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Research paper

Regional oral absorption, hepatic first-pass effect, and non-linear disposition of salmon calcitonin in beagle dogs

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Abstract

The dose-dependent disposition, first pass hepatic elimination, and absorption pharmacokinetics (PK) of salmon calcitonin (sCT) were investigated in a canine Intestinal Vascular Access Port (IVAP) model. The PK of sCT were determined after intravenous (IV), subcutaneous (SC), portal venous (PV), and oral (PO) administration of sCT. Regional oral absorption of unformulated sCT was also evaluated by direct administration into the duodenum (ID), ileum (IL), and colon (IC) by means of surgically implanted, chronic catheters. Plasma samples were collected and analyzed by radioimmunoassay (RIA). Salmon calcitonin PK were evaluated using 2-compartmental and model independent methods. Intravenous sCT PK were non-linear over the dose range studied. High dose groups (100–1000 μg) demonstrated higher total plasma clearance (CL) and V_{dss} than the low dose groups (1–25 μg). However, the MRT did not change for doses ranging from 10 to 1000 μg. After SC administration, the absorption of sCT was rapid with bioavailability (BA) varying from 21.4 to 52.9%. However, the BA of sCT was low after ID, IL, and IC administration (0.039, 0.064, and 0.021%, respectively). The role of hepatic first-pass elimination was negligible. The results of these studies demonstrate that the elimination of sCT is rapid but does not occur in the liver. Enhanced sCT clearance at higher doses was indicated by increasing V_{dss} values, and it is hypothesized that increased renal blood flow and/or saturated plasma protein binding may contribute to the non-linear behavior. The IVAP canine model was found to have utility for probing the absorption and disposition PK of sCT. The combination of high oral bioavailability variability and non-linear disposition of sCT may produce highly variable therapeutic effects. The practical impact of the non-linear disposition of sCT remains to be determined. Based on the current results it appears that the rate-limiting step to the successful oral administration of sCT is its delivery into the portal vein since hepatic metabolism was negligible.

Keywords: Pharmacokinetics; First-pass effect; Intestinal absorption; Dogs; Peptides; Salmon calcitonin

1. 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. 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 [3]. The oral route is a preferred route of administration considering the chronic nature of CT therapy, but the absolute BA is extremely low, 0.022% following intraduodenal administration of sCT [4] and from 0.2 to 0.9% following colonic administration of hCT in rats [5]. In human studies following intracolonic administration, the BA of hCT is from 0.05 to 2.7% [6,7]. Therefore, the current therapeutic use of calcitonin requires SC or intranasal administration which affords higher BA than that reported for oral administration [8,9].

Limited PK studies of the various calcitonins (CTs) have been performed in laboratory animals or humans. Early PK studies in humans evaluated the disposition of sCT following IV, intramuscular (IM), and SC administration [10–13]. CT studies in rhesus monkeys have demonstrated that it is a

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useful animal model for the investigation of the pathophysiology of calcitonin, however, they were not evaluated as an absorption model [14]. While the baseline intestinal regional dependence of sCT absorption has not yet been reported, a few reports focusing on rectal and colonic administration have been described. The rectal absorption of sCT in humans has also been evaluated using a pharmacodynamic, (i.e. calcium lowering) rather than PK model [15]. A similar study in humans evaluated rectal versus nasal absorption using calcium lowering as the outcome variable [16]. Salmon calcitonin plasma concentrations expressed in relation to time zero concentrations peaked quickly after rectal administration and were significantly higher than after nasal administration.

Thus, it is desirable to understand the precise nature of the absorption and disposition of sCT in order to improve its delivery and BA in humans. In the present study, an in vivo canine IVAP model was utilized for evaluating the disposition PK, baseline regional intestinal absorption and first-pass hepatic extraction of sCT.

2. Materials and methods

2.1. Materials

Recombinant salmon calcitonin was obtained from Unigene Laboratories, Fairfield, NJ. Salmon calcitonin antibody (cross reacts less than 1% with mammalian calcitonins) and ^{125}Iodotyrosyl-salmon calcitonin was obtained from Advanced Chem Tech (Louisville, KY). 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). IV catheter, 20G Abbocath was obtained from Abbott Labs (North Chicago, IL). Heparinized syringes, 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 (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.

2.2. Preparation of IVAP dogs

All animal studies were performed under approved protocols (IRB-UCA, Rutgers University and IACUC, University of Medicine and Dentistry of New Jersey). Male beagle dogs (11–16 kg) were surgically fitted with intestinal and vascular access ports. A description of the surgical procedure was detailed elsewhere [17]. Briefly, the ports were implanted along the spine, in the subcutaneous space behind the shoulder blades. Four catheters 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.

Visualization of the portal vasculature by contrast fluoroscopy indicated that blood flow to the liver was not compromised by insertion of the portal vein catheter. The animals were allowed to recover for at least 2 weeks prior to the initiation of the studies. Dogs were fasted overnight prior to/and during the study. Water was allowed ad libitum. During the study, the animals were restrained in a dog sling and the back and foreleg shaved. The ports for IVAP infusions were accessed transcutaneously with a 22G Huber needle. 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. 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. Blood samples were drawn through a 20G IV catheter with a heparin lock which was inserted into the brachial vein. The catheter was flushed with heparinized saline (50 IU/ml) after each blood draw. The IVAP dogs were used once every 1 or 2 weeks.

2.3. IV, SC, and PV administration

Doses were administered IV (1, 10, 25, 100, 500, and 1000 μg) into the brachial vein or SC (10–1000 μg) into the hind leg. For PV administration, 10 and 50 μg were administered into the PV port followed by a 1 ml saline flush. To investigate the hepatic extraction of sCT, 50 μg of sCT was injected into the PV with 9 mg sodium taurodeoxycholate (TDC) or 7 mg lauroyl carnitine chloride (LCC). TDC and LCC were selected because they are present in current oral formulations of sCT as a result of their ability to enhance the oral absorption of sCT. The dosing solution was prepared using bacteriostatic 0.9% Sodium Chloride Inj., USP. The dosing volume was 1–2 ml per dog. 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. Blood samples were drawn at 1, 3, 6, 9, 12, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240, 300, and 360 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 at 3°C. The samples were stored at −20°C pending analysis by radioimmunoassay (RIA).

2.4. PO, ID, IL, and IC administration

For the absorption studies, sCT dosing solution (25 mg/5 ml per dog) was prepared in water. The dosing solution was rapidly infused (12 ml/min) via gavage or through the intestinal ports and followed by a 1 ml sterile water flush. Blood samples were drawn at 3, 6, 9, 12, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, and 240 min. All samples were processed as previously described. Prior to each weekly study, all intestinal ports were accessed and flushed with 1 to 2 ml of sterile water.

2.5. Salmon calcitonin analysis

The concentration of sCT in dog plasma was determined by competitive RIA using sCT antibody and ^{125}Iodotyrosyl-salmon calcitonin as described elsewhere [17,18]. The assay was accurate and reproducible over the concentration range of 100–1500 pg/ml of sCT. The plasma sCT concentration beyond the calibration range was diluted 10 to 500 times with blank dog plasma to fit within calibration range. 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% crossreactivity with calcitonin tryptic fragments [4]. The assay was also highly specific with no immunological reactions caused by repeated studies, and analytical validity of the RIA was confirmed with the blank plasma every sCT study.

2.6. Pharmacokinetic analysis

The IV plasma concentration versus time data were analyzed using a conventional 2-compartmental PK method [19]. Various rate constants (K_{12}, K_{21}, K_{el}, α, and β), terminal half-life (t1/2b), volume of distribution (V_c and V_{dss}), area under the plasma concentration-time curve from time 0 to time infinity (AUC), total plasma clearance (CL), and mean residence time (MRT) were obtained after fitting the IV plasma concentration data using Kinetica™ (version 2.00.200, InnaPhase). The plasma concentration versus time data of PV, SC, and PO dosings were analyzed by non-compartmental PK method [19]. The highest observed concentration and the corresponding sampling time were defined as C_{max} and t_{max}, respectively. The AUC was calculated by a combination of the trapezoidal and log-trapezoidal methods [20], and then extrapolated to infinity. Since the CL increased non-linearly over the dose range of 1–1000 μg, the estimated BA of oral, intestinal, SC, and PV (50 μg) treatments was calculated using mean dose normalized IV AUC values, which are the mean of AUC/Dose for all doses administered.

2.7. Statistical Analysis

All statistical tests were performed using Jandel Sigma Stat (Version 2.0, San Raphael, CA). One way ANOVA was performed, and a minimum P-value of 0.05 was used as the significance. All data are reported as the mean ± standard error (SEM) unless otherwise noted. Linear regression analysis was also performed using the independent variable (log dose) and the dependent variable (AUC/Dose, V_{dss}, and CL).

3. Results and discussion

3.1. Baseline pharmacokinetics of sCT

The mean plasma concentration time profiles of sCT after various IV doses are shown in Fig. 1; the estimated PK parameters for IV administration are listed in Table 1. Salmon calcitonin demonstrated non-linear PK following IV administration in IVAP dogs. There were statistically significant differences in the V_{dss}, normalized AUC, and CL after the various IV doses ($P < 0.05$ by ANOVA). In contrast, MRT was not changed over the dose range studied. The mean CL at low doses (1–25 μg) and at high doses (100–1000 μg) were 4.9–6.4 and 12.6–13.6 ml/min/kg after IV administration, respectively. Previous it was reported that the CL values of sCT were not different in IVAP and normal dogs [17]. V_{dss} values of the high dose groups (100–1000 μg) were significantly higher than those of the low dose groups (1–25 μg). At the 1 μg dose, plasma sCT concentrations after 20 min were below the lower limit of quantification (LLOQ: 80 pg/ml), therefore 2-compartment model rate constants were not determined. In a linear regression analysis, the PK parameters were plotted versus the log dose (Fig. 2). The slopes were greater than 0 for V_{dss} and CL, whereas less than 0 for AUC/Dose with $R^2 > 0.5$. Since the normalized AUC decreased, and V_{dss} and CL

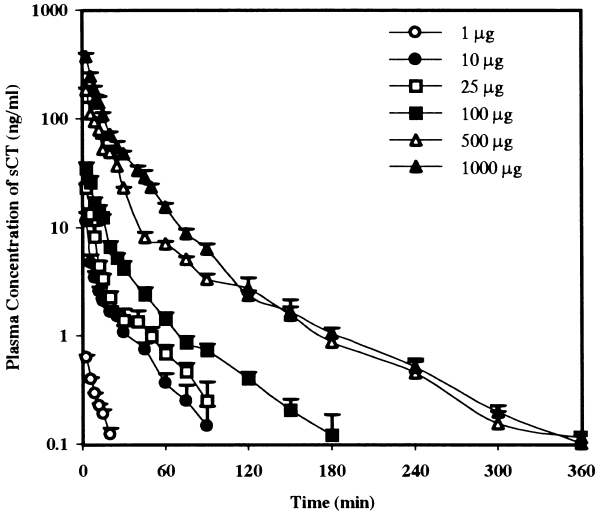

Fig. 1. Plasma concentration (mean ± SEM) versus time curves of sCT after intravenous administration of various sCT doses in IVAP dogs ($n = 3$–12).

Table 1
Pharmokinetic parameters of sCT after IV administration of various sCT doses in IVAP dogs (mean ± SEM)[a]

Parameters	1 μg ($n=4$)	10 μg ($n=6$)	25 μg ($n=3$)	100 μg ($n=4$)	500 μg ($n=3$)	1000 μg ($n=12$)	P
K_{12} (min^{-1})	ND	0.159 ± 0.062	0.079 ± 0.014	0.022 ± 0.003	0.015 ± 0.004	0.007 ± 0.001	<0.05[b]
K_{21} (min^{-1})	ND	0.082 ± 0.025	0.043 ± 0.007	0.028 ± 0.004	0.018 ± 0.001	0.015 ± 0.001	<0.05[b]
K_{el} (min^{-1})	ND	0.173 ± 0.065	0.152 ± 0.016	0.072 ± 0.005	0.063 ± 0.007	0.054 ± 0.003	<0.05[b]
α (min^{-1})	ND	0.383 ± 0.121	0.248 ± 0.030	0.102 ± 0.008	0.081 ± 0.011	0.063 ± 0.004	<0.05[b]
β (min^{-1})	ND	0.031 ± 0.005	0.026 ± 0.003	0.019 ± 0.002	0.014 ± 0.000	0.013 ± 0.001	<0.05[b]
$t_{1/2\beta}$ (min)	ND	32.5 ± 9.2	27.5 ± 3.4	37.6 ± 5.2	50.7 ± 2.0	57.3 ± 6.2	<0.05[b]
V_c (ml/kg)	ND	43.8 ± 19.3	41.5 ± 1.0	187.2 ± 21.0	211.5 ± 61.1	253.0 ± 17.6	<0.05[b]
V_{dss} (ml/kg)	75.2 ± 25.3	108.3 ± 46.8	118.0 ± 3.5	336.9 ± 38.7	372.2 ± 47.0	360.0 ± 25.6	<0.05
AUC (ng/min/ml)	11.8 ± 1.1	158.2 ± 14.0	299.8 ± 33.0	575.2 ± 64.2	3016.8 ± 406.0	5755.4 ± 321.4	<0.05[c]
MRT (min)	ND	29.6 ± 12.1	18.9 ± 1.6	25.4 ± 2.2	29.3 ± 0.4	26.9 ± 1.2	>0.05[b]
CL (ml/min/kg)	6.4 ± 0.6	4.9 ± 0.5	6.3 ± 0.7	13.6 ± 1.9	12.6 ± 1.6	13.4 ± 0.8	<0.05

[a] ND: not determined.
[b] One milligram data.
[c] Statistics performed after dose normalization.

increased with increasing dose, the results clearly demonstrate that the disposition of sCT is non-linear in dogs.

With increasing concentration (or dose) of sCT, it was observed that V_{dss} and CL increase without a change in MRT (using ANOVA and linear regression analysis), suggesting that the non-linearity is not a result of typical dose dependent elimination mechanisms. Typical dose dependent elimination kinetics are observed when a capacity limited process such as biotransformation or excretion is operative [19]. With increasing concentration (or dose), V_{dss} is not affected, but CL decreases and MRT increases. Using a physiologically based organ clearance [19] and the fact that the kidney is the target organ of sCT extraction [21–23], renal blood flow, plasma protein binding, and intrinsic organ clearance could contribute potentially to sCT total body clearance (CL). However, the non-linearity of sCT disposition was not due to intrinsic organ clearance, (i.e. metabolism and elimination) since the MRT was not changed (Table 1). MRT is most closely related to the elimination from the central compartment [19]. The elimination rate constant (K_{el}) decreased rather than increased with increasing dose (Table 1) suggesting that changes in renal blood flow and/or plasma protein binding could contribute to the non-linearity of sCT disposition. sCT has been reported to affect vascular blood flow [24], therefore sCT may enhance its own clearance by increasing distribution into the tissues (predominantly in the central compartment) by blood flow enhancement. The V_c increased from 43.8–41.5 ml/kg at 10–25 μg to 187.2–253.0 ml/kg at 100–1000 μg (Table 1). Salmon calcitonin may also decrease its own plasma protein binding with the saturation mechanism at higher dose groups (100–1000 μg) which can result in an increase in CL and V_{dss}. Although several peptide hormones including calcitonin reportedly bind to plasma proteins [25], reports on the extent of plasma protein binding of sCT are lacking.

PK data after IV administration of sCT in dogs are limited. Recently, PK data in rats were reported by our group [4]. sCT demonstrated linear PK following IV administration of 1, 5, and 10 μg doses in rats. Mean CL and V_{dss} were 12.9 ± 1.7 ml/min/kg and 700 ± 50 ml/kg, respec-

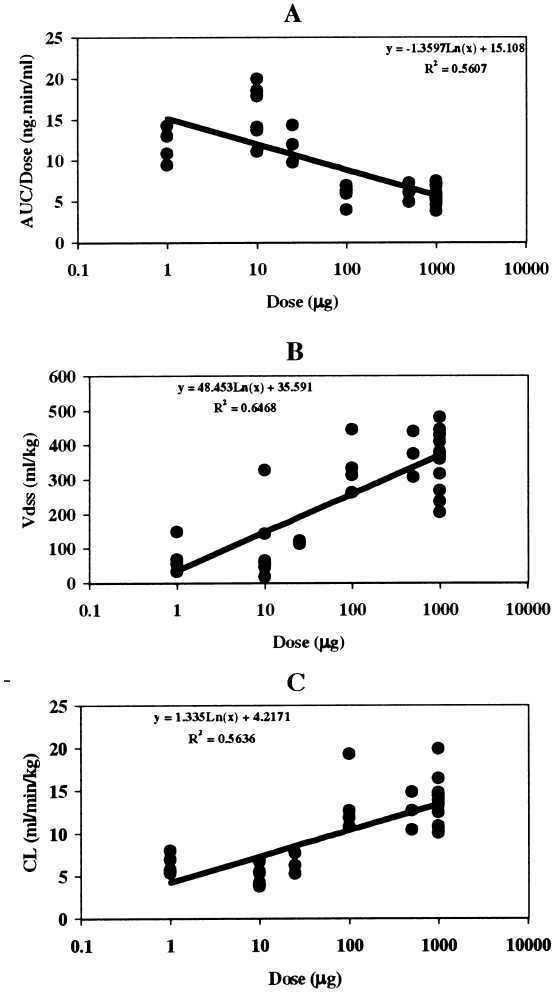

Fig. 2. Linear regression analysis of dose normalized AUC (A), volume of distribution at steady state (B), and total body clearance (C) at intravenous doses from 1 to 1000 μg in IVAP dogs.

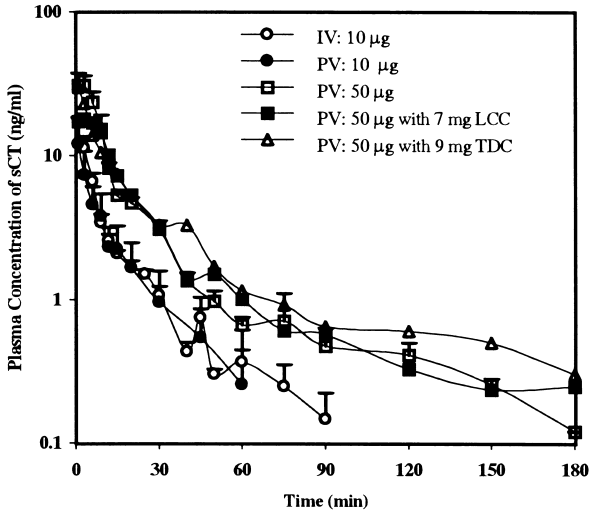

Fig. 3. Plasma concentration (mean ± SEM) versus time curves of sCT after portal venous administration of 10 and 50 μg sCT with 7 mg lauroyl carnitine chloride (LCC) or 9 mg taurodeoxycholic acid (TDC) in IVAP dogs ($n = 3$–6).

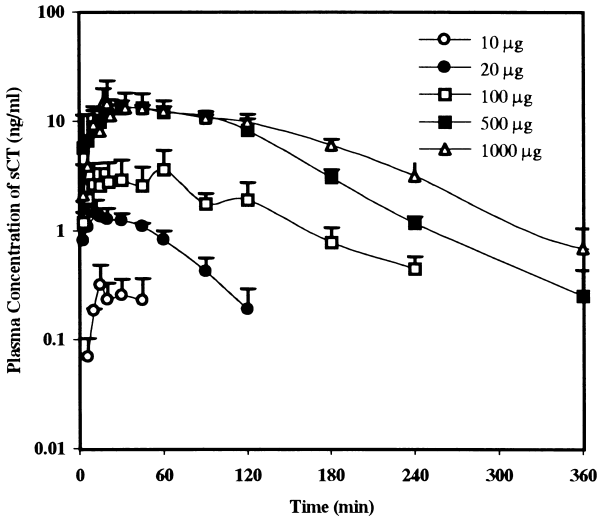

Fig. 4. Plasma concentration (mean ± SEM) versus time curves after subcutaneous administration of various sCT doses in IVAP dogs ($n = 3$–4).

tively, when they were recalculated assuming 200 g body weight rats (100–250 g). When similar doses (μg/kg) are given intravenously to animals (1, 5, and 10 μg/rat or 5, 25, and 50 μg/kg for rats vs. 100, 500, and 1000 μg/dog or 7.4, 37, and 74 μg/kg for dogs), the PK parameters are similar. The terminal half-lives of dogs were also similar to that of rat (40.1 ± 7.8 min). The terminal half-life of hCT was similar to those reported in this study when it was colonic administered [6,7].

3.2. Hepatic extraction of sCT

The mean plasma concentration time profiles of sCT after PV administration of 10 or 50 μg are shown in Fig. 3. Plasma concentrations of sCT were very similar for both IV and PV administration at the 10 μg dose. AUC and BA of sCT after PV administration of 10 μg dose were 132.0 (±72.0) ng.min/ml and 83.3 (±45.5)% when compared IV administration of 10 μg dose, respectively. At 50 μg dose, AUC and BA were 420.0 (±126.0) ng.min/ml and 92.8 (±32.0)% using dose normalized IV AUC, respectively. The hepatic first-pass elimination of sCT was negligible in dogs consistent with previously reported data in rats [21]. It is also known that the structure of sCT protects it against its sequestration in the muscle and bone [26]. Hepatic elimination of sCT after PV administration was not changed by the presence of the surfactants TDC or LCC (Fig. 3), which are used in oral formulations and thought to enhance the hepatic sequestration of sCT. In the presence of 7 mg LCC or 9 mg TDC, the BA of sCT were 87.8 and 102.4%, respectively. 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.

3.3. SC absorption of sCT

One of the most common routes of sCT administration occurs by means of SC injection. Many studies only report the relative sCT BA of the tested dosage form to the SC form. The mean plasma concentration time profiles of sCT after various SC doses are shown in Fig. 4 and the estimated PK parameters for SC administration are listed in Table 2. Plasma concentrations demonstrated proportional increases with increasing doses, but showed large variability. C_{max} and AUC were also dose proportional with BA varying from 21.4 to 52.9%. PK parameters, C_{max}, normalized AUC, and BA were not significantly different for the doses studied ($P > 0.05$ by ANOVA). Peak concentrations following SC

Table 2
Pharmacokinetic parameters of sCT after SC administration of various sCT doses in IVAP dogs (mean ± SEM)

Parameters	10 μg ($n = 4$)	20 μg ($n = 3$)	100 μg ($n = 3$)	500 μg ($n = 3$)	1000 μg ($n = 3$)	P
C_{max} (ng/ml)	0.4 ± 0.1	1.5 ± 0.3	4.0 ± 1.5	14.6 ± 1.4	15.2 ± 2.9	>0.05[a]
t_{max} (min)	26.3 ± 7.2	25.0 ± 10.4	43.3 ± 16.7	26.0 ± 12.3	61.7 ± 30.0	>0.05
AUC (ng.min/ml)	20.4 ± 8.6	100.8 ± 6.4	452.8 ± 89.0	1870.0 ± 242.3	2170.2 ± 169.9	>0.05[a]
BA (%)	21.4 ± 9.0	52.9 ± 3.4	47.5 ± 9.3	39.2 ± 5.1	22.8 ± 1.8	>0.05

[a] Statistics was performed after dose normalization.

Table 3
Pharmacokinetic parameters of sCT after PO and regional intestinal administration of 25 mg sCT in IVAP dogs (mean ± SEM)[a]

Parameters	PO ($n=4$)	Duodenum ($n=4$)	Ileum ($n=6$)	Colon ($n=4$)	P^b
C_{max} (ng/ml)	LLOQ	2.5 ± 0.8	4.7 ± 1.5	1.8 ± 0.3	> 0.05
t_{max} (min)	ND	12.0 ± 4.6	6.8 ± 1.0	7.0 ± 1.0	> 0.05
BA (%)	ND	0.039 ± 0.017	0.064 ± 0.022	0.021 ± 0.004	> 0.05

[a] LLOQ: lower limit of quantification, ND: not determined.
[b] PO data were not included.

administration occurred within 25.0–61.7 min. In rats [4], BA was from 11.2 to 23.1% and peak concentrations occurred within 30–60 min at dose ranges of 1–100 μg. Therefore, the absorption of sCT was rapid and considerable after SC administration in rats and dogs.

3.4. Intestinal absorption of sCT

The typical plasma concentration versus time profiles of sCT following each intestinal route of administration were presented elsewhere [17]; and the estimated PK parameters for each route of unformulated drug are listed in Table 3. Since the CL was increasing (non-linear) over the dose ranges of 1–1000 μg, the estimated BA was calculated using mean dose normalized IV AUC values, which is the mean of AUC/Dose over 1–1000 μg/dog. A method was proposed by Martis and Levy [27] for bioavailability calculations for drugs showing simultaneous first-order and capacity-limited elimination kinetics, but it could not be applied in this study since the observed non-linearity is opposite in direction. In this studies, plasma concentrations of sCT following a PO dose were below the LLOQ (80 pg/ml) in the RIA plasma assay, therefore PK parameters were not included in Table 3. This may be attributed to extensive gastric and intestinal enzymatic degradation. Very little data have been published regarding the oral PK of sCT, possibly because, with current methodology, the required analytical sensitivity is lacking. Absorption of sCT following duodenal administration was rapid with peak concentrations occurring within approximately 10 min. BA was 0.039% following duodenal administration. In duodenal administration of sCT into rats [4], BA was reported to be 0.022% with the peak concentrations occurring within 15 min at 1 and 2 mg doses. The rapid absorption of sCT was probably a function of the extensive enzymatic degradation within the intestine in rats and dogs. BA of sCT was 0.064% for ileal administration and 0.021% for colonic administration. The C_{max} and BA were not significantly different for the routes studied (Table 3, $P > 0.05$ by ANOVA) due to high variability in the data. Compared to other regions, the low BA of sCT from the colon is considered to be related to the combined effects of poor membrane permeability and/or proteolytic degradation by microorganisms specifically residing in the colon. The rational design of colon-specific protein/peptide delivery systems has been based on two premises (1) that the colon compartment has decreased pancreatic proteolytic activity and/or (2) the colon possesses bacterial activities with unique specificities [28,29]. The absolute BA of hCT following colonic administration in rats ranged from 0.2 to 0.9% [5]. The BA of hCT following intracolonic administration in humans was 0.32 ± 0.80% with a range from 0.05 to 2.7% [6,7]. As current and previous studies show, the oral BA of sCT or hCT is extremely limited and highly variable in animals or humans. This is clearly due to extensive proteolytic enzymatic degradation and low intrinsic intestinal membrane permeability, but not to hepatic extraction. In attempts to enhance the oral absorption of sCT by using formulation technology, our group recently enhanced sCT BA from 12 to 31-fold versus control in dogs [17]. The reduction of proteolytic degradation in the GI lumen and permeation enhancement were the two primary mechanisms involved in the augmentation of sCT absorption.

In conclusion, sCT showed non-linear disposition PK in IVAP dogs. The enhanced sCT clearance at higher doses, as indicated by increasing V_{dss}, is hypothesized to be a function of increased renal blood flow and/or saturated plasma protein binding. The intestinal BA of sCT was 0.039, 0.064, and 0.021% for ID, IL, and IC administration, respectively, whereas the BA after SC administration was significantly higher. Hepatic first-pass elimination of sCT was negligible suggesting that the successful oral delivery of sCT depends upon its appearance in the portal vein.

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