Impact of Regional Intestinal pH Modulation on Absorption of Peptide Drugs: Oral Absorption Studies of Salmon Calcitonin in Beagle Dogs

Yong-Hee Lee,[1] Barbara A. Perry,[2] Stacy Labruno,[1] Hee Sang Lee,[1] William Stern,[3] Lisa M. Falzone,[3] and Patrick J. Sinko[1,4]

Received February 10, 1999; accepted May 13, 1999

Purpose. To investigate the relationship between the modulation of intestinal pH and the oral absorption properties of a model peptide drug, salmon calcitonin (sCT), in conscious beagle dogs.

Methods. Studies were performed to characterize the disintegration of the formulation, intestinal pH changes, and the appearance of the peptide in the blood. Enteric-coated formulations containing sCT and various amounts of citric acid (CA) were tethered to a Heidelberg capsule (HC) and given orally to normal beagle dogs. Blood samples were collected and analyzed by radioimmunoassay (RIA). Intestinal pH was continuously monitored using the Heidelberg pH capsule (HC) system. The integrity of the HC-delivery system tether was verified by fluoroscopy.

Results. The intra-individual variation in gastric emptying (GE) of the delivery system was large. There were also large inter-individual differences in the disintegration and absorption properties of the various formulations. However, the peak plasma concentrations of sCT were always observed when the intestinal pH declined. The average baseline intestinal pH was 6.1 ± 0.2 (mean ± SEM, n = 12). The intestinal pH reduction was 2.6 ± 0.4 (mean ± SEM, n = 12, ranged from 0.5 to 4.0 units from baseline). There was a good correlation between the time to reach the trough intestinal pH ($t_{pH,min}$) and time to reach the peak plasma concentration ($t_{conc,max}$) of sCT ($t_{conc,max} = 0.95 \times t_{pH,min} + 14.1$, n = 11, $r^2 = 0.91$). Plasma C_{max} and area under the curve (AUC) increased with increasing amounts of CA in the formulations.

Conclusions. The results of these studies demonstrate that the oral absorption properties of a model peptide drug, sCT, can be modulated by changing intestinal pH. sCT is a substrate for the pancreatic serine protease trypsin which has maximal activity at pH 5 to 6. Reducing intestinal pH presumably stabilizes sCT in the GI tract enabling greater absorption of the intact peptide.

KEY WORDS: intestinal pH recovery; oral absorption; peptide drugs; salmon calcitonin; dogs.

INTRODUCTION

Establishing an oral delivery system for peptides and protein drugs is of great importance because parenteral administration results in poor patient compliance during chronic treatment resulting in limited clinical utility. The clinical development of peptide drugs, however, has been impeded by poor peptide absorption across intestinal membranes and rapid proteolytic degradation which typically result in oral bioavailabilities less than 1–2% (1–3). Although gastric acid and enzymes efficiently degrade peptides, this can be avoided by using an enteric coating to bypass the stomach. Unfortunately, the proteolytic enzymes in the small intestine are equally proficient at degrading peptide drugs (4–6). Potential approaches to limit the activity of intestinal enzymes include delivery of protease inhibitors, adjusting the pH of the intestinal contents, and maintaining high local drug concentrations (7–9). Therefore, it is essential to understand if the intestinal microenvironment can be temporarily modified to enhance drug delivery and, if so, to understand how the intestine responds to changes induced by excipients. Calcitonin is an excellent candidate for the development of alternate delivery routes due to its size and wide therapeutic index (10) with a preference towards the oral delivery route. One requirement for its oral delivery is intestinal stabilization since sCT is a substrate for the pancreatic serine protease trypsin. In our previous report (11), the inclusion of CA significantly improved the oral absorption of sCT in various intestinal regions in Intestinal and Vascular Access Ports (IVAP) dogs. In this report, traditional pharmacokinetic techniques are combined with radiotelemetric measurement of intestinal pH to elucidate the effect of pH modulation on the oral absorption properties of sCT. The results clearly demonstrate that the modulation of intestinal pH can be successfully used as a strategy to enhance the oral absorption properties of peptide drugs.

MATERIALS AND METHODS

Materials

Recombinant salmon calcitonin (sCT) was obtained from Unigene Laboratories, Fairfield, NJ. sCT antibody (cross reacts less than 1% with mammalian calcitonins) and ^{125}Iodotyrosyl-salmon calcitonin were obtained from Advanced Chem Tech (Louisville, KY). Eudragit L30-D55 was obtained from Huls America Inc. (Sommerset, NJ). Surgical thread, 3-0 vicryl was obtained from Ethicon (Somerville, NJ). Surgical adhesive, Krazy glue was obtained from Berden Inc. (Columbus, OH). IV catheter, 20G Abbocath was obtained from Abbott Labs (North Chicago, IL). Heparinized syringe, Monovette® was obtained from Sarstedt (Newton, NC). The Heidelberg radiotelemetry instrument and the Heidelberg capsules were purchased from the Heidelberg International Corporation (Atlanta, GA). Dog slings were obtained from Alice King Chatham Medical Arts (Hawthorne, CA). All other materials were obtained from Fisher Scientific (Fair Lawn, NJ) or Sigma Chemical Co. (St. Louis, MO) and were used as received.

Animals

Male beagle dogs weighing 10 to 15 kg were used and fasted overnight prior to the study. Water was allowed *ad libitum*. All animal studies were performed under approved protocols (IRB-UCA, Rutgers University) in AAALAC accredited facilities.

[1] Department of Pharmaceutics, College of Pharmacy, Rutgers—The State University of New Jersey, Piscataway, New Jersey 08854.
[2] Department of Surgery, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey 08901.
[3] Unigene Laboratories, Fairfield, New Jersey 07004.
[4] To whom correspondence should be addressed at Rutgers University, College of Pharmacy 160 Frelinghuysen Road, Piscataway, New Jersey 08854. (e-mail: sinko@rcirutgers.edu)

0724-8741/99/0800-1233$16.00/0

Oral Formulations

Four oral formulations were manufactured by Unigene Laboratories, Fairfield, NJ (Table 1) and evaluated in these studies. Hard gelatin capsules (#0, 97B-E) containing sCT and various amounts of CA were coated with approximately 12% (by weight) Eudragit L30-D55 to prevent gastric disintegradation. All other additives were identical among the formulations studied.

pH Measuring System

Continuous determination of pH was accomplished using a radiotelemetric device, the Heidelberg capsule (HC) (12–14). The device consists of a battery-operated high frequency radio transmitter and a pH electrode housed in a nondigestible acrylic capsule 7 mm in diameter and 20 mm in length. The dogs wore an antenna strapped around the body to receive the radio signal, which was then recorded on a chart recorder. The capsule battery was activated with normal saline and calibrated in pH 1 and pH 7 buffer solutions maintained at 37°C. The HC was then tethered to the drug capsule using surgical thread (3-0 vicryl) and administered orally to dogs. Because pH values change with location within the gut and the drug capsule dissolution, alterations in pH were interpreted to be indicative of the movement of the HC-drug capsule through the different segments until the drug capsule dissolves. Generally, Heidelberg capsules provide readings with ±0.5 pH unit accuracy and excellent *in vivo* reproducibility in the pH range of 1 to 8 for 22 hr after activation (15).

Oral Absorption Study with Heidelberg Capsule

Four male beagle dogs were used to monitor the disintegration and oral absorption of 4 formulations. A HC tethered to an enteric capsule was given orally with 10 ml water to each dog. Blood samples were drawn through a 20G IV catheter (Abbocath) with a heparin lock which was inserted in the brachial vein. The catheter was flushed with heparinized saline (50 units per mL) after each blood draw. Two baseline samples were drawn prior to dosing and another immediately following GE. From the time the capsules enter the small intestine, blood samples were taken every 10 minutes until the HC showed a drop in pH which signified the disintegration of the test capsule. More frequent blood sampling was performed from the time that disintegration was first detected, blood samples taken at 3, 6, 9, 12, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180 minutes. No more than 25 × 3 ml samples were taken during a particular study. The dogs were used once every one or two weeks based on the maintenance of adequate hematocrit levels, an indicati of anemia.

Table 1. The Composition of sCT Enteric Capsules Tested in Beagle Dogs

Formulations*	sCT (mg)	CA (mg)	LCC (mg)	Talc (mg)	Dextrose (mg)
97B	1.11	0	55.2	55.2	552.4
97C	1.20	145.7	54.7	54.7	400.7
97D	1.15	260.2	51.9	52.2	260.5
97E	1.19	565.1	56.3	56.3	0

* CA, citric acid; LCC, lauroyl carnitine chloride.

Fluoroscopy Study with Heidelberg Capsule

An enteric capsule containing 500 mg of barium sulf powder (BS) was prepared as a test formulation with Eudra L30-D55 to prevent gastric disintegration. Its upper cap w first attached to a 3-0 silk suture with Krazy glue and dri overnight. In the next morning, it was tethered to HC witl suture, and then given orally. To verify the integrity of the H sCT delivery system tether, contrast radiography was perform to trace intestinal spreading of HC and BS radiopaque partic for the first 5 min. Momentary recordings were made perio cally through the next two to three hours. Image intensificati fluoroscopy with television monitoring was used to view t intestinal spreading of capsules and recorded on videota for counting.

sCT Analysis

The concentration of sCT in dog plasma was determir by competitive RIA using the sCT antibody and ^{125}Iodotyros salmon calcitonin as previously described (11,16). The ass was accurate and reproducible over the concentration range 100–1500 pg/mL of sCT. The lower limit of detection of t assay was 50 pg/ml. Interday coefficients of variation w 7–19% and intraday coefficients of variation were 7–24%. T assay was highly specific with less than 1% cross-reactiv with calcitonin tryptic fragments (17).

Data Analysis

Data were analyzed to provide information on the GE a intestinal pH profiles induced by the presence of extrinsica

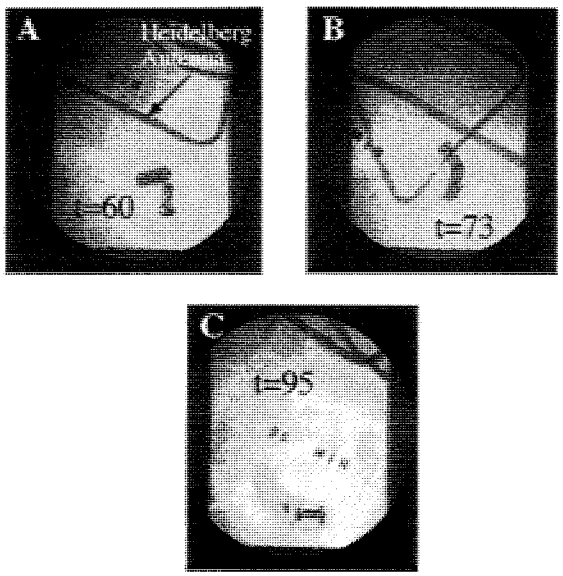

Fig. 1. Radiographic views of intestinal spreading of Heidelberg c sule and radiopaque powder after oral administration of enteric caps tethered to Heidelberg capsule into the normal beagle dogs. The Heic berg capsule and enteric capsule traveled together before disintegrat (A, at 60 min after dosing), during disintegration (B, at 73 min a dosing), and post disintegration (C, at 95 min after dosing). The Heic berg capsule and most of barium sulfate dye were contained in a sin loop of intestine after disintegration (C).

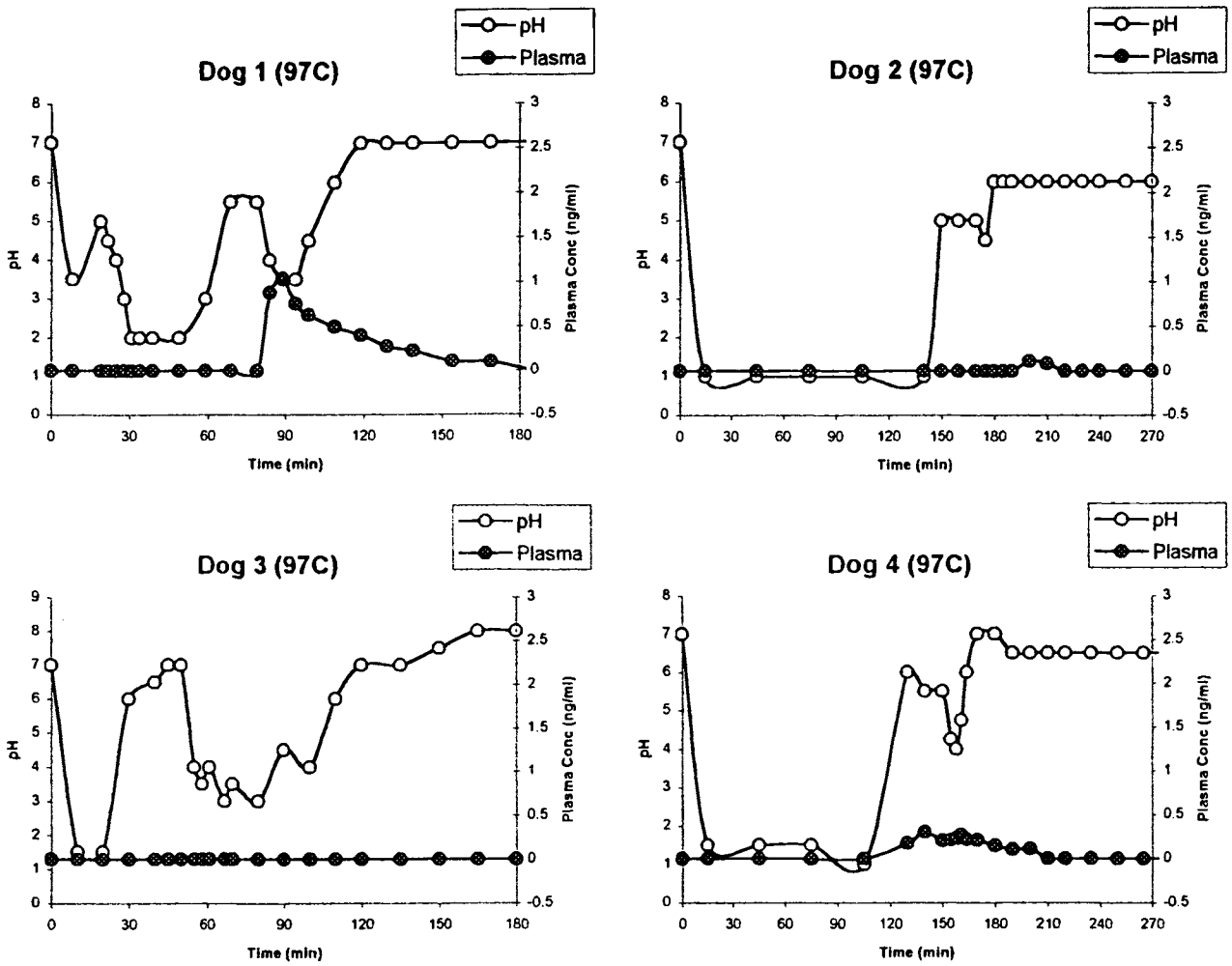

Fig. 2. The pH and plasma sCT concentration-time profiles after oral administration of formulation 97C tethered to Heidelberg capsule in normal beagle dogs.

introduced CA. GE was indicated by a sharp pH increase from acidic to neutral pH. GE was considered immediate if there was no initial record of acidic pH. The observed time to reach the lowest intestinal pH was defined as $t_{pH,min}$ (min). Plasma concentration versus time data were analyzed by non-compartmental pharmacokinetic methods (18). The highest observed concentration and the corresponding sampling time were defined as C_{max} (ng/ml) and $t_{conc,max}$ (min), respectively. The area under the concentration time curve (AUC, ng.min/ml) was calculated by the trapezoidal method.

Statistical Analysis

All statistical tests were performed using Jandel Sigma Stat (Version 2.0, San Raphael, CA). A minimum P-value of 0.05 was used as the significance level for all tests. One way ANOVA test was performed on the *in vivo* C_{max} and AUC data. All data are reported as the mean ± standard error (SEM) unless otherwise noted.

RESULTS AND DISCUSSION

The availability of peptide drug formulations has been limited by low oral bioavailabilities (less than 1–2%) usually accompanied by variable bioavailability (1–3). The poor oral bioavailability (F) of peptides is primarily due to poor absorption across intestinal membranes and rapid proteolytic degradation. sCT, a large peptide drug that has low intestinal permeability and high proteolytic lability (19,20), was selected as a model peptide drug for these studies. Efforts to maximize F have focused on maximizing the extent of peptide that survives degradation in the gut (F_G) and the fraction that enters the portal vein intact after passing through the intestinal tissues (F_A). Our previous work demonstrated that sCT delivery to the portal vein is the rate limiting step in achieving adequate oral bioavailability of the peptide in dogs since first pass hepatic metabolism is negligible (i.e., F_H, the fraction of peptide not extracted by the liver, is approximately equal to 1) (11). Methods to enhance F_G include the use of inhibitors and modulation of pH to alter proteolytic activity (7–9). Methods to enhance F_A include permeability enhancement, stabilization against proteolysis and maximization of local drug concentrations. In the present study, the feasibility of modulating the local intestinal environment to enhance peptide absorption was investigated in conscious beagle dogs. The use of delayed release but rapidly dissolving dosage forms to enhance local intestinal peptide concentrations

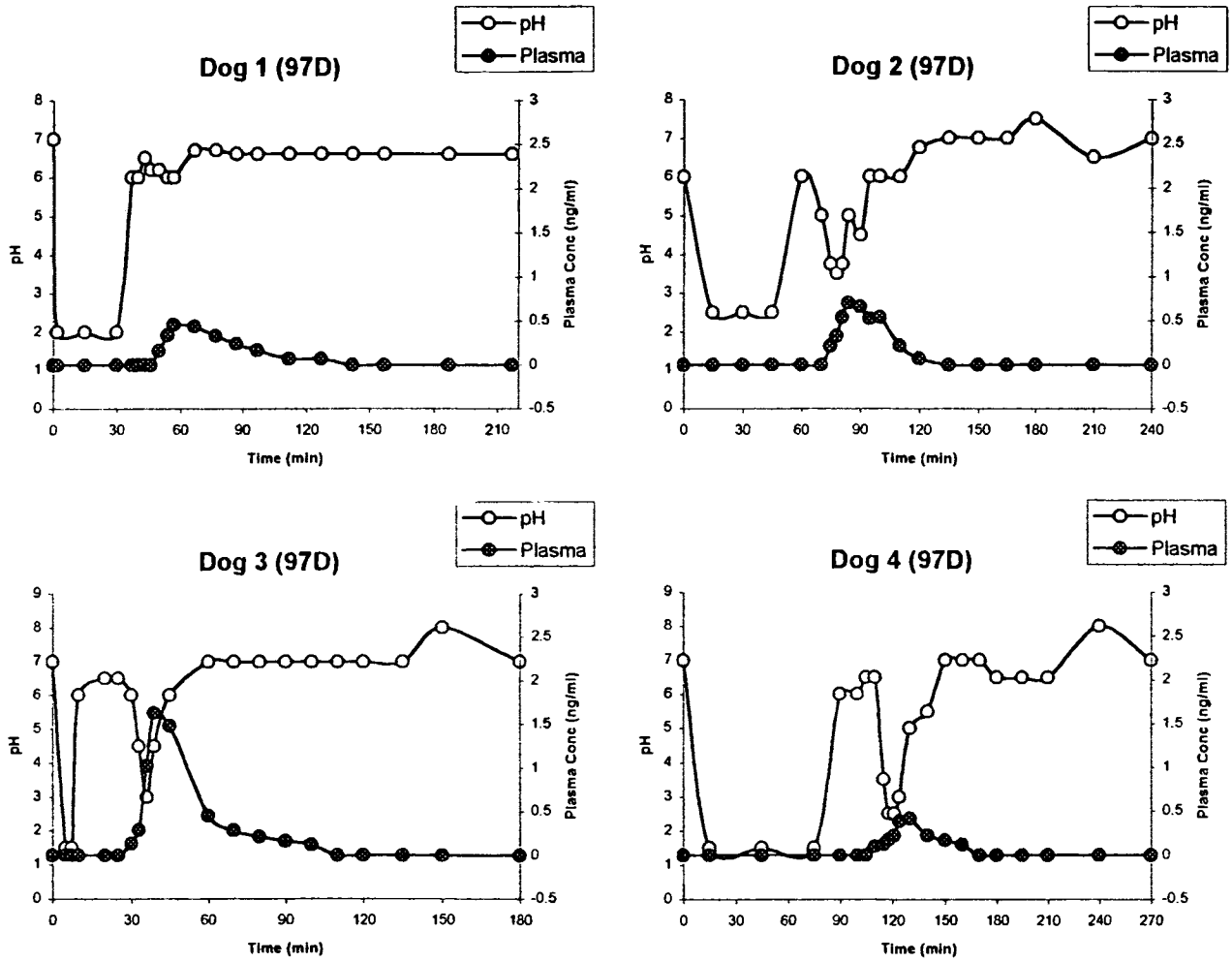

Fig. 3. The pH and plasma sCT concentration-time profiles after oral administration of formulation 97D tethered to Heidelberg capsule in normal beagle dogs.

and local pH modulation were the two strategies specifically examined in the present study.

An enteric-coated formulation containing sCT and CA (see Table 1) was tethered to a HC, and given orally to normal conscious dogs. Blood samples were taken with simultaneous pH measurements. Two goals were achieved by measuring GI pH. First, the sCT plasma level versus time curve was better characterized since the time for gastric emptying (GE) and dosage form disintegration could be determined. Second, a correlation between pH modulation and appearance of peptide in the blood was obtained. Through the belt antenna, the Heidelberg machine records the pH to which the capsule is exposed. Since the dosage form was enteric coated, disintegration was not supposed to occur in the stomach. *In vitro* disintegration studies demonstrated that the enteric coat remained intact for at least 2 hr in 0.1 N hydrochloric acid, and the coat began to dissolve within 30 min after the pH was increased to 6 (data not shown). As a result, it was difficult to establish a blood sampling regimen and the absorption phase of the plasma level versus time curve and the peak (t_{max}, C_{max}) were often missed. However, with the use of the HC pH measurement system, the time for GE and disintegration of formulation became very obvious. Since blood sampling was delayed until the formulation disintegrated, frequent sampling during this time period resulted in a better characterization of the sCT absorption phase. The interpretation of the results could be potentially confounded if the tether to the HC became dislodged. Therefore, several fluoroscopic examinations were performed during the studies that clearly demonstrated that the integrity of the tether was maintained until after disintegration of the dosage form occurred (Fig. 1). BS was used as a contrast agent in gastrointestinal contrast radiography, since it gave a good view of the bowel lumen unless it was significantly diluted. Simultaneous assessment of intestinal pH changes and plasma sCT concentration demonstrated that a clear correlation between pH reduction and appearance of peptide in the plasma existed (Figs. 2–4).

Significant variations in the pH and plasma concentration profiles were observed among dogs, even with the same formulation. This was primarily due to the marked variation of GE and intestinal disintegration of the enteric formulation, although interindividual variations of plasma sCT concentration profiles are possibly due to a variation in proteolytic activity/capacity between dogs. Large interindividual variations in GE were observed (0 to 150 min, Table 2) for the four formulations.

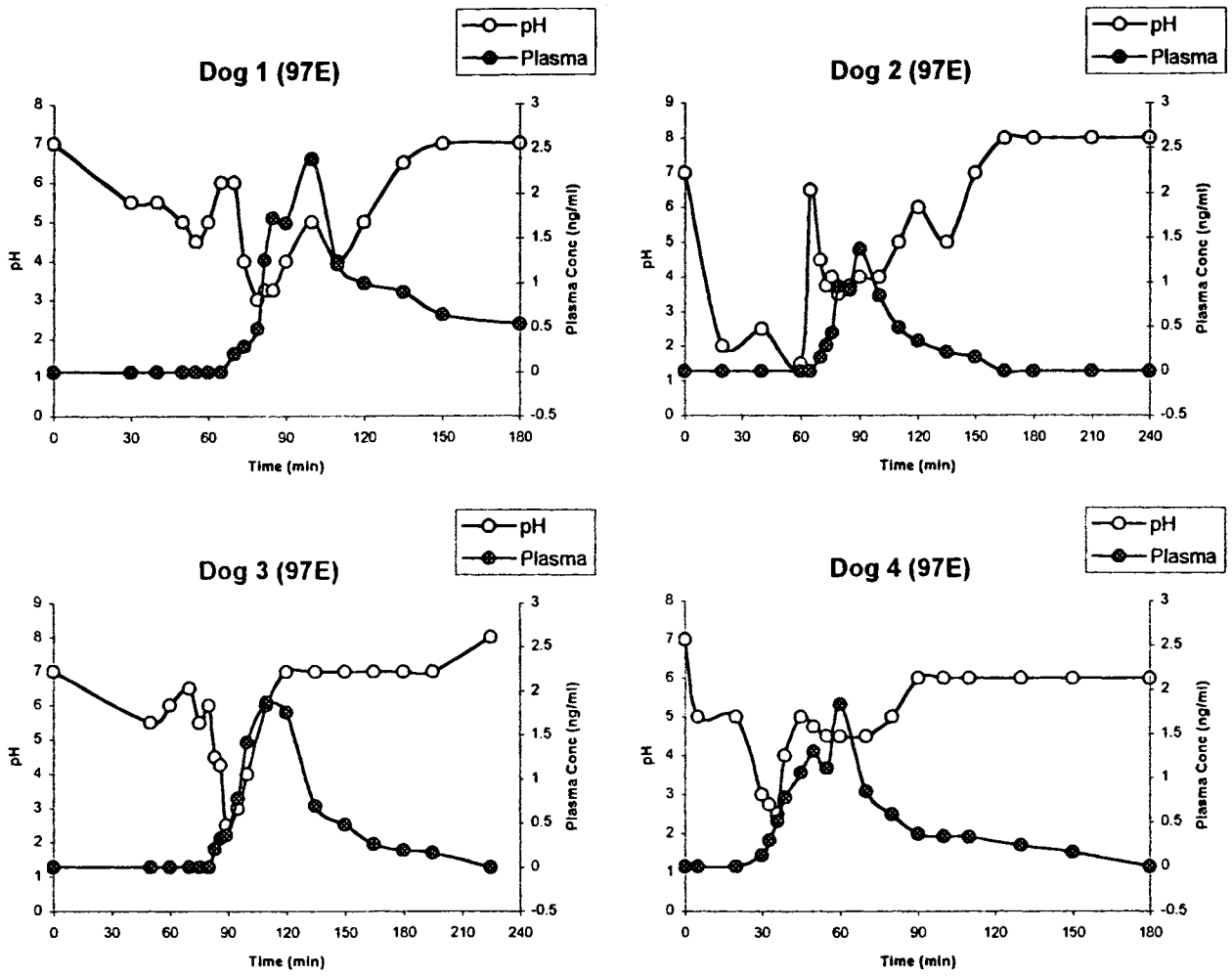

Fig. 4. The pH and plasma sCT concentration-time profiles after oral administration of formulation 97E tethered to Heidelberg capsule in normal beagle dogs.

These results are consistent with previously reported results for enteric coated aspirin dosage forms (21,22). Under fasted conditions, the GE of a nondisintegrating dosage form occurs when a migrating myoelectric complex (MMC) occurs. A MMC occurs approximately every 2 hr (23). It is the contractions in the third phase of the cycle that are important for the GE of nondisintegrating dosage forms. Recent combined scintigraphy and telemetry studies (24) have confirmed that large single-unit devices, such as a HC, are emptied from the stomach only by the large phase 3 contractions of the MMC. The sCT formulation tethered to a HC will empty from the stomach based on the contractile activity of the MMC. However, GE is not always efficient. It has been suggested that dosage forms can remain in the less muscular body of the stomach and not be propelled into the antrum of the stomach from which emptying takes place (25). This may explain the prolonged GE observed for Dog 2 (150 min) and Dog 4 (130 min) following administration of formulation 97C in the fasted state (Table 2).

Since we observed that sCT bioavailability in the ileum was better than the duodenum or colon (11), enteric capsules were prepared to delay disintegration and allow absorption of sCT to occur in the jejunum and/or ileum. However, there were large interindividual differences in regional intestinal disintegration, even for the same formulation. The range of intestinal transit ($t_{pH,min}$-GE, i.e., disintegration time after GE) for formulations 97C, 97D, and 97E were 25–50 min, 20–30 min, and 20–90 min, respectively (Table 2). When disintegration occurs within the first 5 min after GE it is considered to occur in the duodenum; up to 60 min is in the jejunum; and over 75 min is in the ileum, since the fasted dog has an average small intestinal transit time of 111 ± 17 min (mean ± SD) (26). Therefore, disintegration of all sCT formulations is considered to occur in the jejunum and/or ileum regions.

As expected, the intestinal pH was significantly affected by the amount of CA in the formulations. The intestinal pH decrease was not observed when CA was not included (97B, Table 2, figure not shown), but an intestinal pH decrease was obvious in all formulations that included CA. The average baseline intestinal pH before capsule disintegration was 6.1 ± 0.2 (mean ± SEM, n = 12). In formulations 97C, 97D, and 97E, intestinal pH reduction was 2.6 ± 0.4 (mean ± SEM, n = 12, ranged from 0.5 to 4.0 units from baseline). Plasma concentrations of sCT were observed in all formulations that included CA, but they were not detected before capsule disinte-

Table 2. Physiological and Pharmacokinetic Parameters Following Oral Administration of Heidelberg Capsule Tethered to Enteric sCT Capsule into Beagle Dogs

Parameters	Formulations	Dog 1	Dog 2	Dog 3	Dog 4
GE (min)	97B	100	60	ND	NT
	97C	60	150	30	130
	97D	40	60	10	90
	97E	0	60	0	0
$t_{pH,min}$ (min)	97B	ND	ND	ND	NT
	97C	90	175	80	160
	97D	60	80	35	120
	97E	80	80	90	35
$t_{con,max}$ (min)	97B	ND	ND	60	NT
	97C	90	200	ND	140
	97D	60	85	40	130
	97E	100	90	110	60
C_{max} (ng/ml)	97B	0	0	0.19	NT
	97C	1.05	0.11	0	0.30
	97D	0.46	0.7	1.63	0.42
	97E	2.39	1.37	1.87	1.83
AUC (ng.min/ml)	97B	0	0	4.7	NT
	97C	35.8	1.92	0	16.4
	97D	19.1	19.9	40.7	12.5
	97E	111.9	45.3	86.7	73.6

Note: GE, gastric emptying; $t_{pH,min}$, the observed time to reach trough intestinal pH; $t_{conc,max}$, the observed time to reach plasma peak concentration; ND, not detected; NT, not tested.

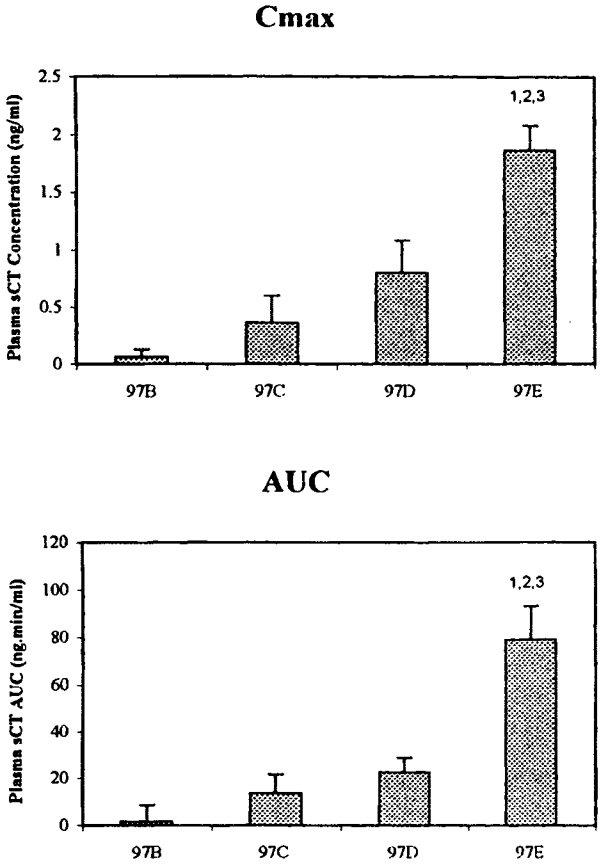

Fig. 5. The plasma C_{max} (up) and AUC (down) of sCT after oral administration of various formulations in normal beagle dogs. Data are expressed as mean ± SEM (n = 3–4). Formulation 97E was significantly different from 97B (1), 97C (2), and 97D (3) by $p < 0.05$.

gration. However, plasma concentrations of sCT were not observed in formulation 97B where CA was not included (Table 2). Only dog 3 showed a slight absorption peak with C_{max}: 0.19 ng/ml and AUC: 4.7 ng.min/ml. As a consequence, the intestinal pH decrease caused by CA appears to be critical for the oral absorption of sCT. When 146 mg of CA was included in the formulation (97C), 3 of 4 dogs showed oral absorption (Fig. 2). By increasing the amount of CA in the formulation, the oral absorption of sCT increased gradually. The results of the ANOVA demonstrated that the plasma C_{max} and AUC from formulations 97B, 97C, 97D, and 97E were increased significantly by increasing CA (Fig. 5). There were significant differences of plasma C_{max} and AUC between formulations ($P < 0.05$ by ANOVA), although formulations 97C and 97D failed to show a significant difference from 97B since two values of 97B were 0 (Table 2). When compared to the IV data (unpublished data), the bioavailability (mean ± SEM) of sCT was increased by increasing CA from 0.02 ± 0.08% for 97B to 0.15 ± 0.09% for 97C, 0.25 ± 0.07% for 97D, and 0.86 ± 0.15% for 97E.

The sCT plasma level results correlated well with the GI transit data. Oral absorption of sCT occurs when disintegration of the dosage forms begins in the small intestine (Figs. 2–4). The peak plasma concentrations of sCT were always observed to occur when intestinal pH declined. As a result, there was a good correlation between $t_{pH,min}$ and $t_{conc,max}$ (Fig. 6). These results indicate that the oral absorption/or enhancement of sCT absorption is directly related to the stabilization of sCT by a reduction in intestinal pH. In our previous report (11), the inclusion of CA improved the oral absorption of sCT in IVAP dogs. There were also reports that proteolytic activity against insulin, calcitonin, and insulin-like growth factor-I was completely inhibited by pH lowering mechanisms using polyacrylic acid polymer (8,9). The pH stability of pancreatic trypsin (human) is optimal at pH 5–6. At pH 4.0 approximately 45%

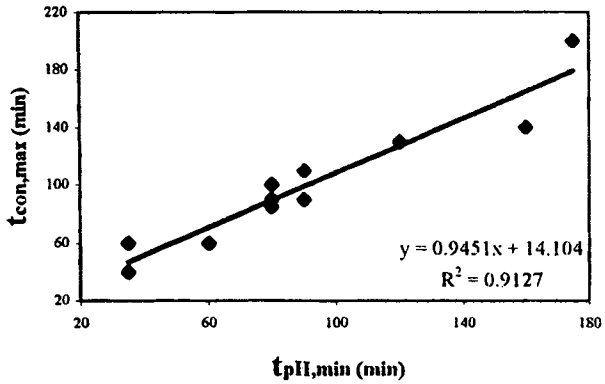

Fig. 6. The correlation between the time to reach the trough intestinal pH ($t_{pH,min}$) and the time to reach the peak plasma sCT concentration ($t_{conc,max}$) after oral administration of various formulations in normal beagle dogs (n = 11). Dog 3 in 97C was excluded from the plot since plasma sCT concentration was undetectable.

of the activity remained while 15% of activity was retained at pH 3.5 (27). It is interesting to note that Dog 3 (formulation 97C) did not show any sCT absorption, although a reduction in intestinal pH was observed (Fig. 2). Although this is an unexpected result, it is possibly due to a suboptimal amount of CA in the formulation since proteolytic activity/capacity may vary between dogs. It was also observed that sCT plasma levels could not be detected for formulation 97B where CA was not included (Table 2).

There are numerous strategies for enhancing the oral delivery of protein and peptide drugs such as sCT. However, the successful absorption of oral medications depends primarily on how the GI tract processes drugs and drug delivery systems. Factors such as regional pH differences, motility (or residence time), brush border membrane permeability, digestive proteolytic enzymatic activity, and colonic microflora enzymatic activity significantly influence the absorbed fraction of many drugs (28). As evidenced in this study, reducing intestinal pH resulted in a significant improvement in sCT absorption. In this report, the pharmacokinetic studies combining the Heidelburg radiotelemetric device technique was conducted to elucidate the CA effect on sCT absorption. It was observed that the HC always followed the enteric dosage form prior to disintegration and followed the dissolved contents after disintegration (Fig. 1).

In conclusion, this study shows a good correlation between intestinal pH modulation and the oral absorption of sCT in conscious beagle dogs. Plasma C_{max} and AUC increased with the amount of CA in the formulations. Since sCT is a substrate for the pancreatic serine protease trypsin, the rate of degradation of sCT in the GI lumen is considered to be dependent upon the pH and concentration of sCT in the intestinal lumen. These results were successfully used for devising delivery strategies and fabricating oral sCT delivery systems. This study demonstrates how the oral absorption properties of a peptide drug are modulated by the intestinal pH changes induced by formulation.

REFERENCES

1. X. H. Zhou. Overcoming enzymatic and absorption barriers to non-parenterally administered protein and peptide drugs. *J. Contr. Rel.* **29**:239–252 (1994).
2. G. L. Amidon and H. J. Lee. Absorption of peptide and peptidomimetic drugs. *Annu. Rev. Pharmacol. Toxicol.* **34**:321–341 (1994).
3. P. Lagguth, V. Bohner, J. Heizmann, H. P. Merckle, S. Wolffram, G. L. Amidon, and S. Yamashita. The challenge of proteolytic enzymes in intestinal peptide delivery. *J. Contr. Rel.* **46**:39–57 (1997).
4. V. H. L. Lee and A. Yamamoto. Penetration and enzymatic barriers to peptide and protein absorption. *Adv. Drug Del. Rev.* **4**:171–207 (1990).
5. J. P. Bai and G. L. Amidon. Structural specificity of mucosal-cell transport and metabolism of peptide drugs: Implications for oral peptide drug delivery. *Pharm. Res.* **9**:969–978 (1992).
6. J. F. Woodley. Enzymatic barriers for GI peptide and protein delivery. *Crit. Rev. Ther. Drug Carrier Syst.* **11**:61–95 (1994).
7. D. I. Friedman and G. L. Amidon. Oral absorption of peptides: Influence of pH and inhibitors on the intestinal hydrolysis of leu-enkephalin and analogues. *Pharm. Res.* **8**:93–96 (1991).
8. J. P. Bai, L. L. Chang, and J. H. Guo. Effects of polyacrylic polymers on the luminal proteolysis of peptide drugs in the colon. *J. Pharm. Sci.* **84**:1291–1294 (1995).
9. J. P. Bai, L. L. Chang, and J. H. Guo. Effects of polyacrylic polymers on the degradation of insulin and peptide drugs by chymotrypsin and trypsin. *J. Pharm. Pharmacol.* **48**:17–21 (1996).
10. M. Mackay. Delivery of recombinant peptide and protein drugs. *Biotechnol. Genet. Eng. Rev.* **8**:251–278 (1991).
11. P. J. Sinko, Y. H. Lee, V. Makhey, G. D. Leesman, J. P. Sutyak, H. Yu, B. Perry, C. L. Smith, P. Hu, E. J. Wagner, L. M. Falzone, L. T. McWhorter, J. P. Gilligan, and W. Stern. Biopharmaceutical approaches for developing and assessing oral peptide delivery strategies and systems: In vitro permeability and in vivo oral absorption of salmon calcitonin (sCT). *Pharm. Res.* **16**:527–533 (1999).
12. P. Mojaverian, R. K. Ferguson, P. H. Vlasses, M. L. Rocci, Jr., A. Oren, J. A. Fix, L. J. Caldwell, and C. Gardner. Estimation of gastric residence time of the Heidelberg capsule in humans: Effect of varying food composition. *Gastroenterology* **89**:392–397 (1985).
13. P. Mojaverian, M. L. Rocci, Jr., D. P. Conner, W. B. Abrams, and P. H. Vlasses. Effect of food on the absorption of enteric-coated aspirin: Correlation with gastric residence time. *Clin. Pharmacol. Ther.* **41**:11–17 (1987).
14. M. L. Rocci, Jr., P. Mojaverian, R. J. Davis, R. K. Ferguson, and P. H. Vlasses. Food-induced gastric retention and absorption of sustained-release procainamide. *Clin. Pharmacol. Ther.* **42**:45–49 (1987).
15. P. Mojaverian and K. Chan. Radiotelemetric determination of gastrointestinal pH: In vitro accuracy and in vivo reproducibility in man. *Pharm. Res.* **5**:S-243 (1988).
16. E. Wagner, J. P. Gilligan, L. T. McWhorter, V. Burkett-Pazel, P. J. Sinko, and W. Stern. Rapid detection and identification of salmon calcitonin in plasma after oral or gastrointestinal administration of recombinant salmon calcitonin. *Pharm. Res.* **11** (suppl):S19 (1994).
17. P. J. Sinko, C. L. Smith, L. T. McWhorter, W. Stern, E. Wagner, and J. P. Gilligan. Utility of Pharmacodynamic measures for assessing the oral bioavailability of peptides. 1. Administration of recombinant salmon calcitonin in rats. *J. Pharm. Sci.* **84**:1374–1378 (1995).
18. M. Gibaldi and D. Perrier. *Pharmacokinetics*, 2nd ed, Marcel Dekker, New York, 1982.
19. P. J. Sinko, P. Hu, E. Wagner, A. Sturmer, J. P. Gilligan, and W. Stern. Determination of the intestinal permeability of recombinant salmon calcitonin. *Pharm. Res.* **10** (suppl):S293 (1993).
20. H. Yu, F. Jiang, W. Stern, and P. J. Sinko. Intestinal binding and degradation of recombinant salmon calcitonin. *Pharm. Res.* **11** (suppl):S254 (1994).
21. J. R. Leonards and G. Levy. Absorption and metabolism of aspirin administered in enteric-coated tablets. *JAMA* **193**:93–98 (1965).
22. P. D. Paull, R. Day, G. G. Graham, and G. D. Champion. Single dose evaluation of a new enteric-coated aspirin preparation. *Med. J. Aust.* **1**:617–619 (1976).
23. S. F. Phillips. Small bowel. In D. Kumar and S. Gustavsson (eds.), *An illustrated guide to gastrointestinal motility*, John Wiley and Sons, Chichester, 1988, pp. 187–206.
24. A. J. Coupe, S. S. Davis, D. F. Evans, and I. R. Wilding. Correlation of the gastric emptying of non-disintegrating tablets with gastrointestinal motility. *Pharm. Res.* **8**:1281–1285 (1991).
25. I. R. Wilding, J. G. Hardy, R. A. Sparrow, S. S. Davis, P. B. Daly, and J. R. English. *In vivo* evaluation of enteric-coated naproxen tablets using gamma scintigraphy. *Pharm. Res.* **9**:1436–1441 (1992).
26. S. S. Davis, E. A. Wilding, and I. R. Wilding. Gastrointestinal transit of a matrix tablet formulation: Comparison of canine and human data. *Int. J. Pharm.* **94**:235–238 (1993).
27. E. F. Legg and A. M. Spencer. Studies on the stability of pancreatic enzymes in duodenal fluid to storage temperature and pH. *Clin. Chim. Acta.* **65**:175–179 (1975).
28. J. B. Dressman, P. Bass, W. A. Ritschel, D. R. Friend, and A. Rubinstein. Gastrointestinal parameters that influence oral medications. *J. Pharm. Sci.* **82**:857–872 (1993).