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Assessment of enhancing ability of medium-chain alkyl saccharides as new absorption enhancers in rat rectum

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Summary

The rectal absorption-enhancing abilities of several medium-chain alkyl saccharides, i.e., *n*-octyl β-glycopyranoside (OG), *n*-decyl β-glucopyranoside (DG), *n*-decyl β-maltopyranoside (DM), *n*-dodecyl β-glucopyranoside (LG) and *n*-dodecyl β-maltopyranoside (LM), were evaluated by in vivo rat studies. Carboxyfluorescein (CF) was used a poorly absorbable model compound in the absorption experiments. The absorption of CF was enhanced about 3–10-fold vs the control by the addition of these compounds at their most appropriate doses; the order of efficacy was: LM > DM ≒ DG > OG > LG. LM possesses excellent properties for use as an absorption enhancer and is superior to the known absorption enhancers caprate and/or laurate. The enhancement effect of LM is reversible and readily restored to the normal level by washing out; LM is also effective in increasing the absorption of high molecular weight compounds such as FITC-dextrans, and no apparent histological change was observed in the rectal mucosa on treatment with LM. The absorption enhancing effect of the alkyl saccharides was reduced by the combined use of HCO-60 and LM whereas the effect was increased on co-administration of HCO-60 and LG. The lack of effect of the addition of Ca^{2+} and Mg^{2+} suggests that chelation can be ruled out as a possible mechanism for the absorption-enhancing action of LM.

Introduction

Considerable attention has been focussed on enhanced rectal delivery as a method of enteral administration to achieve systemic availability of many poorly absorbable therapeutic drugs (Nishihata et al., 1982; Muranishi, 1985; Rytting, 1991). It is generally accepted that drugs are absorbed from the rectum according to their various physicochemical characteristics such as ionization state, lipid-water partitioning (Hogben et al., 1959; Shanker, 1959; Kakemi et al., 1965) and molecular size. In several studies aimed at improving the bioavailability of poorly absorbable drugs that are too hydrophilic or of high molecular weight, various absorption enhancers such as

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bile salts (Windsor et al., 1961; Gibaldi, 1970), surfactants (Engel et al., 1969; Kakemi et al., 1969), anti-inflammatory agents (Yaginuma et al., 1981), enamines (Nishihata et al., 1980), salicylates (Kamada et al., 1981) and liposomes (Hemker et al., 1980) have been investigated. However, in some of these papers, the technology employed has been ineffective for achieving sufficient bioavailability and rather harmful to the intestinal mucosa (Rampton et al., 1981; Nakanishi et al., 1983, 1984; Hashida et al., 1984). We have previously reported that lipoidal adjuvants such as natural fatty acids, and their salts and monoglycerides can be used as potential and innocuous absorption enhancers (Muranishi et al., 1977, 1979; Muranishi, 1985). We have also recently reported that Azone (1-dodecylazacycloheptan-2-one) has a distinct absorption-enhancing effect on the large intestine, which is nearly equivalent to that of unsaturated long-chain fatty acids (Murakami et al., 1986b). Compounds exhibiting a high absorption-enhancing ability commonly involve a medium- or long-chain acyl group in their molecules.

Medium-chain alkyl glucosides fall within the same category of compounds, but have thus far not been studied with respect to their ability to enhance absorption. These compounds are water-soluble nonionic surfactants which are considered to be suitable for use as nontoxic and biodegradable emulsifiers in foodstuffs. Weber and Benning (1984) have reported that β-glycosidic bonds of orally administered alkyl glucosides are rapidly hydrolyzed and converted to common products of mammalian metabolism, i.e., sugars and fatty alcohols, in the intestine and the liver. Therefore, one may consider the application of alkyl saccharides via the enteral route for medical use to be sufficiently safe.

The aims of this work were to evaluate the absorption-enhancing effect of several alkyl saccharides having a hydrocarbon chain length of 8–12 carbons on the rectal absorption of carboxyfluorescein (CF) and FITC-dextrans (FDs), used previously as poorly absorbable model compounds (Yoshikawa et al., 1981; Hashida et al., 1984) in anesthetized rats, and to elucidate the mechanism of absorption enhancement.

Materials and Methods

Materials

O-*n*-Octyl-β-D-glucopyranoside (OG), *O*-*n*-decyl-β-D-glucopyranoside (DG), *O*-*n*-decyl-β-D-maltopyranoside (DM), *O*-*n*-dodecyl-β-D-glucopyranoside (LG), *O*-*n*-dodecyl-β-D-maltopyranoside (LM) and fluorescein isothiocyanate dextrans (FDs) were obtained from Sigma Chemical Co. (St. Louis, U.S.A.). The FDs used were FD-10 (MW 9000), -20 (MW 18900), -40 (MW 40500), -70 (MW 71600) and -150 (MW 154200). Polyoxy 60 caster oil (HCO-60; HLB, 14.0) was kindly supplied by Nikko Chemical Co., Ltd (Tokyo, Japan). 5(6)-Carboxyfluorescein (CF) was purchased from Eastman Kodak Co. (Rochester, NY) and used after treatment with activated charcoal in boiling ethanol followed by passage through a hydrophobic gel column (Sephadex LH-20; Pharmacia, Uppsala, Sweden) (Ralston et al., 1981). All other chemicals and reagents were commercial products of the highest available grade of purity.

Test preparations

CF and FDs were dissolved in pH 7.4 phosphate-buffered saline (Dulbecco's PBS(−), Nissui Pharmaceutical Co., Ltd, Tokyo, Japan), and each alkyl saccharide added when needed. The osmotic pressure of the preparations was checked using a Vogel model OM-801 osmometer (Giessen, Germany) and, if necessary, adjusted to isotonicity with NaCl. In some experiments, mixed micelle solutions were prepared by short sonication after mixing aqueous solutions of alkyl saccharides and HCO-60.

Animal experiments

Male Wistar albino rats weighing 230–280 g, fasted with free access to water for 16 h before experiments, were anesthetized intraperitoneally with sodium pentobarbital (40 mg/kg Somnopentyl®, Pitman-Moore, Washington, U.S.A.) and kept on a hot plate at 37 °C during the experimental period. The body temperature was monitored with a commercial digital thermometer (Omron, Tokyo, Japan), of which the probe was placed in the lower abdominal cavity. The intes-

tine was exposed through a midline incision, and a closed loop was prepared using approx. 6 cm length each of the proximal jejunum, distal ileum, proximal colon or rectum (probably containing part of the distal colon) by ligation. The test solution was introduced into the loop via a vinyl catheter immediately after ligation. The blood samples (~ 0.2 ml) were periodically collected from the carotid artery and immediately centrifuged at $5500 \times g$ for 2 min to obtain the plasma fraction. In some separate experiments, a vinyl catheter was cannulated into the thoracic lymph duct to collect lymph fluid. The doses of CF and FDs were 0.5 and 20 mg per kg rat body weight, respectively.

The intravenous administration of each drug was carried out separately at the equivalent dose via the femoral vein. The extent of bioavailability following rectal administration was calculated as the ratio of the area under the plasma concentration vs time curve (AUC) of rectal administration to that for intravenous administration.

Analytical methods

CF and FDs in plasma and lymph samples were determined by a modified method of Masuda et al. (1986) as reported previously. Plasma samples (50 μl) were obtained by centrifugation of the collected blood, and an equal volume of 10 (w/v) % Triton X-100 solution was added to the plasma samples. Collected lymph samples (0.1 ml) were mixed well with 0.6 ml of 10% trichloroacetic acid solution, followed by centrifugation at $10000 \times g$ for 3 min. The lymph supernatants thus obtained were then neutralized with 0.5 N NaOH aqueous solution. Each of the plasma and lymph solutions was appropriately diluted with 0.2 M carbonate buffered solution (pH 10) for assay of CF and Atkins-Pantin's buffered solution (0.17 M H_3BO_3, 0.17 M KCl, 0.03 M Na_2CO_3; pH 8.2) for assay of FD. The fluorescence of CF and FD was determined spectrofluorometrically using a Hitachi model 650-10S spectrofluorometer (Tokyo, Japan): the respective excitation and emission wavelengths were 520 and 490 nm for CF and 512 and 495 nm for FD.

Histological evaluation

The rectal loops were excised at 30 min after administration of the test solutions. Visual observation was performed with a light microscope on paraffin sections of rectal segments which were stained with hematoxylin and eosin after fixation in 10 (w/v) % isotonic formalin.

TABLE 1

Mean absorption values ± SE of CF after rectal delivery of CF solutions with or without absorption enhancer

Enhancer	Concentration (mM)	AUC_{0-2h} (μg min ml^{-1})	F (%)	No. of animals
None	–	2.5 ± 0.5	7.1 ± 1.3	5
OG	30	17.2 ± 0.7	48.4 ± 2.0	3
	50	19.7 ± 1.5	55.5 ± 4.3	4
DG	5	12.5	35.2	2
	10	17.8 ± 2.7	50.1 ± 7.7	4
	20	21.6 ± 1.5	60.9 ± 4.3	6
DM	5	8.0 ± 0.8	22.5 ± 2.3	6
	10	16.4 ± 1.5	46.3 ± 4.2	6
	20	22.1 ± 3.2	62.3 ± 9.0	3
LG	10	7.0 ± 2.3	19.8 ± 6.6	3
	20	9.2 ± 0.3	25.8 ± 0.8	3
LM	1	10.9 ± 1.8	30.8 ± 5.1	5
	5	23.2 ± 1.9	65.1 ± 5.3	6
	10	25.9 ± 3.3	73.0 ± 9.4	6
Caprate	50	12.5 ± 1.6	35.3 ± 4.5	3
Laurate	50	24.0 ± 3.4	67.7 ± 9.7	3
i.v.	–	35.5 ± 1.3	100	4

Results and Discussion

Effects of alkyl saccharides on CF absorption

Various medium-chain alkyl saccharides were examined in the study of adjuvant effects on rat rectal absorption of CF from pH 7.4 aqueous solution. The concentration range over which the alkyl saccharides under test, namely, OG, DG, DM, LG and LM were observed to exert an enhancement effect was found to be 1–50 mM, as shown in Table 1. Representative plots of plasma CF concentration vs time are presented in Fig. 1A. Although the plasma CF level-time curve was dependent on the concentration of administered alkyl saccharides, the curve corresponding to the highest bioavailability for CF is presented in the figure with each of the alkyl saccharides. The plasma CF concentrations were markedly increased by the addition of LM and DG, and the plasma peaks appeared very early, i.e., within 10 min after dosing. The area under the plasma CF concentration-time curve (AUC) from time 0 to 120 min was calculated on the basis of the trapezoidal rule, the results also being summarized in Table 1. The percent bioavailability (F) of each test solution was determined by comparison with the AUC values established for the i.v. route. The enhancement effects of all the compounds were found to vary in a concentration-dependent manner. By taking the value of F and that corresponding to the minimum concentration of alkyl saccharide required to produce absorption enhancement activity into account, the test compounds were found to conform to the following decreasing order of efficacy: LM > DM ≒ DG > OG > LG. Among the alkyl saccharides, LM is believed to produce the greatest degree of enhancement of CF absorption from rat rectum.

Concerning LG, the time to reach the peak plasma level of CF was delayed to 30 min after administration and the AUC value was rather

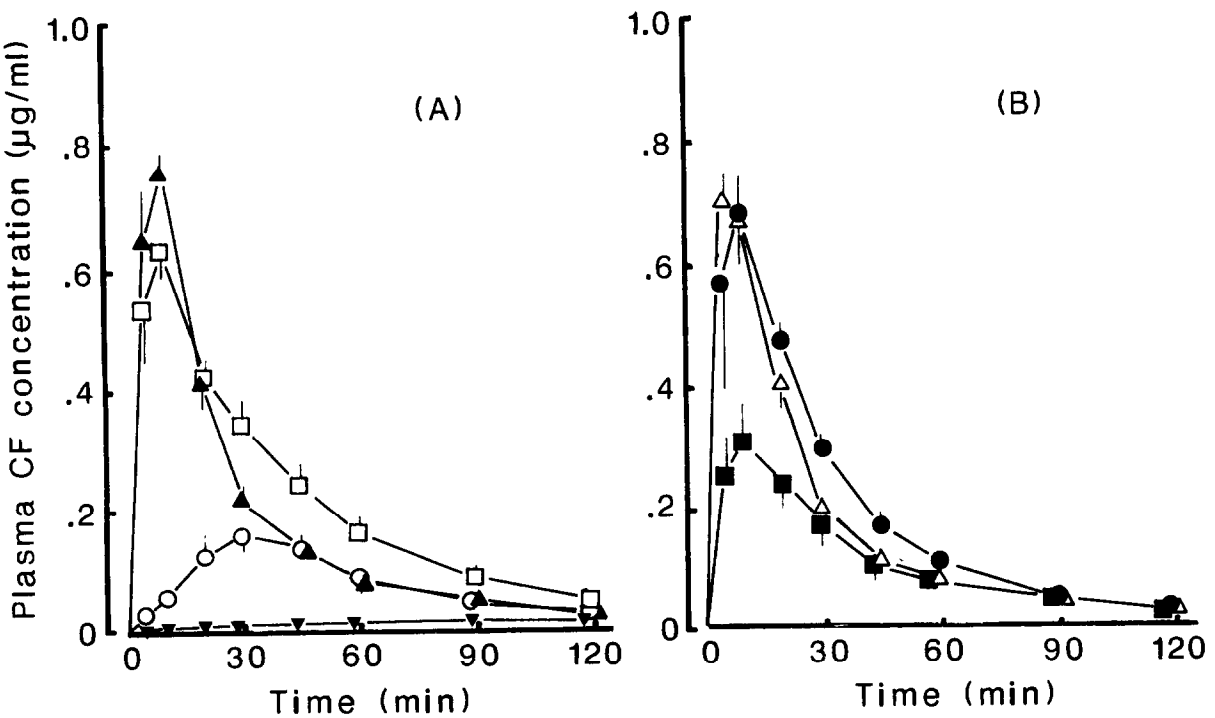

Fig. 1. Plasma level vs time profiles of CF following rectal administration with or without alkyl saccharide. (A) (▼) No additive; (□) 10 mM LM; (▲) 20 mM DG; (○) 20 mM LG. (B) In the presence of HCO-60: (■) 10 mM LM/HCO-60 (w/w, 2 : 1); (△) 20 mM DG/HCO-60 (2 : 1); (●) 20 mM LG/HCO-60 (1 : 1). Each point represents the mean ± SE of 4–5 rats.

low. The weak enhancement by LG appeared to be due to its own low aqueous solubility at concentrations above the critical micelle concentration (CMC, 0.19 mM; De Grip and Bovee-Geurts, 1979). Therefore, the influence of HCO-60 addition on the effect of LG was investigated in rats, since HCO-60 is a non-ionic surfactant and it does not affect the intestinal absorption of CF (Murakami et al., 1986b). The results are illustrated in Fig. 1B, together with the corresponding data for LM and DG. It was observed that the co-administration of HCO-60 with LG resulted in greater increases in plasma levels of CF than in the case of LG alone, reaching values comparable to those for dosing with LM alone; obviously, the AUC value approximated that for LM. In contrast, the LM-induced enhancement was clearly reduced in the presence of HCO-60, although that of DG was not affected.

It appears that the water solubility of the alkyl saccharides and the interaction with HCO-60 may be important factors in determining the extent of absorption enhancement by alkyl saccharides. It is also conceivable that HCO-60 increased the solubility of LG and hence that LG induced a greater increase in absorption enhancement. On the other hand, the possibility exists that HCO-60 might reduce the net concentration of free LM and thus lower the absorption-enhancement activity. Since LM has been shown to be one of the most effective enhancers besides the micellization interaction, it was decided on the basis of the above results to use mainly LM in subsequent studies.

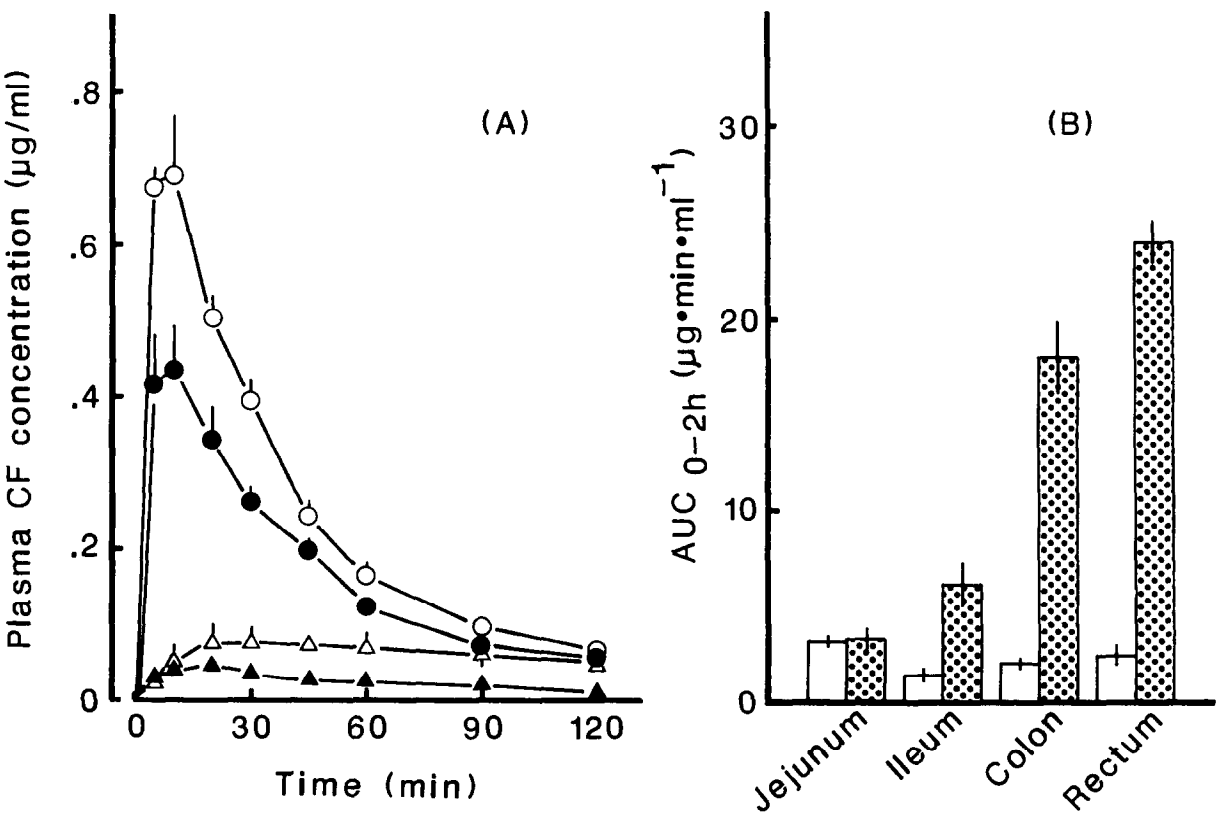

Fig. 2. Plasma levels of CF following administration at various sites of the intestinal tract with 10 mM LM (A) and histogram of AUC_{0-2h} values (B). (A) (▲) Jejunum; (△) ileum; (●) colon; (○) rectum. (B) (□) No additive; (▨) 10 mM LM. Data are expressed as means ± SE of 4–6 rats.

Intestinal regional differences in absorption enhancement by LM

The susceptibility of the intestinal mucosa to absorption enhancers is known to vary considerably according to the particular sites of the gastrointestinal tract, the lower part of which is generally more susceptible to the increase in permeability resulting from absorption enhancers (Muranishi, 1985). For the purpose of comparison with previously established absorption enhancers, the LM-induced absorption enhancement was monitored as a function of the sites within the entire intestine (from the jejunum to the rectum). As shown in Fig. 2, the AUC values observed with LM displayed considerably larger increases in the lower regions: the rectum was determined to be the most effective site. The same tendency was found to occur on administration of the other alkyl glucosides (data not shown).

The influence of alkyl saccharides, which also follows a general trend towards greater susceptibility of the lower intestinal regions, was almost identical to that reported earlier for other absorption enhancers, such as fatty acids (Muranishi et al., 1977) and Azone (Murakami et al., 1986b). The site dependence of the susceptibility to absorption enhancers may, at least in part, be attributable to distinct physicochemical differences (e.g., in lipid dynamics) between the plasma membranes of epithelial cells at those sites, and partly to physiological variations including those in the absorption and secretion of water, electrolytes and bile salts (Brasitus and Dudeja, 1988).

Recovery of mucosal barrier function

A number of absorption-enhancing adjuvants, including ionic surfactants and chelating agents, exert their effect of enhancement together with

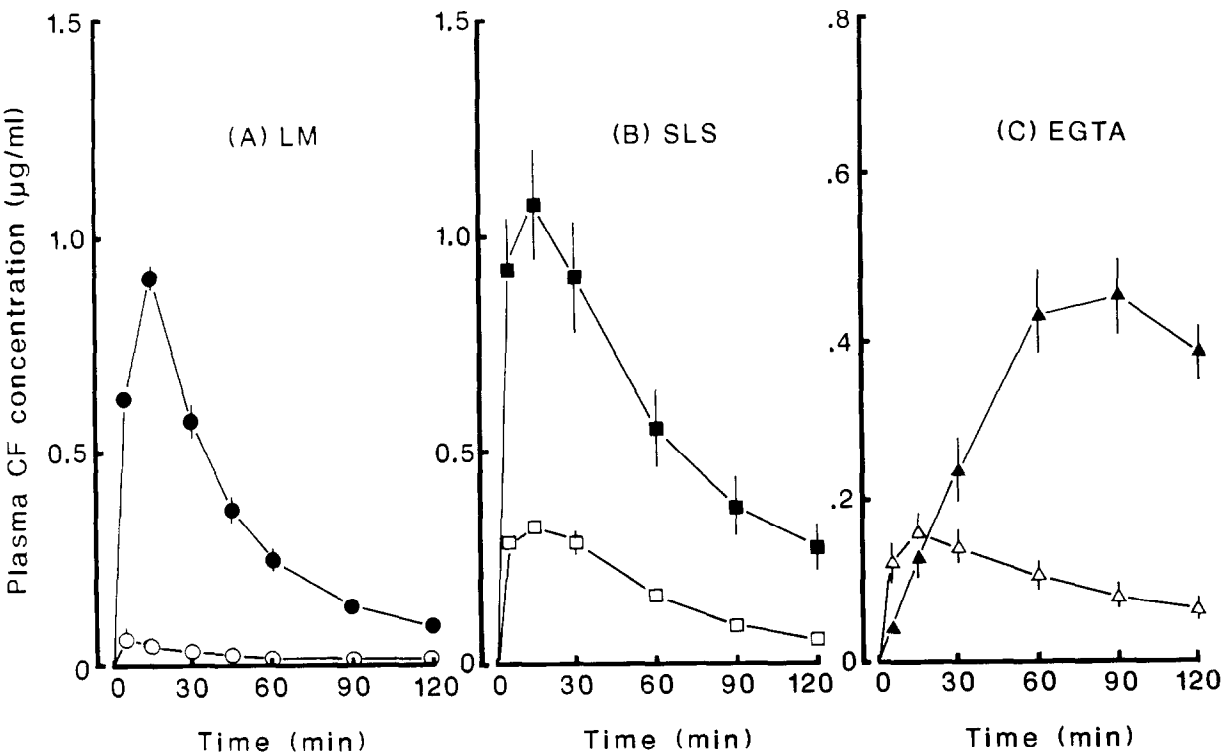

Fig. 3. Effect of in situ pretreatment with absorption enhancers (open symbols) prior to the administration of CF. Reference (closed symbols) was co-administration of absorption enhancers after pretreatment with the same medium solution. (A) (○) No additive; (●) 5 mM LM. (B) (□) No additive; (■) 10 mM SLS. (C) (△) No additive; (▲) 50 mM EGTA. The pretreatment was carried out for 30 min in the rectal loop followed by triplicate washing with 10 ml saline before dosing with CF. Each point represents the mean ± SE of at least three rats.

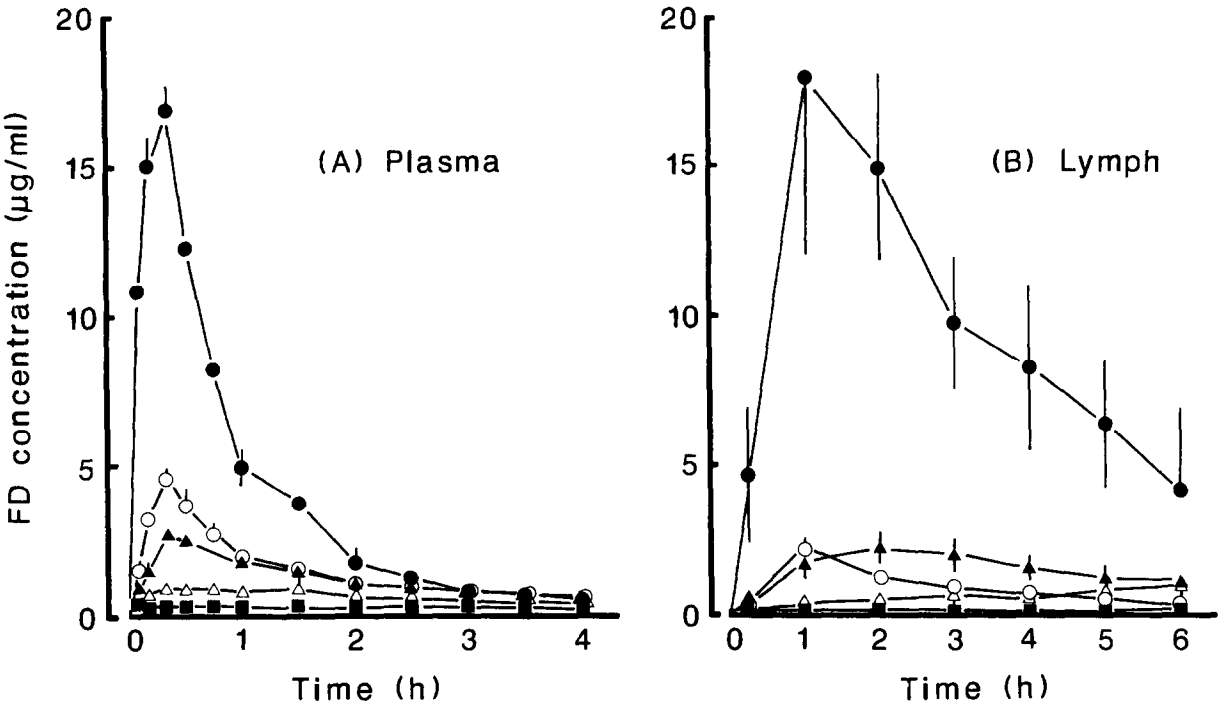

Fig. 4. Plasma (A) and lymph (B) levels of FD following rectal administration with 10 mM LM. (●) FD-10; (○) FD-20; (▲) FD-40; (△) FD-70; (■) FD-150. Each point represents the mean ± SE of 3–4 rats.

inducing irreversible mucosal damage (Nakanishi et al., 1983, 1984; Sakai et al., 1986; Muranishi, 1988). The state of a permeable membrane as induced by treatment with absorption enhancers should be quickly restored to the physiologically impermeable epithelial membrane to avoid such damage.

As sodium lauryl sulfate (SLS) and EGTA are harmful adjuvants which cause long-lasting damage in the epithelium, SLS and EGTA were used as positive controls in this study. The plasma concentrations of CF administered after treatment with 10 mM SLS and 50 mM EGTA apparently increased, although to a lower extent than in the case of concomitant administration. This suggests that irreversible mucosal trauma may be produced (Fig. 3B and C). In contrast, pretreatment with 5 mM LM failed to cause an increase in the plasma concentration of CF (Fig. 3A), indicating that the permeable state disappeared immediately following the removal of LM and that the treatment caused no irreversible damage to the barrier function of the rectal mucosa.

Morphological integrity of the rectal mucosa

In order to evaluate the histological changes induced by alkyl saccharides, microscopy was performed on the rectal mucosa following pretreatment with alkyl saccharides in the same manner as that described above.

In rectal mucosa pretreated with 10 mM LM or 50 mM OG, no overt deformation or desquamation of the epithelium was detectable, however, appreciable goblet cell vacuoles and slight edema were observed. As a result of the pretreatment of LM, some goblet cell vacuoles disappeared in the crypt, indicating that LM might stimulate mucus secretion, although in a less irritative manner compared to OG. However, such histological changes rapidly vanished within 2 h after treatment with LM. On the other hand, treatment with 10 mM SLS led to generalized

local edema which was more extensive than in the cases of LM and OG, leading to the desquamation of epithelial cells. The severity of the SLS-induced morphological changes became even greater with lapse of time. Treatment with 25 and 50 mM EGTA also caused desquamation of epithelial cells with passage of time, corresponding to an increase in mucosal permeability. On pretreatment with DG, the number of cuboidal epithelial cells was observed to increase and an edema in the mucosa was found.

Consequently, LM and OG are considered to be safe, but DG rather harmful, to the rectal mucosa. It should be borne in mind that the enhancement in mucosal permeability as a result of the alkyl saccharides was not correlated with the degree of morphological integrity of the mucosa.

Effect of LM on the bioavailability of dextrans with different molecular sizes

To obtain information on the effect of the new absorption enhancer on compounds having much higher molecular weights, we employed FITC-labeled dextrans (FDs), of average molecular weights ranging from 9000 to 154200, as polar macromolecular model compounds (Yoshikawa et al., 1981; Masuda et al., 1986).

Fig. 4A shows the plasma dextran levels vs time curves following co-administration of 10 mM LM. The enhanced plasma levels of FDs on co-administration of LM diminished with increase in the molecular size. The reduction in the efficacy of enhancement was pronounced between FD-10 and -20: the calculated systemic availabilities were 38.0% (FD-10) and 7.4% (FD-20), respectively, and that of FD-40 was estimated to be approx. 3.5%. Only small amounts of FD-70 and -150 were detected in plasma following co-administration with LM, resulting in a systemic availability determined to be less than 0.5%. These results were consistent with previous data on unsaturated fatty acid mixed micelles (Muranishi, 1984). Masuda et al. (1986) also showed, in an in vitro experiment in the presence of linoleic acid-HCO-60 mixed micelles, that FDs larger than 70 kDa in molecular mass were almost totally prevented from passing across the brush-border membrane (transcellular route) of epithelial cells to be transported, and that they might be transported via a paracellular route.

Furthermore, the lymphatic transport of FDs was studied under the same conditions as those described above (Fig. 4B). The significantly higher lymph levels of FD-40 compared to those in plasma resulted in lymph-plasma (L/P) concentration ratios of 2–3. The L/P values determined were slightly lower than those (3–7) reported previously in the case of linoleic acid-HCO-60 mixed micelles (Muranishi, 1984). No apparent lymphotropic transport was observed for FD-70 and -150. The difference in L/P ratios is probably due to variations in the rate of absorption, biochemical metabolism and disposition of absorption enhancers, and/or their association with dextrans and the formation of chylomicrons.

Effect of divalent cations on absorption enhancement by LM

Divalent cations such as Ca^{2+} and Mg^{2+} are known to play an important role in cell-cell binding at tight junctions, regulating the lipid fluidity of cell membranes, the viscosity of the mucus layer lining of the epithelial surface and the permeability of the intestine (Tidball, 1964; Allen, 1981). However it remains unknown as to whether the promoting action of absorption enhancers is correlated with the sequestration ability of Ca^{2+} and other divalent cations.

We investigated the LM-induced changes in permeation enhancement of CF in the rectal area on the addition of various concentrations of divalent cations, the results being displayed in Fig. 5. The presence of 2 mM $CaCl_2$ led to a small but significant reduction in the extent of absorption enhancement of CF induced by LM ($p < 0.05$), however, the decreases observed at 10 and 20 mM $CaCl_2$were not significant. Moreover, in the presence of 2 mM $MgSO_4$, such reduction was not seen, although the addition of 10 mM $MgSO_4$ produced a significant reduction in the absorption enhancement to about 70% of the control level. Nonetheless, summarizing the above data, neither cation appears to result in a substantial decrease and it is unlikely that a divalent cation-based mechanism, e.g., chelation, is involved in

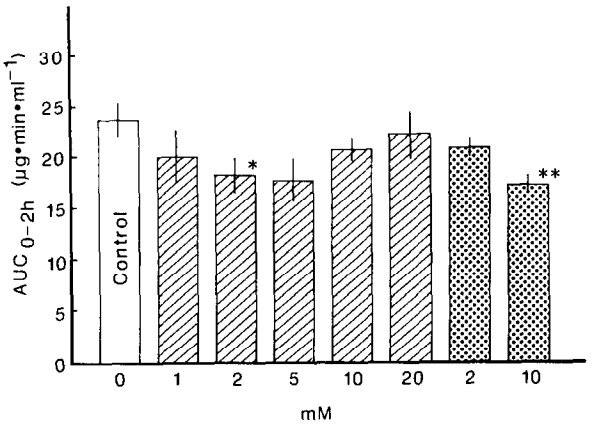

Fig. 5. Histogram of the mean $AUC_{0-2h} \pm SE$ of CF after administration with 10 mM LM and various concentrations of $CaCl_2$ (hatched bars) or $MgSO_4$ (stippled bars). Statistical comparisons were performed by Student's t-test; * $p < 0.05$; ** $p < 0.01$ vs control (without divalent cations).

the absorption enhancement induced by alkyl saccharides.

The minor reduction in absorption enhancement induced by the divalent cations was assumed to be due to changes in the viscosity of the mucous layer or in the lipid dynamics of the mucosal membrane being caused by the presence of a relatively higher concentration of Ca^{2+} in the lumen (Brasitus and Dudeja, 1988). However, our pretreatment study of mucosa with 10–50 mM $CaCl_2$ revealed no reproducible effect of reduction in the absorption enhancement caused by LM (data not shown). Therefore, it is considered that the divalent cations increase the association between LM molecules or facilitate the micellization through the lowering of the CMC. The increase in the degree of formation of micelles may lead to a smaller monomer fraction of LM and lower diffusibility of LM micelles in the mucous layer. An interesting observation was made on addition of 10 mM $CaCl_2$ to a mixed micelle solution composed of LM and HCO-60 (2:1 w/w), for which a considerable degree of inhibition of absorption enhancement was determined (Fig. 6). The turbidity of the mixed micelle solution was found to be slightly greater on visual examination. Therefore, an increase in the size of the micelles or the formation of microparticles as a result of the lower solubility of mixed micelles was suspected. The reduced degree of absorption enhancement on treatment with $CaCl_2$, as described in the present paper, was not observed for a combined solution of Azone and HCO-60 in a previous study (Murakami et al., 1986). In the latter article, a weak interaction was suggested as taking place between HCO-60 and Ca^{2+}, and thus, that a definite interaction between HCO-60 and LM should be considered to occur. Consequently, it appears that the reduction in the presence of Ca^{2+} may result from a modification in the physical state of LM micelles in the bulk within the lumen rather than being due to a biophysical change in the mucosa.

In conclusion, the present work has demonstrated that medium-chain alkyl saccharides exert an absorption-enhancing action in rat rectum. LM is both the most effective and the least harmful among those compounds tested here. The mechanism of absorption enhancement on addition of alkyl saccharides is believed to bear no relation to that of chelation by divalent cations.

The applicability of the alkyl saccharides for practical use as pharmaceutical adjuvants is currently under investigation.

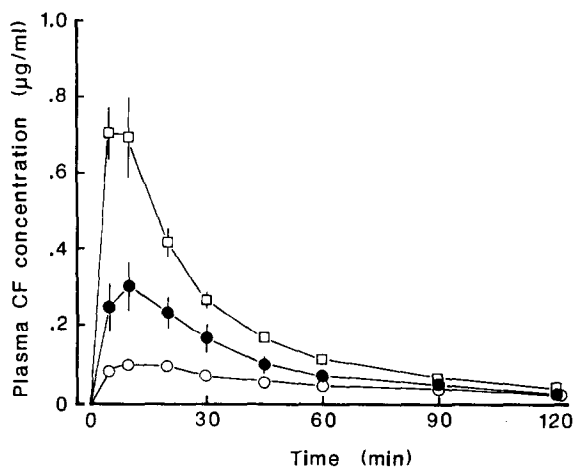

Fig. 6. Effect of $CaCl_2$ on the plasma level of CF following rectal administration of LM or LM-HCO-60 solution of CF. (□) 10 mM LM+7.5 mM NaCl; (●) 10 mM LM/HCO-60 (w/w, 2:1) (shown in Fig. 2); (○) 10 mM LM/HCO-60 (2:1)+10 mM $CaCl_2$. Each point represents the mean ± SE of 3–4 rats.

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