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Mechanism of promotion of lymphatic drug absorption by milk fat globule membrane

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Abstract

A soybean oil emulsion containing vitamin D_3, which was prepared using MFGM as an emulsifier, was administered to rats. The percentage of vitamin D_3 recovered in lymph over 12 h was 19.2%. This was reduced to 2.05% when rats were treated with colchicine, a chylomicron synthesis inhibitor, and further to only 0.27% when pancreatic ducts were ligated. When each of these MFGM micro-dispersions (micro-emulsions or mixed micelles of 40–150 nm diameter), i.e., without taurocholate (TC) or pancreatic lipase (PL); with TC alone; and with both TC and PL, was administered to pancreatic duct-ligated rats, the recovered percentages of vitamin D_3 in lymph were 3.45, 10.6 and 20.4%, respectively. These results suggest that pancreatic lipases and bile salts are critical factors for the absorption of vitamin D_3 in MFGM dosage forms and the promotive effect of MFGM takes place in the lumen of the intestine rather than the epithelial cells of the intestine. The particle morphology and physical characteristics of MFGM micro-dispersion were also analyzed by electron microscopy and electron spin resonance (ESR) spectroscopy. The results suggest that the micro-dispersion was a mixed micelle when TC was present, and as an emulsion when TC was absent. Furthermore, although the size and form of the mixed micelles with and without PL were very similar, the membrane fluidity evaluated from the ESR experiment for the micelles with PL was higher than that for the micelles without PL. This suggests that PL plays an important role in modifying micelle characteristics. It is concluded that mixed micelle formation by MFGM and bile salts in the lumen is a dominant mechanism of promotion of lymphatic drug absorption by MFGM.

Keywords: Milk fat globule membrane; Lymphatic absorption; Mixed micelle; Bile salt; Pancreatic lipase; Vitamin D_3; Colchicine

1. Introduction

The milk fat globule membrane (MFGM) is membrane material derived from the apical plasma membrane of secretory cells of the lactating mammary gland (Patton and Keenan, 1975; McPherson and Kitchen, 1983). It plays an important role in the delivery of nutrition and stabilization of the dispersion phase in milk. We have previously reported that the emulsifiability of MFGM was as strong as that of Tween 80, and

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that the lymphatic absorption of vitamin D_3 (Liu et al., 1991) and of epidermal growth factor (EGF) (Adachi et al., 1993) was enhanced by MFGM. Recently, enhancement of the intestinal absorption of drugs by MFGM has been reported, for example, vitamin A (Moriwaki et al., 1990) and insulin (Honda et al., 1992). Since MFGM, a natural product, is expected to be much less harmful especially to the intestinal epithelial cells, in contrast to synthetic surface-active agents, it would be applicable in clinical use as a novel emulsifier or absorption promoter. However, the mechanism of enhancement of intestinal absorption in the MFGM dosage form is not clear. The purpose of this study, therefore, was to provide some insight into the mechanism of promotion of lymphatic drug absorption by MFGM using vitamin D_3 as a model drug.

For the lymphatic absorption of lipophilic drugs, for example, vitamin D_3, we must consider the following two physiological processes: (1) absorption of the drug from the intestinal lumen into the epithelial cells; and (2) transfer from the epithelial cells into the mesenteric lymph. In the former process, solubilization of lipophilic drugs in micelles is considered to be the rate-limiting step in lipophilic drug absorption, and in the latter, chylomicrons play a role in transporting the lipophilic drugs from the absorption site into the lymph. Kanno (1980) reported that the MFGM was mainly composed of structural lipoprotein consisting of 44% of protein and 55% of lipid, and most lipids were present as glycerides (including mono, di- and triglycerides). Therefore, when MFGM is administered to rats orally as an emulsifier, it should be hydrolyzed from the surface of emulsion by pancreatic lipases, and the lipolytic products possibly contribute to mixed micelle formation in the presence of bile salts in the small intestine. Furthermore, Dueland et al. (1982) reported that about 90% of vitamin D_3 absorbed in lymph was associated with chylomicrons, suggesting that chylomicrons play an important role in the transport of vitamin D_3 from the absorption site into the lymph.

Following this, the present investigation was focused on: (1) the effects of lipase and bile salt on the lymphatic absorption of vitamin D_3 in MFGM dosage forms; (2) the formation of mixed micelles and the effect of the characteristics of the micelles on lymphatic absorption of vitamin D_3; and (3) whether the promotion site of lymphatic absorption by MFGM is in the lumen of the intestine or the absorption cell.

Although the promotion mechanism for drug absorption by MFGM may be different with drug variety and MFGM dosage forms, research in this subject is very interesting for biopharmaceutics, and would also be important from the viewpoint of safety and effectiveness in applying MFGM as an emulsifier or absorption promoter in clinical medicine.

2. Materials and methods

2.1. Materials

Milk fat globule membrane (MFGM) was generously supplied by Kyodo Nyugyo Co., Ltd, Tokyo. Lipase from porcine pancreas (specific activity 50 U/mg protein) and 5-doxyl stearate as a spin label reagent were purchased from Sigma Chemical Co., Ltd, USA. Other reagents were of commercial analytical grade or HPLC grade.

2.2. Preparation of MFGM emulsion and MFGM micro-dispersion

Emulsion containing soybean oil (25% v/v), vitamin D_3 (450 IU/ml, 1 IU = 0.025 μg) and MFGM or Tween 80 (27 mg/ml) as an emulsifier, in phosphate buffer (10 mM, pH 7.0), of total volume 50 ml, was prepared as follows: The mixture was homogenized with a Polytron homogenizer (type PT 10/35, Kinematica, GmbH) for 1 min and sonicated with an ultrasonicator (UR-200P, Tomy Seiko Co., Ltd, Tokyo, Japan) at 200 W output for 30 min at 4°C.

The MFGM emulsion was diluted 10-fold with a phosphate buffer or taurocholate solution (20 mM) or taurocholate solution (20 mM) plus lipase (0.741 mg/ml), and then centrifuged at 160 000 × g, for 30 min at 25°C using an ultracentrifuge (Hitachi 55P-72, Tokyo, Japan). The middle

phase, which was a transparent or semitransparent dispersion, was aspirated gently from the fixed position of the centrifuge tube and used as a micro-dispersion sample.

2.3. Animal experiments

Male Wistar rats (weighing 230–270 g) were fasted overnight but had free access to water. A right subcostal incision was carried back to the flank under anesthesia with nembutal sodium solution (40 mg/kg). A polyethylene tube (Intramedic PE 50, Clay Adams, Parsippany, NJ, USA) was inserted into the major intestinal lymphatic according to the procedures of Warshaw (1972). A drop of tissue cement (Aron Alpha, Sunkyo Co., Ltd) was applied to the hole in the lymphatic to seal it and fix the cannula in place. The accessory lymphatic was intentionally disrupted with forceps and occluded with the cement to increase the return through the cannulated main lymphatic. Another polyethylene tube (Intramedic PE 60) was inserted into the duodenum 2 cm below the pylorus, and anchored correspondingly. In some experiments, the pancreatic duct and accessory pancreatic duct were ligated with a silk suture near the entrance of the duodenum. After surgery, the rats were kept restrained in cages and allowed to recuperate for at least 3 h. Physiological saline was continuously infused via a duodenal tube at a rate of 3 ml/h (Tso et al., 1981) using a syringe infusion pump (Harvard Apparatus USA).

For intraduodenal injection (i.d.) of the drug, 1 ml of MFGM emulsion or micro-dispersion containing vitamin D_3 (7500 IU/kg) was administered to rats via the duodenal cannula. Lymphatic fluid was collected at the indicated times (i.e., 0–1, 1–2, 2–4, 4–6, 6–8, 8–10, and 10–12 h), and the amount of vitamin D_3 in the lymphatic fluid was determined by HPLC.

To examine the effect of chylomicrons on the lymphatic absorption of vitamin D_3 in lymph, a solution of colchicine was administered intravenously (5 mg/kg) 1 h before the MFGM emulsion, or Tween 80 emulsion was administered intraduodenally.

2.4. Electron microscopy

The samples of micro-dispersions were fixed with 2.5% glutaraldehyde and 1% malachite green (1:1 v/v) for 30 min, and stained in 3% uranyl acetate for 5 s as previously described (Pattinson et al., 1991). The samples were air-dried and observed with an electron microscope (JEM-200, JEOL Ltd, Japan).

2.5. Particle size evaluation

The particle size of the micro-dispersions was determined by electron micrography and quasi-elastic light scattering using Otsuka Electronics LPA 3000/3100 equipment.

2.6. ESR experiments

Preparation of the samples was as follows: 100 μl of stock solution of 5-doxyl stearate (1.25 mM) in chloroform was evaporated under N_2, then the MFGM suspension was added to the residue and mixed. The weight ratio of MFGM to the probe was 500:1. After soybean oil was added, the MFGM micro-dispersion was prepared according to the method described above.

ESR spectra were recorded on an ESR spectrometer (JEOL JM-3X, Japan) at room temperature (22 ± 2°C). The order parameter (S), used for characterization of the membrane fluidity of MFGM micro-dispersions, was calculated according to the following equation (Hubbell and McConnell, 1971):

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{zz} - 1/2(A_{xx} + A_{yy})}$$

where $A_{\parallel}$ and $A_{\perp}$ are the parallel and perpendicular hyperfine splitting parameters, respectively, and A_{xx}, A_{yy}, and A_{zz} denote the hyperfine tensor constants.

2.7. Assay methods

A high-performance liquid chromatography (HPLC) system with UV detector (Shimadzu LC-6A, Japan) was used for determining the amount

of vitamin D_3. Aliquots of 0.5 ml of sample were mixed with 1.2 ml of DMSO and extracted with 0.8 ml of hexane. After centrifugation at 3000 rpm for 10 min at 4°C, the hexane phase was evaporated under N_2 gas, and the residue was redissolved with 200 μl methanol. 50 μl of the resulting solution was injected into the HPLC system. The HPLC conditions were as follows: column, Shimpack CLC-ODS (15 cm × 6.0 mm); mobile phase, methanol-distilled water (96:64 v/v); detection wavelength, 264 nm; flow rate, 2 ml/h; and column temperature, room temperature.

Protein and fatty acid contents in micro-dispersions were determined by the method of Lowry et al. (1951) and as desribed by Duncombe (1963), respectively. The amount of phospholipid was determined according to the method of Bartlett, 1959.

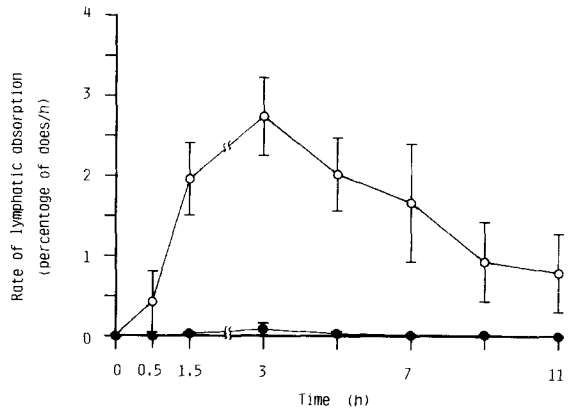

Fig. 1. Lymphatic absorption of vitamin D_3 in rat intestinal lymph after vitamin D_3 (7500 IU/kg) administration. Soybean oil-in-water emulsion was prepared using MFGM as an emulsifier and administered to pancreatic duct ligated rats (●) or normal rats (○) intraduodenally. Values are the mean ± S.D. ($n = 4$).

3. Results

3.1. Effect of ligation of pancreatic duct on lymphatic absorption of vitamin D_3 in MFGM emulsion

Absorption rate-time profiles of vitamin D_3 in lymph after i.d. administration of MFGM emulsion containing vitamin D_3 to pancreatic duct-intact or ligated rats are represented in Fig. 1. In the pancreatic duct-intact rats, the maximum absorption rate of vitamin D_3 was observed at 2–4 h after administration and the value was 2.74% of dose/h. The percentage amount of vitamin D_3 transferred into the lymphatics over 12 h of the experimental period was 19.2%. However, when the pancreatic duct was ligated, the vitamin D_3 in the MFGM dosage form was hardly absorbed. The percentage of vitamin D_3 recovered in the lymph over 12 h was only 0.27%.

3.2. Effect of various micro-dispersions on lymphatic absorption of vitamin D_3

Hofmann and Borgstrom (1962, 1964) first reported that, during establishment of fat digestion in human beings, luminal lipids, following ultracentrifugation, constituted two or three 'phases': an upper oily or emulsion phase, a middle aqueous mixed micellar phase, and, frequently, a lower precipitate phase. Since aqueous solubilities of vitamin D_3 are extremely low, mixed micelles can be considered as the sole vehicles for solubilization and transport of these from the emulsion surfaces to the enterocytes. To clarify whether the mixed micelle can be formed by MFGM with bile salts or by lipolytic products of MFGM with bile salts, the middle phase under centrifugation was submitted to succeeding experiments. The phase was designated a 'micro-dispersion' in this study. The term micro-dispersion includes mixed micelles and micro-emulsions of 40–150 nm diameter.

Three kinds of MFGM micro-dispersion dosage forms, i.e., without TC or PL, with TC alone, and with both TC and PL, all containing vitamin D_3, were administered to pancreatic duct-ligated rats. The absorption profiles of vitamin D_3 in lymph are shown in Fig. 2. The maximum absorption rate of vitamin D_3 was 0.76% of dose/h in the micro-dispersion without TC or PL, 1.46% in that with TC alone, and 3.25% in that with both TC and PL. The cumulative percentages of vitamin D_3 absorbed in the lymph were 3.45, 10.6%and 20.4% in the same micro-

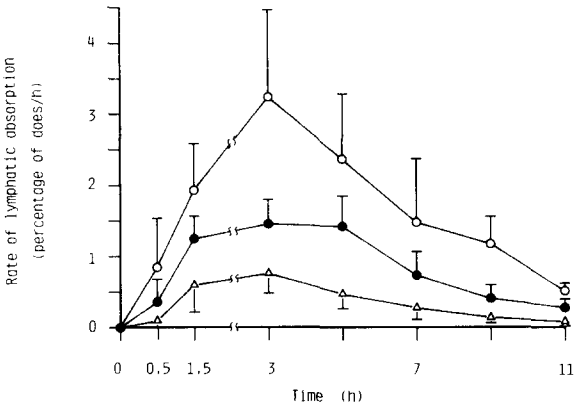

Fig. 2. Effect of various micro-dispersions on lymphatic absorption of vitamin D_3. Three kinds of micro-dispersions, i.e., without taurocholate (TC) or pancreatic lipase (PL) (△), with TC alone (●) and with both TC and PL (○), were prepared and administered to pancreatic duct ligated rats intraduodenally. Values are the mean ± S.D. ($n = 4$).

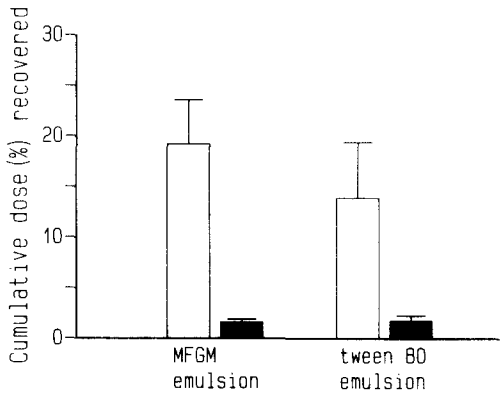

Fig. 3. Effect of colchicine on lymphatic absorption of vitamin D_3. MFGM emulsion or Tween 80 emulsion was administered to colchicine (5 mg/kg) treated (■) or control (□) rats. Values are the mean ± S.D. ($n = 4$).

dispersions, respectively. Significant differences were found among the three micro-dispersion dosage forms ($p < 0.01$).

3.3. Effect of colchicine on lymphatic absorption of vitamin D_3

Treatment of rats with colchicine to block chylomicron synthesis and transport in the enterocyte (Glickman et al., 1976) led to a significant decrease of lymphatic absorption of vitamin D_3 as shown in Fig. 3. When vitamin D_3 was administered to colchicine-untreated rats using MFGM emulsion and Tween 80 emulsion as the dosage forms, the cumulative percentages of vitamin D_3 absorbed in lymph at 12 h post-dose were 19.2% and 13.8% of the dose, respectively. When a solution of colchicine (5 mg/kg) was administered to rats intravenously before MFGM or the Tween 80 dosage form was administered, the cumulative percentages of vitamin D_3 in the lymph were significantly decreased to 2.05 and 2.23% in each case, respectively. A significant difference ($p < 0.01$) was found between colchicine-treated and untreated rats either in the MFGM dosage form or the Tween 80 dosage form.

3.4. Analysis of the characteristics of micro-dispersions

MFGM emulsion containing vitamin D_3 (1875 IU/ml) was diluted using various concentrations of TC with or without PL. After centrifugation, the concentration of vitamin D_3 in the phase of micro-dispersion was determined (Fig. 4).

The concentration of vitamin D_3 in the micro-dispersion varied as the concentration of TC increased. When TC was absent, the concentration of vitamin D_3 was 8.36 IU/ml, and when TC was

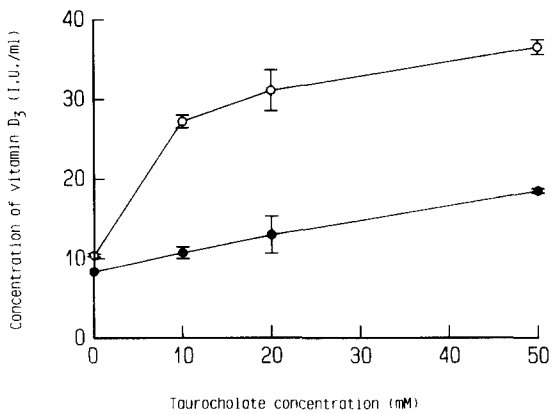

Fig. 4. Effects of TC and PL on the concentration of vitamin D_3 in micro-dispersion. MFGM micro-dispersions containing vitamin D_3 were prepared with various concentrations of TC in the presence (○) or absence (●) of PL. Values are the mean ± S.D. ($n = 3$).

present at 10, 20 or 50 mM, this increased to 10.8, 13.0 and 18.5 IU/ml, respectively. The relationship between the concentration of vitamin D_3 and TC was linear. Furthermore, when PL coexisted with TC, the concentration of vitamin D_3 in the micro-dispersion was 3–4-fold greater than that when they were absent, and 2–2.5-fold higher than when TC was present alone. However, the effect of PL on the concentration of vitamin D_3 in the micro-dispersion was very small or negligible when TC was absent.

The contents of protein, phospholipids, and fatty acid in three kinds of micro-dispersions are listed in Table 1. The contents of protein and phosphorus in micro-dispersions without TC or PL were lower than those in the other types of micro-dispersion. The content of fatty acid in the micro-dispersion with both TC and PL was 2-fold higher than that in the other micro-dispersions.

Fig. 5 demonstrates electron micrographs of three kinds of micro-dispersion. The particle morphology of the micro-dispersion without TC or PL (A) is very different from that with TC alone (B) or with both TC and PL (C). Panel A looks like emulsion particles, and B and C appear as aggregates of worm-like (or rod-like) mixed micelles with a diameter of 5–10 nm. The particle sizes of micro-dispersions from the micrographs and quasi-elastic light scattering (QELS) are summarized in Table 2.

Fig. 6 shows ESR spectra of 5-doxyl stearate incorporated into three kinds of MFGM micro-dispersions. The profiles of the spectra obtained from the micro-dispersion without TC or PL (A), with TC alone (B) and with both TC and PL (C)

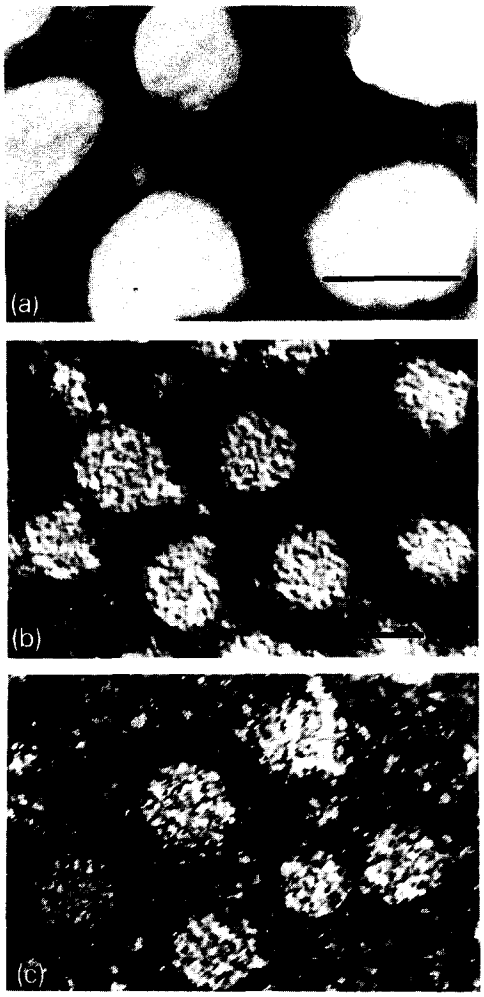

Fig. 5. Electron negative staining micrographs of micro-dispersions. (A) Micro-dispersion without TC or PL. (B) Micro-dispersion with TC alone. (C) Micro-dispersion with both TC and PL. Each bar represents 100 nm.

Table 1
Composition of MFGM micro-dispersions [a]

Composition	Without TC or PL	With TC alone	With TC and PL
Protein (mg/ml)	0.245	0.705	1.039
Phospholipid (mM)	0.048	0.506	0.503
Fatty acid (mM)	8.432	9.172	19.920

[a] Middle phase in ultracentrifuge (particle size approx. 100 nm).

Table 2
Particle size of micro-dispersions

Method	Mean particle diameter (nm)		
	Without TC or PL	With PL alone	With TC and PL
QELS [a]	150	51	40
TEM [b,c]	120 ± 25	45 ± 10	41 ± 12

[a] Quasi-elastic light scattering.
[b] Transmission electron microscopy.
[c] Mean ± S.D. (n = 20–40).

were different. The order parameters (S) calculated from the spectra were 0.611 for A, 0.544 for B and 0.440 for C. These results suggest that the properties and structures of the particle membranes in the three kinds of micro-dispersion were different, and the membrane fluidity of the micro-dispersions was in the order of C > B > A.

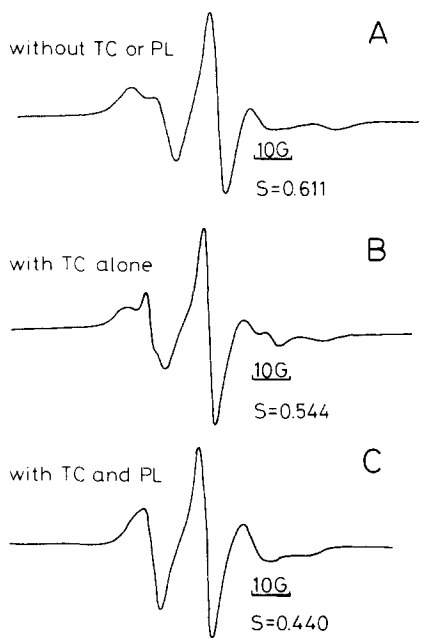

Fig. 6. ESR spectra of 5-doxyl stearate in micro-dispersions. (A) Micro-dispersion without TC or PL. (B) Micro-dispersion with TC alone. (C) Micro-dispersion with both TC and PL.

4. Discussion

We reported earlier (Liu et al., 1991) that the MFGM promoted lymphatic absorption of vitamin D_3. The recovered percentage of vitamin D_3 emulsified with MFGM in lymph was 2.5-fold greater than that of oil-in-water dispersion. When a rat bile duct was fistulated, the percentage of lymphatic absorption of vitamin D_3 was decreased to 1/32 of that of a normal rat, but this recovered completely when taurocholate was constantly infused into the duodenum (Liu et al., 1993). When a rat pancreatic duct was ligated, the recovered percentage of vitamin D_3 in the lymph also decreased (Fig. 1). These observations suggest that the promotion of lymphatic vitamin D_3 absorption by MFGM may be related to the process of the formation of mixed micelles in the intestinal lumen, since bile salts and lipases (Hernell et al., 1990; Staggers et al., 1990) play an important role in this process.

Formation of mixed micelles requires at least two kinds of surface-active materials. Since MFGM, a natural product, is a complex consisting of various kinds of proteins and lipids, the behavior of MFGM will be more complicated than that of artificial surface-active agents in the lumen of the intestine. It is possible that mixed micelles may be formed by MFGM and bile salts, or by the lipolytic products of MFGM and bile salts. To examine these possibilities, three kinds of micro-dispersion were prepared and their particle forms and membrane characteristics were examined in this study.

When TC and PL were not present, the micro-dispersion was characterized as an emulsion. This was supported by observation of electron micrograph A in Fig. 5 and ESR spectrum A in Fig. 6, where the spectrum was similar to that of the Tween 80 emulsion with a diameter of 2–3 μm (spectrum not shown). However, when TC or both TC and PL were present, the appearance of the dispersed particles looked like aggregates of worm-like (or rod-like) micelles, as shown in the electron micrograph (Fig. 5B and C). Considering that the chain length of the phospholipid, constituting the membrane, is around 2 nm, and that the section diameter of the worm-like micelles is

about 5 nm, there is no space to form oil droplets inside. This suggests, therefore, that the micro-dispersion with TC alone or with both TC and PL was mixed micelles rather than an emulsion. Although the appearance of the particles in the micro-dispersions with TC and with both TC and PL was similar, the particles' characteristics were very different: for example, the ESR spectra of 5-doxyl stearate in the micro-dispersion with TC alone and with both TC and PL were different. The spectrum in the latter was similar to that in a typical mixed-micelle system, as reported previously (Ernandes et al., 1976). The above observations suggest that PL plays an important role in modification of the characteristics of the mixed micelles.

Carey and Small (1970) reported that the amount of lipid solubilized in bile salt micelles is usually low, but when another amphiphile, for example, phospholipid, is present, mixed micelles will be formed. Mixed micelles can incorporate large amounts of insoluble molecules. As shown in Fig. 4, the amount of vitamin D_3 in micro-dispersions was directly proportional to the concentration of TC, and increased by about 2-fold in the presence of PL with TC. The increase in vitamin D_3 in the micro-dispersion is considered to be due to incorporation of lipolysis products into the bile-salt mixed micelles. In contrast, since there were no micelles formed when TC was absent, the effect of PL on the amount of vitamin D_3 in the micro-dispersion was negligible.

The presence of PL also affected the composition of the micro-dispersions (Table 1). The content of fatty acid in the micro-dispersions with TC and PL was double that in the two other kinds of micro-dispersion. Although the content of protein in the micro-dispersions with TC and PL was also higher, due to hydrolysis by PL, the proteins were separated out from the dispersed particles in the micro-dispersions, as shown in our previous study using gel filtration (Liu et al., 1993). Thus, the membrane characteristics of the micro-dispersions with both TC and PL were very much different from those of the others. The membrane fluidity of the micro-dispersion with both TC and PL, evaluated from ESR experiments, was the highest among the three kinds of micro-dispersions (Fig. 6). Westergaard and Dietschy (1976) reported that the absorption mechanism of lipid incorporated into micelles was through lipid molecules in the bulk phase in equilibrium with the micelles, not by uptake of the whole micelles. Vitamin D_3, therefore, cannot be absorbed directly in the micellar state. Release of vitamin D_3 from the carriers is a limiting process for its absorption, and this may be closely related to the membrane fluidity of micro-dispersions. When three kinds of micro-dispersions containing vitamin D_3 were administered to pancreatic duct-ligated rats, the extent of lymphatic absorption of vitamin D_3 was different in each case (Fig. 1). It is considered that the poor absorption of vitamin D_3 in micro-dispersions without TC or PL was mainly due to the large particle size (about 100 nm as shown in Table 2) and the difference in absorption between micro-dispersions with TC alone and with both TC and PL was due to the different characteristics of the membranes, which were dependent on the presence or absence of PL.

In our previous study (Liu et al., 1991), the lymphatic absorption of vitamin D_3 in MFGM emulsion dosage form was 1.4-fold higher than that in the Tween 80 emulsion. It was found that the MFGM emulsion was much more strongly subject to lipolysis by PL, and the membrane fluidity of MFGM micro-dispersion was greater than that of the Tween 80 micro-dispersion in the presence of both TC and PL (Liu et al., 1993). The results in this study suggest that the difference between the promotion effects of lymphatic absorption of vitamin D_3 by MFGM and Tween 80 may be due to the formation of MFGM-bile salt mixed micelles, which have a higher membrane fluidity. As is well known, mixed micelles play an important role in solubilization of lipophilic drugs in water milieu of gut lumen. Furthermore, a small size and large number of micelles can carry drugs across the unstirred layer to efficiently access the mucosal surface. The results in this study, therefore, strongly emphasize that promotion of lymphatic drug absorption by MFGM is closely related to formation of MFGM-bile salt mixed micelles in the lumen of the intestine.

Palin et al. (1982) and Palin and Wilson (1984) found that highly lipophilic compounds, such as 2,2-bis(*p*-chlorophenyl)-1,1,1-trichloroethane (DDT) and probocol, having a high lymphotropic selectivity, also have an affinity to chylomicrons. In this study, it was found that when vitamin D_3 was administered to colchicine-treated rats using MFGM emulsion as a dosage form, the recovered percentage of vitamin D_3 in lymph decreased to 1/40 of that in untreated rats (Fig. 3). In the case of using Tween 80 in place of MFGM, the results were the same. Since colchicine is known to block the synthesis and transfer of chylomicrons, this result suggests that chylomicrons are also necessary to transport vitamin D_3 from the absorption site to the lymph, and that MFGM itself has no effect on this process.

We investigated the process of absorption of vitamin D_3 in MFGM dosage form and tried to explain the mechanism for promotion of lymphatic drug absorption by MFGM. The results showed that MFGM, modified by lipase, contributed to formation of mixed micelles with bile salts in the intestinal lumen. The formation of mixed micelles and their characteristics affected the absorption of vitamin D_3. It is concluded that promotion of lymphatic absorption of vitamin D_3 by MFGM takes place in the lumen of the intestine, rather than in the absorption cells.

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