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Absorption enhancement of poorly absorbed hydrophilic compounds from various mucosal sites by combination of mucolytic agent and non-ionic surfactant

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Abstract

Absorption enhancement of poorly absorbed hydrophilic compounds from various mucosal sites by co-administration of a mucolytic agent and a non-ionic surfactant was examined in rats. Fluorescein isothiocyanate-labeled dextran with average molecular weight of ca. 4.4 kDa (FD-4), and salmon calcitonin (SCT) were used as model compounds. *N*-acetylcysteine (NAC) and *p-t*-octyl phenol polyoxyethylene-9.5 (Triton X®-100, TX-100) were selected as a mucolytic agent and a non-ionic surfactant, respectively. Dosing solutions containing these agents were administered into various mucosal sites including the nose, the lung and the large intestine, and the bioavailabilities were determined. The combination of 5% NAC and 5% TX-100 significantly enhanced the nasal, the pulmonary and the large intestinal absorption of FD-4 compared to the control, and the enhancement ratios relative to the control were 7.2-, 2.8- and 4.5-fold, respectively. The different enhancement ratio among the administration sites explored indicates that the absorption enhancing effect of the combination of NAC and TX-100 is site-dependent. This combination also improved the nasal and the pulmonary absorption of SCT, and the enhancement ratios relative to the control were 6.1- and 8.1-fold, respectively. All these results suggest that the combination strategy of a mucolytic agent and a non-ionic surfactant may be widely applicable to various mucosal deliveries of poorly absorbed hydrophilic compounds.

Keywords: Mucolytic agent; Non-ionic surfactant; Combination; Mucosal; Poorly absorbed hydrophilic compounds

1. Introduction

Recently, numerous protein and peptide drugs have been used as therapeutic agents for the clinical treatment of various chronic diseases (Struck, 1994; Walsh, 2003). Due to their susceptibility to enzymatic degradation and low permeability across the epithelium via the paracellular pathway, however, absorption of these drugs from mucosal sites is generally poor. Therefore, the administration of these drugs is mostly limited to invasive injections, which can be painful and inconvenient. Significant efforts have been directed to explore the development of alternative delivery routes such as oral (Mahato et al., 2003; Hamman et al., 2005), pulmonary (Agu et al., 2001), nasal (Illum, 2003), colonic (Haupt and Rubinstein, 2002).

For the successful development of these alternatives, the poor absorption of these drugs from the mucosal sites should be one of the most important problems to be solved. Recently, we have proved that the combination of a mucolytic agent and a non-ionic surfactant enhanced the small intestinal absorption of poorly absorbed hydrophilic compounds in a synergistic manner, which was demonstrated using model compounds like fluorescein isothiocyanate-labeled dextran with an average molecular weight of ca. 4.4 kDa (FD-4) (Takatsuka et al., 2006a) and salmon calcitonin (SCT) (Takatsuka et al., 2006b). As a speculative mechanism, a mucolytic agent reduces the mucus viscosity, which enables the target drug as well as the surfactant molecules to diffuse more efficiently onto the epithelial membrane. This enhanced accessibility of both the target drug and the surfactant molecules leads to the synergistic absorption enhancement. In other mucosal sites such as the nose, the lung and the large intestine, the mucus layer is known to exist and may act as a diffusion barrier (Edwards, 1997; Agu et al., 2001; Ugwoke et al.,

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2005). In addition, the epithelium itself would act as a penetration barrier. Considering the physiological barriers mentioned above, the strategy of the simultaneous perturbation of those barriers might be widely applicable to various mucosal deliveries of poorly absorbed hydrophilic compounds. In other words, the combination of a mucolytic agent and a non-ionic surfactant may possess a potential as an effective strategy for enhancing the mucosal absorption.

In this study, therefore, the effects of the combination of a mucolytic agent and a non-ionic surfactant on the nasal, pulmonary and large intestinal absorption of FD-4 were examined in rats. Also, the applicability of this absorption enhancing strategy to the nasal and pulmonary absorption of SCT was investigated.

2. Materials and methods

2.1. Materials

Fluorescein isothiocyanate-labeled dextran (MW, ca. 4.4 kDa, FD-4) and *N*-acetylcysteine (NAC) were purchased from Sigma–Aldrich Chemical Co. (MO, USA). *p-t*-Octyl phenol polyoxyethylene-9.5 (Triton® X-100, TX-100) was purchased from Nacalai Tesque (Kyoto, Japan). Salmon calcitonin (SCT) was purchased from Bachem (Bubendorf, Switzerland). SCT enzyme immunoassay kit was obtained from Peninsula Laboratories (CA, USA). Ethylcellulose was purchased from Dow Chemical Company (MI, USA). Carbopol 974P was obtained as a gift from BFGoodrich (OH, USA). All other materials were of reagent grade.

2.2. Preparation of dosing solution

The dosing solution was prepared as follows. FD-4 or SCT was dissolved in saline to a concentration of 1 g/mL and 40 mg/mL, respectively. Separately, NAC and TX-100 together, or either of them were dissolved in saline to a concentration of 10% (w/v), i.e. enhancer solution. An aliquot of drug solution was mixed with the same volume of enhancer solution, and kept cool until administration to rats. Thus, in case of FD-4, dosing solution finally contained 500 mg/mL of FD-4 and 5% (w/v) of NAC and/or TX-100. Also, in case of SCT, dosing solution finally contained 20 mg/mL of SCT and 5% (w/v) of NAC and/or TX-100. The pH of both dosing solution was 2.1.

2.3. Animal experiments

All animal experiments were carried out in accordance with the ethical guidelines established by the Animal Experimental Ethical Committee of Tanabe Seiyaku Co. Ltd. Male Wistar rats (Nippon SLC, Hamamatsu, Japan), weighing 170–240 g, were fasted for about 20 h and anesthetized by intraperitoneal injection of 50 mg/kg sodium pentobarbital (50 mg/mL in saline).

Nasal administration was performed according to Hirai's method (Hirai et al., 1981) with minor modification. Briefly, the rats were tracheotomized and the outlets of the nasal cavity were sealed by another blind-ended cannula placed in the esophagus to prevent drainage of the drug into the esophagus or trachea and thus avoid overestimation of drug absorption. Five microliters of dosing solution with or without enhancers (5% NAC, 5% TX-100) was administered (FD-4; 2.5 mg/head, SCT; 0.1 mg/head) into the nasal cavity with a micropipette.

Pulmonary administration was performed according to the method of Enna and Schanker (Enna and Schanker, 1972). Briefly, the trachea was exposed through a ventral middle incision in the neck. A 2.5cm length of polyethylene tubing (i.d., 2.5 mm; o.d., 2.3 mm), which served as a tracheal cannula, was inserted through an incision between the fourth and fifth tracheal rings caudal to the thyroid cartilage to a depth of 0.6 cm. Five microliters of dosing solution with or without enhancers (5% NAC, 5% TX-100) was administered intratracheally (FD-4; 2.5 mg/head, SCT; 0.1 mg/head) through a tube inserted in the trachea by a Hamilton micro-syringe.

Large intestinal administration was performed using a reservoir made with a gelatin capsule. The reservoir was prepared using a part of gelatin capsule. A body part of capsule was coated with ethylcellulose, and the bottom of the brim of the capsule was covered with Carbopol 974P for adhesion with mucosa. Finally, the top of the capsule was pierced using an 18-G needle for applying the dosing solution. The large intestine was exteriorized through a midline abdominal incision. The exposed large intestine was incised, and the empty reservoir was adhered to the mucosal surface. Five microliters of dosing solution with or without enhancers (5% NAC, 5% TX-100) was instilled (FD-4; 2.5 mg/head) into the capsule reservoir through the small hole.

On each animal experiment, blood samples (200 μL) were taken directly from jugular vein with heparinized syringes at predetermined time intervals. For nasal administration, blood samples were at 5, 10, 20, 30, 45, 60, 90 and 120 min. Also, the blank blood sample was taken prior to the administration of dosing solution. For pulmonary administration, blood samples were taken at 1, 5, 10, 20, 30, 45, 60, 90 and 120 min. For large intestinal administration, blood samples were taken at 10, 20, 30, 45, 60, 90 and 120 min. In these experiments, the blank blood sample was also taken prior to the administration of dosing solution. The plasma sample was collected after centrifugation at 12,000 rpm for 3 min.

2.4. Determination of plasma concentration

For the determination of FD-4, the plasma samples (20 μL) were diluted with 680 μL of 0.1 M sodium hydrogen carbonate solution. FD-4 concentrations in plasma were determined by a spectrofluorometer (HITACHI model F-4010, Tokyo, Japan) at excitation wavelength of 495 nm and emission wavelength of 512 nm. For the determination of SCT, plasma concentrations of SCT were assayed with an enzyme immunoassay kit using 10 μL of rat plasma. The testing was performed according to the protocol shown in the kit (the limit of qualification: 0.32 ng/mL, the limit of detection: 0.16 ng/mL).

2.5. Kinetic calculation

The maximum plasma concentration (C_{max}), and the time to reach C_{max} (T_{max}) were taken directly from the observed plasma

concentration versus time data. The area under the plasma concentration versus time curve based on the period 0–2 h ($AUC_{0–2\,h}$) was calculated according to the trapezoidal rule. Absolute bioavailability based on the period 0–2 h ($BA_{0–2\,h}$) was calculated as follows:

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

The area under the plasma concentration versus time (0–∞) curve after i.v. administration ($AUC_{i.v.\infty}$) was cited from our previous report. $AUC_{i.v.\infty}$ s at 50 mg/kg of FD-4 (Takatsuka et al., 2006a) and at 0.1 mg/head of SCT (Takatsuka et al., 2006b) were 6087.4 ± 911.8 μg min/mL and 30437.2 ± 3147.3 ng min/mL, respectively.

2.6. Statistical analysis

Statistical analysis of $AUC_{0–2\,h}$ was performed with the Student's *t*-test or 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

3.1. Nasal absorption study with FD-4 and SCT

Fig. 1(A) shows the plasma concentration–time profile of FD-4 after nasal administration with or without 5% NAC and/or 5% TX-100. Also, Table 1 summarizes the pharmacokinetic parameters of FD-4 after administration. When FD-4 was administered without enhancer, a slow absorption was observed (T_{max}: 105.0 min). The $AUC_{0–2\,h}$ was 126.8 μg min/mL, and the absolute bioavailability was 8.3%. The administration of FD-4 with NAC slightly enhanced the absorption of FD-4 (1.6-fold). When FD-4 was administered with TX-100, a rapid absorption and a clear improvement of absorption were observed. The $AUC_{0–2\,h}$ was increased to 390.9 μg min/mL, which was significantly higher than that of FD-4 alone (3.1-fold). The administration of FD-4 in combination with NAC and TX-100 provided a remarkable and a synergistic absorption enhancement, and the absolute bioavailability was 7.2-fold significantly higher than that of FD-4 alone.

Fig. 1(B) shows the plasma concentration–time profile of SCT after nasal administration with or without 5% NAC and/or 5% TX-100. Also, Table 2 summarizes the pharmacokinetic parameters of SCT after administration. When SCT was administered alone, the $AUC_{0–2\,h}$ was 2591.5 ng min/mL, and the absolute bioavailability was 8.5%. The administration of SCT with NAC slightly but significantly enhanced the absorption (2.1-fold). When SCT was administered with TX-100, a significant absorption enhancement was also observed (3.7-fold). The administration of SCT in combination with NAC and TX-100 provided a remarkable and an additive absorption enhancement, and the absolute bioavailability was 6.1-fold higher than that of SCT alone.

3.2. Pulmonary absorption study with FD-4 and SCT

Fig. 2(A) shows the plasma concentration–time profile of FD-4 after pulmonary administration with or without 5% NAC

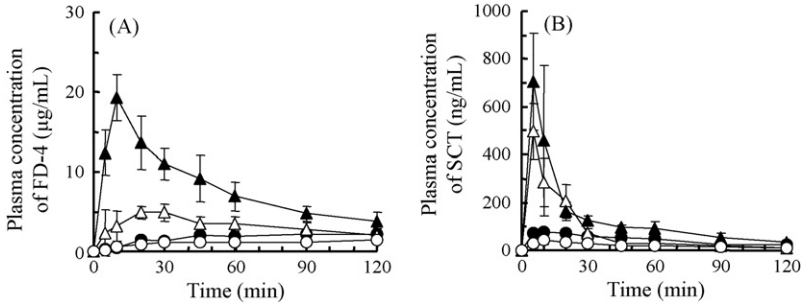

Fig. 1. Plasma concentration profiles of (A) FD-4 and (B) SCT after nasal co-administration with NAC and/or TX-100. NAC and TX-100 were given at 5% (w/v). Each point presents the mean ± S.D. (n = 3–5). Control (◯), NAC (●), TX-100 (△); NAC + TX-100 (▲).

Table 1
Pharmacokinetic parameters of FD-4 after nasal co-administration with 5% NAC and/or 5% TX-100

Agent	C_{max} (μg/mL)	T_{max} (min)	$AUC_{0–2\,h}$ (μg min/mL)	$BA_{0–2\,h}$[a] (%)	Enhancement ratio[b]
None (control)	1.5 ± 0.1	105.0 ± 30.0	126.8 ± 7.4	8.3 ± 0.5	–
NAC	2.2 ± 0.6	85.0 ± 37.7	205.4 ± 45.7	13.5 ± 3.0	1.6
TX-100	5.0 ± 0.9	26.7 ± 5.8	390.9 ± 115.7*	25.7 ± 7.6	3.1
NAC + TX-100	17.9 ± 183	14.0 ± 8.9	917.9 ± 183.1*	60.3 ± 12.0	7.2

Each value presents the mean ± S.D. (n = 3–5).
[a] $BA_{0–2\,h}$ was determined using AUC after i.v. administration at 50 mg/kg ($AUC_{i.v.\infty}$) of 6087.4 μg min/mL.
[b] Enhancement ratio was determined as the $AUC_{0–2\,h}$ increase relative to the control.
* Significantly different from the control ($P < 0.05$).

Table 2
Pharmacokinetic parameters of SCT after nasal co-administration with 5% NAC and/or 5% TX-100

Agent	C_{max} (ng/mL)	T_{max} (min)	$AUC_{0-2\,h}$ (ng min/mL)	$BA_{0-2\,h}$[a] (%)	Enhancement ratio[b]
None (control)	48.9 ± 14.9	1.5 ± 417.8	2591.5 ± 417.8	8.5 ± 1.4	–
NAC	90.3 ± 3.1	20.0 ± 10.0	5452.3 ± 915.4*	17.9 ± 3.0	2.1
TX-100	498.9 ± 116.7	5	9527.5 ± 1278.1*	31.3 ± 4.2	3.7
NAC + TX-100	706.3 ± 203.4	5	15714.2 ± 2395.8*	51.6 ± 7.9	6.1

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

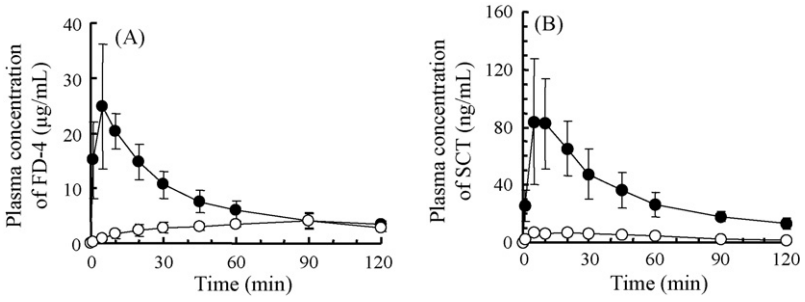

Fig. 2. Plasma concentration profiles of (A) FD-4 and (B) SCT after pulmonary co-administration with NAC and TX-100. NAC and TX-100 were given at 5% (w/v). Each point presents the mean ± S.D. ($n = 3$). Control (◯); NAC + TX-100 (●).

Table 3
Pharmacokinetic parameters of FD-4 after pulmonary co-administration with 5% NAC and 5% TX-100

Agent	C_{max} (μg/mL)	T_{max} (min)	$AUC_{0-2\,h}$ (μg min/mL)	$BA_{0-2\,h}$[a] (%)	Enhancement ratio[b]
None (control)	4.3 ± 1.2	80.0 ± 17.3	353.9 ± 102.5	23.3 ± 6.7	–
NAC + TX-100	26.2 ± 10.5	6.7 ± 2.9	1002.9 ± 204.2*	65.9 ± 13.4	2.8

Each value presents the mean ± S.D. ($n = 3$).
[a] $BA_{0-2\,h}$ was determined using AUC after i.v. administration at 50 mg/kg ($AUC_{i.v.\infty}$) of 6087.4 μg min/mL.
[b] Enhancement ratio was determined as the $AUC_{0-2\,h}$ increase relative to the control.
* Significantly different from the control ($P < 0.05$).

and 5% TX-100. Also, Table 3 summarizes the pharmacokinetic parameters of FD-4 after administration. FD-4 alone showed a slow absorption (T_{max}: 80.0 min), but provided the high bioavailability. The $AUC_{0-2\,h}$ was 353.9 μg min/mL, and the absolute bioavailability was 23.3%. The administration of FD-4 in combination with NAC and TX-100 showed a rapid absorption (T_{max}: 6.7 min), and significantly enhanced the FD-4 absorption. The $AUC_{0-2\,h}$ was 1002.9 μg min/mL, which is 2.8-fold higher than that of FD-4 alone.

Fig. 2(B) shows the plasma concentration–time profile of SCT after pulmonary administration with or without 5% NAC and 5% TX-100. Also, Table 4 summarizes the pharmacokinetic parameters of SCT after administration. When SCT was administered alone, the absolute bioavailability was low (1.7%). The administration of SCT in combination with NAC and TX-100 showed a significant enhancement, and its bioavailability was 8.1-fold higher than that of SCT alone.

3.3. Large intestinal absorption study with FD-4

Fig. 3 shows the plasma concentration–time profile of FD-4 after large intestinal administration with or without 5% NAC and 5% TX-100. Also, Table 5 summarizes the pharmacokinetic parameters of FD-4 after administration. When administered

Table 4
Pharmacokinetic parameters of SCT after pulmonary co-administration with 5% NAC and 5% TX-100

Agent	C_{max} (ng/mL)	T_{max} (min)	$AUC_{0-2\,h}$ (ng min/mL)	$BA_{0-2\,h}$[a] (%)	Enhancement ratio[b]
None (control)	7.3 ± 1.2	10.0 ± 8.7	516.3 ± 35.1	1.7 ± 0.1	–
NAC + TX-100	87.9 ± 40.6	11.7 ± 7.6	4177.3 ± 1367.7*	13.7 ± 4.5	8.1

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

Table 5
Pharmacokinetic parameters of FD-4 after large intestinal co-administration with 5% NAC and 5% TX-100

Agent	C_{max} (μg/mL)	T_{max} (min)	$AUC_{0-2\,h}$ (μg min/mL)	$BA_{0-2\,h}$[a] (%)	Enhancement ratio[b]
None (control)	0.3 ± 0.1	110.0 ± 17.3	27.5 ± 11.7	1.8 ± 0.8	–
NAC + TX-100	1.5 ± 0.4	60.0 ± 26.0	122.9 ± 28.7*	8.1 ± 1.9	4.5

Each value presents the mean ± S.D. ($n = 3$).
[a] $BA_{0-2\,h}$ was determined using AUC after i.v. administration at 50 mg/kg ($AUQ_{i.v.\infty}$) of 6087.4 μg min/mL.
[b] Enhancement ratio was determined as the $AUC_{0-2\,h}$ increase relative to the control.
* Significantly different from the control ($P < 0.05$).

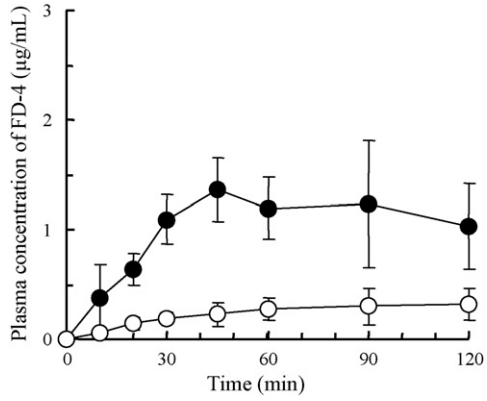

Fig. 3. Plasma concentration profiles of FD-4 after large intestinal co-administration with NAC and TX-100. NAC and TX-100 were given at 5% (w/v). Each point presents the mean ± S.D. ($n = 3$). Control (◯); NAC + TX-100 (●).

alone, FD-4 was slowly absorbed (T_{max}: 110.0 min), and its bioavailability was not so high (1.8%). The combination of NAC and TX-100 significantly enhanced the large intestinal absorption of FD-4, and its bioavailability was 4.5-fold higher than that of FD-4 alone.

4. Discussion

4.1. Absorption enhancement from various mucosal sites by the combination of NAC and TX-100

The present study investigated the absorption enhancement of poorly absorbed hydrophilic compounds from various mucosal sites by co-administration of NAC and TX-100. Bioavailabilities and enhancement ratios of FD-4 and SCT from various mucosal sites by the combination of NAC and TX-100 are summarized in Table 6. The results of the small intestinal absorption are cited from our previous reports (Takatsuka et al., 2006a,b).

When FD-4 was administered alone, the lung showed the highest bioavailability among the administration sites explored. It is well known that the lung has large absorptive area, extensive vasculature, thin layer alveolar epithelium and short distance of air-blood exchange passage (Hussain et al., 2004). These favorable physiological characteristics may explain the highest bioavailability after pulmonary administration. In our present finding, the rank order of FD-4 absorption was lung > nose > large intestine ≥ small intestine. Hosoya et al. reported that the permeability of FD-4 in the nose was higher than those in the small intestine and the large intestine (Hosoya et al., 1993), which was consistent with our present result. On the contrary, Yamamoto et al. showed the rank order of FD-4 absorption was lung > small intestine ≥ nose ≥ large intestine (Yamamoto et al., 2001). The fact that the highest bioavailability was obtained in the lung was consistent with our present result, but the other rank order was different from our result. Yamamoto et al. performed the small and large intestinal absorption study using an in situ closed loop. On the contrary, we selected a direct injection method and a capsule reservoir method for the small intestinal absorption study and the large intestinal absorption study, respectively. This difference in the experimental technique may explain the discrepancy in the rank order of FD-4 bioavailability.

When SCT was administered alone, the rank order of bioavailability was nose > lung > small intestine. Pulmonary delivery of peptide drugs generally results in higher bioavail-

Table 6
Bioavailabilities and enhancement ratios of FD-4 and SCT after administration from various mucosal sites

Mucosal sites	Compounds	Bioavailability (%)		Enhancement ratio	Thickness of mucus layer (μm)
		No enhancer	NAC + TX-100		
Nose	FD-4	8.3	60.3	7.2	5[a]
	SCT	8.5	51.6	6.1	
Lung	FD-4	23.3	65.9	2.8	1–10[b]
	SCT	1.7	13.7	8.1	
Small intestine[c]	FD-4	0.52	11.68	22.5	120[d]
	SCT	0.12	1.56	12.5	
Large intestine	FD-4	1.8	8.1	4.5	830[d]

[a] Referred from Ugwoke et al. (2005).
[b] Referred from Agu et al. (2001).
[c] Data were cited from our previous reports (FD-4: Takatsuka et al., 2006a; SCT: Takatsuka et al., 2006b).
[d] Referred from Atuma et al. (2001).

ability than nasal delivery (Byron and Patton, 1994). Moreover, some previous studies show that the bioavailability of SCT after pulmonary administration is much higher than that after nasal administration (Patton et al., 1994; Kagatani et al., 1996; Kobayashi et al., 1996; Sinswat and Tengamnuay, 2003). Therefore, the pulmonary bioavailability of SCT in this study is considered to be extremely low. Although the detailed reasons are not clearly known, the difference in an experimental condition may lead to the discrepancy in the pulmonary bioavailability of SCT. Namely, in those previous studies, about 50–100 μL of dosing solution was instilled into the lung. On the other hand, 5 μL of dosing solution was administered in the present study for increasing local drug concentration. This difference in the volume of dosing solution may influence on the bioavailability of SCT after pulmonary administration.

The combination of NAC and TX-100 significantly enhanced the nasal, the pulmonary and the large intestinal absorption of FD-4 compared with the control. This combination also improved the nasal and the pulmonary absorption of SCT effectively. Previously, we showed that the combination of NAC and TX-100 improved the small intestinal absorption of FD-4 (Takatsuka et al., 2006a) and SCT (Takatsuka et al., 2006b) dramatically. Therefore, the present study, along with these previous findings, suggests that the combination strategy of a mucolytic agent and a non-ionic surfactant would be widely applicable to various mucosal deliveries of poorly absorbed hydrophilic compounds.

4.2. Site dependency of the absorption enhancement by the combination of NAC and TX-100

The degree of the absorption enhancement of FD-4 and SCT from various mucosal sites by the combination of NAC and TX-100 was largely different among the administration sites explored, indicating that the effect of co-administration of NAC and TX-100 is site-dependent. The rank order of enhancement ratio of FD-4 absorption was small intestine ≫ nose > large intestine > lung. In addition, the rank order of enhancement ratio of SCT absorption was small intestine > lung > nose. For both compounds, the highest absorption enhancement was obtained after the small intestinal administration, which may be due to the lower bioavailability when compounds were administered alone. On the contrary, lower enhancement ratios obtained after the nasal and the pulmonary administration may result from the higher bioavailabilities when compounds were administered without enhancers.

In a previous study, TX-100 alone showed essentially no enhancing effect on the intestinal absorption of FD-4, although it was a strong potent absorption enhancer. When TX-100 was applied together with NAC, however, the enhancing ratio relative to the control was dramatically increased (Takatsuka et al., 2006a). Also, NAC is reported to reduce the mucus glycoprotein to smaller subunits and disrupt the surface network of the mucus layer (Livingstone et al., 1990). From these findings, the mucus layer is considered to act as a strict barrier against the absorption enhancing ability of TX-100. In addition, it is known that the mucus layer thickness is much different among the mucosal sites. So, we attempt the explanation of site-dependent absorption enhancement from a viewpoint of the difference of the mucus layer thickness. The mucus layer thickness is similar in the nose and in the lung (Ugwoke et al., 2005; Agu et al., 2001). When SCT was administered with the combination of NAC and TX-100, the enhancement ratios of SCT absorption were comparable in the nose and the lung. However, the enhancement ratios of FD-4 absorption were quite different between the nose and the lung. In addition, although the mucus layer thickness of large intestine is much thicker than that of small intestine (Atuma et al., 2001), the enhancement ratio of FD-4 absorption from large intestine was considerably lower than that from the small intestine. Given these results, the difference in the mucus layer thickness alone cannot explain the site-dependency. Besides the mucus layer thickness, the thickness of epithelium, the tightness of tight junction, the absorption area after instilling of dosing solution, the composition of mucus layer may be involved. Nevertheless, it is difficult to discuss the detailed mechanism of all observed phenomena on the basis of the present data only, and further investigations are needed for deeply understanding of the site-dependent absorption enhancement.

5. Conclusions

The present study showed the absorption enhancing effect of the combination of NAC and TX-100 on the FD-4 absorption from various mucosal sites including the nose, the lung and the large intestine. This combination also improved the nasal and the pulmonary absorption of SCT. The degree of absorption enhancement was found to be different among the administration sites explored, indicating that the absorption enhancing effect of the combination of NAC and TX-100 is site-dependent. These results suggest that the combination strategy of a mucolytic agent and a non-ionic surfactant may be widely applicable to various mucosal deliveries of poorly absorbed hydrophilic compounds.

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