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European Journal of Pharmaceutics and Biopharmaceutics 62 (2006) 52–58
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Enhancement of intestinal absorption of poorly absorbed hydrophilic compounds by simultaneous use of mucolytic agent and non-ionic surfactant

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Received 29 March 2005; accepted in revised form 27 July 2005

Abstract

The effect of co-administration of a mucolytic agent with a penetration enhancer was assessed on the intestinal absorption of poorly absorbed hydrophilic compounds. Fluorescein isothiocyanate-labeled dextran with average molecular weight of ca. 4.4 kDa (FD-4) was used as a model compound, and *N*-acetylcysteine (NAC) was used as a mucolytic agent. Sodium caprate (C10), tartaric acid (TA), sodium taurodeoxycholate (TDC), sodium dodecyl sulfate (SDS), *p-t*-octyl phenol polyoxyethylene-9.5 (Triton X® − 100, TX-100) were selected as penetration enhancers with different mechanisms of action. Various dosing solutions containing a penetration enhancer in the absence or in the presence of NAC were directly administered into the exposed rat jejunum, and the bioavailability of FD-4 up to 2 h was determined. The extent of improvement by co-administration was highly dependent on the penetration enhancer species applied. The observed enhancement was thought to result from the mucolytic activity of NAC, which can reduce the mucus viscosity and facilitate the penetration of FD-4 to mucosal membrane. Among the combinations tested, the simultaneous administration of NAC and TX-100 provided the highest enhancement (22.5-fold) of intestinal FD-4 absorption compared to the control. Although the detailed mechanism for the observed drastic improvement is unclear, one possible reason was thought to be due to the improved diffusivity of TX-100 micellar system in the mucus layer. All these results suggest that the combination of a mucolytic agent and a non-ionic surfactant may have potential as an enhancing system for peroral delivery of poorly absorbed hydrophilic compounds like protein and peptide drugs.

Keywords: Mucolytic agent; Penetration enhancer; Intestinal absorption; Poorly absorbed hydrophilic compounds; Non-ionic surfactant

1. Introduction

Recent great progress in the fields of biotechnology and genetic engineering has enabled the increasing use of protein and peptide drugs for the clinical treatment of various chronic diseases [1,2]. Due to their poor permeability in the intestinal epithelium and rapid metabolism by proteolytic enzymes, however, the administration of these drugs is mostly limited to invasive injections, which can be painful and inconvenient. Although much effort has been made to develop less invasive delivery system such as pulmonary delivery, nasal delivery and other transmucosal deliveries [3], there is no doubt that peroral administration would offer the greatest ease of medication for patients.

For intestinal absorption following peroral administration, peptide and protein drugs cross at least three physical or physiological barriers, i.e. the mucous layer on the intestinal wall acts as a diffusion barrier because of its high viscosity to hinder drug diffusion [4,5]; the intestinal epithelium itself acts as a penetration barrier to limit the uptake of drug depending on molecular weight and hydrophilic/lipophilic characteristics; and various digestive enzymes present in the intestinal lumen and metabolic enzymes located in the intestinal walls can be regarded as a sort of enzymatic barrier to degrade or transform drugs into inactive forms [6]. Much research has been focused on how to overcome the penetration barrier of the mucosal membrane, and various penetration enhancers were found to increase membrane permeability through in vitro or in situ experiments [7]. Other interesting approaches involve the use of delivery agents with low molecular weight, which interact non-covalently with drug to promote membrane permeation [8]. Some researchers have focused on the enzymatic barrier, and showed the effect of enzyme inhibitors on the intestinal absorption enhancement [9]. Also, the potential use of mucolytic agents for peroral administration of peptide and protein drugs, reducing the diffusion resistance in the mucus

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doi:10.1016/j.ejpb.2005.07.008

layer, has also been investigated [10,11]. However, most of the previous research has focused on how to overcome these barriers. Considering the anatomical and physical barriers in the GI tract mentioned above, the simultaneous perturbation of these barriers is considered to be a successful approach. In other words, the appropriate combination of enhancers with different mechanisms of action may be a useful formulation strategy for peroral protein and peptide drug delivery. If the synergism of absorption enhancement can be achieved, enhancers could be used at lower concentrations, which may avoid the risk of local toxicity of enhancers.

The objectives of the present study were firstly to evaluate the enhancing effect of mucolytic agent on intestinal absorption of poorly absorbed drug in rats, and secondly to examine the synergistic effect after simultaneous administration of a mucolytic agent and a penetration enhancer, and then to explore the possibility of creating a new formulation strategy. In this study, fluorescein isothiocyanate-labeled dextran (FD-4), which does not undergo enzymatic degradation in the body, was chosen as a poorly absorbed hydrophilic model compound in order to neglect the influence of enzymatic barrier. *N*-acetylcysteine (NAC) was used as the mucolytic agent, which has been widely used for clinical medication. Also, various types of penetration enhancers were used, each of which can improve the intestinal absorption through transcellular and/or paracellular pathway.

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 (St Louis, MO, USA). Sodium caprate (C10), sodium taurodeoxycholate (TDC), sodium dodecyl sulfate (SDS) and *p-t*-octyl phenol polyoxyethylene-9.5 (Triton® X-100, TX-100) were purchased from Nacalai Tesque (Kyoto, Japan). Tartaric acid (TA) was purchased from Katayama Chemical (Osaka, Japan). Nonylphenoxy polyoxyethylene-10 (NP-10) and Polyoxyethylene-9-lauryl ether (BL-9) were supplied from Nikko Chemicals (Tokyo, Japan). All other materials were of reagent grade.

2.2. Preparation of dosing solution

For intraintestinal administration study, FD-4 was dissolved in saline to a concentration of 1 g/mL. Separately, a mucolytic agent and/or penetration enhancer were dissolved in saline to a concentration of 10% (w/v). An aliquot of FD-4 solution was mixed with the same volume of enhancer solution, and kept cool until administration to rats. Thus, dosing solution finally contained 500 mg/mL of FD-4 and 5% (w/v) of a mucolytic agent and/or penetration enhancer. For intravenous (i.v.) administration study, FD-4 was dissolved in saline to a concentration of 25 mg/mL.

2.3. Rat studies

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 the previous report [12] with minor modification. Male Wistar rats (Nippon SLC, Hamamatsu, Japan), weighing 180–230 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, jejunum was exposed through a midline abdominal incision. FD-4 dosing solution was instilled into the exposed rat jejunum (50 mg/0.1 mL/kg) using a Hamilton micro-syringe. For another group of rats, a dose of 50 mg/2 mL/kg FD-4 was intravenously administered into jugular vein. Blood samples (200 μL) were taken from 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 FD-4 concentration

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.

2.5. Kinetic calculation

The i.v. plasma concentration data was analyzed based on a conventional two-compartmental PK model using a computer program (WinNonlin ®, Scientific Consulting). The calculated PK parameters such as total clearance (CL_{tot}) and volume of distribution (Vd_{ss}) were 8.3 ± 1.3 mL/min/kg and 202.5 ± 26.9 mL/kg, respectively. These parameters were comparable to those described in the Miyamoto's report [13]. Also, the area under the plasma concentration versus time (0–∞) curve after i.v. administration ($AUC_{i.v.\infty}$) at 50 mg/kg was calculated to 6087.4 ± 911.8 μg min/mL, which was used to calculate the absolute bioavailability of FD-4.

The area under the plasma concentration versus time (0–2 h) curve after intraintestinal administration ($AUC_{0-2\ h}$) was calculated according to the trapezoidal rule. The absolute bioavailability of FD-4 based on the period 0–2 h ($BA_{0-2\ h}$) was calculated as follows:

$$BA_{0-2\ h}\ (\%) = (AUC_{0-2\ h})/(AUC_{i.v.\infty}) \times 100$$

2.6. Statistical analysis

Statistical analysis was performed with Fisher pairing *t*-test. *P*-value of 0.05 was used as the significant level for all tests. All data are presented as the mean ± standard deviation (SD) unless otherwise noted.

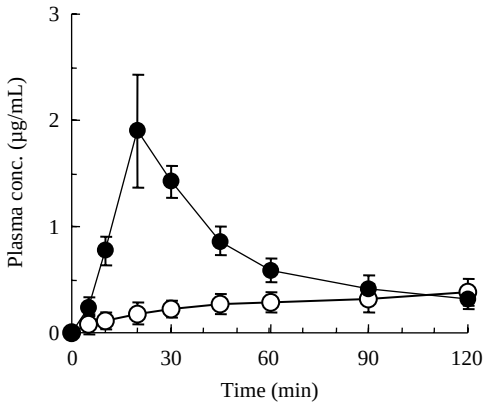

Fig. 1. Plasma concentration profile of FD-4 after intraintestinal co-administration with a mucoytic agent. A mucolytic agent was given at 5% (w/v). Each point represents the mean ± SD (n = 3–11). Control (open circle), NAC (closed circle).

3. Results and discussion

3.1. Effect of a mucolytic agent on intestinal FD-4 absorption

The absorption enhancing effect of NAC was evaluated by performing the rat study, in which 50 mg/kg of FD-4 and 5 mg/kg of NAC were co-administered intraintestinally to rats. FD-4 solution alone was also administered as the control. The plasma concentration profiles of FD-4 after administration are depicted in Fig. 1. The $AUC_{0-2\ h}$s and the absolute bioavailabilities are listed in Table 1. The administration of FD-4 solution alone provided a considerably low plasma concentration. The $AUC_{0-2\ h}$ was 31.6 ± 10.3 μg min/mL, and the absolute bioavailability was only 0.5 ± 0.2%, proving that the absorbability of FD-4 was very poor in this experimental model. When FD-4 was administered in combination with NAC, a clear increase of plasma concentration was observed within 20 min, after which it decreased. The $AUC_{0-2\ h}$ was 87.2 ± 6.5 μg min/mL, which is 2.8-fold higher than that of FD-4 solution alone, and the statistic calculation provided a significant difference between both values ($P < 0.05$). These results suggest that NAC is a potential absorption enhancer. According to Bernkop-Schnüch's finding, the diffusion rates of polypeptides (MW 3.4 and 6.5 kDa) into the native mucus gel of porcine intestine increased 2 or 3-fold after NAC treatment [11]. From the similarity between the enhancing rate and the diffusion rate, the observed enhancing effect could be due to the mucolytic activity of NAC, which can reduce the mucus viscosity and facilitate the diffusion of FD-4 into the mucosal membrane.

Table 1
Bioavailability of FD-4 after intraintestinal co-administration with a mucolytic agent

Agent	$AUC_{0-2\ h}$ (μg min/mL)	$BA_{0-2\ h}$ (%)	Enhancing ratio[a]
Control	31.6 ± 10.3	0.5 ± 0.2	–
NAC	87.2 ± 6.5*	1.5 ± 0.1	2.8

Each values represents the mean ± SD (n = 3–11). *Significantly different from the control ($P < 0.05$).

[a] Enhancing ratio was determined as the $AUC_{0-2\ h}$ increase relative to the control.

3.2. Effect of co-administration of a mucolytic agent and a penetration enhancer

Effect of co-administration of NAC and a penetration enhancer on the intestinal absorption of FD-4 was examined. In this study, 50 mg/kg of FD-4 and 5 mg/kg of penetration enhancer were intraintestinally administered with or without 5 mg/kg of NAC. The plasma concentration profiles of FD-4 after administration are individually depicted in Fig. 2. The $AUC_{0-2\ h}$s and the absolute bioavailabilities are listed in Table 2.

In the present study, five classical penetration enhancers with different mechanisms of action were chosen from fatty acids [14–16], organic acids [17], bile salts [18–20], anionic surfactants [18–21], and non-ionic surfactants [18–20,22]. C10, selected from fatty acids, did not show any apparent absorption enhancing effect, with or without NAC, under the present experimental condition applied. TDC, representative of bile salts, provided a distinct enhancement of FD-4 absorption (3.9-fold greater than control). SDS, representative of anionic surfactants, also provided absorption enhancement (4.6-fold). In both cases, however, co-administration with NAC resulted in no significant enhancement of FD-4 absorption. TA, selected from organic acids, showed a distinct enhancing effect as large as 6.4-fold compared to the control, and this effect tended to increase in combination with NAC (10.4-fold). TX-100, selected from non-ionic surfactants, showed essentially no enhancing effect on FD-4 absorption. When co-administered with NAC, however the enhancing ratio relative to the control was drastically increased up to 22.5-fold compared to the control and the absolute bioavailability became 11.7%. Many factors could be involved in the observed phenomena, and also the reasons may be different in individual cases. Possible reasons may include the difference in the mechanism of action of penetration enhancer, the suitability of dosing amount. For example, TDC and SDS are reported to reduce the viscosity of the mucus layer [23], which may explain the reason why no additional enhancement was observed when they were simultaneously applied with NAC. It is difficult to discuss the detailed mechanism of all observed phenomena on the basis of the present data only. Nevertheless, the obtained results may at least suggest one important fact that the appropriate combination of a penetration enhancer and a mucolytic agent is effective to enhance the intestinal absorption of poorly absorbed hydrophilic compound like FD-4. In particular, the synergistic effect between NAC and TX-100 seemed to be quite extraordinary.

The present study clearly demonstrated the effectiveness of simultaneous use of a mucolytic agent and a penetration enhancer at a concentration of 5% (w/v). This concentration was higher than those used in the previous studies, thus further investigations to clarify the extent of mucosal toxicity at the concentration used are necessary.

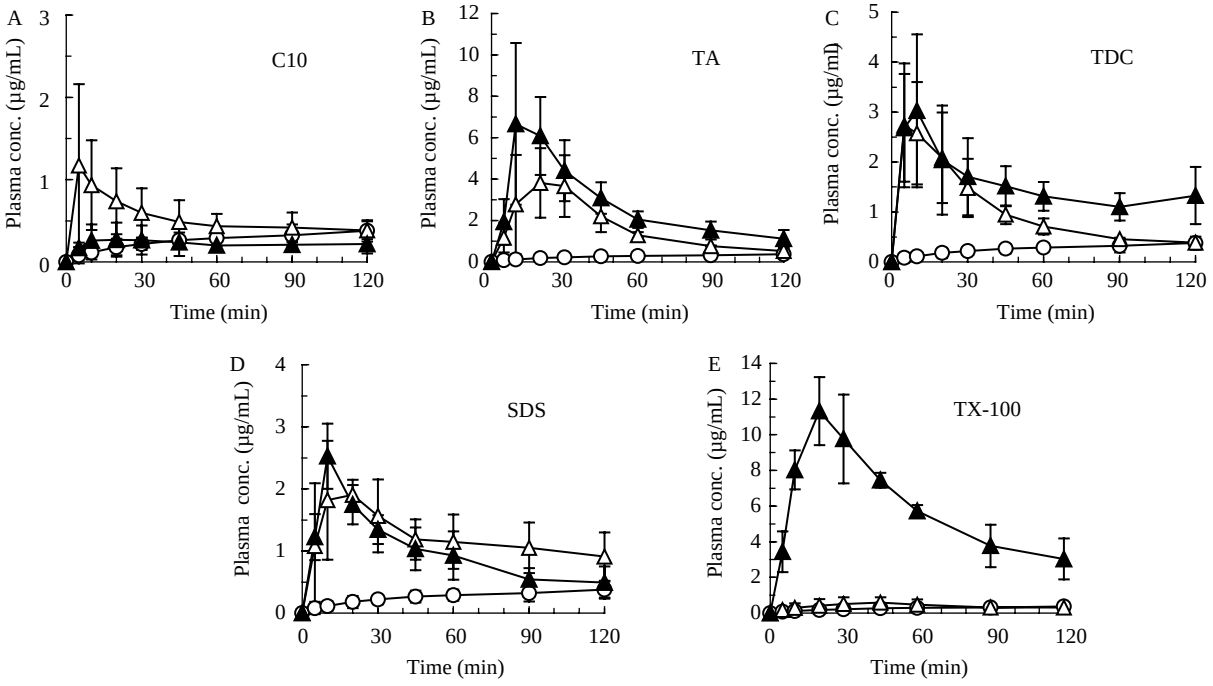

Fig. 2. Plasma concentration profiles of FD-4 after intraintestinal co-administration with a penetration enhancer in the absence or in the presence of NAC. A penetration enhancer and NAC were given at 5% (w/v). (A) C10, (B) TA, (C) TDC, (D) SDS, (E) TX-100. Each point represents the mean ± S.D. (n = 3–4). Control (open circle), penetration enhancer alone (open triangle), NAC + penetration enhancer (closed triangle).

3.3. Effect of co-administration of a mucolytic agent and a non-ionic surfactant

Among the penetration enhancers tested, only TX-100 enhanced intestinal FD-4 absorption in a synergistic manner when co-administered with NAC, suggesting that the combination of a mucolytic agent and a non-ionic surfactant may be appropriate. Therefore, the effect of other non-ionic surfactants on the intestinal FD-4 absorption, with or without NAC, was examined. Nonylphenoxy polyoxyethylene-10 (NP-10) and polyoxyethylene-9-lauryl ether (BL-9) were chosen as non-ionic surfactants, which are known to enhance the transmucosal absorption of hydrophilic compounds [24,25]. In the present study, 50 mg/kg of FD-4 and 5 mg/kg of non-ionic surfactant were intraintestinally administered with or without 5 mg/kg of NAC. The plasma concentration profiles of FD-4 after administration are individually depicted in Fig. 3. The $AUC_{0-2\ h}$s and the absolute bioavailabilities are listed in Table 3. Both non-ionic surfactants showed essentially no enhancing effect when they were used alone. However, they showed a clear enhancement in the presence of NAC, and enhancement increased up to 5.5-fold (NP-10) and 3.3-fold (BL-9) compared to the control, respectively. Although the enhancement effects obtained by NP-10 and BL-9 were not so drastic as TX-100, these results demonstrate that non-ionic surfactants would commonly show the absorption enhancing ability when they are co-administered with a mucolytic agent.

Table 2
Bioavailabilities of FD-4 after intraintestinal co-administration with a penetration enhancer in the absence or in the presence of NAC

Agent	AUC_{0-2h} (μg min/mL)	BA_{0-2h} (%)	Enhancing ratio[a]
Control	31.6 ± 10.3	0.5 ± 0.2	-
C10	63.2 ± 29.4	1.0 ± 0.5	2.0
NAC + C10	26.5 ± 9.6	0.4 ± 0.2	0.8
TA	203.4 ± 14.8*	3.3 ± 0.2	6.4
NAC + TA	330.1 ± 89.7*,**	5.4 ± 1.5	10.4
TDC	122.1 ± 34.5*	2.0 ± 0.6	3.9
NAC + TDC	183.1 ± 44.8*	3.0 ± 0.7	5.8
SDS	146.5 ± 30.2*	2.4 ± 0.5	4.6
NAC + SDS	127.7 ± 23.4*	2.2 ± 0.5	4.0
TX-100	47.4 ± 28.3	0.8 ± 0.5	1.5
NAC + TX-100	711.9 ± 92.4*,**	11.7 ± 1.5	22.5

Each value represents the mean ± S.D.(n = 3-4); *Significantly different from the control ($P < 0.05$). **Significantly different from corresponding penetration enhancer alone ($P < 0.05$).

[a] Enhancing ratio was determined as the AUC_{0-2h} increase relative to the control.

3.4. Mechanistic consideration for the synergistic effect of NAC and TX-100

It is notable that the considerably large synergistic effect was shown only by the combination of TX-100 and NAC. The mucolytic activity of NAC is thought be the major reason for this effect. Considering the chemical and physical characteristics of NAC, however, other factors such as its pH-lowering effect and unique chemical structure may also be contributing factors. Thus, in order to investigate the contribution of such factors to the absorption enhancing effect, additional rat experiments were performed using 0.1 M HCl solution as a pH-lowering agent or cysteine hydrochloride (cysteine·HCl) as a non-mucolytic agent with an analogous chemical structure to

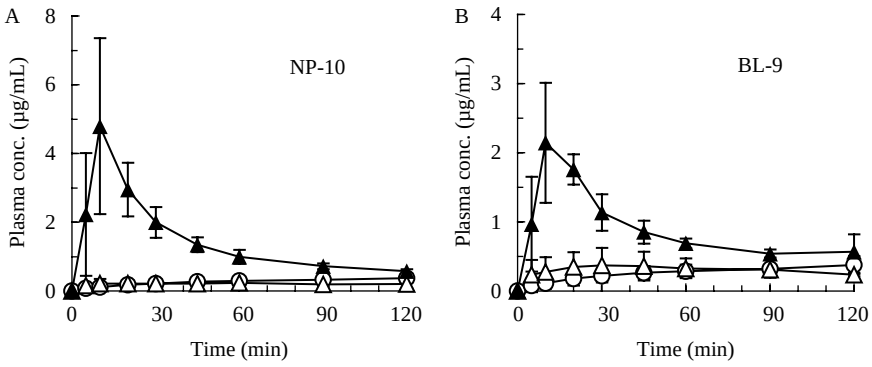

Fig. 3. Plasma concentration profiles of FD-4 after intraintestinal co-administration with a non-ionic surfactant in the absence or in the presence of NAC. A non-ionic surfactant and NAC were given at 5% (w/v). Each point represents the mean ± SD ($n = 3$). (A) NP-10, (B) BL-9. Control (open circle), non-ionic surfactant alone (open triangle), NAC + non-ionic surfactant (closed triangle).

NAC. The pH values of TX-100 in 0.1 M HCl and TX-100 in NAC solution were about 1.1 and 2.1, respectively. The plasma concentration profiles of FD-4 after administration are depicted in Fig. 4. The $AUC_{0-2\ h}$s and the absolute bioavailabilities are listed in Table 4. When TX-100 was co-administered with either 0.1 M HCl solution or cysteine·HCl, both combinations enhanced the intestinal absorption of FD-4; calculated bioavailabilities were 3.0 ± 0.7% and 2.4 ± 0.8%, respectively. Although the enhancement was observed in each case, the absolute bioavailability is far lower than that obtained by the combination of NAC and TX-100 (11.7 ± 1.5%). This result may be positive evidence supporting the hypothesis that the enhancement effect was mainly attributed to the mucolytic activity of NAC.

TX-100 is known as a strong non-ionic surfactant and has been frequently used as the solubilizing agent for biological tissues or cellular components. It is curious that the use of TX-100 alone was not effective whereas the combination with NAC resulted in the strong enhancement of FD-4 absorption. This interesting phenomenon may be related to the characteristics of TX-100 as a non-ionic surfactant. The critical micelle concentration of TX-100 is very low (about 0.24 mM), therefore, at the concentration employed in the present study, most of TX-100 molecular is thought to exist as micelle form. Also, non-ionic surfactants form stable micelles in physiological condition. These characteristics may be associated with the observed drastic change in absorption enhancement. Due to geometrical size of micelle and high viscosity of mucous layer in vivo, TX-100 micelles hardly access the intestinal epithelial membrane. In addition, very few TX-100 free molecules, which physicochemically interact with the mucosal membrane to increase the permeability [26], exist on the intestinal wall. This may be a possible reason why TX-100 alone did not show sufficient enhancing effect. When NAC is administered together with TX-100, the mucolytic activity of NAC reduces the mucus viscosity to facilitate the movement of the micelles onto epithelial membrane. The micelles reaching the membrane can act as reservoir to supply the free surfactant molecule more effectively to alter the membrane permeability [26]. Furthermore, with reducing viscosity, the accessibility of FD-4 to the mucosal membrane considerably increased. Both effects may synergistically increase the intestinal absorption. This may be a possible reason why the combination with NAC provided the drastic enhancement of FD-4 absorption. These proposed processes are illustrated in Fig. 5.

Table 3
Bioavailabilities of FD-4 after intraintestinal co-administration with a non-ionic surfactant in the absence or in the presence of NAC

Agent	AUC_{0-2h} (μg min/mL)	BA_{0-2h} (%)	Enhancing ratio[a]
Control	31.6 ± 10.3	0.5 ± 0.2	–
NP-10	24.7 ± 12.5	0.4 ± 0.2	0.8
NAC + NP-10	174.1 ± 52.0*,**	2.9 ± 0.9	5.5
Bl-9	37.0 ± 15.4	0.6 ± 0.3	1.2
NAC + BL-9	105.7 ± 15.1*,**	1.7 ± 0.3	3.3

Each value represents the mean ± S.D.($n = 3$); *Significantly different from the control ($P < 0.05$). **Significantly different from corresponding non-ionic surfactant alone ($P < 0.05$).

[a] Enhancing ratio was determined as the AUC_{0-2h} increase relative to the control.

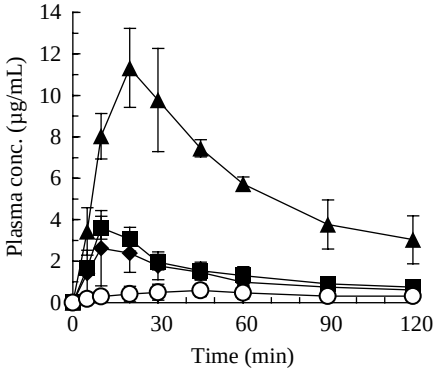

Fig. 4. Plasma concentration profiles of FD-4 after intraintestinal co-administration with a pH-lowering agent or non-mucolytic agent in the presence of TX-100. Non-mucolytic agent and TX-100 were given at 5% (w/v). Each point represents the mean ± SD ($n = 3$). TX-100 (open circle), 0.1 M HCl + TX-100 (closed square), cysteine·HCl + TX-100 (closed diamond), NAC + TX-100 (closed triangle). The result from NAC + TX-100 is shown for comparison.

Table 4
Bioavailabilities of FD-4 after intraintestinal co-administration with pH-lowering agent or non-mucolytic agent in the presence of TX-100

Agent	AUC_{0-2h} (μg min/mL)	BA_{0-2h} (%)	Enhancing ratio[a]
TX-100	47.4±28.3	0.8±0.5	–
0.1M HCl+TX-100	180.9±40.0*	3.0±0.7	3.8
Cysteine HCl+TX-100	148.8±44.9	2.4±0.7	3.1
NAC+TX-100[b]	711.9±92.4*	11.7±1.5	15.0

Each value represents the mean±S.D.($n=3$); *Significantly different TX-100 alone ($P<0.05$).
[a] Enhancing ratio was determined as the AUC_{0-2h} increase relative to TX-100 alone.
[b] The result from NAC+TX-100 is show for comparison.

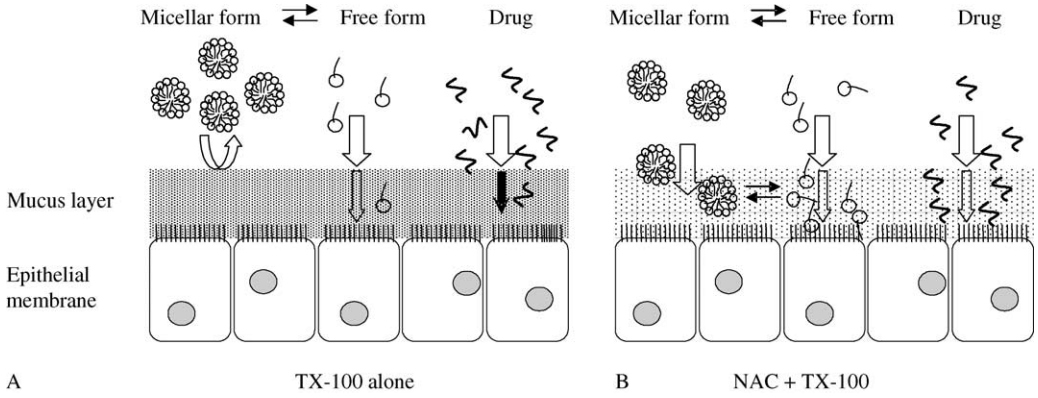

Fig. 5. Proposed mechanism on the absorption enhancing ability of the simultaneous use of NAC and TX-100. (A) When FD-4 was co-administered with TX-100 alone, (B) When FD-4 was co-administered with NAC and TX-100.

4. Conclusions

The present study has shown the effectiveness of co-administration of NAC and a penetration enhancer for the intestinal absorption of FD-4. The extent of enhancement was found to be highly dependent on the penetration enhancer species applied, and the synergistic effect was obtained when NAC was co-administered with a non-ionic surfactant. The combination of NAC and TX-100 provided the highest bioavailability of FD-4. These results suggest that the combination of a mucolytic agent and a non-ionic surfactant may have potential as an enhancing system for peroral delivery of poorly absorbed hydrophilic compounds like protein and peptide drugs. Further investigations to demonstrate the enhancement for protein and peptide drugs and to clarify the extent of mucosal toxicity are needed for practical application of this absorption enhancing system.

Acknowledgements

The authors would like to thank Dr Mitsunori Harada sincerely for his thoughtful discussion and suggestion.

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