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Improvement of absorption enhancing effects of *n*-dodecyl-β-D-maltopyranoside by its colon-specific delivery using chitosan capsules

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Abstract

In general, absorption enhancing effects of various absorption enhancers were greater in the large intestine than those in the small intestinal regions. Therefore, the effectiveness of absorption enhancers is expected to be remarkably observed, if these enhancers can be delivered to the large intestine with some poorly absorbable drugs after oral administration. In this study, therefore, we examined whether chitosan capsules were effective for the colon-specific delivery of a certain absorption enhancer and can improve the absorption enhancing action of the absorption enhancer after oral administration. 5(6)-Carboxyfluorescein (CF) was used as a model drug to investigate the site-dependent effectiveness of various absorption enhancers by an in situ closed loop method. Sodium glycocholate (NaGC), *n*-dodecyl-β-D-maltopyranoside (LM), sodium salicylate (NaSal) and sodium caprate (NaCap) were used as models of absorption enhancers in this study. Overall, the absorption enhancing effects of these enhancers for intestinal absorption of CF were greater in the colon than those in the jejunum and the ileum. Especially, among these enhancers tested in this study, LM showed much greater absorption enhancing effect in the colon than in the jejunum and the ileum. Therefore, LM was selected as a model absorption enhancer to examine the effect of chitosan capsules on the absorption enhancing effect of LM. When CF and LM were orally administered to rats using chitosan capsules, the plasma concentration of CF was much higher than those in other dosage forms including solution and gelatin capsules. Therefore, chitosan capsules may be useful carriers for colon-specific delivery of LM, thereby increasing its absorption enhancing effect from the intestinal membranes.

Keywords: Intestinal absorption; Oral absorption; Absorption enhancer; *n*-Dodecyl-β-D-maltopyranoside; Chitosan capsule; Colon-specific delivery

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1. Introduction

The intestinal absorption of hydrophilic and macromolecular drugs is generally low by their poor intestinal permeability. The use of absorption enhancers has been shown to improve the absorption characteristics of these drugs. Various kinds of absorption enhancers have been investigated regarding their effectiveness, toxicity and mechanism of action (Aungst and Rogers, 1988; Nishihata et al., 1983; Uchiyama et al., 1996, 1999; Yamamoto et al., 1992, 1996, 2001. These absorption enhancers include surfactants, bile salts, chelating agents, and fatty acids. As for surfactants and bile salts, our previous study indicated that rectal permeability of insulin was enhanced by the co-administration of various bile salts such as sodium glycocholate (NaGC), sodium taurocholate, and sodium deoxycholate (NaDC) (Yamamoto et al., 1992). In addition, Uchiyama et al. (1999) demonstrated that *n*-dodecyl-β-D-maltopyranoside (LM), a non-ionic surfactant, and bile salts such as NaGC and NaDC enhanced the permeability of insulin across the intestinal membrane. With regard to chelating agents, it was found that sodium salicylate and 5-methoxysalicylate remarkably enhanced the rectal absorption of insulin in rats (Aungst and Rogers, 1988; Nishihata et al., 1981, 1983). Furthermore, it was reported that linoleic acid (fatty acid)-surfactant-mixed micelles improved the intestinal absorption of streptomycin and gentamicin in rats (Muranishi, 1985, 1990). These different types of enhancers have been known to increase the intestinal absorption of poorly absorbable drugs by various mechanisms.

As for the regional differences in the effect of various absorption enhancers in the intestine, it was generally known that the absorption enhancing effects of these absorption enhancers were greater in the large intestine than those in the small intestine. For example, our previous studies demonstrated that the absorption enhancing effects of sodium caprate (NaCap) and LM for the intestinal absorption of insulin and ebiratide in the large intestine were much greater than those in the small intestine (Uchiyama et al., 1999; Yamamoto et al., 1997), although the reason was not fully understood. Therefore, the effectiveness of absorption enhancers is expected to be more remarkably observed, if these enhancers can be specifically delivered to the large intestine with drugs after oral administration.

On the other hand, we previously demonstrated that chitosan capsules could be useful carriers for the colon-specific delivery of peptide and anti-inflammatory drugs including insulin, 5-aminosalicylic acid and ridogrel, and intestinal absorption of insulin was enhanced using chitosan capsules (Tozaki et al., 1997, 1999a,b, 2002). Chitosan is a high molecular weight cationic polysaccharide derived from naturally occurring chitin in crab and shrimp shells by deacetylation. It has previously been employed as a pharmaceutical excipient in oral drug formulations to improve the dissolution of poorly soluble drugs or for the sustained release of drugs by a process of slow erosion from a hydrated compressed matrix (Kristl et al., 1993). This biopolymer is considered to be non-toxic, with an oral LD_{50} in mice of >16 g/kg. It was reported that this compound is degraded by microflora, which are richly distributed in the colon (Tozaki et al., 1997, 1999a,b, 2002). This character might make it a suitable carrier to deliver drugs and other compounds specifically to the colon. However, no study has been done to examine the effect of chitosan capsules on the colon-specific delivery of a certain absorption enhancer and to improve its absorption enhancing action.

In this study, therefore, we examined whether the chitosan capsules can be effective for delivering LM, a typical absorption enhancer to the colon and improving its absorption enhancing effect for the intestinal absorption of CF, a poorly absorbable compound.

2. Materials and methods

2.1. Materials

CF was obtained from Eastman Kodak Company (Rochester, NY). LM and NaGC were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). NaSal and NaCap were obtained from Nacalai Tesque, Inc. (Kyoto, Japan). All other reagents used were of analytical grade.

2.2. Preparation of drug solution

For the absorption studies, CF was dissolved in a phosphate buffered saline solution (PBS) adjusted to pH 7.4 to yield a final concentration of 0.25 mg/rat. In certain experiments, the dosing solutions were added to

20 mM absorption enhancers including NaGC, NaCap, NaSal and LM.

2.3. Absorption experiments

Absorption experiments were performed by an in situ closed loop method (Asada et al., 1995; Yamamoto et al., 1996; Yodoya et al., 1994). Male Wistar albino rats (250–280 g) were anaesthetized with sodium pentobarbital (32 mg/kg body weight i.p.). Animals were fasted for about 16 h before the experiment. Studies in this article have been carried out in accordance with the guidelines of the Animal Ethics Committee at Kyoto Pharmaceutical University. The intestine was exposed through a midline abdominal incision and flushed by passing phosphate buffered saline (PBS, pH 7.4). The remaining buffer was expelled with air and a closed loop of jejunum, ileum and colon was prepared. Segments were identified in the following method. In the small intestine, the first 10 cm of the top of the small intestine was not used, the next 10 cm was used as the jejunum and the final 10 cm was considered to be the ileum. For the large intestine, the first 3 cm of the large intestine was not used and the next 5 cm was used as the colon. The distal part of the loop was cannulated with polyethylene tubing then closed by clipping with a forceps. Drug solution (2 ml), kept at 37 °C was introduced into the loop through a cannulated opening in the proximal part of the loop, which was then closed by closing the tubing with forceps. The jugular vein was exposed and blood samples (∼0.3 ml) were collected into heparinized syringes at predetermined time intervals up until 240 min. Samples were immediately centrifuged at 10,000 rpm for 5 min to obtain the plasma fraction (100 μl), which was then kept in ice until determination. The plasma samples of CF were determined by a modified method of Masuda et al. (1986). An equal volume of 10% (w/v) Triton X-100 solution was added to each plasma sample, and samples were then determined spectrofluorometrically. The intravenous administration of an equivalent dose was carried out separately via the femoral vein. The peak concentration (C_{max}) and the time to reach the peak concentration (T_{max}) were determined directly from the plasma concentration–time curves. The area under the curve (AUC) was calculated by the trapezoidal method from zero to the final sampling time (240 min). The extent of bioavailability was calculated as follows:

$$F = \frac{\mathrm{AUC}_{(\mathrm{intestine})}}{\mathrm{AUC}_{(\mathrm{i.v.})}} \times 100.$$

2.4. Preparation of chitosan capsules

The chitosan capsules were obtained from Aicello Chemical Company Ltd. (Toyohashi, Japan). The scheme of chitosan capsule used in this study is shown in Fig. 1. The mean dimensions and weight of these capsules were 3.5 mm × 1.6 mm and about 1.0 mg respectively. Each chitosan capsule or gelatin capsule contains 0.1 mg CF and 1 mg LM. The surface of these capsules was coated with hydroxypropyl methylcellulose phthalate (HPMCP) as an enteric coating material. The HPMCP was applied to the capsule by the following procedure. First, HPMCP was dissolved in acetone/ethanol solvent (w/w = 1.1), and then the capsules were dipped in this solution to obtain capsules

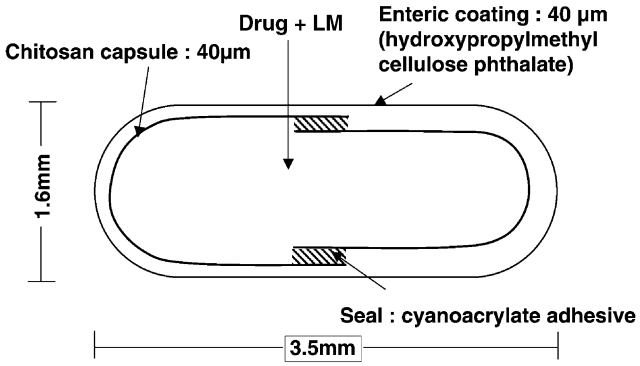

Fig. 1. Scheme of chitosan capsules for colon-specific delivery of *n*-dodecyl-β-D-maltopyranoside (LM).

coated with HPMCP. The PBS solution containing CF (1 mg/ml) and LM (10 mg/ml) was also prepared.

2.5. In vivo oral administration of chitosan capsules

Male Wistar rats (250–280 g) were fasted for more than 8 h before the experiments. The capsules (10 capsules) were orally administered to stomach via polyethylene tubing (2 mm in diameter) followed by 1 ml of distilled water under light ether anesthesia. A volume of 200 μl of distilled water was administered to rats every 3 h, and 1 ml of drug solution (1 mg/rat) was administered the same manner. Blood samples (∼0.3 ml) were collected from the jugular vein at predetermined time intervals for up to 24 h. Samples were centrifuged at 10,000 rpm and the plasma fractions were obtained.

2.6. Determination (assay) of drug

CF was determined spectrofluorometrically using microplate reader fluorescence spectrophotometer (Spectrafluor Plus, TECAN, Austria GmbH) at an excitation wavelength of 485 nm and an emission wavelength of 535 nm.

2.7. Statistical analyses

Results were expressed as the mean ± S.E. and statistical significance was assessed by the Student's *t*-test or Dunnett's test for multiple comparison with $P < 0.05$, as the minimum level of significance.

3. Results

3.1. Effect of various absorption enhancers on the intestinal absorption of 5(6)-carboxyfluorescein (CF) in different regions

Figs. 2–4 show the plasma concentration–time profiles of CF after administration of different intestinal loops of rats with or without various absorption enhancers. CF alone was poorly absorbed from all intestinal regions, although the absorption of CF from the jejunal loops was slightly greater than that in the other two regions. In the jejunum, the plasma concentrations of CF were markedly increased by the addition of NaGC, while LM, NaSal and NaCap did have a little absorption enhancing effect after administration of CF with these enhancers to the jejunal loops of rats (Fig. 2). The peak plasma concentration (C_{max}) of CF with NaGC was the greatest, but the time to reach the peak plasma concentration (T_{max}) of CF with NaGC was slightly delayed as compared with the control (Fig. 2). The plasma concentrations of CF were also remarkably enhanced by the addition of NaGC in the ileum (Fig. 3). The C_{max} value of CF with NaGC was the greatest and the T_{max} of CF with NaGC was 45 min (Fig. 3). On the other hand, a moderate absorption enhancing effect was observed for the ileal intestinal absorption of CF in the presence of NaSal, NaCap and LM (Fig. 3). The absorption of CF from the colonic region was remarkably enhanced by the addition of NaCap, LM and NaGC and the absorption enhancing effects of these enhancers for the absorption of CF were the

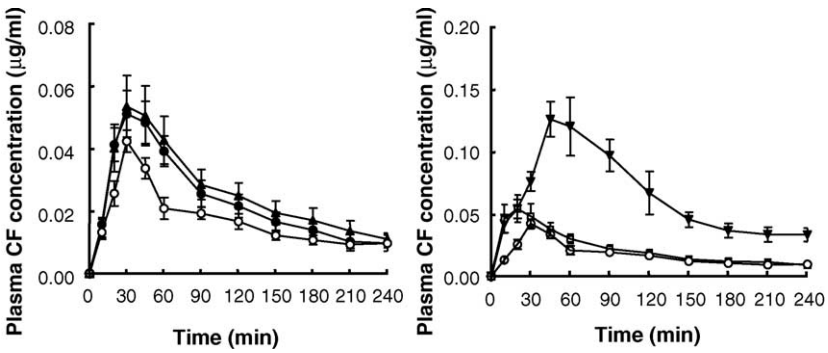

Fig. 2. Plasma concentration–time profiles of 5(6)-carboxyfluorescein (CF) following administration to the rat jejunal loops of rats in the presence or absence of various absorption enhancers. Each value represents the mean ±S.E. of five experiments. Keys: (◯) no additive, (▲) 20 mM sodium salicylate (NaSal), (●) 20 mM sodium caprate (NaCap), (▼) sodium glycocholate (NaGC), (□) 20 mM *n*-dodecyl-β-D-maltopyranoside (LM).

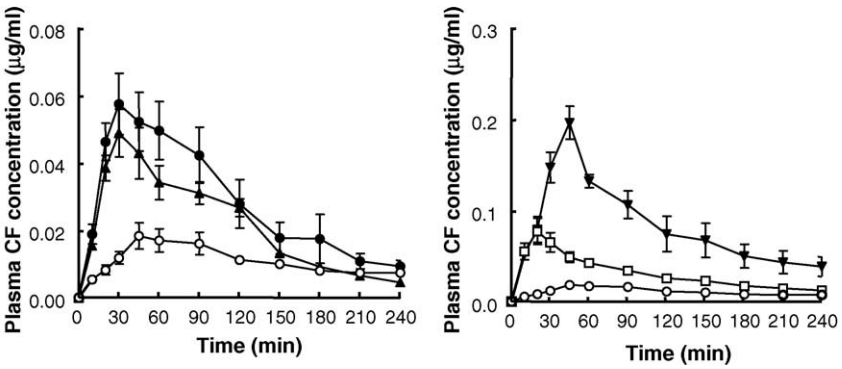

Fig. 3. Plasma concentration–time profiles of 5(6)-carboxyfluorescein (CF) following administration to the rat ileal loops of rats in the presence or absence of various absorption enhancers. Each value represents the mean ± S.E. of five experiments. Keys: (◯) no additive, (▲) 20 mM sodium salicylate (NaSal), (●) 20 mM sodium caprate (NaCap), (▼) sodium glycocholate (NaGC), (□) 20 mM *n*-dodecyl-β-D-maltopyranoside (LM).

greatest in the colon (Fig. 4). The C_{max} value of CF was greatest in the presence of LM. However, the absorption enhancing effect of NaSal in the colon was marginal for the absorption of CF (Fig. 4).

Table 1 summarizes the pharmacokinetic parameters (C_{max}, T_{max}, AUC and *F*%) of CF after administration of CF with various absorption enhancers to the different intestinal loops of rats. As shown in Table 1, in the jejunum, the AUC values and *F*% of CF in the presence of NaGC were significantly higher than those in the control, while the other three enhancers did not increase the AUC values and *F*% of CF significantly. In the ileum, the AUC value and *F*% of CF significantly increased by the addition of all the enhancers, especially in the presence of NaGC. On the other hand, the AUC value and *F*% of CF significantly increased in the colon with NaGC, NaCap and LM, especially LM. However, NaSal did not improve the AUC value and *F*% of CF significantly in the colon. Overall, the effectiveness of these absorption enhancers except for NaSal was greatest in the colon among these intestinal regions.

Fig. 5 summarizes the absorption enhancing ratios of these absorption enhancers in jejunum, ileum and colon. Absorption enhancement ratios of these absorption enhancers in different regions were estimated by calculating the enhancement ratio ($AUC_{enhancer}/AUC_{control}$) of these enhancers in the intestinal regions. It was found that all the enhancers were more effective in the colon, although we found almost

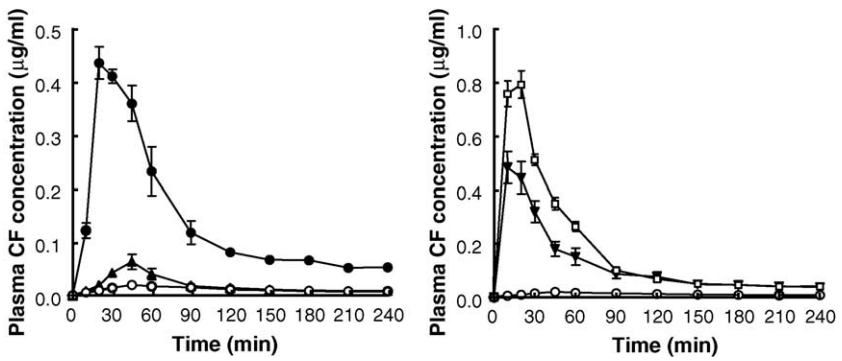

Fig. 4. Plasma concentration–time profiles of 5(6)-carboxyfluorescein (CF) following administration to the rat colonic loops of rats in the presence or absence of various absorption enhancers. Each value represents the mean ± S.E. of five experiments. Keys: (◯) no additive, (▲) 20 mM sodium salicylate (NaSal), (●) 20 mM sodium caprate (NaCap), (▼) sodium glycocholate (NaGC), (□) 20 mM *n*-dodecyl-β-D-maltopyranoside (LM).

Table 1
Pharmacokinetic parameters after intestinal absorption of 5(6)-carboxyfluorescein (0.25 mg/rat) with or without absorption enhancers

Enhancer	Concentration (mM)	C_{max} (μg/ml)	T_{max} (min)	AUC (μg/min ml^{-1})	F(%)
Jejunum					
None	–	0.042 ± 0.004	30	4.07 ± 0.48	5.43 ± 0.65
NaGC	20	0.138 ± 0.021	45	14.93 ± 2.33**	19.93 ± 3.11**
NaCap	20	0.051 ± 0.007	30	5.59 ± 0.86$^{n.s.}$	7.45 ± 1.15$^{n.s.}$
NaSal	20	0.053 ± 0.008	30	6.30 ± 1.12$^{n.s.}$	8.41 ± 1.49$^{n.s.}$
LM	20	0.054 ± 0.010	20	5.31 ± 0.83$^{n.s.}$	7.09 ± 1.10$^{n.s.}$
Ileum					
None	–	0.018 ± 0.004	45	2.62 ± 0.38	3.49 ± 0.51
NaGC	20	0.194 ± 0.018	45	19.84 ± 3.19***	26.49 ± 4.26***
NaCap	20	0.057 ± 0.009	30	6.43 ± 1.40**	8.58 ± 1.87**
NaSal	20	0.059 ± 0.007	30	5.21 ± 0.58*	6.96 ± 0.87*
LM	20	0.077 ± 0.014	20	7.41 ± 0.89***	9.89 ± 1.20***
Colon					
None	–	0.019 ± 0.002	45	2.79 ± 0.09	3.72 ± 0.12
NaGC	20	0.482 ± 0.060	10	28.41 ± 2.10***	37.93 ± 2.81***
NaCap	20	0.594 ± 0.030	30	36.76 ± 3.16***	49.07 ± 4.22***
NaSal	20	0.105 ± 0.014	45	5.17 ± 0.65$^{n.s.}$	6.91 ± 0.87$^{n.s.}$
LM	20	0.790 ± 0.030	20	42.72 ± 2.32***	57.02 ± 3.10***

Each value represents the mean ± S.E. of five experiments. (*) $P < 0.05$, (**) $P < 0.01$, (***) $P < 0.001$, significantly different compared with the control. (n.s.) Not significantly different compared with the control.

no significant regional difference in the absorption enhancing effect of NaSal. The values of enhancement effect ($AUC_{enhancer}/AUC_{control}$) were highest in the colon for all the tested enhancers except for NaSal (Fig. 5). Of all these absorption enhancers, LM and NaCap have a large regional difference in the absorption enhancing effect between the small and large intestine. Therefore, in the following studies, LM was selected as a model absorption enhancer to examine the effect of chitosan capsules on the absorption enhancing effect of LM.

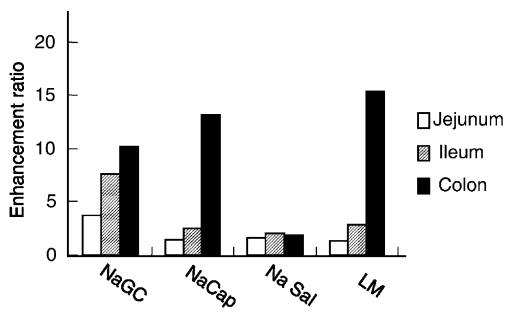

Fig. 5. Absorption enhancement ratios of various absorption enhancers in jejunum, ileum and colon. Absorption enhancement ratios of these absorption enhancers in different regions were calculated from the equation. Enhancement ratio = $(AUC_{enhancer})/(AUC_{control})$. Each value represents the mean of five experiments.

3.2. Effect of different dosage form (solution, gelatin capsules and chitosan capsules) on the intestinal absorption of CF and the absorption enhancing effect of LM

Fig. 6 indicates the comparison of absorption enhancing effect of LM for intestinal absorption of CF in different dosage forms (solution, gelatin capsules and chitosan capsules). When CF and LM were orally administered using chitosan capsules, the plasma concentration of CF was much greater than those in other dosage forms including solution and gelatin capsules, although the T_{max} value of CF using chitosan capsules was delayed as compared with the CF solution and gelatin capsule groups. Table 2 shows the pharmacokinetic parameters of CF in different dosage forms. The T_{max} values of CF using chitosan capsules and gelatin capsules were longer than that of control study due to the lag time of drug release from the capsules, especially in the case of chitosan capsules. However, the AUC values and F% of CF using chitosan capsules was significantly higher than those in solution and

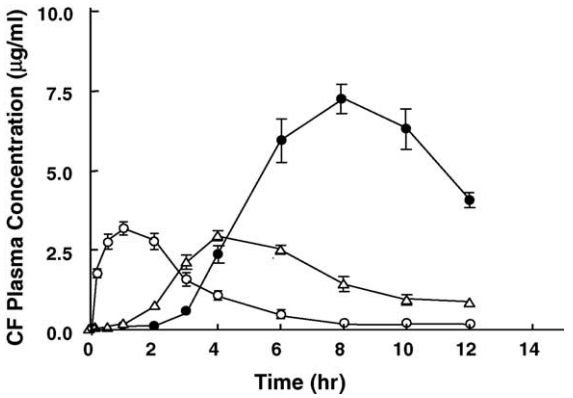

Fig. 6. Plasma concentration–time profiles of 5(6)-carboxyfluorescein (CF) following oral administration of CF with *n*-dodecyl-β-D-maltopyranoside (LM) in different formulations. Each value represents the mean ± S.E. of five experiments. Keys: (◯) CF solution with LM, (△) gelatin capsules containing CF and LM, (●) chitosan capsules containing CF and LM.

gelatin capsules. These findings suggest that LM can be delivered to the large intestinal region using chitosan capsules and this colon-specific delivery of LM might increase the absorption of CF from the colon.

Fig. 7 demonstrates the proposed mechanism for the colon-specific delivery of LM using chitosan capsules and intestinal absorption enhancing effect of LM in this study. When CF and LM were orally administered in solution or in gelatin capsules, CF was mainly absorbed from the small intestinal region where the absorption enhancing effect of LM was considerably small. Therefore, the absorption enhancing effect of LM was not clearly observed in this case. However, when CF and LM were orally administered using chitosan capsules, CF could be delivered to the large intestine where the absorption enhancing effect of LM was remarkably observed. Consequently, we observed higher intestinal absorption of CF with LM using chitosan capsules, as compared with the other dosage forms.

4. Discussion

The present study demonstrated that the absorption enhancing effects of various absorption enhancers were generally site-dependent, but their regional different effectiveness in the intestine was depend on the type of absorption enhancers. Of all these enhancers tested in this study, we found that the absorption enhancing effect of NaGC was not so site-dependent and its effectiveness was observed in the jejunum and the ileum as well as in the colon. Our previous study indicated that the transport of insulin with NaGC in the jejunum and the colon was 2.5 times and 2 times greater than that in the control group without any absorption enhancer (Uchiyama et al., 1999). That is, the transport of insulin across the jejunal membrane was enhanced by the addition of NaGC in a similar manner as was the case of colonic membrane (Uchiyama et al., 1999). Therefore, our present finding was well correlated with our previous finding.

We found almost no significant enhancement effect of NaSal in the present study, irrespective of administration site of NaSal. In a previous study, Nishihata et al. (1983) reported that sodium salicylate remarkably enhanced insulin absorption from the rectal membranes and they usually used higher concentrations of NaSal to enhance the intestinal absorption of drugs. Therefore, the negative absorption enhancing effect of NaSal in the intestine may be due to the low concentration (20 mM) of NaSal used in this study, and higher concentrations might be need to enhance the intestinal absorption of drugs.

On the other hand, we observed a remarkable absorption enhancing effect of NaCap for the intestinal absorption of CF in this study and the absorption enhancing effect of NaCap was greater in the colon than in the jejunum and ileum. Tomita et al. (1988) reported that the jejunal absorption of cefmetazole, a poorly absorbable antibiotic, was significantly enhanced by

Table 2
Pharmacokinetic parameters after oral administration of CF (1 mg/rat) with LM (10 mg/rat) in different dosage forms

Dosage from	C_{max} (μg/ml)	T_{max} (min)	AUC (μg min ml^{-1})	F%
Solution	3.17 ± 0.21	60	704.10 ± 68.32	16.94 ± 1.64
Gelatin capsule	3.31 ± 0.37	240	1216.15 ± 144.37$^{n.s.}$	29.25 ± 3.74$^{n.s.}$
Chitosan capsule	7.24 ± 0.52	480	2892.12 ± 262.13**	68.36 ± 6.30**

Each value represents the mean ± S.E. of four to five experiments. (**) $P < 0.01$, significantly different compared with the solution, (n.s.) not significantly different compared with the solution.

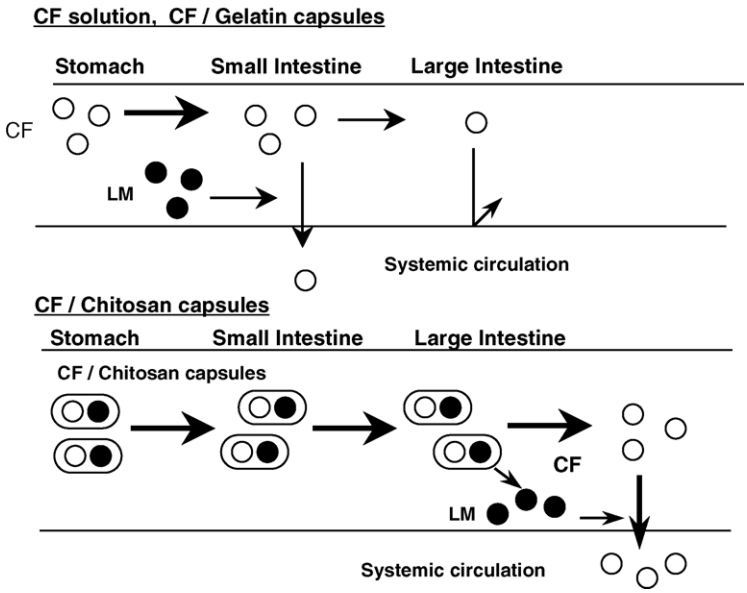

Fig. 7. Proposed mechanisms for the colon-specific delivery of LM and the absorption enhancing effect of LM with CF using chitosan capsules.

NaCap, but to a lesser extent than colonic membrane. Morishita et al. (1993) reported that the enhancement effect of NaCap on insulin absorption was greater in the colon than in the jejunum. Furthermore, our previous study indicated that the transport of insulin across the colonic membrane was enhanced by the addition of NaCap, while we observed no significant effect of NaCap in the jejunal membrane. Thus, our current findings concur with those previous findings and we conclude that the absorption enhancing effect of NaCap in the intestine was site-dependent and was greater in the large intestine than that in the small intestine.

Regarding the regional differences in the absorption enhancing effect of LM, our present study indicated that LM had a large regional difference in the absorption enhancing effect between the small and large intestine as well as NaCap. Uchiyama et al. (1999) reported that LM significantly enhanced the transport of insulin in the colon, but in the jejunum. That is, the permeability of insulin co-administered with LM in the colonic membrane was about six times higher than in the control, although its jejunal permeability was almost the same as that in the control. Similar regional differences were also observed in the effect of LM on the transport of phenol red and ebiratide across the jejunal and colonic membranes by in vitro transport studies (Sugiyama et al., 1997; Yamamoto et al., 1997). Therefore, because of the clear regional different effectiveness of LM between the small and large intestine, we selected LM as a suitable absorption enhancer to examine the effect of chitosan capsules on the intestinal absorption of CF with LM in different dosage forms in this study.

We previously demonstrated that chitosan capsules could be useful carriers for the colon-specific delivery of peptide and anti-inflammatory drugs including insulin, 5-aminosalicylic acid and ridogrel (Tozaki et al., 1997, 1999a,b, 2002). As for peptide and protein drugs, it was reported that insulin can be delivered specifically to the colon using chitosan capsules and intestinal absorption of insulin from the colon was enhanced. With respect to the anti-inflammatory drugs, chitosan capsules were effective to deliver the 5-aminosalicylic acid and ridogrel to the colon and their anti-inflammatory effects were elevated in trinitrobenzene sulfonic acid (TNBS)-induced colitis rats. It was also reported that the chitosan capsules are degraded by microflora, which are richly distributed in the colon (Tozaki et al., 1997, 1999a,b, 2002). We suppose that this character of chitosan capsules might make it a suitable to deliver a certain absorption enhancer specifically to the colon. As a result, the intestinal absorption

of CF with LM was much improved using chitosan capsules, as compared with the control. Therefore, LM might be delivered specifically to the colon using chitosan capsules where it might increase the intestinal absorption of poorly absorbable drugs after oral administration.

In conclusion, these results suggest that chitosan capsules may be useful carriers for colon-specific delivery of absorption enhancers including LM, thereby increasing the absorption enhancing effect of LM.

References

Asada, H., Douen, T., Waki, M., Adachi, S., Fujita, T., Yamamoto, A., Muranishi, S., 1995. Absorption characteristics of chemically modified-insulin derivatives with various fatty acids in the small and large intestine. J. Pharm. Sci. 84, 682–687.

Aungst, B.J., Rogers, N.J., 1988. Site dependence of absorption-promoting actions of laureth-9, Na salicylate, Na_2EDTA, and aprotinin on rectal, nasal, and buccal insulin delivery. Pharm. Res. 5, 305–308.

Kristl, J., Smid-Korbar, J., Struc, E., Schara, M., Rupprecht, H., 1993. Hydrocolloids and gels of chitosan as drug carriers. Int. J. Pharm. 99, 13–19.

Masuda, Y., Yoshikawa, H., Takada, K., Muranishi, S., 1986. The mode of enhanced enteral absorption of macromolecules by lipid-surfactant mixed micelles. I. J. Pharmacobiodyn. 9, 793–798.

Morishita, M., Morishita, I., Takayama, K., Machida, Y., Nagai, T., 1993. Site-dependent effect of aprotinin, sodium caprate, Na_2EDTA, and sodium glycocholate on intestinal absorption of insulin. Biol. Pharm. Bull. 16, 68–72.

Muranishi, S., 1985. Modification of intestinal absorption of drugs by lipoidal adjuvants. Pharm. Res. 2, 108–118.

Muranishi, S., 1990. Absorption enhancers. Crit. Rev. Ther. Drug Carrier Syst. 7, 1–33.

Nishihata, T., Rytting, J.H., Kamada, A., Higuchi, T., 1981. Enhanced intestinal absorption of insulin in rats in the presence of sodium 5-methoxysalicylate. Diabetes 30, 1065–1067.

Nishihata, T., Rytting, J.H., Kamada, A., Higuchi, T., Routh, M., Caldwell, L., 1983. Enhancement of rectal absorption of insulin using salicylates in dogs. J. Pharm. Pharmacol. 35, 148–151.

Sugiyama, T., Yamamoto, A., Kawabe, Y., Uchiyama, T., Quan, Y.-S., Muranishi, S., 1997. Effects of various absorption enhancers on the intestinal absorption of water soluble drugs by in vitro Ussing chamber method: correlation with an in situ absorption experiment. Biol. Pharm. Bull. 20, 812–814.

Tomita, M., Shiga, M., Hayashi, M., Awazu, S., 1988. Enhancement of colonic drug absorption by the paracellualr permeation route. Pharm. Res. 5, 341–346.

Tozaki, H., Komoike, J., Tada, C., Maruyama, T., Terabe, A., Suzuki, T., Yamamoto, A., Muranishi, S., 1997. Chitosan capsules for colon-specific drug delivery: improvement of insulin absorption from the rat colon. J. Pharm. Sci. 86, 1016–1021.

Tozaki, H., Fujita, T., Odoriba, T., Terabe, A., Suzuki, T., Tanaka, T., Okabe, S., Muranishi, S., Yamamoto, A., 1999a. Colon-specific delivery of R68070, a new thromboxane synthase inhibitor, using chitosan capsules: therapeutic effects against 2,4,6-trinitrobenzene sulfonic acid-induced ulcerative colitis in rats. Life Sci. 64, 1155–1162.

Tozaki, H., Fujita, T., Odoriba, T., Terabe, A., Okabe, S., Muranishi, S., Yamamoto, A., 1999b. Validation of a pharmacokinetic model of colon-specific drug delivery and the therapeutic effects of chitosan capsules containing 5-aminosalicylic acid on 2,4,6-trinitrobenzenesulphonic acid-induced colitis in rats. J. Pharm. Pharmacol. 51, 1107–1112.

Tozaki, H., Odoriba, T., Okada, N., Fujita, T., Terabe, A., Suzuki, T., Okabe, S., Muranishi, S., Yamamoto, A., 2002. Chitosan capsules for colon-specific drug delivery: enhanced localization of 5-aminosalicylic acid in the large intestine accelerates healing of TNBS-induced colitis in rats. J. Control. Release 82, 51–61.

Uchiyama, T., Yamamoto, A., Hatano, H., Fujita, T., Muranishi, S., 1996. Effectiveness and toxicity screening of various absorption enhancers in the large intestine: intestinal absorption of phenol red and protein and phospholipid release from the intestinal membrane. Biol. Pharm. Bull. 19, 1618–1621.

Uchiyama, T., Sugiyama, T., Quan, Y.S., Kotani, A., Okada, N., Fujita, T., Muranishi, S., Yamamoto, A., 1999. Enhanced permeability of insulin across the rat intestinal membrane by various absorption enhancers: their intestinal mucosal toxicity and absorption-enhancing mechanism of *n*-lauryl-β-D-maltopyranoside. J. Pharm. Pharmacol. 51, 1241–1250.

Yamamoto, A., Hayakawa, E., Kato, Y., Nishiura, A., Lee, V.H.L., 1992. A mechanistic study on enhancement of rectal permeability to insulin in the albino rabbit. J. Pharmacol. Exp. Ther. 263, 25–31.

Yamamoto, A., Uchiyama, T., Nishikawa, R., Fujita, T., Muranishi, S., 1996. Effectiveness and toxicity screening of various absorption enhancers in the rat small intestine: effects of absorption enhancers on the intestinal absorption of phenol red and the release of protein and phospholipids from the intestinal membrane. J. Pharm. Pharmacol. 48, 1285–1289.

Yamamoto, A., Okagawa, T., Kotani, A., Uchiyama, T., Shimura, T., Tabata, S., Kondo, S., Muranishi, S., 1997. Effects of different absorption enhancers on the permeation of ebiratide, an ACTH analogue, across intestinal membranes. J. Pharm. Pharmacol. 49, 1057–1061.

Yamamoto, A., Tatsumi, H., Maruyama, M., Uchiyama, T., Okada, N., Fujita, T., 2001. Modulation of intestinal permeability by nitric oxide donors: implications in intestinal delivery of poorly absorbable drugs. J. Pharmacol. Exp. Ther. 296, 84–90.

Yodoya, E., Uemura, K., Tenma, T., Fujita, T., Murakami, M., Yamamoto, A., Muranishi, S., 1994. Enhanced permeability of tetragastrin across the rat intestinal membrane and its reduced degradation by acylation with various fatty acids. J. Pharmacol. Exp. Ther. 271, 1509–1513.