Biopharmaceutical Approaches for Developing and Assessing Oral Peptide Delivery Strategies and Systems: *In Vitro* Permeability and *In Vivo* Oral Absorption of Salmon Calcitonin (sCT)

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Purpose. To evaluate a biopharmaceutical approach for selecting formulation additives and establishing the performance specifications of an oral peptide delivery system using sCT as a model peptide.

Methods. The effect of formulation additives on sCT effective permeability and transepithelial electrical resistance (TEER) was evaluated in side-by-side diffusion chambers using rat intestinal segments. Baseline regional oral absorption of sCT was evaluated in an Intestinal and Vascular Access Port (IVAP) dog model by administration directly into the duodenum, ileum, and colon by means of surgically implanted, chronic catheters. The effect of varying the input rate and volume of the administered solution on the extent of sCT absorption was also evaluated. Citric acid (CA) was utilized in all studies to cause a transient reduction in local pH. *In vitro* samples and plasma samples were analyzed by radioimmunoassay (RIA). Two oral delivery systems were prepared based on the results of the *in vitro* and IVAP studies, and evaluated in normal dogs.

Results. Maximal permeability enhancement of sCT was observed using taurodeoxycholate (TDC) or lauroyl carnitine (LC) *in vitro*. Ileal absorption of sCT was higher than in other regions of the intestine. Low volume and bolus input of solution formulations was selected as the optimal condition for the IVAP studies since larger volumes or slower input rates resulted in significantly lower sCT bioavailability (BA). Much lower BA of sCT was observed when CA was not used in the formulation. The absolute oral bioavailability (mean ± SD) in dogs for the control (sCT + CA) and two proprietary sCT delivery systems was 0.30% ± 0.05%, 1.10 ± 0.18%, and 1.31 ± 0.56%, respectively.

Conclusions. These studies demonstrate the utility of *in vitro* evaluation and controlled *in vivo* studies for developing oral peptide delivery strategies. Formulation additives were selected, the optimal intestinal region for delivery identified, and the optimal release kinetics of additives and actives from the delivery system were characterized. These methods were successfully used for devising delivery strategies and fabricating and evaluating oral sCT delivery systems in animals. Based on these studies, sCT delivery systems have been fabricated and tested in humans with favorable results.

KEY WORDS: permeability; bioavailability; rats; dogs; humans; oral delivery; peptides; and salmon calcitonin.

INTRODUCTION

Calcitonin (CT), an endogenous polypeptide hormone composed of 32 amino acids, plays a crucial role in both calcium homeostasis and bone remodeling (1,2). Four forms of CT are used clinically, namely synthetic human CT (hCT), synthetic salmon CT (sCT), natural porcine CT (pCT), and a synthetic analogue of eel calcitonin. Currently CT is administered parenterally or nasally (3,4). In order to effectively inhibit the manifestations of metabolic bone disorders such as Paget's disease and osteoporosis, a frequent and relatively high dosage of CT is administered (5). The oral route is a preferred route of administration considering the chronic nature of CT therapy. However, due to extensive proteolytic degradation in the GI lumen and low intrinsic intestinal membrane permeability, insufficient oral BA of CT necessitates the use of high doses (4000–6000 IU/mg) of sCT, although sCT is 20–30 times more potent than hCT (150–200IU/mg) (6–8). The unique structure of sCT protects it against its sequestration in the liver, muscle, and bone (9). Although sCT has been found to be resistant to breakdown by liver homogenates, the liver plays a significant role in the metabolism of pCT (10). The hepatic metabolism of sCT at the subcellular level is not yet fully resolved because a NADPH generating system was not activated in that study. sCT is metabolized in the kidney in the microsomal fraction (10). If, as the evidence suggests, the hepatic metabolism of sCT is minimal, the rate limiting step to the successful oral administration of sCT is its delivery into the portal vein.

Limited pharmacokinetic studies of the various calcitonins (CTs) have been performed in laboratory animals or humans. Early pharmacokinetic studies in humans evaluated the disposition of sCT following intravenous (IV), intramuscular (IM), and subcutaneous (SC) administration (11–14). CT studies in rhesus monkey have demonstrated that it is a useful animal model for the investigation of the pathophysiology of calcitonin, however, they were not evaluated as an absorption model (15). While the intestinal regional dependence of sCT absorption has not yet been reported, a few reports focusing on rectal and colonic administration have been described. The absolute bioavailability of hCT following colonic administration in rats ranged from 0.2% to 0.9% (16). In two studies, the bioavailability of hCT following intra colonic administration in humans was investigated (17,18). In both of these studies, 10 mg of hCT

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was administered to human subjects by colonoscopy. Relative to an IV dose of hCT, bioavailability was 0.32 ± 0.80% with a range from 0.05% to 2.7%. The rectal absorption of sCT in humans has also been evaluated using pharmacodynamic (i.e., calcium lowering) rather than pharmacokinetic data (19). A similar study in humans evaluated rectal versus nasal absorption using calcium lowering as the outcome variable (20). sCT plasma concentrations expressed in relation to time zero concentrations peaked quickly after rectal administration and were significantly higher than after nasal administration.

In the present study, *in vitro* and *in vivo* methods were utilized for evaluating the performance of formulation additives to enhance the intestinal uptake of sCT. Furthermore, optimal delivery system characteristics (e.g., intestinal region and release kinetics) were selected based on *in vivo* studies in a dog model. The results demonstrate the utility of the approach for developing and evaluating oral peptide delivery strategies and systems.

MATERIALS AND METHODS

Materials

Recombinant salmon calcitonin (sCT) was obtained from Unigene Laboratories, Fairfield, NJ. sCT antibody was obtained from Advanced Chem Tech (cross reacts less than 1% with mammalian calcitonins, Louisville, KY). ^{125}Iodotyrosyl-salmon calcitonin (^{125}I-sCT) was obtained from Amersham Laboratories (Arlington Heights, IL). Hydroxypropylmethylcellulose phthalate (NF, HPMCP 55) was obtained from Eastman Chemical Company (Kingsport, TN). Bacteriostatic 0.9% Sodium Chloride Injection, USP, was obtained from Abbott Laboratories (North Chicago, IL). Millipore filter, Millex-GV$_{13}$ was obtained from Millipore Corporation (Bedford, MA). Heparinized syringe, Monovette® was obtained from Sarstedt (Newton, NC). Intestinal and Vascular Access Ports and 22G Huber needles were obtained from Access Technologies (Skokie, IL). Dog slings were obtained from Alice King Chatham Medical Arts (Hawthron, 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 Sprague-Dawley rats (250–350 g) and male beagle dogs (11–16 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 and IACUC, University of Medicine and Dentistry of New Jersey) in AAALAC accredited facilities.

In Vitro Studies

Diffusion Studies

Side-by-side diffusion chambers were used (Navicyte, Inc., Sparks, NV) in these studies as previously described (21). Briefly, the exposed tissue surface area was 0.636 cm^2 and the volume of each half-chamber was 1.7 mL. Mixing in the chambers was controlled using a gas lift mechanism. The temperature was maintained at 37°C throughout the experiment. Fasted rats were anesthetized by intramuscular injection of 80 mg/kg ketamine (Phoenix, St. Joseph, MO) and 10 mg/kg xylazine (Phoenix, St. Joseph, MO). A 1.5 to 2 cm strip of intestinal tissue was excised from the animals, rinsed free of luminal contents using Ringers buffer (pH 7.4), and mounted onto a diffusion half-chamber maintained at 37°C. Tissues were bathed in 15 mM Mes Ringers buffer containing 50 μM sCT with or without formulation additives on the mucosal side (pH 5, 290 mOsm/kg) and Ringers buffer without additives on the serosal side (pH 7.4, 290 mOsm/kg). Small aliquots (0.5 mL) were taken from the serosal chamber at 30, 45, 60, 75, 90, and 105 min and stored at −20°C for subsequent determination of sCT content by radioimmunoassay (RIA). The effective permeabilities were calculated from the experimental data using the following equation based on Fick's First Law:

$$P_{eff} = (V_r/A*C_0) * dC/dt \quad (1)$$

where V_r is the volume of the receiver chamber, A is the absorbing surface, C_0 is the initial drug concentration in the donor (mucosal) phase, and dC/dt is the change in drug concentration in the receptor (basolateral) phase per unit time.

TEER Studies

Side-by-side diffusion chambers were used in these studies as previously described. Chambers connected to a voltage-current clamp (Physiological Instruments, San Diego, CA) operated with the ACQUIRE AND ANALYZE v. 1.05 software was used for measuring transepithelial electrical resistance (TEER) of the intact tissue. The exposed tissue surface area was 1.5 cm^2 and the volume of each half-chamber was 7 mL. Rat intestinal tissue was mounted in chambers and then two sets of Ag/AgCl electrodes were connected to the voltage clamp system to pass the current through the membrane. Tissues mounted in chambers usually required about 20 min to reach temperature and TEER equilibria. Experiments with formulation additives were not initiated until this time. The mucosal strips were then exposed to formulation additives at concentrations ranging from 0.01% to 1% at pH 4 Mes Ringers buffer. After the initial equilibrium period, TEER was measured and subsequently recorded at regular time intervals. In order to determine the reversibility of additive effects on TEER, the buffer was replaced with fresh buffer without additives, and the TEER was monitored until a stable reading was observed.

IVAP Studies

IVAP Dog Model Preparation

Six dogs were surgically fitted with Intestinal and Vascular Access Ports (IVAP). The ports were implanted along the spine, in the subcutaneous space behind the shoulder blades. Four catheters (Access Technologies, Skokies, IL) were tunneled under the skin and through the abdominal wall. The distal end of each catheter was implanted into a different portion of the intestinal tract. The first was 10 cm distal to the pyloric sphincter in the duodenum. The second port was placed in the lower third of the small intestine and the third port was implanted 10 cm distal to the ileocecal valve in the colon. The fourth tubing was inserted into the portal vein. The animals were allowed to recover at least two weeks prior to the initiation of the studies.

IVAP Infusion

During the study, the animal was restrained in a dog sling and the back and foreleg shaved. EMLA cream (Astra Inc., MA) was applied to the skin over the ports and foreleg in order to allow for easier and less stressful access to the ports. After 30 min, a 20G IV catheter (Abbocath, Abbott Labs, IL) with a heparin lock was inserted in the brachial vein. Three baseline blood samples were drawn 10 min apart and the catheter was flushed with heparinized saline (50 units per mL) after each blood draw. The ports for IVAP infusions were accessed transcutaneously with a 22G Huber needle (Access Technologies, Skokies, IL). The EMLA cream was removed, skin scrubbed with Povidone-Iodine solution, and finally wiped with an alcohol soaked gauze pad prior to access the ports. The sCT formulation (25 mg/5 mL/dog) was rapidly infused (12 mL/min) and the port was cleared with a final flush of 1 mL sterile water. Blood samples were drawn at 1 (IV and PV only), 3, 6, 9, 12, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, and 240 min. Samples were drawn directly into 4.5 mL Monovette heparinized syringes and placed on ice. Plasma was prepared within 30 min of sampling by centrifuging the blood sample for 10 min at 2750 × g RCF and 3°C. The samples were stored at −20°C pending analysis by radioimmunoassay (RIA). Prior to each weekly study, all intestinal ports were accessed and flushed with 1 to 2 mL of sterile water for injection.

Baseline IV and PV Administration

The sCT (10 μg/dog) was administered IV or PV infused (through PV port) as previously described. The dosing solution was prepared using bacteriostatic 0.9% Sodium Chloride Inj., USP. To insure sterility, all intravenous solutions were filtered through Millipore filter (Millex-GV_{13}) prior to administration. Since sCT is known to adhere to glass and certain membranes, the binding of sCT to the filter membrane was evaluated by HPLC and gamma counting prior to use. Since the mean recovery of sCT after a single pass through the filter was greater than 90% and consistent in all cases, the filters were deemed acceptable for these studies. Test tubes, transfer pipettes, and syringes used throughout these studies were made of polypropylene or polyethylene to prevent loss of sCT due to adherence. All samples were processed as previously described.

Oral Dog Studies

Delivery Systems

Two oral formulations were evaluated in these studies. Two proprietary sCT delivery systems, designated DDS1 and DDS2, were prepared. DDS1 and DDS2 contained sCT, citric acid (CA), and lauroyl carnitine chloride (LC) or sodium taurodeoxycholate (TDC), respectively. The DDS1 and DDS2 gelatin capsules were coated with 4.5% (by weight) HPMCP 55 to retard release in the stomach. 25 mg sCT and corresponding amount of CA were weighed and placed in a gelatin capsule with an appropriate amount of lactose. The capsule was also coated with HPMCP 55 and it was used as a control. Other details of the delivery systems can be found in the US Patent (#08/616,250, "Oral Salmon Calcitonin Pharmaceutical Products").

Oral Administration

The normal dogs were prepared in the same manner as described for the IVAP infusion. After insertion of the IV catheter, two baseline blood samples were drawn 10 min apart and the catheter was flushed with heparinized saline (50 units per mL) after each draw. The capsule was then administered by placing it at the base of the tongue. Water (10–15 mL) was then administered to assist the dog in swallowing the capsule. Samples were drawn at 15 min intervals for a period of four hours and processed as previously described.

sCT Analysis

The concentration of sCT in dog plasma or in the *in vitro* samples was determined by competitive RIA using sCT antibody and ^{125}I-sCT as described (22). Briefly, a 100 μL aliquot of dog plasma that was undiluted or dog plasma that was diluted in RIA buffer consisting of 0.2% bovine serum albumin, 0.02% NaN_3, 0.1% Triton X-100 was mixed with 100 μL of sCT antibody (diluted 1:20,000 in RIA buffer) and incubated at room temperature. After 4 hr, 25 μL of ^{125}I-sCT (15,000 cpm/mL) in RIA buffer was added to the antibody-sample mixtures, mixed, and the incubation continued overnight at 4°C. At the end of the incubation, 100 μL nonspecific rabbit serum and 100 μL goat anti-rabbit serum were added to the mixture and allowed to incubate for 2 hr at room temperature. 500 μL phosphate buffer was then added to the samples which were then centrifuged at 1700 × g RCF for 10 min at 4°C. The supernatants were carefully removed by aspiration and the pellets were counted in a Beckman 5500 gamma counter. Standards consisting of known amounts of sCT diluted in either plasma or a corresponding dilution of plasma were treated in the same manner as the experimental samples. A standard curve comparing the fraction of counts precipitated versus a known amount of sCT was generated using the LOGISFIT curve fitting program (Origin, Microcal Software, Inc., Northampton, MA). From the standard curve, the fraction of bound counts in the experimental samples was used to quantitate sCT. Validation studies demonstrated that the assay was accurate and reproducible over the concentration range of 100–1500 pg/mL of sCT (22). The lower limit of detection of the assay was 80 pg/ml. Interday coefficients of variation was 7–19% and intraday coefficients of variation was 7–24%. The assay was highly specific with less than 1% cross-reactivity with calcitonin tryptic fragments (23).

Pharmacokinetic Analysis

The area under the sCT concentration versus time curve (AUC_{0-t}) was calculated by the linear trapezoidal method from time 0 to the last time point. The area under the concentration versus time curve from time 0 to time infinity ($AUC_{0-\infty}$) was calculated as follows:

$$AUC_{0-\infty} = AUC_{0-t} + C_t/k_{el} \quad (2)$$

where k_{el} is the rate constant of the terminal disposition phase and t is the time of the last plasma concentration measured. Clearance (CL) after IV administration and bioavailability (BA) after oral (PO) administration were calculated as follows:

$$CL = Dose/AUC \quad (3)$$

$$BA = (AUC_{PO}/AUC_{IV}) * (Dose_{IV}/Dose_{PO}) * 100 \quad (4)$$

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 and Tukey tests were performed on the *in vitro* permeability and *in vivo* BA data. All data are reported as the mean ± standard deviation (SD) unless otherwise noted.

RESULTS AND DISCUSSION

In Vitro Evaluation of Formulation Additives

In order to evaluate the effectiveness of formulation additives in modulating the transport of sCT *in vitro*, various combinations of additives and sCT were dissolved in pH 5 MES Ringers buffer. The mucosal chamber was loaded with the formulation solution at the beginning of the experiment. Prior to permeation study, adherence (binding) and stability of sCT were investigated in the low volume (1.7 mL) mucosal and serosal chambers in the absence or presence of rat intestinal tissue, respectively. In the case of the stability studies, equal concentrations of sCT were placed on both sides of the tissue. The chamber concentration (50 μM) of sCT was constant for 105 min indicating sCT stability and a lack of significant binding under the conditions of the experiment (data not shown). Various additives such as sodium taurodeoycholate (TDC), sodium taurocholate, sucrose sterate, sucrose ester-15, tween 80, lauroyl carnitine chloride (LC), myristoyl carnitine chloride, cetyl pyridinium chloride, and cetrimide were evaluated in rat jejunum at concentrations ranging from 0.01% to 1%. The typical permeability enhancement of sCT by TDC is shown in Fig. 1. The effective permeability of sCT was greatest in the presence of TDC (2.73 ± 0.54 E-5 cm/s), increasing up to 14 times over the control (1.91 ± 0.45 E-6 cm/s) in a concentration dependent manner. The permeability enhancement relative to control was 5 times for LC, 3.9 times for tween 80, 3.2 times for sodium taurocholate, 2.6 times for myristoyl carnitine chloride, 2.3 times for sucrose stearate, 1.7 times for sucrose ester-15, 2.0 times for cetyl pyridinium chloride, and 1.3 times for cetrimide at 1% additives. The order of enhancement on the basis of EC_{50} value (concentration of enhancer for 50% enhancement) was TDC, LC > sodium taurocholate > tween 80 > myristoyl carnitine chloride > sucrose stearate > sucrose ester-15 > cetyl pyridinium chloride > cetrimide. The EC_{50} values of the additives were calculated from the plot of enhancement in effective permeability versus log concentration.

TEER measures the permeability of the membrane to ions. Since the major route of transport of ions occurs paracellularly, TEER has been used as an indicator for the permeability of the tight junction. In order to evaluate the immediate or acute toxic effects of additives on tissue membranes *in vitro*, various additives such as TDC, LC, and cetrimide at concentration ranging from 0.1% to 1% were dissolved in pH 4 MES Ringers buffer as described previously. The mucosal chamber was loaded with the formulation and the change of TEER was monitored. Also, the reversibility of additive effects on TEER was evaluated after washing the tissue with buffer free of additives. Following exposure to additives, the TEER was reduced in a concentration- and contact time-dependent manner for all additives, whereas the TEER increased when the tissue was washed free of additives. The typical TEER versus time curves with/or without 1% TDC in rat jejunum is shown in Fig. 2. When 1% formulation additives were washed after approximately 100 min exposure, the TEER was returned to 92% of initial value for TDC and about 80% of initial value for the others. This result indicated that the acute toxic effect of TDC is probably less than the other additives tested

In Vivo Evaluation of sCT in IVAP Dogs

In order to validate the IVAP dogs, the pharmacokinetics of sCT were evaluated after IV and PV administration. The clearance (CL) of sCT was calculated to be 5.2 L/hr after IV administration and the bioavailability of sCT was close to 100% after PV infusion (data not shown). Based on these results, the hepatic first-pass elimination of sCT was considered to be

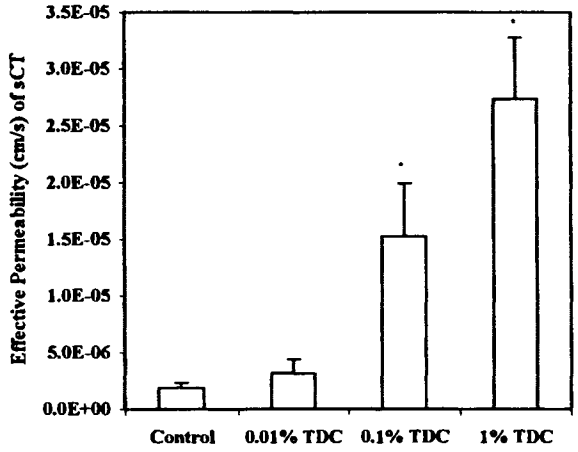

Fig. 1. Plot of effective permeability (mean ± SD) of sCT in rat jejunum. Effect of TDC was investigated at various concentrations ranging from 0.01% to 1% (n = 3). * indicates a significant difference from control by $P < 0.05$.

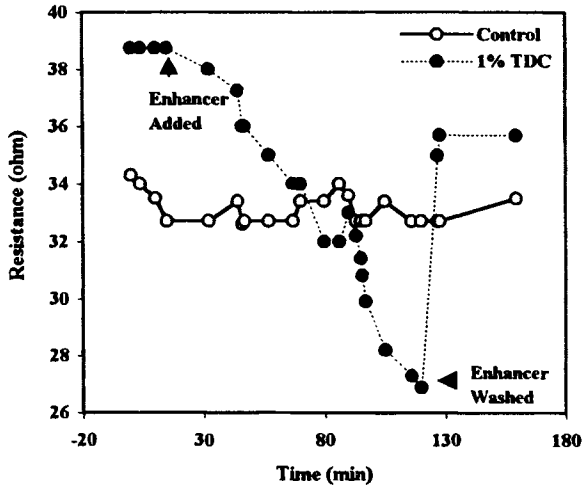

Fig. 2. Transepithelial electrical resistance (TEER) versus time curves with/or without 1% TDC in rat jejunum.

negligible most likely due to its limited hepatic uptake. This result was consistent with previous reports (9,10) clearly indicating that the delivery of sCT into the portal vein is the rate-limiting step in the successful oral delivery of sCT. In another study (data not shown), sCT was IV administered into normal dogs. The total body clearances of sCT were not different in IVAP and normal dogs.

In order to evaluate the regional difference of oral absorption, sCT was administered into intestinal ports at low volume (25 mg/5 ml/dog) and with a fast infusion rate (12 ml/min). Absorption of sCT from the ileum was better than the other regions studied (Fig. 3). Interestingly, the BA (mean ± SD) of unformulated sCT from colon (0.021% ± 0.004%) was lower than the duodenum (0.039% ± 0.017%) and ileum (0.064 ± 0.022). However they were not significantly different. Compared to other regions, the low BA of sCT from the colon is considered to be related to the hybrid effect of poor membrane permeability by "tighter" colonic paracellular spaces and/or by proteolytic degradation of sCT by microorganisms present in the colon. Interestingly, the rational design of colon-specific protein delivery strategies has been based on the premise that the colon compartment has decreased pancreatic proteolytic activity and/or bacterial activities with unique specificities (24,25).

Since sCT is an excellent substrate for the pancreatic serine protease trypsin, the rate of degradation of sCT in the GI lumen is dependent upon the concentration of sCT in the intestinal lumen. If degradation is the controlling factor, sCT absorption would be much more affected at low concentrations (i.e., in the first order region of the Michaelis-Menten curve). To evaluate the intestinal dilution and spreading on oral bioavailability, slow infusion (2 mL/min) and high dilution (25 mg/20 ml/dog) were evaluated *in vivo*. In IVAP studies, the slower and larger volume infusion of sCT showed lower absorption (data not shown). Therefore, intestinal dilution and spreading significantly affect the oral bioavailability of sCT since the rate of degradation will be highest at low sCT. Also, citric acid improved the absorption in all regions probably due to the inhibition of proteolytic degradation by pancreatic enzymes and/or microorganisms. In other reports, it has been demonstrated that the microbial proteolytic activity against insulin, calcitonin, and insulin-like growth factor-I was completely inhibited by polyacrylic acid polymer by pH lowering mechanisms (26).

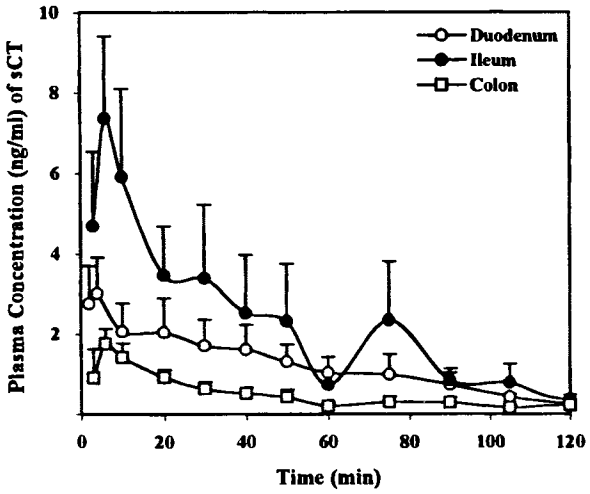

Fig. 3. Plasma concentration (mean ± SEM) of sCT versus time curves after bolus duodenal, ileal, and colonal administration of 25 mg/5 mL/dog in IVAP dogs (n = 5–6).

In Vitro and *In Vivo* Evaluation of Formulation DDS1 and DDS2

Based on the effectiveness of formulation additives in modulating the transport of sCT *in vitro* and baseline pharmacokinetic data in IVAP dogs *in vivo*, two proprietary formulations: DDS1 (containing LC) and DDS2 (containing TDC) were designed and evaluated *in vitro* and *in vivo*. Since formulation additives undergo dilution and spreading in the GI lumen prior to the absorption of sCT, the effect of formulation additives on the membrane permeability of sCT is dependent upon the dilution of formulation. Therefore, the concentration dependence (i.e., "dilution" effect) of sCT intestinal permeability in the presence of formulation additives was evaluated. The *in vitro* diffusion experiments were carried out at concentrations equivalent to the dilution of sCT and the DDS formulation ingredients in 10, 50, and 150 mL of buffer solution. These values were selected since the intestinal volume for the dilution of an orally administered formulation is approximately in the range of 10 to 150 mL, depending on how much fluid is taken with the dosage form. Table 1 lists the effective permeability of sCT; and Fig. 4 shows the relative percent increase in the effective permeability of sCT in rat jejunum and ileum, compared to control. It was observed that formulations containing TDC or LC had a significantly higher effective permeability as compared to their respective controls. Permeability enhancement was most in 50 mL dilution, and ranged from 88% to 422% for all treatments and regions studied. The enhancement in the ileal segment was significantly higher than in the jejunal segment for DDS1 and DDS2. DDS1 had higher permeability enhancement compared to DDS2 at low intestinal dilutions (10 and 50 mL). At higher intestinal dilution (150 mL), there was no significant difference in the enhancement between DDS1 and DDS2. Therefore, intestinal dilution and spreading may significantly affect the oral bioavailability of sCT in DDS additives by modulating the membrane permeability. Other post hoc statistical comparisons are shown in Table 2.

The absolute intestinal (in IVAP dogs) and oral (in normal dogs) bioavailability of sCT control and as formulated in DDS1 and DDS2 are shown in Fig. 5. The intestinal bioavailability

Table 1. The Effective Permeability (P_{eff}, E-7 cm/s, Mean ± SD of n = 3) of sCT in the Simulated Dilution of Formulation DDS1 and DDS2 in the Jejunum and Ileum of Rats

	Dilution	10 mL	50 mL	150 mL
Jejunum	Control	10.8 ± 2.1	3.5 ± 1.6	3.2 ± 0.5
	DDS1	23.1 ± 0.8	11.9 ± 1.5	6.2 ± 1.2
Ileum	Control	11.1 ± 2.0	2.8 ± 2.0	2.4 ± 1.4
	DDS1	28.2 ± 3.3	14.4 ± 1.0	7.0 ± 0.3
Jejunum	Control	11.0 ± 0.8	4.4 ± 0.8	2.8 ± 0.9
	DDS2	20.6 ± 0.8	8.3 ± 0.6	5.3 ± 1.6
Ileum	Control	10.7 ± 1.3	2.5 ± 1.5	2.1 ± 0.8
	DDS2	22.9 ± 0.5	9.0 ± 2.7	5.9 ± 1.6

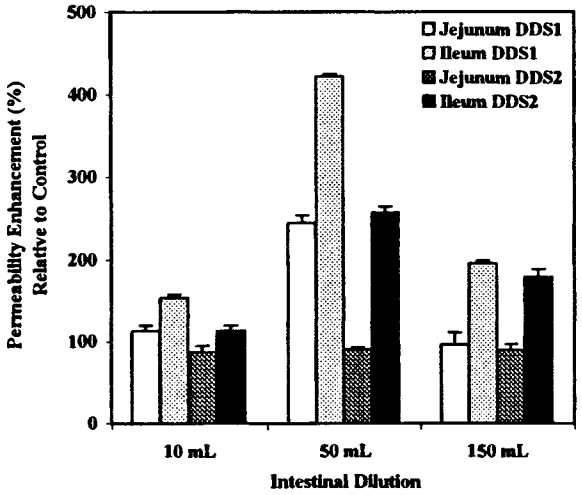

Fig. 4. Plot of the relative enhancement (mean ± SD) of the effective permeability of sCT versus the simulated dilution of formulation ingredients (including sCT). "Dilutions" were simulated by dissolving all formulation ingredients in 10, 50, or 150 mL of pH 5 buffer. Effective permeability enhancement was studied in the jejunum and ileum of rats in side-by-side diffusion chambers (n = 3).

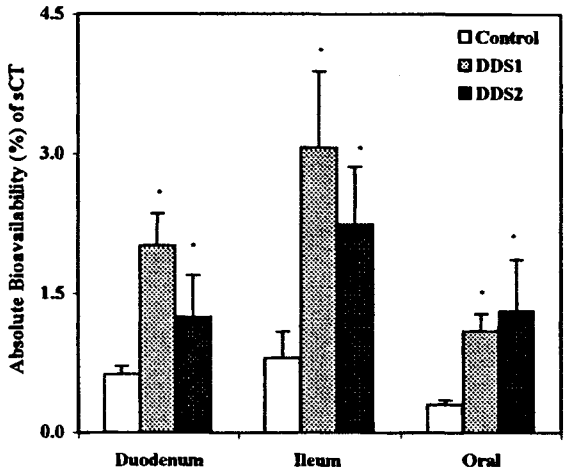

Fig. 5. Absolute bioavailability (mean ± SD) of sCT formulations after duodenal and ileal administration as a solution in IVAP dogs (n = 5–6), and oral administration as an enteric capsule in normal dogs (n = 4). * indicates the significant difference from control by $P < 0.05$.

experiment of formulations in IVAP dogs was carried out at concentrations of sCT and DDS additives in 5 mL of buffer solution. The results of the ANOVA demonstrated that the regional (oral, duodenal, ileal) bioavailabilities of sCT from formulations DDS1 and DDS2 were significantly enhanced as compared to their respective controls. Bioavailability enhancement ranged from 98% to 337% for all treatments and regions studied. When compared to sCT alone (without CA), bioavailability enhancement ranged from 1220% to 3070% for DDS1 or DDS2. These results suggest that two mechanisms of enhancement, a reduction in the proteolytic degradation in the GI lumen and a permeation enhancement, augmented sCT absorption. The *in vivo* augmentation of sCT absorption by DDS1 or DDS2 correlated well to the *in vitro* permeation enhancement (Fig. 6). The current results and correlation suggest that the *in vitro* studies and the *in vivo* IVAP dog studies serve as good screening tools for sCT delivery systems.

In a recent Phase 1 clinical study (27), two formulations (28) of sCT in combination with two formulation additives were administered to 10 normal healthy volunteers. Formulation additives were selected based on the results of the current studies in dogs and rats. Following oral administration, there was a significant reduction in circulating calcium and phosphate levels at both doses that persisted for at least three hours. Plasma sCT levels peaked between 30 and 60 min. In an earlier study (unpublished), sCT was administered without formulation additives by means of a nasogastric tube inserted into the duodenum of human volunteers, sCT was not observed in the plasma. These two studies demonstrate that sCT can be successfully

Table 2. Tukey Test Results: A Comparison of the Permeability Enhancing Effects of Formulations DDS1 and DDS2 in the Jejunum and Ileum of Rats

	Jejunum (DDS1)			Ileum (DDS1)			Jejunum (DDS2)			Ileum (DDS2)		
Intestinal Dilution (mL)	10	50	150	10	50	150	10	50	150	10	50	150
Jejunum: 10 mL (DDS1)		SD	SD	SD	SD	SD	SD	SD	SD	NS	SD	SD
Jejunum: 50 mL (DDS1)			SD	SD	SD	SD	SD	SD	SD	SD	NS	SD
Jejunum: 150 mL (DDS1)				SD	SD	SD	NS	NS	NS	SD	SD	SD
Ileum: 10 mL (DDS1)					SD	SD	SD	SD	SD	SD	SD	SD
Ileum: 50 mL (DDS1)						SD	SD	SD	SD	SD	SD	SD
Ileum: 150 mL (DDS1)							SD	SD	SD	SD	SD	NS
Jejunum: 10 mL (DDS2)								NS	NS	SD	SD	SD
Jejunum: 50 mL (DDS2)									NS	SD	SD	SD
Jejunum: 150 mL (DDS2)										SD	SD	SD
Ileum: 10 mL (DDS2)											SD	SD
Ileum: 50 mL (DDS2)												SD
Ileum: 150 mL (DDS2)												

Note: 1. Permeability enhancement at all concentrations and in all regions was significantly different from their respective controls. 2. SD: Significantly different by $p < 0.05$. 3. NS: Not significantly different.

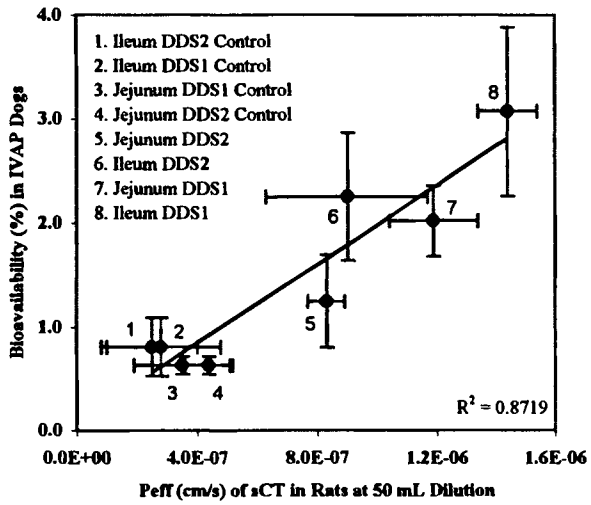

Fig. 6. Plot of the effective permeability of sCT in rats (n = 3) versus absolute bioavailability of sCT formulations in IVAP dogs (n = 5–6). The *in vitro* diffusion study was carried out at concentrations equivalent to the dilution of sCT control and DDS formulation ingredients in 50 mL of buffer solution.

delivered orally and that the approach for developing the delivery strategy and delivery system detailed in this paper has utility.

The current investigation demonstrates that satisfactory sCT BA can be obtained by using suitable formulation additives. For the purpose of choosing optimal formulation additives, efficient and reliable screening tools are required. The current results demonstrate that *in vitro* studies and the IVAP dog studies serve as good screening tools for the determination of optimal formulation additives, selecting the optimal site of intestinal delivery, and for assessing the release kinetics of oral sCT delivery systems.

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