastrointestinal tract. Protease and/or peptidase activities exist in the intestinal lumen juice, brush border and cytosol. The determination of permeability coefficients could be inaccurate if significant metabolism of a compound occurred during the process of diffu-

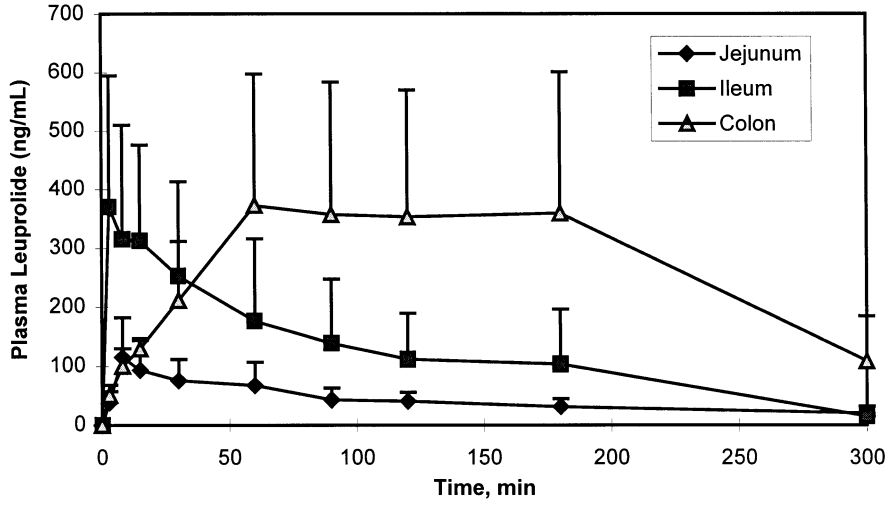

Fig. 1. Site-specific absorption of leuprolide in the intestinal loops of anesthetized rats. Leuprolide was administered at a dose of 10 mg/kg into the rat intestinal loops (mean ± SD, $n = 5$).

sion. Previous studies have shown that leuprolide was enzymatically metabolized in the intestinal mucosa of rats, and the apparent K_m and V_{max} were 898 μM and 3.4 nmol/min/mg protein, respectively (Zheng et al., 1998). To determine the potential metabolism of leuprolide in the in vitro diffusion system, the HPLC analyses of donor side solutions were performed at the end of the experiment. The results showed that the remaining amount of leuprolide was 97% or higher after 3.5-h contact with the rabbit intact jejunum, ileum or colon segments. This indicates that the enzymatic degradation of leuprolide did not occur to any extent as to significantly influence the amount of uptake. However, metabolism of leuprolide by membrane-bound or intracellular enzymes can not be ruled out.

3.3. Site-specific absorption of leuprolide in anesthetized rats

Fig. 1 shows the plasma profile following oral absorption of leuprolide from the jejunum, ileum or colon loop in anesthetized rats at a dose of 10 mg/kg. Absorption of leuprolide from jejunum was poor with the maximum plasma level (C_{max}) of 114.7 ± 67.8 ng/ml at 8 min. However, C_{max} values of leuprolide following administration to the ileum and colon were 369.9 ± 225.1 ng/ml at 3 min and 372.7 ± 225.0 ng/ml at 60 min, respectively. The mean areas under the leuprolide plasma level versus time curve (AUC) in the ileum and colon were significantly higher than that for the jejunum ($p < 0.01$, Table 2). The absorption rate constant (K_a) of leuprolide was significantly lower compared with theophyline, an orally well absorbed drug (Table 2). The order of K_a for leuprolide in the GI tract was ileum > colon ≫ jejunum. The average bioavailability estimates of leuprolide in ileum and colon compared with intravenous injection in anesthetized rats were 5.62 and 9.59%, respectively, which were significantly higher than that in jejunum (1.28%, $p < 0.05$, Table 2). The results, supported by the findings from the permeability studies, indicate that ileum and colon may provide better absorption of leuprolide following oral administration compared with the jejunum.

Ileum and colon are recognized sites in the GI tract to possess lower enzymatic activity compared with the jejunum. Hence the dosing of a labile drug like leuprolide to ileum or colon may improve its bioavailability. In order to evaluate the effect of proteolytic enzymes on the bioavailability of leuprolide, the amount of drug remaining at each dosing site was determined at

Table 2
Site-specific absorption of leuprolide in the gastrointestinal tract of anesthetized rats[a]

Dosing route	Number of rats	AUC (ng/ml/min)	Bioavailability (%)	Absorbed rate constant K_a (h^{-1})	
				Leuprolide	Theophyline
IV	5	9870 ± 1156	–	–	–
Jejunum	5	12629 ± 5850	1.28 ± 0.59	0.0056 ± 0.0021	3.54 ± 0.48
Ileum	5	55447 ± 37595	5.62 ± 3.81[b]	0.0734 ± 0.0569	2.68 ± 0.13
Colon	5	70374 ± 33671	9.59 ± 7.23[b,c]	0.0205 ± 0.0063	2.06 ± 0.12

[a] Data are expressed as mean ± SD from three anesthetized rats as described in Section 2. Leuprolide was given to rats at 0.1 mg/kg for i.v., and 10 mg/kg for jejunal, ileal and colonic administration.
[b] t-Test; $p < 0.05$ compared with jejunum indicates significant differences at the 5% level.
[c] t-Test; $p > 0.05$ compared with ileum indicates no significant differences at the 5% level.

the end of the experiment. As seen in Table 3, the dose recovery from jejunum, ileum and ascending colon in anesthetized rats was 10.7, 24.5 and 40.7%, respectively. Note that only a small portion of the drug was absorbed into the blood circulation. Thus, the data suggest that the enzymatic degradation of leuprolide in the colon was much less than that in the ileum and jejunum as the recovery rate of drug from the colon was significantly greater than results from the ileum and jejunum. In addition, there is some correlation between in vivo absorption of leuprolide in anesthetized rats and in vitro permeability in rabbit intestinal segments, i.e. the intestinal segment with higher in vitro permeability showed greater in vivo absorption using the intestinal loop model in anesthetized rats.

3.4. Site-specific absorption of leuprolide in conscious rats

The state of anesthesia is a drug-induced absence of perception of all sensations. In general, anesthesia does have effects on the circulation, respiration, the nervous system, muscle, the liver and the GI tract. Thus, the absorption, distribution and metabolism of a drug could be changed due to anesthesia. Although the data obtained from the above intestinal loop model showed a promising way for oral delivery of leuprolide, the model did not represent an actual absorption rate when the drug is administered into the GI tract. The intestinal loop constituted a drug reservoir, thus increasing the local drug concentration. Therefore, the site-directed delivery of leuprolide into the GI tract was further evaluated using a conscious rat model. The results of the study are shown in Table 4. Unexpectedly, bioavailabilities of less than 0.6% were found when conscious rats were given 1, 3 or 6 mg/kg of leuprolide to the ileum or the ascending colon (Table 4). At the same dose level, the bioavailability in ileum was slightly higher than that in colon even though the difference was insignificant ($p > 0.05$). The plasma drug concentrations in all of the samples tested were less than 13 ng/ml, and the variability among rats was high (Figs. 2 and 3). Following ileal administration, the C_{max} at the dose of 1 and 3 mg/kg was 3.19 ± 1.49 and 5.26 ± 4.94 ng/ml, respectively, and the AUC was 263 ± 97 and 231 ± 264 ng/ml/min. In the case of colonic administration, the C_{max} values at the dose of 3 and 6 mg/kg were 1.95 ± 0.18 and 1.44 ± 0.74 ng/ml, respectively, and the AUC values were 124 ± 87 and 83 ± 38 ng/ml/min.

Table 3
Dose recovery of leuprolide from intestine loop in anesthetized rats

Region of the gut	Number of rats	Dose recovery (%)[a]
Jejunum	5	10.7 ± 3.4
Ileum	5	24.5 ± 8.0
Colon	5	40.7 ± 16.5

[a] Data are expressed as mean ± SD. Leuprolide was given at a dose of 10 mg/kg to different intestinal regions of anesthetized rats, and the concentration of leuprolide in the tissue rinsing solution was determined by an HPLC method.

Table 4
Site-specific absorption of leuprolide in the gastrointestinal tract of conscious rats (mean ± SD)

Dosing route	Drug	Dose (mg/kg)	Number of rats	$AUC_{(0-5h)}$ (ng/ml/min)	Bioavailability (%)
IV[a]	Leuprolide	0.1	4	4518 ± 802	–
Ileum	Leuprolide	1	4	263 ± 97	0.58 ± 0.21[b]
		3	4	231 ± 264	0.17 ± 0.19[b]
Colon	Leuprolide	1	4	187 ± 100	0.41 ± 0.22
		3	4	124 ± 87	0.09 ± 0.06
		6	5	83 ± 38	0.03 ± 0.01
	Leuprolide +Salicylate Na	3 +3	5	505 ± 96	0.37 ± 0.07[c]

[a] The area under the curve (AUC) was calculated from 0–∞.
[b] *t*-Test; $p > 0.05$ compared with colon at same dose level indicates no significant differences at the 5% level.
[c] *t*-Test; $p < 0.05$ compared with leuprolide alone in colon (3 mg/kg) indicates significant differences at the 5% level.

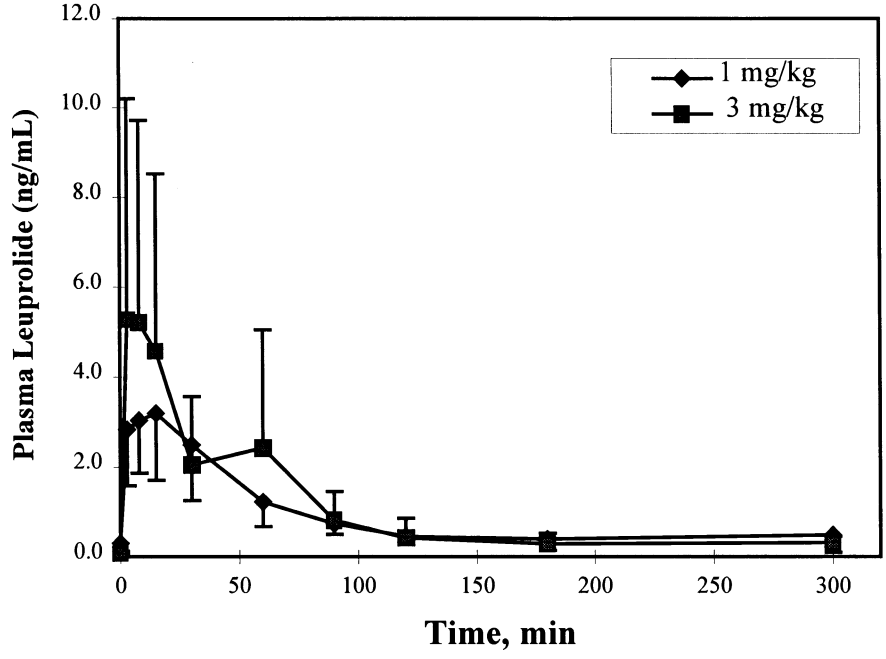

Fig. 2. Ileal absorption of leuprolide in conscious rats (mean ± SD).

Sodium salicylate has been postulated to interact with membrane proteins and reduce the levels of membrane non-protein thiols. Thus, sodium salicylate increases transcellular absorption, and may also increase paracellular transport by calcium chelation (Aungst and Rogers, 1988). Sodium salicylate has been used mostly to promote rectal and intestinal permeability. In this study, sodium salicylate seems to be effective in its ability to increase the colonic absorption of leuprolide as shown in Fig. 3. The C_{max} for leuprolide with sodium salicylate was 4.78 ± 4.62 ng/ml, about 3-fold higher compared with that for leuprolide alone (Table 4). The mean AUC for leuprolide with sodium salicylate also increased to 505 ± 96 ng/ml/min compared with 124 ± 87 ng/ml/min for leuprolide alone (Fig. 3). The mean bioavailability significantly increased from 0.09 to

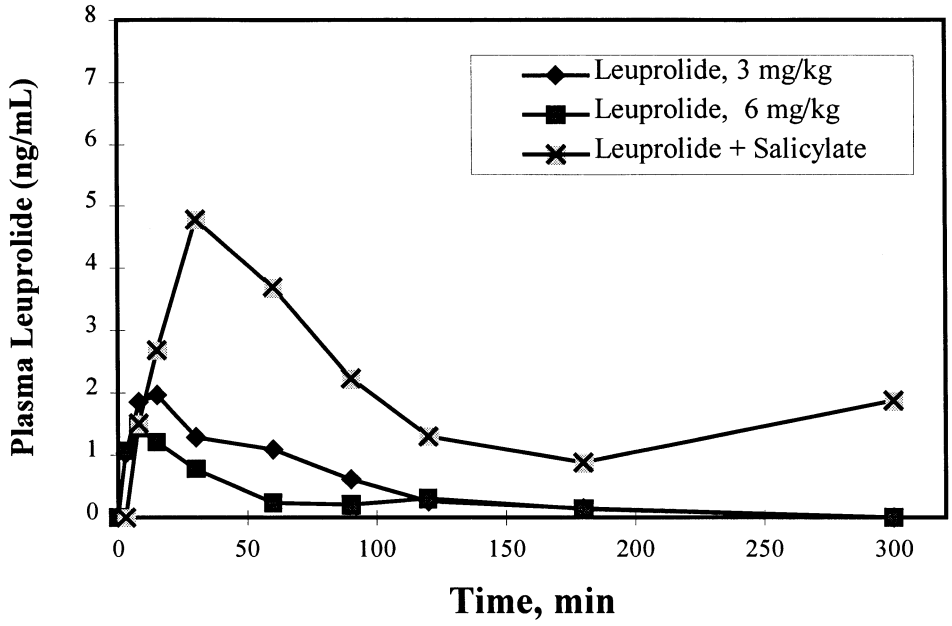

Fig. 3. Colonic absorption of leuprolide in conscious rats. [◆] leuprolide 3 mg/kg; [■] leuprolide 6 mg/kg; [×] leuprolide + sodium salicylate, both leuprolide and sodium salicylate were 3 mg/kg.

0.37% when sodium salicylate (3 mg/kg) was co-administered with leuprolide into the ascending colon of conscious rats. Overall, those results suggest that the lower GI tract should be a targeting site for oral delivery of leuprolide should effective absorption enhancers and suitable enzyme inhibitors be explored.

4. Conclusions

Permeability and bioavailability studies were conducted in rabbit intestinal segments, anesthetized and unanesthetized Sprague–Dawley rats. The conclusions are summarized below.

1. Leuprolide acetate (Abbott-43818) demonstrated very low in vitro permeability in the rabbit GI tract. The permeability of leuprolide in the ileum and colon was about six times higher than that in the jejunum.
2. Absorption for the ileum and colon was significantly higher than that from the jejunum using a rat intestine loop model and a conscious rat model. The degradation of leuprolide in jejunum was much faster than that in ileum and colon.

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