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Influence of various combinations of mucolytic agent and non-ionic surfactant on intestinal absorption of poorly absorbed hydrophilic compounds

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Abstract

The absorption enhancing effects of various combinations of a mucolytic agent and a non-ionic surfactant on the intestinal absorption of poorly absorbed hydrophilic compounds were examined. Fluorescein isothiocyanate-labeled dextran with an average molecular weight of ca. 4.4 kDa (FD-4) was used as a model compound. Cysteine derivatives such as *N*-acetylcysteine (NAC), *S*-carboxymethylcysteine (SCMC), *S*-ethylcysteine (SEC), and *S*-methylcysteine (SMC) were selected as mucolytic agents. A homogeneous series of single chain polyoxyethylene alkyl ethers were employed as non-ionic surfactants. Various dosing solutions were administered into rat jejunum, and the bioavailability of FD-4 was determined. Unlike NAC, the agents such as SCMC, SEC, and SMC, which do not possess a free thiol group, did not show any apparent enhancement of intestinal FD-4 absorption, when they were co-administered with *p-t*-octyl phenol polyoxyethylene-9.5 (Triton® X-100, TX-100). In addition, the absorption enhancement was dependent on the kinds of polyoxyethylene alkyl ethers used, when used in combination with NAC. For a constant alkyl chain of 12 with a varying polyoxyethylene (POE) chain length, the surfactant with a short to medium POE chain length such as lauryl poly (4.2) oxyethylene ether (BL-4.2) and lauryl poly (9) oxyethylene ether (BL-9) were effective. In addition, for a constant alkyl chain of 18 with a varying POE chain length, the surfactants with a longer POE chain length such as oleyl poly (15) oxyethylene ether (BO-15) and stearyl poly (20) oxyethylene ether (BS-20) showed the effective enhancement.

All these results suggest that a mucolytic agent not possessing a free thiol group is not effective for enhancing the intestinal absorption of poorly absorbed hydrophilic compounds. Also, they indicate that the combination of a mucolytic agent possessing a free thiol group and a non-ionic surfactant either with a short to medium POE chain length and a medium alkyl chain length, or with a longer POE chain length and a longer alkyl chain length shows the effective enhancement. This fundamental information might be useful for finding the optimal combination.

Keywords: Intestinal absorption; Combination; Mucolytic agent; Polyoxyethylene alkyl ethers; Poorly absorbed hydrophilic compounds

1. Introduction

Numerous protein and peptide drugs have been used recently as therapeutic agents for the clinical treatment of various chronic diseases (Walsh, 2003). There is no doubt that peroral delivery of protein and peptide drugs can offer the greatest ease of medication for patients, and therefore significant efforts have been directed to explore the development of an oral delivery system (Mahato et al., 2003; Hamman et al., 2005). However, the successful development has not been obtained yet, and the administration of those drugs was mostly limited to invasive route by injection.

Recently, we have shown that the co-administration of a mucolytic agent and a non-ionic surfactant improved the intestinal absorption of poorly absorbed hydrophilic compounds in a synergistic manner (Takatsuka et al., 2006a,b). A mucolytic agent, *N*-acetylcysteine (NAC), is known to reduce the mucus viscosity by reducing the disulfide bond to a sulfhydryl bond of the mucus glycoproteins (Sheffner, 1963). In addition, it is reported that NAC treatment increased the diffusion rate of polypeptides in native intestinal mucus gel (Bernkop-Schnürch and Fragner, 1996). As a speculative mechanism, therefore, a

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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 locally high concentration of both the target drug and the surfactant molecules, thereby providing the synergistic absorption enhancement. In our recent studies mentioned above, NAC was used as a mucolytic agent, which has been widely used for clinical medication. In addition, *p-t*-octyl phenol polyoxyethylene-9.5 (Triton® X-100, TX-100) and nonylphenoxy polyoxyethylene-10 (NP-10) were selected for use as non-ionic surfactants. In order to gain a deeper understanding of the absorption enhancement of FD-4 by this combination, i.e. to elucidate the chemical structural or physicochemical property of mucolytic agent and a non-ionic surfactant needed for effective agents of the intestinal absorption, an extensive study using various combinations of mucolytic agent and non-ionic surfactant should be performed.

In this study, therefore, cysteine derivatives such as *S*-carboxymethylcysteine, *S*-ethylcysteine, and *S*-methylcysteine, which are analogous to NAC in chemical structure but do not possess a free thiol group, were selected for use as mucolytic agents. As non-ionic surfactants, a homogeneous series of single chain polyoxyethylene alkyl ethers were applied. Using the agents, the enhancing effects of various combinations of a mucolytic agent and a non-ionic surfactant on the intestinal absorption of FD-4 were examined and discussed.

2. Materials and methods

2.1. Materials

Fluorescein isothiocyanate-labeled dextran (MW, ca. 4.4 kDa, FD-4), *N*-acetylcysteine (NAC), *S*-methylcysteine (SMC), *S*-ethylcysteine (SEC), and *S*-carboxymethylcysteine (SCMC) were purchased from Sigma (St. Louis, MO, USA). *p-t*-Octyl phenol polyoxyethylene-9.5 (Triton® X-100, TX-100) was purchased from Nacalai Tesque (Kyoto, Japan). The following polyoxyethylene alkyl ethers were gifted from Nikko Chemicals (Tokyo, Japan): lauryl poly (4.2) oxyethylene ether (BL-4.2), lauryl poly (9) oxyethylene ether (BL-9), lauryl poly (21) oxyethylene ether (BL-21), lauryl poly (25) oxyethylene ether (BL-25), cetyl poly (10) oxyethylene ether (BC-10), cetyl poly (20) oxyethylene ether (BC-20), oleyl poly (7) oxyethylene ether (BO-7), oleyl poly (10) oxyethylene ether (BO-10), oleyl poly (15) oxyethylene ether (BO-15), and stearyl poly (20) oxyethylene ether (BS-20). All other materials were of reagent grade.

2.2. Preparation of dosing solution

FD-4 was dissolved in saline to a concentration of 1 g/mL. Separately, a mucolytic agent and/or a non-ionic surfactant 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, the dosing solution finally contained 500 mg/mL of FD-4 and 5% (w/v) of a mucolytic agent and/or a non-ionic surfactant. Previously, we examined the dose effect of the combination of NAC and TX-100 on the intestinal absorption of salmon calcitonin (SCT), and found that the highest bioavailability of SCT was obtained when 20 μL of dosing solution containing 5% of NAC and TX-100 was applied (Takatsuka et al., 2006b). Based on this result, we selected 5% of a mucolytic agent and a non-ionic surfactant.

2.3. Animal experiments

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 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, the jejunum was exposed through a midline abdominal incision. The exposed jejunum was fixed with tweezers, and FD-4 dosing solution was directly instilled into the exposed rat jejunum (50 mg/0.1 mL/kg) using a Hamilton micro-syringe. In general, the overall absorption in the small intestine is higher than that in the large intestine. Also, our previous study showed that the combination of NAC and TX-100 showed the higher absorption in jejunum than in the large intestine (Takatsuka et al., 2007). Therefore, we selected the jejunum as a target absorption site.

Blood samples (200 μL) were directly taken from the jugular vein with heparinized syringes 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. The plasma sample was collected after centrifugation at 12,000 rpm for 3 min.

2.4. Determination of plasma concentration

The plasma samples (20 μL) were diluted with 680 μL of 0.1 N 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 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. The absolute bioavailability of FD-4 based on the period 0–2 h ($BA_{0-2\,h}$) was calculated as follows:

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

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

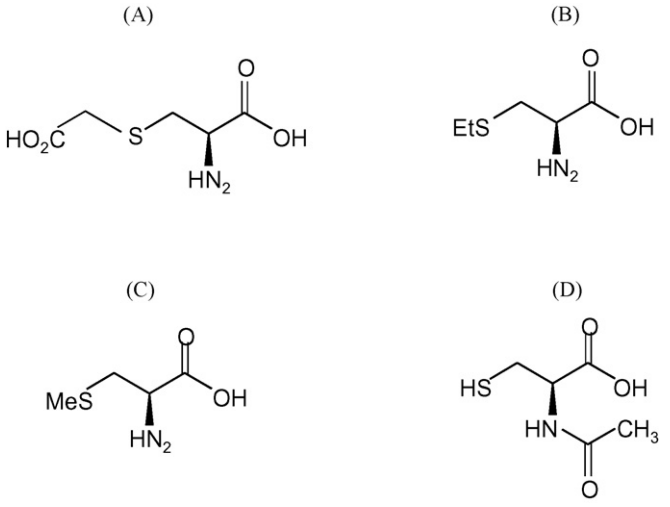

Fig. 1. Chemical structures of mucolyitc agents used in the present study. (A) SCMC, (B) SEC, (C) SMC, (D) NAC.

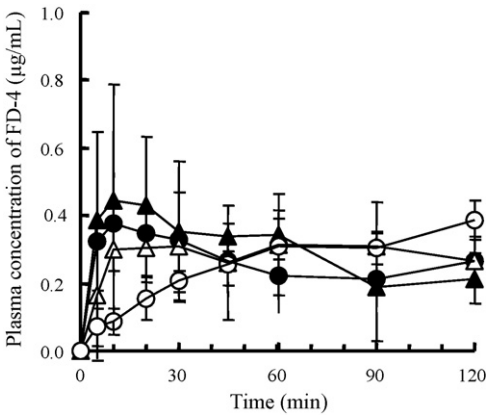

Fig. 2. Plasma concentration profiles of FD-4 after intraintestinal co-administration with a mucolytic agent in the presence of TX-100. Mucolytic agent and TX-100 were given at 5% (w/v). Each point presents the mean ± S.D. (n = 3–6). Control (◯), SCMC (●), SEC (△), SMC (▲).

2.6. Statistical analysis

Statistical analysis of $AUC_{0–2\,h}$ was performed with the Dunnett's test for multiple comparisons. The 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 cysteine derivatives on the intestinal absorption of FD-4 in the presence of TX-100

Previously, we proved that the combination of a mucolytic agent and a non-ionic surfactant enhanced the intestinal absorption of FD-4 effectively. *N*-Acetylcysteine (NAC), which has been clinically used in bronchopulmonary diseases, was selected to use as a mucolytic agent. Another cysteine derivative, *S*-carboxymethylcysteine (SCMC), has also been used as a mucolytic agent in the treatment of respiratory disease. SCMC are analogous to NAC in chemical structure, but do not possess a free thiol group. In order to elucidate the chemical structural requirement of a mucolytic agent, the effect of SCMC, *S*-methylcysteine (SMC), and *S*-ethylcysteine (SEC) on the intestinal absorption of FD-4 in the presence of TX-100 was examined. The chemical structures of mucolytic agents used are shown in Fig. 1. In this study, 50 mg/kg of FD-4 was intraintestinally administered with and a 5% mucolytic agent and 5% TX-100. The plasma concentration profiles of FD-4 after administration are individually depicted in Fig. 2. The pharmacokinetic parameters of FD-4 after administration are listed in Table 1.

When FD-4 was administered together with the combination of SCMC and TX-100, its absorption was very low. The $AUC_{0–2\,h}$ was 31.2 μg min/mL and the absolute bioavailability was 0.5%. When FD-4 was administered together with SMC or SEC in the presence of TX-100, its absorption was also very low. The absolute bioavailabilities obtained from these cysteine derivatives were 0.6%. There was no significant difference between those cysteine derivatives and the control (FD-4 solution alone) in bioavailabilities. In our previous study, NAC alone provided the significant absorption enhancement of FD-4 compared with the control. Also, the combination of NAC and TX-100 drastically enhanced the intestinal absorption of FD-4, and the bioavailability became more than 10% (Takatsuka et al., 2006a), which is much higher than those obtained using SCMC, SEC, and SMC in this study. The present finding, therefore, indicates that the cysteine derivatives such as SCMC, SEC, and SMC were not effective for the synergistic absorption enhancement.

It is interesting that the use of SCMC, SEC, and SMC did not provide any apparent absorption enhancement of FD-4, whereas NAC showed a considerably good enhancement. Livingstone et al. reported that the NAC treatment disrupted the surface pore network of the purified mucus glycoprotein gel, but SCMC did not cause any structural change (Livingstone et al., 1990). Furthermore, Martin et al. showed that SCMC and SEC had no effect

Table 1
Pharmacokinetic parameters of FD-4 after intraintestinal co-administration with 5% mucolytic agent in the presence of 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)	0.4 ± 0.1	105.0 ± 25.1	30.8 ± 5.4	0.5 ± 0.1	–
SCMC	0.4 ± 0.2	17.0 ± 12.0	31.2 ± 11.6	0.5 ± 0.2	1.0
SEC	0.4 ± 0.1	20.0 ± 10.0	34.2 ± 14.2	0.6 ± 0.2	1.1
SMC	0.5 ± 0.3	43.3 ± 41.6	35.6 ± 3.4	0.6 ± 0.1	1.2

Each value presents the mean ± S.D. (n = 3–6).
[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.

Table 2
Properties of the non-ionic surfactants used

Chemical name	Trade name	HLB	Poly oxyethylene chain length	Alkyl chain length
Lauryl poly (4.2) oxyethylene ether	BL-4.2	11.5	4.2	12
Lauryl poly (9) oxyethylene ether	BL-9	14.5	9	12
Lauryl poly (21) oxyethylene ether	BL-21	19.0	21	12
Lauryl poly (25) oxyethylene ether	BL-25	19.5	25	12
Cetyl poly (10) oxyethylene ether	BC-10	13.5	10	16
Cetyl poly (20) oxyethylene ether	BC-20	17.0	20	16
Oleyl poly (7) oxyethylene ether	BO-7	10.5	7	18
Oleyl poly (10) oxyethylene ether	BO-10	14.0	10	18
Oleyl poly (15) oxyethylene ether	BO-15	16.0	15	18
Stearyl poly (20) oxyethylene ether	BS-20	18.0	20	18

on the rheological properties of canine tracheal mucus (Martin et al., 1980). Possible reasons may be explained by the chemical structural difference among these agents, which would affect the rheological property of the mucus layer. NAC has a free thiol group in its chemical structure, which can reduce the disulfide bond to a sulfhydryl bond. This breakage of the disulfide bond leads to a reduction of the molecular size of mucin, resulting in the reduction of the elasticity and the viscosity of the mucus. On the other hand, SMC, SEC, and SCMC do not possess a free thiol group, which apparently renders it unable to reduce the mucus glycoprotein to smaller subunits.

All these findings suggest that a mucolytic agent possessing a free thiol group shows the ability to enhance the intestinal absorption of the poorly absorbed hydrophilic compounds, and also that this enhancing ability would come from the direct effect on the rheological property of the intestinal mucus.

3.2. Effect of non-ionic surfactant on the intestinal FD-4 absorption in the presence of NAC

Previously, we showed that non-ionic surfactants such as TX-100 and NP-10 had the ability to enhance the intestinal absorption of FD-4 when they were used together with NAC. In order to examine the influence of the physicochemical property of non-ionic surfactants on absorption enhancement, an extensive study was carried out using a homologous series of single chain polyoxyethylene alkyl ether. The properties of non-ionic surfactants are represented in Table 2. In the present study, 50 mg/kg of FD-4 and 5% non-ionic surfactant were intraintestinally administered without or with 5% NAC. The plasma concentration profiles of FD-4 after the administration are depicted in Fig. 3 (without NAC) and Fig. 4 (with NAC). The pharmacokinetic parameters after FD-4 administration are listed in Table 3 (without NAC) and Table 4 (with NAC).

When non-ionic surfactants were applied without NAC, the $AUC_{0-2\,h}$ values obtained from them were comparable to or a little higher than that from the control, suggesting that they had little or no effect on the intestinal absorption of FD-4 in the absence of NAC. This result was consistent with our previous findings, which was obtained using TX-100 and NP-10 (Takatsuka et al., 2006a). When concurrently administered with NAC, some non-ionic surfactants such as BL-4.2, BL-9, BC-10, BC-20, BO-10, BO-15, and BS-20 significantly improved the intestinal FD-4 absorption, and the enhancement ratios of the $AUC_{0-2\,h}$ values were ranging from 2.2 to 3.7. On the other hand, other surfactants such as BL-21, BL-25, and BO-7 and did not enhance the absorption significantly, and the enhancement ratios of the $AUC_{0-2\,h}$ values were ranging from 1.0 to 1.9. These results indicate that non-ionic surfactants such as BL-4.2, BL-9, BC-10, BC-20, BO-10, BO-15, and BS-20 had potential enhancing ability, and that NAC treatment should be necessary for exerting their absorption enhancing effect. In addition, non-ionic surfactants such as BL-21, BL-25, and BO-7 possess essentially no absorption enhancing ability, since there was no apparent difference in the bioavailability between those with NAC and without NAC.

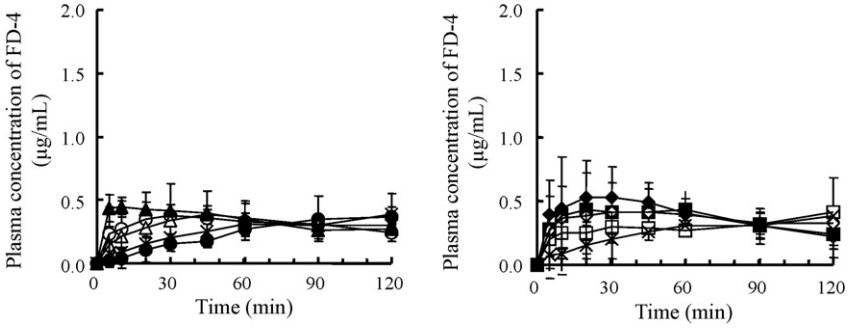

Fig. 3. Plasma concentration profiles of FD-4 after intraintestinal co-administration with a non-ionic surfactant in the absence of NAC. Non-ionic surfactant was given at 5% (w/v). Each point presents the mean ± S.D. (n = 3–6). Control (×), BL-9 (○), BL-21 (●), BC-10 (△), BC-20 (▲), BO-7 (□), BO-10 (■), BO-15 (◇), BS-20 (◆).

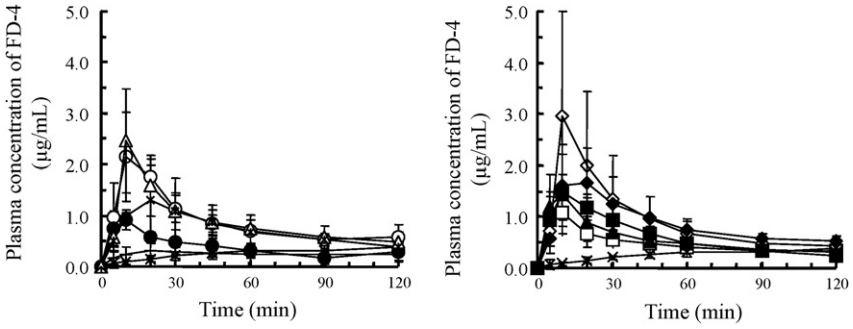

Fig. 4. Plasma concentration profiles of FD-4 after intraintestinal co-administration with a non-ionic surfactant in the presence of NAC. Non-ionic surfactant and NAC were given at 5% (w/v). Each point presents the mean ± S.D. (n = 3–6). Control (×), BL-4.2 (*), BL-9 (◯), BL-21 (●), BL-25 (+), BC-10 (△), BC-20 (▲), BO-7 (□), BO-10 (■), BO-15 (◊), BS-20 (♦).

Table 3
Pharmacokinetic parameters of FD-4 after intraintestinal co-administration with non-ionic surfactant in the absence of NAC

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)	0.4 ± 0.1	105.0 ± 25.1	30.8 ± 5.4	0.5 ± 0.1	–
BL-9	0.4 ± 0.2	50.0 ± 34.6	37.0 ± 15.4	0.6 ± 0.3	1.2
BL-21	0.4 ± 0.2	110.0 ± 17.3	28.0 ± 7.2	0.5 ± 0.1	0.9
BC-10	0.4 ± 0.0	38.3 ± 25.7	36.6 ± 2.8	0.6 ± 0.0	1.2
BC-20	0.5 ± 0.1	11.7 ± 7.6	40.4 ± 1.6	0.7 ± 0.0	1.3
BO-7	0.4 ± 0.2	85.0 ± 37.7	35.1 ± 9.7	0.6 ± 0.2	1.1
BO-10	0.6 ± 0.3	43.3 ± 28.9	42.5 ± 13.3	0.7 ± 0.2	1.4
BO-15	0.4 ± 0.1	40.0 ± 8.7	42.9 ± 13.6	0.7 ± 0.2	1.4
BS-20	0.6 ± 0.1	36.7 ± 14.4	46.1 ± 2.9	0.8 ± 0.0	1.5

Each value presents the mean ± S.D. (n = 3–6).
[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.

3.3. Relationship between the absorption enhancement and the physical parameter of non-ionic surfactant

The results obtained earlier clearly showed that the degree of absorption enhancement was dependent on the kind of non-ionic surfactants applied, which might come from the difference of the physicochemical property of the non-ionic surfactant. Therefore, the relationship between the physicochemical property of non-ionic surfactant and the enhancement ratio was discussed.

Non-ionic surfactants are generally known to possess the specific hydrophilic/lipophilic balance (HLB) value dependent on structure. The relationship between the HLB of the surfactant and the enhancing ability has been often discussed. Therefore, we attempted the explanation of different absorption enhancements by non-ionic surfactants from a viewpoint of HLB value. The HLB values of non-ionic surfactants ranged from 10.5 to 19.5. As shown in Fig. 5, HLB value was not simply related to the absorption enhancement of FD-4. Wal-

Table 4
Pharmacokinetic parameters of FD-4 after intraintestinal co-administration with non-ionic surfactant in the presence of NAC

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)	0.4 ± 0.1	105.0 ± 25.1	30.8 ± 5.4	0.5 ± 0.1	–
BL-4.2	1.4 ± 0.7	13.3 ± 5.8	86.7 ± 28.3*	1.4 ± 0.5	2.8
BL-9	2.3 ± 0.6	13.3 ± 5.8	105.7 ± 15.1*	1.7 ± 0.2	3.4
BL-21	1.0 ± 0.2	8.3 ± 2.9	45.3 ± 10.8	0.7 ± 0.2	1.5
BL-25	0.3 ± 0.1	36.7 ± 20.8	29.6 ± 13.0	0.5 ± 0.2	1.0
BC-10	2.5 ± 1.0	10.0	104.9 ± 26.8*	1.9 ± 0.4	3.4
BC-20	1.4 ± 0.8	10.0	68.9 ± 19.1*	1.1 ± 0.3	2.2
BO-7	1.1 ± 0.5	8.3 ± 2.9	57.7 ± 13.3	0.9 ± 0.2	1.9
BO-10	1.5 ± 0.3	10.0	74.4 ± 17.3*	1.2 ± 0.3	2.4
BO-15	3.0 ± 2.0	10.0	112.9 ± 54.5*	1.9 ± 0.9	3.7
BS-20	1.9 ± 0.8	15.0 ± 5.8	104.5 ± 30.7*	1.7 ± 0.5	3.4

Each value presents the mean ± S.D. (n = 3–6).
[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 ($P < 0.05$).

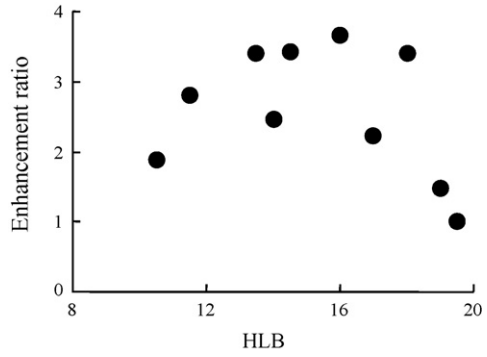

Fig. 5. Enhancement of intestinal FD-4 absorption as a function of non-ionic surfactant HLB. Each point presents the mean ($n = 3$–4).

ters et al. studied the effect of the surfactant structure on the gastric mucosal transport of paraquat, and showed that there is no simple relationship between HLB and absorption enhancement, which was consistent with our present result (Walters et al., 1981). Therefore, it would be concluded that HLB might not be a physicochemical parameter, which can be used as a reliable predictor for the enhancement of intestinal FD-4 absorption.

A series of single chain polyoxyethylene alkyl ethers, which were used in this study, consisted of the alkyl chain portion and the polyoxyethylene (POE) chain portion. The alkyl chain length, an indicator of hydrophobic property, could be involved in the effective partition into biological membrane. In addition, the POE chain length, an indicator of hydrophilic property, could lead to the aqueous solubility. It has been generally observed that surfactants, which are too hydrophobic to be water-soluble, are poor enhancers, while surfactants, which are very hydrophilic cannot partition into the hydrophobic environment of the lipid bilayer. This general observation suggests that the balance between the alkyl chain length and the POE chain length should be important. Therefore, the relationship between the alkyl chain length/the POE chain length and the absorption enhancement was examined.

As shown in Fig. 6, for a fixed POE chain length of 9–10, the rank order of absorption enhancement was C-12/POE-9 = C-16/POE-10 > C-18/POE-10, which was mostly in agreement with the previous findings that non-ionic surfactants with a medium alkyl chain length such as C-12 is effective (Florence, 1981; Ishizawa et al., 1987; Dimitrijevic et al., 2000). However, for a fixed POE chain length of 20, the rank order of absorption enhancement was C-18/POE-20 > C-16/POE-20 > C-12/POE-20, which was a reversed trend compared with the case of a fixed POE chain length of 9–10. Therefore, the POE chain length alone was not a predictor for the absorption enhancement.

When analyzing the results of non-ionic surfactants with a constant alkyl chain length, some interesting patterns could be found. As shown in Fig. 6, for a constant alkyl chain of 12 with a varying POE chain length, the parabolic relation between the POE chain length and the enhancement ratio was observed. The surfactants with short to medium POE chain lengths such as BL-4.2 and BL-9 were effective, whereas the surfactants

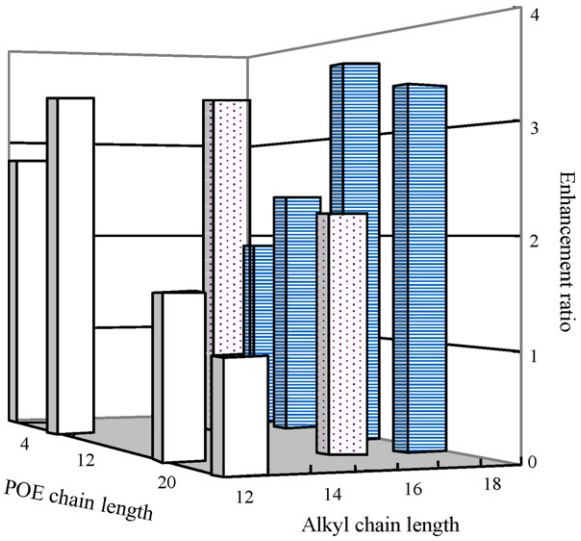

Fig. 6. Enhancement of intestinal FD-4 absorption as a function of the POE chain length and the alkyl chain length. Each column presents the mean ($n = 3$–4).

with longer alkyl chain lengths such as BL-21 and BL-25 were not. Similar trend was also observed in a constant alkyl chain of 18 with a varying POE chain length. The surfactants with longer POE chain lengths such as BO-15 and BS-20 showed the effective enhancement, whereas the surfactant with medium POE chain lengths such as BO-7 and BO-10 were less effective. Okuyama et al. reported the parabolic relation between the POE chain length and the enhancement ratio in the percutaneous absorption of piroxicam (Okuyama et al., 1999). In addition, Ichikawa et al. found that for a fixed alkyl chain length of 12, C-12/POE-9 and C-12/POE-6 were effective for rectal absorption of insulin, while C-12/POE-3, C-12/POE-25 and C-12/POE-40 were not (Ichikawa et al., 1980). These previous findings by other researchers could support our present result.

It is unclear why the absorption enhancements obtained from a series of single chain polyoxyethylene alkyl ethers were much smaller than those from TX-100 and NP-10. It is known that the size and shape of both the alkyl chain and the polar group influence absorption enhancing ability (Swenson and Curatolo, 1992). The hydrophobic regions of TX-100 and NP-10 possess aromatic moiety and are a little more bulky than those of a series of single chain polyoxyethylene alkyl ethers. This slightly bulky structure of the hydrophobic region might improve the interaction with epithelial membranes and provide a higher enhancement.

The present study focused on the absorption enhancement only and did not investigate the intestinal mucosal damage. The combination of NAC and TX-100 provided the highest bioavailability among the combination applied. Since our previous study showed that the combination of NAC and TX-100 caused the reversible mucosal injury, however, the application of this combination to practical use might be difficult. Therefore, the combination of a mucolytic agent and a non-ionic surfactant should be evaluated in terms of both absorption enhancement and toxicity, and optimized for practical use.

4. Conclusions

Cysteine derivatives such as SMC, SEC, and SCMC did not show any apparent enhancement of intestinal FD-4 absorption, when they were co-administered with TX-100. The absorption enhancement was dependent on the kind of polyoxyethylene alkyl ethers used, when they were used in combination with NAC. For a constant alkyl chain of 12 with a varying POE chain length, the surfactants with short to medium POE chain lengths were effective. In addition, for a constant alkyl chain of 18 with a varying POE chain length, the surfactants with the longer POE chain length showed the effective enhancement. All these results suggest that the combination of a mucolytic agent possessing a free thiol group and a non-ionic surfactant either with a short to medium POE chain length and a medium alkyl chain length, or with a longer POE chain length and a longer alkyl chain length shows the effective enhancement.

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