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ORIGINAL ARTICLE

Evaluation of salmon calcitonin (sCT) enteric-coated capsule for enhanced absorption and GI tolerability in rats

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Abstract

Background: Considering the chronic and repeated nature of salmon calcitonin (sCT) therapy, the oral route is a preferred route of administration. But, the oral bioavailability of sCT is very low due to enzymatic degradation and poor permeation across intestinal epithelial cells. It was the aim of this study to investigate the pharmacodynamic (PD), pharmacokinetic (PK), and mucosal injury characteristic of sCT oral delivery system. *Method*: In this study, PD experiments were performed to find a suitable releasing region of sCT, an effect absorption enhancer, and an optimal mass ratio of sCT/enhancer. In addition, the PK experiments were designed to validate the absorption enhancement of this oral delivery system. Histopathological evaluations on the intestinal mucosa were carried out to assess any potential toxicity of the absorption enhancer. *Results*: Through the PD research, we determined that oral sCT enteric-coated capsules containing sCT and citric acid (CA) with a ratio of 1:20 may be an adaptable delivery. PK study further proved that the oral absorption of sCT was enhanced from this delivery system. Finally, no damage on intestinal mucosa was observed when rats received the delivery system containing CA for up to 7 days. *Conclusion*: These results suggested that enteric-coated capsules with a certain amount of CA might give enhanced oral delivery of peptide drugs like sCT.

Key words: *Enteric-coated capsule; oral delivery; pharmacodynamics; pharmacokinetics; salmon calcitonin*

Introduction

Calcitonin (CT), a cyclic and endogenous polypeptide hormone composed of 32 amino acids (molecular weight ~3500 Da), plays a crucial physiological role in the regulation of calcium homeostasis and bone remodeling[1]. CT is found widely in different species. Four forms of CTs are used clinically: synthetic human CT (hCT), synthetic salmon CT (sCT), natural porcine CT (pCT), and a synthetic analogue of eel CT[2]. sCT is most widely used as it is readily available and has good tolerability.

sCT is most commonly used parenterally or nasally[3,4]. It can effectively inhibit manifestation of metabolic bone disorders. It is widely used in the therapy of Paget's disease and osteoporosis[5,6]. However, frequent and relatively high dosages of sCT need to be administered. Daily injection of sCT has poor patient compliance for long-term therapy and sCT chronic nasal therapy may cause irritation of mucosa[7].

Considering the chronic and repeated use of sCT, the oral route is a preferred route of administration[8]. However, like many other proteins or peptides, the oral bioavailability (BA) of sCT is very low because of enzymatic degradation in the gastrointestinal tract and poor permeation across intestinal epithelial cells[9,10]. It has been reported from several animal studies that the absolute BA is 0.022% following intra-duodenal administration of sCT and from 0.2% to 0.9% following colonic administration of CT in rats[11]. In human studies, the BA of hCT ranges from 0.05% to 2.7% after intra-colonic administration[12].

Recently, some advanced technological approaches have been adopted to increase BA and pharmacodynamic

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(PD) effects after sCT oral administration. The new formulations included sCT enteric-coating tablets, sCT liposome[13], sCT microsphere[14], sCT dextran hydrogel[15], sCT nanostructures[16], PEGylation of sCT[17], and w/o/w multiple emulsion of sCT. Despite such dedicated efforts to the design of sCT oral delivery systems, the BA of sCT remains a challenge and the oral delivery systems are costly[18].

In this study, PD experiments were performed to find a suitable releasing region of sCT, an effect absorption enhancer, and an optimal mass ratio of sCT/enhancer. Oral sCT enteric-coated capsules containing sCT and CA with a ratio of 1:20 were prepared for the experiments. In addition, the PK experiments were designed to validate the absorption enhancement of this oral delivery system. As previous reports had shown correlation between the absorption enhancers and the intestinal mucosal toxicity[19,20], histopathological evaluations on the intestinal mucosa were carried out to assess any potential toxicity of CA.

Materials and methods

Materials

sCT (sCT; 3431.9 Da, purity 99%) was purchased from Soho-Yiming Pharmaceuticals Co. (Shanghai, China). CA was purchased from Yixin Chemical Reagents Factory (Yixin, China). Enteric-coated capsules for animals were purchased from Qiangji Pharmaceuticals Co. (Guangzhou, China). Ca^{2+} kit was obtained from Nanjing Bioengineering Institution (Nanjing, China). sCT enzyme immunoassay kit was obtained from Peninsula Laboratories (San Carlos, CA, USA). All other materials were obtained from Nanjing No. 1 Chemical Co. (Nanjing, China).

Preparation of dosing solution

sCT and absorption enhancers were dissolved in sodium dihydrogen phosphate solution (0.1 M, pH 4.4).

For intra-intestinal administration, the concentration of sCT solution was 0.4 mg/mL. The concentration of absorption enhancers was 20% (w/v). An aliquot of sCT solution was mixed with the same volume of enhancer solution and kept cool until administration to rats. The final dosing solution contained 0.2 mg/mL of sCT and 10% (w/v) of an absorption enhancer.

For subcutaneous (s.c.) and intra-gastric (i.g.) administration, sCT concentration of dosing solution was 0.2 mg/mL. For intravenous (i.v.) administration, sCT concentration of dosing solution was 0.01 mg/mL.

Preparation of sCT enteric-coated capsule

For the CA proportion study, each enteric-coated capsule was filled with 0.25 mg sCT and 0, 0.1, 2.5, 5, and 10 mg of CA, respectively (starch as bulking agent).

For PK/PD experiments, a certain amount of sCT and CA were loaded into each enteric-coated capsule. The strengths of capsules were 0.5 mg sCT mixture and 10 mg CA, 0.25 mg sCT mixture and 5 mg CA, and 0.125 mg sCT mixture and 2.5 mg CA, respectively (starch as bulking agent).

For histopathological evaluation, 0.25 mg sCT and 5 mg CA were filled into each enteric-coated capsule (starch as bulking agent).

Animal studies

Animal experiments were carried out in compliance with the guidelines for animal experimentation of China Pharmaceutical University (Nanjing, China) and protocol was approved by the Animal Ethics Committee of this institution.

Intestinal absorption studies in rats

Four female Sprague–Dawley rats (Qinglongshan Animal Center, Nanjing, China), weighing 180–240 g, were fasted for 12 hours. Before the administration of sCT, each rat was anesthetized by an intra-peritoneal injection of urethane (20%) at a dose of 1 mg/kg. The abdominal cavity of rats was opened along linea alba abdominis till the intestine and stomach were exposed. The intestine (15 cm) was ligated away from pylorus. sCT solution was injected into the ligation site (0.1 mg/0.5 mL), and the intestinal segment was then quickly ligated at the injection site. After injection, abdominal cavity of rat was sutured[21]. The second group of rats was given a s.c. sCT dose of 0.1 mg/0.5 mL. The third group of rats was administered an i.g. sCT dose of 0.1 mg/0.5 mL.

Blood samples (0.2 mL) were taken from retro-orbital sinus with syringes before dose administration and at 15, 30, 60, 90, 120, 180, 240, and 360 minutes post-dose. Plasma samples (20 μL) were collected after centrifugation at 12,000 × *g* for 3 minutes.

Oral administration of sCT enteric-coated capsules

Four female Sprague–Dawley rats (Qinglongshan Animal Center), weighing 180–240 g, were fasted for 12 hours before experiments but allowed water ab libitum. Animals were kept conscious during capsule administration. The rat's head and four limbs were fixed to the iron board. Then the board was placed and the rat's tongue was pulled out with forceps till its esophagus was exposed. Meanwhile, a capsule carrying sCT was put into the esophagus and the capsule was pushed

down along the esophagus with a blunt forceps. When the capsule could not be seen, the rat was given several drops of water to complete a comfortable swallowing. The rat was unclamped and it was observed for 2 minutes, ensuring that the capsule was not spat out.

Blood samples were collected from retro-orbital sinus 30 minutes before the capsule administration in order to establish the baseline Ca^{2+} and sCT levels. Blood samples (0.4 mL) were collected at 5, 10, 20, 30, 60, 90, 120, 180, 240, 360, 480, and 720 minutes post-dose. Every 1 hour, each animal was given 1 mL water to make up the decrease in blood volume. Plasma samples (150 μL) were collected after centrifugation at $12{,}000 \times g$ for 3 minutes.

Determination of plasma Ca^{2+} and sCT concentrations

Plasma concentrations of Ca^{2+} were assayed with colorimetric Ca^{2+} assay kits using 20 μL rat plasma. The principles are based on the ability of methyl-thymol blue to form blue-colored complex with calcium in an alkaline medium, which can be measured at 610 nm. The assay procedure is as follows: Add 1 mL methyl-thymol blue and 2 mL alkaline medium into each sample (20 μL distilled water, 20 μL standard Ca^{2+} sample, and 20 μL rat plasma sample, respectively). Vortex until it is well-mixed. Five minutes later, read absorbance (A) at 610 nm with a 721 ultra-violet spectrophotometer. Plasma concentrations of Ca^{2+} were calculated by the formula as follows:

Plasma concentrations of Ca^{2+}(mmol/L) = $(A_{\text{plasma sample}} - A_{\text{distilled water}}) \times$ standard Ca^{2+} concentration (2.5 mmol/L)/$(A_{\text{standard } Ca^{2+} \text{ sample}} - A_{\text{distilled water}})$.

Plasma concentrations of sCT in rats were assayed with sCT enzyme immunoassay kits using 100 μL rat plasma. The kit is designed for the measurement of salmon rat serum or plasma without the use of an extraction procedure. Protocols provided by the vendors were listed as follows: First, 100 μL/well primary antibody was added into the 96-well immunoplate and incubated at room temperature for 2 hours. Then the immunoplate was washed 3 times with 300 μL/well of assay buffer. A standard/sample (100 μL/well) was added and incubated at room temperature for 2 hours. The plate should not be washed and the standard/sample solution should be kept in plate wells. Then 25-μL biotinylated peptide (incubated overnight at 2–8°C) was added. The immunoplate was washed 5 times with 300 μL/well of assay buffer. Streptavidin-HRP (100 μL/well) was added and incubated at room temperature for 1 hour. Then the immunoplate was washed 5 times with 300 μL/well of assay buffer. Finally, 100 μL/well of TMB solution was added and incubated at room temperature for 0.5–1 hour. The solution was terminated with 100 μL/well of 2 N HCl. Optical density was read at 450 nm by ELISA reader within 10 minutes and results were calculated. The standard curve was plotted on semi-log graph paper. The known concentrations of peptide were plotted on a log scale (on the x-axis) and the corresponding optical density on a linear scale (on the y-axis). The peptide concentration in the unknown sample was determined by the standard curve.

Date analysis

PD calculation

The baseline Ca^{2+} concentrations of the blank plasma samples were designated as 100%. All other data were expressed as percentage of the baseline values. Concentrations of Ca^{2+} versus time and the PD parameters, such as the Ca^{2+} concentration after the maximal reduction ($C_{\max}$), the time to reach the maximal reduction ($T_{\max}$) in Ca^{2+} concentration, and the area above the curve of reduced plasma Ca^{2+} concentrations (AAC) were calculated.

Kinetic calculation

For i.v. administration, plasma concentration data were analyzed using a noncompartmental PK model with WinNonlin5.00.0101 (Pharsight Corporation, Mountain View, CA, USA). PK parameters calculated included half-life ($t_{1/2}$) and the area under the plasma concentration–time curve ($AUC_{0-\infty}$). For oral administration of enteric-coated sCT capsules, the maximum plasma concentration of sCT ($C_{\max}$) and the time to reach $C_{\max}$ ($T_{\max}$) were taken directly from the observed plasma sCT concentrations versus time data. The area under the plasma sCT concentrations versus time curve ($AUC_{0-\infty}$) and half-life ($t_{1/2}$) were also calculated using a noncompartmental PK model. Absolute BA of sCT was calculated as follows:

$$\text{BA} = \frac{\text{AUC}_{0-\infty}}{\text{AUC}_{\text{i.v.}\infty}} \times \frac{\text{Dose}_{\text{i.v.}}}{\text{Dose}_{\text{oral}}} \times 100\%$$

Histopathological evaluation

Intestinal mucosal injury caused by combination of CA was evaluated. Each of the 14 female SD rats (Qinglongshan Animal Center), weighing 180–240 g, was given a sCT capsule daily and two of them were killed each day at 6 hours after capsule administration. The jejunum segments were collected and exposed to 10% formaldehyde phosphate buffer (0.1 M, pH 7.4). Hematoxylin–eosin-stained cross-sections were prepared

by routine histological processing and they were examined using light microscopy (×100). The jejunum segments of two rats administered with daily saline were prepared with the same method as control.

Statistical analysis

The results were expressed as mean ± SD. All data were processed with SPSS Software (SPSS v13.0). One-way analysis of variance (ANOVA) with LSD/Dunnett for post hoc analysis was used to compare results between different groups. A probability (P) of less than 0.05 was considered statistically significant.

Results

Sites of absorption

To find sCT absorption site, sCT (0.1 mg/0.5 mL) was administered subcutaneously, intra-intestinally, and intra-gastrically. The hypocalcemic effect was used for evaluation. The plasma concentration profiles of Ca^{2+} are presented in Figure 1. PD parameters are listed in Table 1.

The s.c. control group showed a significant reduction in Ca^{2+} levels and the Ca^{2+}concentrations decreased to 68.2 ± 4.7% of the baseline Ca^{2+} levels. Moreover, the low Ca^{2+} level was maintained for at least 6 hours.

Remarkable and durative (6 hours) decreases in plasma Ca^{2+} concentrations were also observed when sCT was intra-intestinally administered. The $AAC_{0–6\ hours}$ was 5639 ± 1911 minutes, which reached up to 59.8% of the s.c. control. The maximal reduction in Ca^{2+} level brought the Ca^{2+} concentrations to 77.5 ± 10.5% of the baseline. This extent of reduction was much higher than that of sCT i.g. group (86.5 ± 17.8%), indicating that intestine was a better sCT absorption site and oral enteric-coated capsule might be a useful delivery system.

Table 1. sCT PD parameters of different administration ways.

Group/parameter	T_{max} (minutes)	C_{max} (%)	$AAC_{0–6\ hours}$ (minutes)
s.c. (0.1 mg/head)	165.0 ± 17.3	68.2 ± 4.7	9434 ± 1881*
i.g. (0.1 mg/head)	110.0 ± 17.3	86.5 ± 17.8	1151 ± 911
Intestinal injection (0.1 mg/head)	120.0 ± 54.8	77.5 ± 10.5	5639 ± 1911*

Each value presents the mean ± SD (n = 4). T_{max} represents the time to reach the maximal reduction in Ca^{2+} concentration. C_{max} represents the Ca^{2+} concentration after the maximal reduction. $AAC_{0–6\ hours}$ represents the area above the curve of reduced plasma Ca^{2+} concentrations. *Significantly different from the i.g. control ($P < 0.05$).

The statistic calculation provided significant difference in Ca^{2+} levels between sCT intra-intestinal group and sCT i.g. group, with $AAC_{0–6\ hours}$ values of 5639 ± 1911 and 1151 ± 911 minutes, respectively, suggesting that sCT was nearly destroyed in stomach because of pepsins and pH effect.

Selection of absorption enhancer

In this study, four typical absorption enhancers assessed included taurocholic acid (TAU), CA, salicylic acid (SA), and Polysorbate 80. The absorption-enhancing capability of these enhancers was compared through PD parameters after intra-intestinal administrations. sCT intra-intestinal group was used as control. The profiles of plasma Ca^{2+} concentrations after sCT administration are illustrated in Figure 2. PD parameters are presented in Table 2.

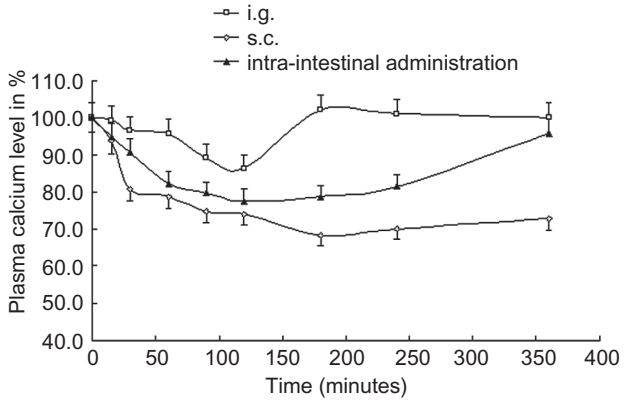

Figure 1. Plasma concentration profiles of Ca^{2+} after administration with 0.5 mL sodium dihydrogen phosphate solution (containing 0.1 mg sCT). Each point presents the mean ± SD (n = 4). sCT intragastric administration (□), sCT intra-intestinal administration (▲), and sCT subcutaneous administration (◇).

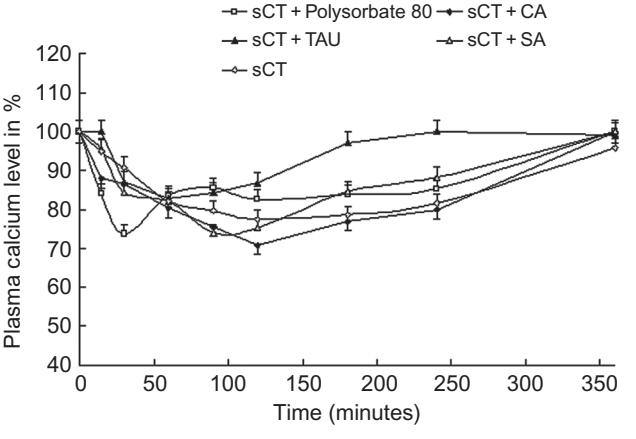

Figure 2. Plasma concentration profiles of Ca^{2+} after sCT (0.1 mg) intra-intestinal administration with various classical absorption enhancers. Each absorption enhancer was given at 10% (w/v). Each point presents the mean ± SD (n = 4). sCT + CA (◆), sCT + TAU (▲), sCT + SA (△), sCT + Polysorbate 80 (□), and sCT intra-intestinal administration as the control (◇).

Table 2. PD parameters after sCT (0.1 mg) intra-intestinal co-administration with various classical enhancers (w/v = 10%).

Group/parameter	T_{max} (minutes)	C_{max} (%)	$AAC_{0-6\ hours}$ (minutes)
sCT	120.0 ± 54.8	77.5 ± 10.5	5639 ± 1911
sCT + CA	120.0 ± 35.6	70.8 ± 2.1	6320 ± 1512
sCT + TAU	57.3 ± 12.8	82.8 ± 11.0	2096 ± 957
sCT + SA	75.5 ± 15.0	73.9 ± 0.5	4847 ± 1809
sCT + Polysorbate 80	30.0 ± 16.2	73.8 ± 10.1	4838 ± 1517

Each value presents the mean ± SD ($n = 4$). T_{max} represents the time to reach the maximal reduction in Ca^{2+} concentration. C_{max} represents the Ca^{2+} concentration after the maximal reduction. $AAC_{0-6\ hours}$ represents the area above the curve of reduced plasma Ca^{2+} concentrations.

TAU did not show any absorption-enhancing effect under the experimental conditions employed in this study. $AAC_{0-6\ hours}$ was only 2096 ± 957 minutes. Polysorbate 80 only exhibited a weak decrease in plasma Ca^{2+} level with the $AAC_{0-6\ hours}$ value of 4838 ± 1517 minutes. The time to reach the maximal Ca^{2+}-lowering effect was less than 0.5 hour.

CA demonstrated the distinct enhancement in sCT absorption. $AAC_{0-6\ hours}$ was 6320 ± 1512 minutes, close to the sCT intra-intestinal administration group. Plasma Ca^{2+} concentrations decreased to 70.8 ± 2.1% of the baseline, which was lower than that of sCT intra-intestinal administration alone. The Ca^{2+}-lowering effect was durative (about 6 hours).

SA also provided a clear enhancement in sCT absorption. Plasma Ca^{2+} concentrations decreased to 73.9 ± 0.5% of the baseline level. However, its $AAC_{0-6\ hours}$ value of 4847 ± 1809 minutes was lower than that of the CA group. Moreover, the SA group reached the maximal reduction in Ca^{2+} concentration at 75 minutes after administration, and the plasma Ca^{2+} level recovered faster than the CA group. These results indicated that the absorption-enhancing capability of SA was not comparable with CA. Therefore, CA was selected as the absorption enhancer in the following research.

sCT to CA ratios in enteric-coated capsules

Intestine absorption enhancement of sCT was obtained when 0.1 mg of sCT and 10% (w/v) of CA were co-administered intra-intestinally. To optimize the enhancing potency of CA, the relation between CA doses and its enhancing capability was investigated with sCT enteric-coated capsules (0.25 mg/capsule). CA was given at 0.1–10 mg and the ratios of sCT to CA were 2.5:1, 1:10, 1:20, and 1:40. sCT enteric-coated capsules (0.25 mg/capsule) with no CA were used as control. The plasma concentration profiles of Ca^{2+} after the oral capsule administration are compared in Figure 3. PD parameters are listed in Table 3.

When CA was administered at 0.1 mg, a very small decrease in Ca^{2+} level was observed, and $AAC_{0-12\ hours}$ was 7887 ± 3080 minutes. A statistic calculation indicated that there was no significant difference between this group and the control group.

At CA doses ranging from 2.5 to 10 mg, a clear increase in plasma Ca^{2+} concentration reductions was observed. The maximal reduction of the Ca^{2+} concentration obtained at CA doses of 2.5, 5, and 10 mg were 58.3 ± 9.7%, 54.6 ± 5.0%, and 60.6 ± 10.7% of the baseline, respectively, and the $AAC_{0-12\ hours}$ were 16110 ± 6006, 20554 ± 6625, and 19630 ± 6850 minutes, respectively. These values were significantly higher than that of control, especially at 5 mg. Compared with 2.5- and 10-mg groups, 5-mg group had the best maximal reduction of the Ca^{2+} concentration. Besides, we found that 10-mg group did not show a better C_{max} and $AAC_{0-12\ hours}$ than 5-mg group, suggesting that increasing the content of CA had no significant effect on the elevation of absorption enhancement when CA dose reached 5 mg. So we considered that the optimal mass ratio of sCT to CA was 1:20.

Pharmacokinetic study of sCT enteric-coated capsule

Through the PD research above, we determined absorption site, absorption enhancer, and sCT to CA mass ratio. sCT enteric-coated capsules containing sCT and CA with a mass ratio of 1:20 were prepared for the following pharmacokinetic (PK) study. Three sCT doses: 0.125, 0.25, and 0.5 mg/head were chosen as low-, middle-, and high-dose groups, respectively. The i.v. group was served as control. The plasma concentration profiles of sCT after the administration are compared in Figure 4. PK parameters are listed in Table 4.

After i.v. administration, plasma concentration of sCT decreased rapidly. The terminal elimination half-life was 91.85 ± 18.76 minutes. $AUC_{0-\infty}$ was 807.7 ± 365.7 ng min/mL.

After oral administration, the mean plasma concentration of sCT increased rapidly and reached the maximum level at 4.301 ± 1.01, 7.24 ± 0.15, and 14.07 ± 0.55 ng/mL for the 0.125-, 0.25-, and 0.5-mg dose groups, respectively, in less than 30 minutes post-dose. At 1.5 hours, the oral profiles started to decline quickly. The half-lives were 63.67 ± 10.29, 106.8 ± 10.44, and 90.12 ± 6.06 minutes for the 0.125-, 0.25-, and 0.5-mg dose groups, respectively.

$AUC_{0-\infty}$ of the three groups appeared to be dose-dependent (Figure 5) and was 391.1 ± 60.41, 827.0 ± 241.8, and 22420 ± 3878.0 ng min/mL for low-, middle-, and high-dose groups, respectively. These results

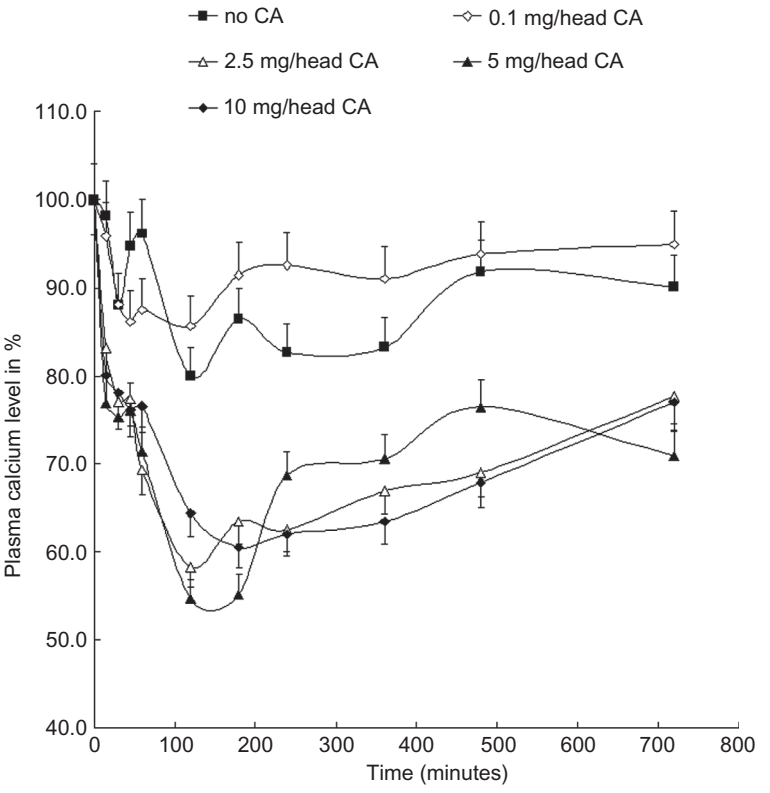

Figure 3. Plasma concentration profiles of Ca^{2+} after oral administration of enteric-coated capsules containing sCT 0.25 mg and various CA doses. Each point represents the mean ± SD ($n = 4$). No CA (■), 0.1 mg/head (◇), 2.5 mg/head (△), 5 mg/head (▲), and 10 mg/head (◆).

Table 3. PD parameters after sCT (0.25 mg) oral administration of enteric-coated capsules and various CA doses.

Group/ parameter	Ratio (sCT/CA)	T_{max} (minutes)	C_{max} (%)	$AAC_{0-12\ hours}$ (minutes)
No CA	—	135.0 ± 53.0	80.0 ± 5.8	7813 ± 2916
0.1 mg CA	2.5:1	105.0 ± 56.5	85.7 ± 4.4	7887 ± 3080
2.5 mg CA	1:10	135.0 ± 30.0	58.3 ± 9.7*	16110 ± 6006*
5 mg CA	1:20	129.0 ± 55.7	54.7 ± 5.0*	20550 ± 6625*
10 mg CA	1:40	150.0 ± 42.4	60.6 ± 10.7*	19630 ± 6850*

Each value presents the mean ± SD ($n = 4$). T_{max} represents the time to reach the maximal reduction in Ca^{2+} concentration. C_{max} represents the Ca^{2+} concentration after the maximal reduction. $AAC_{0-12\ hours}$ represents the area above the curve of reduced plasma Ca^{2+} concentrations. *Significantly different from the no CA group ($P < 0.05$).

showed that sCT exhibited linear kinetics following oral administration over the dose range studied.

The mean plasma concentration versus time curve after i.v. administration was similar to that of the middle-dose group after oral administration. The absolute BA was rather low and appeared to be dose-dependent. At the high dose (0.5 mg/head), the extent of absolute oral BA was 1.80%, higher than those of lower doses (0.25 and 0.125 mg/head).

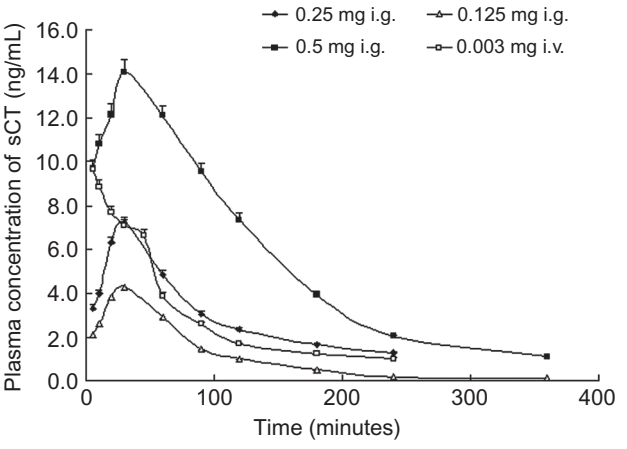

Figure 4. Plasma concentration profiles of sCT after sCT enteric-coated capsule administration at various doses. Each point represents the mean ± SD ($n = 4$). i.v. control (□), 0.125 mg (△), 0.25 mg (◆), and 0.5 mg (■).

Mucosa injury effect of absorption enhancer

In this study, the intestine was assessed for potential mucosal damage by long-term administration of CA.

Histopathological evaluation was carried out on the segments of the small intestine after daily administration

Table 4. Pharmacokinetic parameters of sCT after sCT enteric-coated capsule oral administration at various doses.

Dose		*C*max	*T*max	$T_{1/2}$	$AUC_{0-\infty}$	
sCT	CA	(ng/mL)	(minutes)	(minutes)	(ng min/mL)	BA (%)
0.003 mg (i.v)	—	—	—	91.85 ± 18.76	807.7 ± 365.7	—
0.125 mg	2.5 mg	4.30 ± 1.01	30.00 ± 0.00	63.67 ± 10.29	391.1 ± 60.40	1.16
0.25 mg	5 mg	7.24 ± 0.15	30.00 ± 0.00	106.8 ± 10.44	827.0 ± 241.8	1.23
0.5 mg	10 mg	14.07 ± 0.55	26.67 ± 5.77	90.12 ± 6.06	2242 ± 388.0	1.80

Each value presents the mean ± SD (n = 4). BA was determined using AUC after i.v. administration at 0.003 mg/head ($AUC_{i.v.\infty}$) of 807.66 ng min/mL.

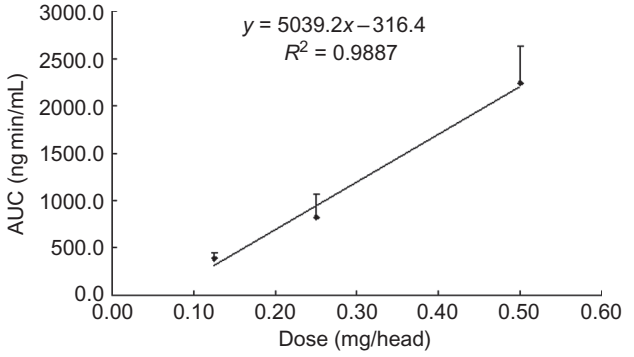

Figure 5. The relation of AUC and dose after sCT enteric-coated capsule oral administration. Each point represents the mean ± SD (n = 4).

for up to 7 days. An electron microscope was used to take representative histological images of rat jejunum mucosal epithelium. After saline (control) administration, jejunum mucosa showed no abnormality at all exposure times (Figure 6a). For oral CA group, after the first, fourth, and last administration, no desquamated epithelial cell or severe inflammation was observed (Figure 6b–d), suggesting that exposure to CA at the doses used in this study did not cause any serious mucosal damage.

Discussion

Recently, several approaches for enhancing the oral absorption of sCT have been presented, including the use of formulation additives in the drug product to transiently modulate the intestinal environment or targeting specific intestinal regions that may have favorable peptide delivery properties (e.g., low residual volume, high absorptive surface area, or reduced enzymatic activity)[1].

Our data provided evidence that sCT has a better absorption in intestine compared with stomach (Table 1). Compared with intestine, stomach could secrete lots of pepsin and gastric acid. Most of sCTs were degraded when passing through stomach. sCT got deactivated before it was absorbed by stomach. The regional dependence of sCT absorption in intestine has not been extensively reported. A few articles focused on rectal and colonic administration have been published. There has been increasing interest in targeting peptide and protein drugs to the colon because of the relatively low activities of proteolytic enzymes in the colon[22]. But through sCT PK experiments in dogs, Lee reported that, compared with other intestine regions, the colonic BA of sCT was lower, probably related to the combined effects of poor membrane permeability and/or proteolytic degradation by microorganisms specifically residing in the colon[23]. As current and previous studies show, the accurate intestine absorption site of sCT is highly variable in animals or humans. Further efforts may be required.

Various kinds of absorption enhancers were reported to enhance the intestinal absorption of peptide drugs, including bile salts, dihydrofusidates, cyclodextrins, surfactants, and chelating agents[24,25]. We observed that the absorption-enhancing capability of four classic absorption enhancers were generally in the rank order of CA > SA > Polysorbate 80 > TAU (Figure 2). The absorption of sCT was significantly increased when co-administered with CA. As an organic acid, CA may temporarily modify intestinal pH. Lower pH may be useful for sCT stabilization. This stabilization is mostly determined by enzymes distributed on intestine. Among these enzymes, luminally secreted serine proteases seem to be mainly responsible for the digestion of sCT. Sakuma, for instance, demonstrated a high cleavage rate of CT caused by trypsin[26]. Furthermore, Dohi showed that the peptide is digested not only by trypsin but also by α-chymotrypsin[27]. Low pH may limit the activity of these intestinal enzymes, thus stabilizing sCT and improving the absorption of sCT[28,29].

Data from above-mentioned experiments suggest that the sCT enteric-coated capsule with CA in the sCT/CA mass ratio of 1:20 may be a good and simple delivery system of sCT. Our PK data validated the oral absorption enhancement of the new sCT delivery system. The PK parameters of i.v. group were at equal pace with previous reports[30]. Compared with the previous research[11], oral BA of this sCT delivery system had a significant elevation, nearly twofolds than that of sCT

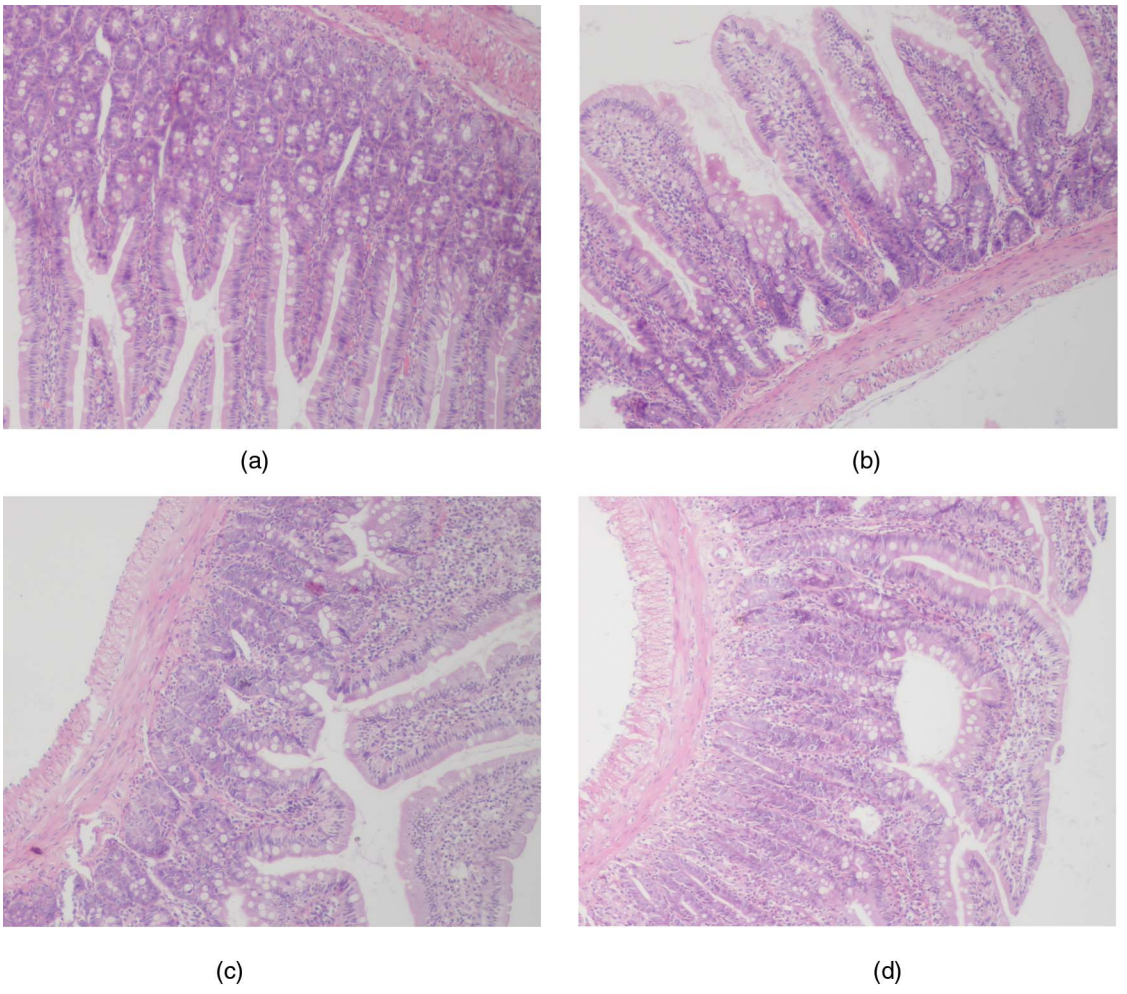

Figure 6. Electron micrographs (×100) of jejunum mucosal epithelium after long-term administration of sCT enteric-coated capsules (each contain CA 5 mg). (a) Control, (b) Day 1, (c) Day 4, and (d) Day 7.

administration alone. $AUC_{0-\infty}$ values of three dose groups appeared to be dose-dependent (Figure 5), indicating linear kinetics for sCT following oral administration over the dose range 0.125–0.5 mg. The PK results were consistent with previous PD study, in which the enhancement of intestinal absorption was observed, indicating that CA was an effective absorption enhancer for sCT. Filling a peptide drug into controlled release capsules with an absorption enhancer may be an effective strategy for enhancing the oral absorption of peptide drugs.

It was reported that certain absorption enhancers, including organic acids and surfactants, cause intestinal mucosal damage, although they showed higher intestinal absorption enhancement[31,32]. Oberle reported that TX-100 induced the intestinal damage, although it was rapidly repaired[33]. Our results showed that the use of CA induced no acute or chronic damage on the jejunum epithelium in the 7 days. CA was proved to be an effective and nonvenomous absorption enhancer, which may be applied extensively in drug delivery systems for polypeptide and others with poor absorption.

In conclusion, compared with stomach, sCT was better absorbed in intestine. CA is a better absorption enhancer than TAU, SA, and Polysorbate 80 for sCT enteric absorption. In sCT enteric-coated capsules, CA displayed significant enhancement in sCT absorption in PD and PK experiments, when the mass ratio of sCT to CA was 1:20. Histopathological evaluation demonstrated that CA caused no intestine mucosal damage. These results showed that enteric-coated capsules with a ration of CA and sCT may be an effective sCT oral delivery system.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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