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Synergistic absorption enhancement of salmon calcitonin and reversible mucosal injury by applying a mucolytic agent and a non-ionic surfactant

Shinya Takatsuka [a,*], Takahiro Morita [a], Atsushi Koguchi [b], Yuji Horikiri [a], Hiroshi Yamahara [a], Hiroyuki Yoshino [a]

[a] *Pharmaceutical Development Laboratories, Tanabe Seiyaku Co. Ltd., 3-16-89 Kashima, Yodogawa-ku, Osaka 532-8505, Japan*
[b] *Drug Safety Research Laboratories, Tanabe Seiyaku Co. Ltd., 3-16-89 Kashima, Yodogawa-ku, Osaka 532-8505, Japan*

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Abstract

The present study investigated the intestinal absorption enhancement of salmon calcitonin (SCT) and the intestinal mucosal damage when a mucolytic agent and a non-ionic surfactant were administered simultaneously to rats. *N*-acetylcysteine (NAC) and *p-t*-octyl phenol polyoxyethylene-9.5 (Triton X®-100, TX-100) were chosen as the model mucolytic agent and the non-ionic surfactant, respectively. Dosing solutions containing these agents were administered directly into the rat jejunum, and the bioavailability of SCT up to 2 h was determined. NAC and TX-100, when they were used alone at a dose of 1 mg/head, did not show the apparent enhancement compared to the control. However, simultaneous use of NAC and TX-100 enhanced the intestinal absorption of SCT in a synergistic manner, and absolute bioavailability increased 12.5-fold compared to the control. The effect of NAC and TX-100 on SCT absorption was not dependent on their doses over the range of 0.2–2 mg/head, and the maximum effect was obtained at a dose of 1 mg/head. Absorption enhancement of SCT by a combination of NAC and TX-100 was compared to those from the classical absorption enhancers. Absorption-enhancing ability of the combination of NAC and TX-100 was significantly higher than those of sodium deoxycholate, citrate, and the combination of citrate and taurocholate, and was comparable with that of the combination of citrate and taurodeoxycholate. Finally, the intestinal mucosal damage caused by the combination of NAC and TX-100 was assessed using a capsule device. Acute damage on intestinal mucosa was observed when they were exposed into rat intestine, but this morphological damage was found to be reversible. All these results suggest that simultaneous use of a mucolytic agent and a non-ionic surfactant would offer a potentiality for peroral delivery of peptide drugs like SCT.

Keywords: Peroral delivery; Salmon calcitonin; Mucolytic agent; Non-ionic surfactant; Simultaneous use

1. Introduction

Peroral delivery of peptide drugs can offer the greatest ease of medication for patients. However, the development of this delivery system is considered to be a great challenge due to their poor permeability in the intestinal epithelium and rapid metabolism by proteolytic enzymes. Several approaches such as absorption enhancers (Lee and Yamamoto, 1990; Aungst, 2000), protease inhibitors (Lee and Yamamoto, 1990; Bernkop-Schnürch, 1998), chemical modification (Asada et al., 1995; Wang et al., 2003) and dosage forms (Janes et al., 2001; Sakuma et al., 2001) have been explored in order to attain peroral delivery of peptide drugs. Of these approaches, the use of absorption enhancers has been often adapted to enhance the intestinal absorption of peptide drugs. Various kinds of absorption enhancers including surfactants, bile salts, chelating agents, and fatty acids have been found to enhance their intestinal absorption (Lee et al., 1991; Aungst, 2000). Furthermore, recent reports have demonstrated the effectiveness of new absorption enhancers including nitric oxide donor (Yamamoto et al., 2001; Takahashi et al., 2004) and chitosan (Thanou et al., 2001). Unfortunately, only limited success was achieved because these absorption enhancers could hardly possess both sufficient absorption-enhancing ability and lower intestinal mucosal toxicity.

We have recently demonstrated that the combination of a mucolytic agent and a non-ionic surfactant could provide the

* Corresponding author. Tel.: +81 6 6300 2778; fax: +81 6 6300 2582.
E-mail address: s-taka@tanabe.co.jp (S. Takatsuka).

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drastic enhancement of the intestinal absorption of a poorly absorbed hydrophilic compound like fluorescein isothiocyanate-labeled dextran with an average molecular weight of ca. 4.4 kDa (FD-4) (Takatsuka et al., 2006). In order to find a potentiality for practical use, the applicability of a combination of a mucolytic agent and a non-ionic surfactant to peptide drugs was examined. Salmon calcitonin (SCT) was selected to use as a peptide drug, which suffers from enzymatic degradation and low permeability in the intestinal mucosa and thus has to be delivered mostly by injection (Torres-Lugo and Peppas, 2000). Although the development of a peroral delivery system of SCT has been keenly pursued, very limited success was achieved. Therefore, in the present study we have firstly examined the intestinal absorption enhancement of SCT by the combination of a mucolytic agent and a non-ionic surfactant. We have secondarily compared its enhancing ability with those of several classical absorption enhancers in a single study, in order to rank their absorption-enhancing abilities directly. Potential intestinal mucosal damage caused by this combination should be clarified because the previous reports have shown the correlation between the absorption enhancement and the intestinal mucosal toxicity (Quan et al., 1998; Swenson et al., 1994b). Therefore, we have finally carried out the histopathological evaluation on the access site of the intestinal mucosa, in order to reveal the potential toxicity of the combination of a mucolytic agent and a non-ionic surfactant.

2. Materials and methods

2.1. Materials

Salmon calcitonin (SCT) was purchased from Bachem (Bubendorf, Switzerland). *N*-acetyl-L-cysteine (NAC) was from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). TritonX-100, sodium deoxycholate (DC), sodium taurocholate (TC), and sodium taurodeoxycholate (TDC) were purchased from Nacalai Tesque (Kyoto, Japan). Citric acid (CA) was purchased from Katayama Chemical (Osaka, Japan). Salmon calcitonin enzyme immunoassay kit was obtained from Peninsula Laboratories (Belmont, CA, USA). All other materials were of reagent grade.

2.2. Preparation of dosing solution

For intraintestinal administration study, SCT was dissolved in saline to a concentration of 25 mg/mL. Separately, a mucolytic agent and/or a penetration enhancer were dissolved in saline to a concentration of 10% (w/v). An aliquot of SCT solution was mixed with the same volume of enhancer solution, and kept cool until administration to rats. Thus, the dosing solution finally contained 12.5 mg/mL of SCT and 5% (w/v) of a mucolytic agent and/or a penetration enhancer. For intravenous (i.v.) administration study, SCT was dissolved in saline to a concentration of 0.5 mg/mL.

2.3. Intestinal absorption studies in rats

Animal experiments were carried out in accordance with the ethical guidelines established by the Animal Experimental Ethical Committee of Tanabe Seiyaku Co. Ltd. The intraintestinal administration experiment was performed according to the method described in a previous report (Michael et al., 2000) with minor modification. Male Wistar rats (Nippon SLC, Hamamatsu, Japan), weighing 190–240 g, were fasted for about 20 h and anesthetized by intraperitoneal injection of 50 mg/kg sodium pentobarbital (50 mg/mL in saline). For one group of rats, the jejunum was exteriorized through a midline abdominal incision. SCT dosing solution was instilled into the exposed rat jejunum (0.25 mg/20 μL/head) using a Hamilton micro-syringe. For another group of rats, a dose of 0.1 mg/0.2 mL/head SCT was intravenously administered into the jugular vein. Blood samples (200 μL) were taken from the jugular vein with heparinized syringes at predetermined time intervals. The plasma sample was collected after centrifugation at 12,000 rpm for 3 min.

2.4. Determination of plasma SCT concentration

Plasma concentrations of SCT in rats were assayed with salmon calcitonin enzyme immunoassay kit using 10 μL of rat plasma. The testing was performed according to the protocol shown in the kit.

2.5. Kinetic calculation

For i.v. administration, plasma concentration data was analyzed based on a conventional two-compartmental PK model using a computer program (WinNonlin®, Scientific Consulting). The PK parameters such as the plasma concentration versus time (0–∞) curve after administration ($AUC_{i.v.\infty}$) were calculated.

For intraintestinal administration, maximum plasma SCT concentration (C_{max}), and the time to reach C_{max} (T_{max}) were taken directly from the observed plasma SCT concentration versus time data. The area under the plasma SCT concentration versus time curve based on the period 0–2 h ($AUC_{0–2h}$) and the area under the first-moment curve based on the period 0–2 h ($AUMC_{0–2h}$) after administration were calculated according to the trapezoidal rule. The mean plasma residence time (MRT) was determined by dividing $AUMC_{0–2h}$ by $AUC_{0–2h}$. Absolute bioavailability of SCT based on the period 0–2 h ($BA_{0–2h}$) was calculated as follows:

$$BA_{0–2h}\,(\%) = \left(\frac{AUC_{0–2h}}{AUC_{i.v.\infty}}\right) \times \left(\frac{\text{Dose i.v.}}{\text{Dose intraintestinal}}\right) \times 100$$

2.6. Histopathological evaluation

Intestinal mucosal injury caused by combination of NAC and TX-100 was evaluated as follows. In order to evaluate the toxicity in the closed local area on the intestinal mucosa, we developed an administration capsule device. This device was prepared using a gelatin capsule, with a surface coated with a water-insoluble polymer to give a shape like a hat. The bottom of the brim of the capsule was coated with an adhesive polymer for adhesion with the intestinal mucosa. Finally, the top of the capsule was pierced using an 18-G needle for applying the solution containing NAC and TX-100.

Male Wistar rats (Nippon SLC, Hamamatsu, Japan), weighing 200–240 g, were fasted for about 20 h and anesthetized by intraperitoneal injection of 50 mg/kg sodium pentobarbital (50 mg/mL in saline). The jejunum was exteriorized through a midline abdominal incision. The exposed jejunum was incised, and two administration devices were adhered to the mucosal surface. One hundred microlitres of solution containing 5 mg of NAC and 5 mg of TX-100 was injected into one administration device through the small hole. Also, saline solution was injected into the other administration device as the control.

At 20 min, 1 h, 2 h, 4 h, or 6 h after administration, collected jejunum segments exposed to the solutions were fixed with 10% formaldehyde in 0.1 M phosphate buffer (pH 7.4). Subsequently, hematoxylin-eosin-stained cross-sections were prepared by routine histological processing, and they were examined by light microscopy.

2.7. Statistical analysis

Statistical analysis was performed with Dunnett's test for multiple comparisons. *P*-value of 0.05 was used as the significant level for all tests. All data are presented as the mean ± standard deviation (S.D.) unless otherwise noted.

3. Results and discussion

3.1. Effect of combination of a mucolytic agent and a non-ionic surfactant on intestinal SCT absorption

Enhancement of intestinal SCT absorption was assessed when a mucolytic agent and a non-ionic surfactant were simultaneously applied. *N*-acetyl-L-cysteine (NAC) was selected as the mucolytic agent, and *p-t*-octyl phenol polyoxyethylene-9.5 (Triton X®-100, TX-100) was the non-ionic surfactant. The intestinal absorption enhancement of SCT was evaluated by rat study, in which 0.25 mg/head of SCT and 1 mg/head of NAC and/or 1 mg/head of TX-100 were co-administered intraintestinally to rats. SCT solution alone was administered as the control. The plasma concentration profiles of SCT after the administration are compared in Fig. 1. Pharmacokinetic parameters are listed in Table 1.

The SCT solution alone (control) showed a low absorbability, and $AUC_{0–2h}$ was 95.1 ± 17.5 ng min/mL. An increase of plasma SCT concentration was observed when SCT was co-administered with NAC or TX-100, and the $AUC_{0–2h}$s were 263.6 ± 58.9 ng min/mL (2.8-fold higher than the control) and 177.5 ± 38.1 ng min/mL (1.9-fold), respectively. The statistic calculation provided a significant difference between $AUC_{0–2h}$ obtained from SCT alone and that from NAC treatment ($P < 0.05$). However, no significant difference was observed between $AUC_{0–2h}$ obtained from SCT alone and that from TX-100 treatment. These experimental results confirmed that NAC was a potential absorption enhancer for peptide drugs in an in vivo situation. According to a previous finding, the diffusion rates of smaller peptides (M_W 3.4 and 6.5 kDa) into the native mucus gel of porcine intestine were increased up to two- to three-fold after NAC treatment (Bernkop-Schnürch and Fragner, 1996). Therefore, the observed enhancing effect, which was not so drastic under the applied experimental condition, was thought to be due to the improved diffusivity of SCT in the mucus layer.

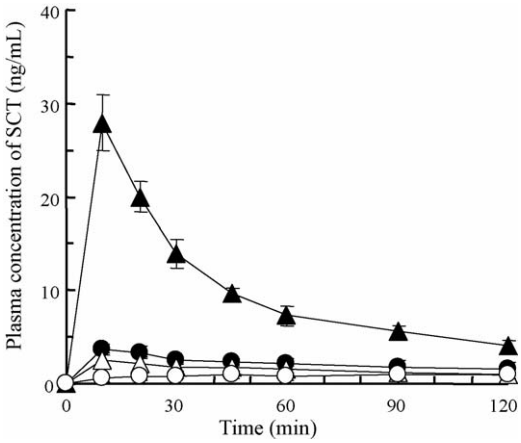

Fig. 1. Plasma concentration profiles of SCT after intraintestinal co-administration with a mucolytic agent and/or a non-ionic surfactant. A mucolytic agent (NAC) and a non-ionic surfactant (TX-100) were given at 1 mg/head. Each point presents the mean ± S.D. ($n = 3$). Control (◯), NAC (●), TX-100 (△), and NAC + TX-100 (▲).

TX-100 is known as a strong non-ionic surfactant and has been used as a typical absorption enhancer. It is interesting that TX-100 alone did not show any apparent enhancement. At the concentration employed in the present study, most of TX-100 molecular is thought to exist as micelle form. When TX-100 was used alone, TX-100 micelles hardly access the intestinal epithelial membrane due to the high viscosity of mucous layer. This would be a possible reason why TX-100 alone did not show sufficient enhancing effect. This consideration would be supported by some previous reports, which evaluate the absorp-

Table 1
Pharmacokinetic parameters of SCT after intraintestinal co-administration with a mucolytic agent and/or a non-ionic surfactant

Agent	C_{max} (ng/mL)	T_{max} (min)	$MRT_{0–2h}$ (min)	$AUC_{0–2h}$ (ng min/mL)	$BA_{0–2h}$[a] (%)	Enhancing ratio[b]
None (control)	1.0 ± 0.1	75.0 ± 39.7	67.0 ± 2.7	95.1 ± 17.5	0.12 ± 0.02	–
NAC	3.8 ± 0.6	13.3 ± 5.8	53.4 ± 5.9	263.6 ± 58.9*	0.35 ± 0.08	2.8
TX-100	2.5 ± 0.7	10	52.7 ± 2.9	177.5 ± 38.1	0.23 ± 0.05	1.9
NAC + TX-100	27.9 ± 2.9	10	42.6 ± 1.0	1189.7 ± 63.0*	1.56 ± 0.08	12.5

Each value presents the mean ± S.D. ($n = 3$).
[a] $B_{0–2h}$ was determined using AUC after i.v. administration at 0.1 mg/head ($AUC_{i.v.\infty}$) of 30437.2 ± 3147.3 ng min/mL.
[b] Enhancing ratio was determined as the $AUC_{0–2h}$ increase relative to the control.
* Significantly different from the control ($P < 0.05$).

tion enhancement of non-ionic surfactants using Caco-2 cells. In the case of Caco-2 cells, non-ionic surfactants freely access the epithelia cells because of the lack of the mucus layer and can exert the enhancing ability, even though they are used alone.

A remarkable increase of plasma SCT concentration was observed when SCT was co-administered with NAC and TX-100. The $AUC_{0–2h}$ was 1189.7 ± 63.0 ng min/mL, which was 12.5-fold higher than the control. This enhancing ratio was much higher than expected from the results of NAC alone (2.8-fold) and TX-100 alone (1.9-fold), indicating that simultaneous use of NAC and TX-100 enhanced the intestinal SCT absorption in a synergistic manner.

This result was consistent with our previous study, in which the synergistic enhancement of intestinal FD-4 absorption was observed. Mechanistic consideration for the synergistic effect was described in our previous report (Takatsuka et al., 2006). Briefly, when TX-100 is administered together with NAC, the mucolytic activity of NAC reduces the mucus viscosity to facilitate the movement of TX-100 micelles onto epithelial membrane. Furthermore, with reducing viscosity, the accessibility of SCT to the mucosal membrane also increased. The increased accessibilities of SCT and TX-100 at the same time could synergistically enhance the intestinal SCT absorption. This would be a possible reason why the combination of NAC and TX-100 provided the synergistic enhancement.

The present experimental result demonstrates that the mucus layer barrier and the penetration barrier encountered with the intestine largely contribute to the intestinal absorption of peptide drugs. Also, it shows that simultaneous use of a mucolytic agent and a non-ionic surfactant would be an effective strategy for enhancing the intestinal absorption of peptide drugs.

3.2. Effect of doses of NAC and TX-100 on intestinal SCT absorption

Synergistic absorption enhancement of SCT was obtained when NAC and TX-100 were simultaneously applied at the prefixed amounts, in which 0.25 mg/head of SCT and 1 mg/head of NAC and TX-100 were co-administered intraintestinally to rats. In order to clarify the enhancing potency of the simultaneous use of NAC and TX-100, the effect of doses of these agents on intestinal SCT absorption was investigated. In this study, NAC and TX-100 were applied at doses ranging from 0.2 to 2 mg/head to rats. The plasma concentration profiles of SCT after the administration are compared in Fig. 2. Pharmacokinetic parameters are listed in Table 2.

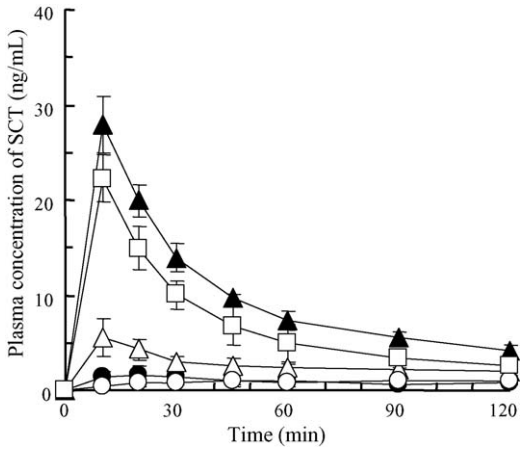

Fig. 2. Plasma concentration profiles of SCT after intraintestinal co-administration with the combination of NAC and TX-100 at various doses. Each point represents the mean ± S.D. (n = 3–4). Control (◯), 0.2 mg/head (●), 0.4 mg/head (△), 1 mg/head (▲), and 2 mg/head (□).

When NAC and TX-100 were administered at 0.2 mg/head, a very low absorbability was observed, and $AUC_{0–2h}$ was 118.7 ± 45.3 ng min/mL. A statistic calculation indicated that there was no significant difference between the control and 0.2 mg/head group. At doses of ranging from 0.4 to 2 mg/head, a clear increase of plasma SCT concentration was observed. AUC_{0-2h}s obtained at doses of 0.4, 1 and 2 mg/head were 325.5 ± 79.1 ng min/mL, 1189.7 ± 63.0 ng min/mL, and 845.3 ± 162.0 ng min/mL, respectively. These values were significantly higher than that from the control, suggesting that the minimum dose for exerting its enhancing ability would be 0.4 mg/head. Interestingly, intestinal SCT absorption at 2 mg/head was lower than that at 1 mg/head. These results showed that the enhancing effect of NAC and TX-100 was not dose-dependent over the range of 0.2–2 mg/head, and the maximum effect was obtained at a dose of 1 mg/head. As mentioned, the interesting phenomenon was observed that intestinal SCT absorption at 2 mg/head was lower than that at 1 mg/head. Possible reasons for this observed phenomenon may include the influence of NAC on the physiological condition of the mucus layer. According to a previous report, NAC can stimulate mucin release by the airway directly acting on goblet cells at a higher concentration (Lee et al., 2004). Another report showed that

Table 2
Pharmacokinetic parameters of SCT after intraintestinal co-administration with a mucolytic agent and a non-ionic surfactant at various doses

Dose	C_{max} (ng/mL)	T_{max} (min)	$MRT_{0–2h}$ (min)	$AUC_{0–2h}$ (ng min/mL)	$BA_{0–2h}$[a] (%)	Enhancing ratio[b]
None (control)	1.0 ± 0.1	75.0 ± 39.7	67.0 ± 2.7	95.1 ± 17.5	0.12 ± 0.02	–
0.2 mg	1.8 ± 0.9	13.3 ± 5.8	54.1 ± 7.2	118.7 ± 45.3	0.13 ± 0.05	1.2
0.4 mg	5.5 ± 1.9	10	52.7 ± 2.9	325.5 ± 79.1*	0.36 ± 0.09	3.4
1 mg	27.9 ± 2.9	10	42.6 ± 1.0	1189.7 ± 63.0*	1.56 ± 0.08	12.5
2 mg	22.2 ± 2.5	10	22.9 ± 12.4	845.3 ± 162.0*	0.94 ± 0.18	8.9

Each value presents the mean ± S.D. (n = 3).
[a] $BA_{0–2h}$ was determined using AUC after i.v. administration at 0.1 mg/head ($AUC_{i.v.\infty}$) of 30437.2 ± 3147.3 ng min/mL.
[b] Enhancing ratio was determined as the $AUC_{0–2h}$ increase relative to the control.
* Significantly different from the control ($P < 0.05$).

the co-administration of NAC and colchicine, antimicrotubular agent suppressing the mucus production in goblet cells, provided the higher intestinal absorption of FITC dextran 70,000 compared to the NAC treatment alone (Iiboshi et al., 1996). Based on these previous findings, the use of NAC at 2 mg/head may stimulate the secretion of mucin from goblet cells, thereby decreasing the absorption enhancement of SCT compared to 1 mg/head.

3.3. Comparison of enhancing ability of simultaneous use of NAC and TX-100 with classical absorption enhancers

Various kinds of absorption enhancers were reported to enhance the intestinal absorption of peptide drugs. Of these enhancers, a few bile salts and an organic acid were selected for use because they enhanced the intestinal absorption of FITC-dextran (FD-4) in our previous study, in which the same experimental method was employed (Takatsuka et al., 2006). Besides these enhancers, the combination of an organic acid and a bile salt was chosen because it was known to enhance the intestinal absorption of SCT effectively (Sinko et al., 1999). In the present study, the absorption-enhancing ability of the combination of NAC and TX-100 (NAC + TX-100) was compared with those from these absorption enhancers in order to rank the absorption-enhancing ability directly. The plasma concentration profiles of SCT after the administration are compared in Fig. 3. Pharmacokinetic parameters are listed in Table 3.

Sodium deoxycholate (DC) did not show the absorption-enhancing effect under the experimental condition employed in this study (bioavailability was 0.18 ± 0.02%). However, citric acid (CA) provided the distinct enhancement of SCT absorption (bioavailability was 1.05 ± 0.22%). The combination of CA and taurocholate (CA + TC) also provided the clear enhancement (bioavailability was 1.05 ± 0.23%), but their enhancements were significantly lower than that of NAC + TX-100. The combination of CA and taurodeoxycholate (CA + TDC) showed the remarkable enhancement of SCT absorption, with a bioavailability (1.68 ± 0.09%) comparable to that from NAC + TX-100. These experimental results revealed that the absorption-enhancing ability of NAC + TX-100 was comparable with or higher than those of classical absorption enhancers.

It is curious that DC was not effective in our present study. In our preliminary study, employed in the same experimental method, DC could enhance the intestinal absorption of FD-4. FD-4 is known to be stable in the intestine, while SCT is reported to be susceptible to the enzyme located in the intestine. Also, the formulations containing citric acid (CA), which might improve the stability of SCT in the intestine, showed the significant enhancement. Therefore, no enhancement of SCT absorption by DC would be due to the low stability of SCT in the intestine.

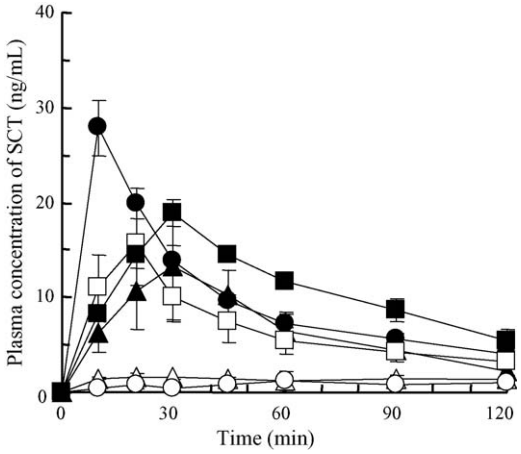

Fig. 3. Plasma concentration profiles of SCT after intraintestinal co-administration with various classical absorption enhancers. Enhancers except for CA were given at 1 mg/head, and CA was at 3 mg/head. Each point represents the mean ± S.D. (n = 3–4). Control (○), NAC + TX-100 (●), DC (△), CA (▲), CA + TC (□), and CA + TDC (■).

Although bioavalabilities of SCT were comparable between NAC + TX-100 and CA + TDC, the profiles of plasma SCT concentration were clearly different. SCT was rapidly absorbed when NAC + TX-100 was applied, but was slowly absorbed when CA + TDC was used. Such a slower intestinal absorption of SCT was also observed when CA and CA + TC were applied. SCT is known to suffer from enzymatic degradation in the intestine, indicating that the stability of SCT is essential for enhancing its absorption. Lee et al. (1999) proposed that the use of CA might stabilize SCT in the intestine by reducing local pH, thereby enhancing intestinal SCT absorption.

The plasma concentration profile of SCT after co-administration with NAC + TX-100 is compared with that of FD-4, which was reported in our previous report (Takatsuka et al., 2006). FD-4, known as the compound with no enzymatic degradation in the body, showed a little longer absorption than SCT, although the molecular weights of both compounds are

Table 3
Pharmacokinetic parameters of SCT after intraintestinal co-administration with various several classical enhancers

Agent	C_{max} (ng/mL)	T_{max} (min)	$MRT_{0–2h}$ (min)	$AUC_{0–2h}$ (ng min/mL)	$BA_{0–2h}$[a] (%)
None (control)	1.0 ± 0.1	75.0 ± 39.7	67.0 ± 2.7	95.1 ± 17.5	0.12 ± 0.02
NAC + TX-100	27.9 ± 2.9	10	42.6 ± 1.0	1189.7 ± 63.0	1.56 ± 0.08
DC	1.9 ± 0.3	43.3 ± 40.4	60.9 ± 4.4	161.1 ± 19.0*	0.18 ± 0.02
CA	13.2 ± 5.7	30	49.5 ± 4.5	798.4 ± 163.6*	1.05 ± 0.22
CA + TC	15.6 ± 4.3	20	46.9 ± 2.2	801.6 ± 175.5*	1.05 ± 0.23
CA + TDC	18.9 ± 1.9	30	54.0 ± 1.4	1282.0 ± 65.5	1.68 ± 0.09

Each value presents the mean ± S.D. (n = 3).
[a] $BA_{0–2h}$ was determined using AUC after i.v. administration at 0.1 mg/head ($AUC_{i.v.\infty}$) of 30437.2 ± 3147.3 ng min/mL.
* Significantly different from NAC + TX-100 ($P < 0.05$). Control was shown as a reference and not used for statistic calculation.

similar. The slower absorption of SCT may be largely due to the low stability of SCT in the intestine. Another possible reason, although less likely, would be the different absorption rate of these compounds.

3.4. Intestinal mucosal injury caused by combination of NAC and TX-100

Certain absorption enhancers including surfactants were reported to cause the intestinal mucosal damage, although they showed the higher intestinal absorption enhancement (Swenson and Curatolo, 1992; Yamamoto et al., 1996; Sakai et al., 1998). Therefore, in the present study, the extent of intestinal mucosal damage caused by combination of NAC and TX-100 (NAC + TX-100) was evaluated. Assessment of intestinal mucosal damage caused by NAC + TX-100 was done using the adhesive administration device, which offers the advantage that the site exposed to enhancers and one exposed to saline (without an enhancer) can be evaluated concurrently in a single animal. Histopathological evaluation was carried out on the segments of the small intestine exposed to NAC + TX-100 solution for 20 min, 1 h, 2 h, 4 h, or 6 h. Fig. 4 represents the representative histological images of rat intestinal mucosal epithelium.

Desquamated epithelial cells were observed in all exposure times, but no severe inflammation occurred. Furthermore, the degrees of mucosal damage were not different among the exposure times, suggesting that the exposure time to NAC + TX-100 does not affect the extent of mucosal damage. At 20 min exposure, the recovery process had already started, and the recovery of damaged epithelium was completed at 1 h after treatment. Therefore, these results indicate that the intestinal mucosal damage caused by NAC + TX-100 was reversible. After the rapid repair of the intestinal mucosa, the top of the villi was lost, and the villus height became shortened. Intestinal mucosa exposed to saline solution (the control) showed no abnormality in all exposure times. These reversible damages observed in the present study were consistent with the previous study, in which the intestinal damage by a non-ionic surfactant and bile salts was examined (Swenson et al., 1994a). In addition, Oberle et al. (1995) reported that the TX-100 induced the intestinal damage and that it was rapidly repaired, These previous findings suggest that the intestinal mucosal damage caused by NAC + TX-100 probably arises from TX-100 alone, and that its damage was not as severe as those induced by conventional absorption enhancers. The present experimental results indicate that the combination of NAC and TX-100 induces acute damage on the intestinal epithelium, but this damage recovers quickly, within 1 h.

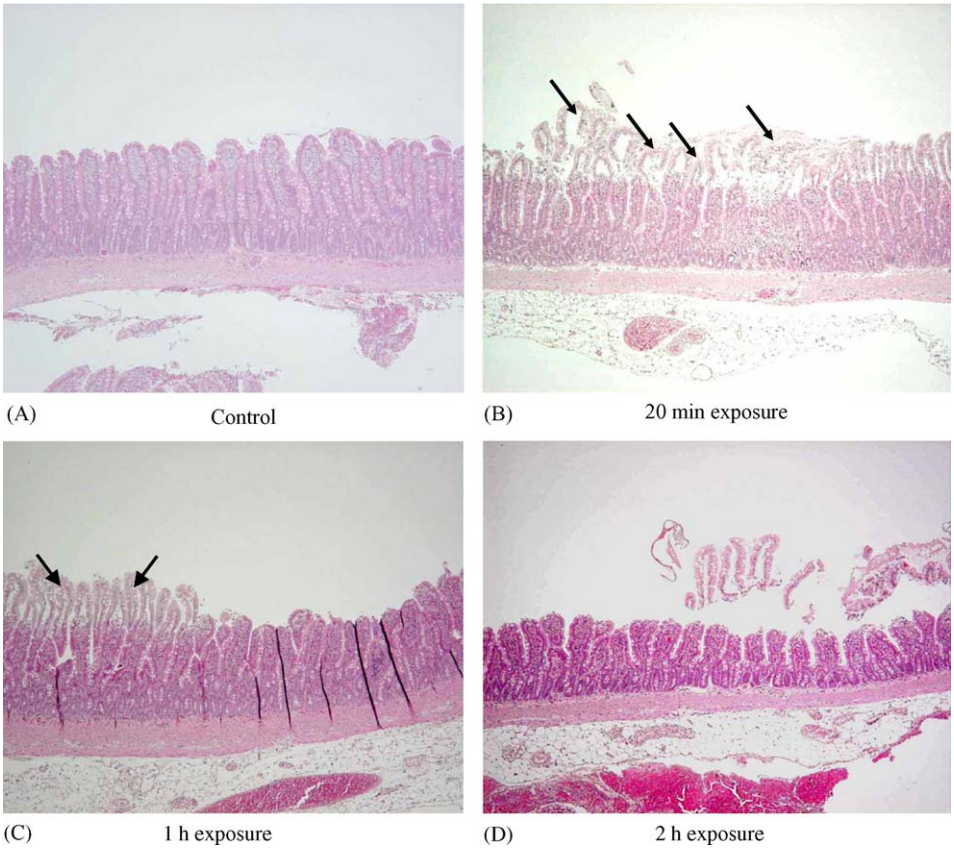

Fig. 4. Light micrographs of jejunum mucosal epithelium exposed to NAC + TX-100 at a dose of 5 mg/site. (A) Control, (B) 20 min exposure, (C) 1 h exposure, and (D) 2 h exposure. (A) Normal villi are seen. (B) Necrotic and desquamated villi are seen (arrows). Recovery of villus surfaces has started (C) Necrotic and desquamated villi are seen (arrows). The right half of the villus surface has already recovered, except for the shortening of the villus height. (D) Recovery of the villus surfaces is complete, except for the shortening of the villus height.

4. Conclusions

The present study has investigated the applicability of simultaneous use of a mucolytic agent and a non-ionic surfactant to peroral delivery of salmon calcitonin (SCT). Synergistic enhancement of intestinal SCT absorption was observed when NAC was co-administered with TX-100. The enhancing effect by a combination of NAC and TX-100 was not dependent on their doses over the range of 0.2–2 mg/head, and the maximum effect was obtained at a dose of 1 mg/head. The absorption-enhancing ability of the simultaneous use of NAC and TX-100 was comparable with or higher than those of classical absorption enhancers. Combination of NAC and TX-100 caused the acute intestinal mucosal damage, but this damage was reversible. All these results suggest that simultaneous use of a mucolytic agent and a non-ionic surfactant would offer a potentiality for peroral delivery of peptide drugs like SCT, although further investigations should be necessary to find the optimal combination with higher enhancement and lower toxicity.

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