Regular Article

Influence of Polysorbate 60 on Formulation Properties and Bioavailability of Morin-Loaded Nanoemulsions with and without Low-Saponification-Degree Polyvinyl Alcohol

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The aim of the present study was to investigate the influence of polysorbate 60 (Tween 60) on the development of morin-loaded nanoemulsions to improve the oral bioavailability of morin. Nanoemulsions were prepared using Tween 60 and polyvinyl alcohol (PVA) as emulsifiers, and medium chain triglycerides (MCT) as the lipid base. Low-saponification-degree PVA (LL-810) was also added to stabilize dispersed droplets. MCT-LL810 nanoemulsion containing LL-810 was prepared with a reduced amount of Tween 60. However, the area under the blood concentration–time curve (*AUC*) of MCT-LL810 (0.18) nanoemulsion containing a small amount of Tween 60 did not increase because the absorption of morin was limited by P-glycoprotein (P-gp)-mediated efflux. MCT-LL810 (0.24) nanoemulsion containing a large amount of Tween 60 showed the highest *AUC*, dispersed droplets containing Tween 60 may have been transported into epithelial cells in the small intestine, and P-gp transport activity appeared to be suppressed by permeated Tween 60. Based on the plasma concentration profile, dispersed droplets in MCT-LL810 (0.24) nanoemulsion permeated more rapidly through the mucus layer and the intestinal membrane than MCT (0.24) nanoemulsion without LL-810. In conclusion, a novel feature of Tween 60 incorporated into the dispersed droplets of a nanoemulsion interacting with P-gp was demonstrated herein. Dispersed droplets in MCT-LL810 (0.24) nanoemulsion containing LL-810 permeated rapidly through the mucus layer and intestinal membrane, and Tween 60 incorporated in dispersed droplets interacted with P-gp-mediated efflux, increasing the bioavailability of morin.

Key words nanoemulsion; morin; polyvinyl alcohol; bioavailability; P-glycoprotein

Morin (2′,4′,3,5,7-pentahydroxyflavonoid) is one of the flavonoids widely found in fruits, vegetables, tea, and numerous therapeutic herbs.[1] Its wide spectrum of biological activities, including anti-inflammatory, anti-cancer, antioxidant, anti-angiogenic, anti-hypertensive, and anti-clastogenic activities, have been reported.[2–8] We previously demonstrated that a treatment with morin induced basal nitric oxide production and rapid improvements in endothelial function in diabetic mice.[9] Morin has been suggested to improve the development of endothelial dysfunction by diabetes. However, the absolute bioavailability of morin after its oral administration is very low (less than 1%), and this may be attributed to its low aqueous solubility, P-glycoprotein (P-gp)-mediated efflux, and low intestinal permeability.[10–12]

Various approaches have been attempted in order to increase the area under the blood concentration–time curve (*AUC*) of low bioavailability drugs after their oral administration. Nanoemulsion systems have received increasing attention as appropriate carriers for insoluble active compounds to increase bioavailability and modify drug release characteristics.[13–15] Improvements in the solubility of drugs into nanoemulsion components such as the oil phase and surfactants represent an effective strategy for drugs for which low water solubility dissolution is the rate limiting step in absorption and bioavailability. However, non-ionic surfactants are inhibitors of intestinal P-gp.[16,17] Regarding the well-known P-gp substrate digoxin, Tween 80 was reported to improve its intestinal absorption *in vitro* and *in vivo*.[18] Zhang *et al.* concluded that this effect was most likely caused by the inhibition of P-gp in the gastrointestinal tract and that a similar enhancement may be achieved for other P-gp substrates.[19] Hence, in nanoemulsions containing drugs with low aqueous solubility that serve as a substrate for P-gp, their pharmacokinetics after their oral administration may be modified by improving drug solubility and inhibiting P-gp using surfactants.

The present study was performed in order to investigate the influence of non-ionic surfactants on the development of morin-loaded nanoemulsions to improve the oral bioavailability of morin. We prepared morin-loaded nanoemulsions using Tween 60 or Tween 80 in a preliminary experiment. Since the dispersed droplet sizes of nanoemulsions containing Tween 60 were smaller than those containing Tween 80, Tween 60 was used as the non-ionic surfactant in the present study. Polyvinyl alcohol (PVA) has hydrophilic hydroxyl groups and hydrophobic acetic acid groups, and is the most commonly used emulsifier during the formulation of poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles.[20–22] In the present study, PVA was used as a surfactant in addition to Tween 60. Medium chain triglycerides (MCT) was used as the lipid base. Furthermore, with the aim of stabilizing dispersed droplets, nanoemulsions with the addition of low-saponification-degree PVA were also prepared. The influence of the amount of Tween 60 added on the formulation properties of morin-loaded nanoemulsions with and without low-saponification-degree PVA was investigated. The *AUC* after the oral administration of morin-loaded nanoemulsions was measured and the influence of the amount of Tween 60 added on bioavailability was investigated.

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MATERIALS AND METHODS

Materials Morin was purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Two grades of PVA, GOHSENOL™ EG-25T (EG-25T) and GOHSENX™ LL-810 (LL-810), were supplied by the Nippon Synthetic Chemical Industry Co., Ltd. (Osaka, Japan). The Saponification degree (mol%) of PVA (EG-25T) and PVA (LL-810) are 87.6 and 48.7, respectively. Equation (1) was used to calculate the degree of saponification:

$$\text{Saponification degree} = n/(n+m)\times 100 \quad (1)$$

where m and n, described in Fig. 1, denote the degree of polymerization of vinyl acetate and vinyl alcohol, respectively. Tween 60 was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). MCT (COCONAD® MT) was supplied by Kao Corporation (Tokyo, Japan). All other chemicals were obtained commercially at the purest grade available.

Preparation of MCT Nanoemulsions The compositions of MCT nanoemulsions are shown in Table 1. Regarding the amount of Tween 60 added, nanoemulsions with small (0.18 g) and large (0.24 g) amounts of Tween 60 were prepared. PVA has various grades with different characteristics in the degree of saponification and viscosity. We previously attempted to prepare emulsions containing morin using several grades of PVA. The use of PVA (EG-25T) was shown to minimize dispersed droplet sizes in emulsions.[23] Hence, in the present study, PVA (EG-25T) was used as an emulsifier.

MCT (0.18) and MCT (0.24) nanoemulsions were prepared as follows. Morin was added to MCT and mixed well. Tween 60 was added to the mixture and stirred well. The morin mixture and 1% (w/w) PVA (EG-25T) solution were separately heated to 70°C, and the PVA solution was gradually added to the morin mixture. The mixture was then stirred at 25000 rpm using a Physcotron® NS-10 homogenizer (Microtect Co., Ltd., Chiba, Japan) for 90 s, followed by sonication using a Smurt NR-50M ultrasonic homogenizer (Microtect Co., Ltd.) for 120 s.

Preparation of MCT-LL810 Nanoemulsions The compositions of MCT-LL810 nanoemulsions are shown in Table 1. As with MCT nanoemulsions, nanoemulsions with small (0.18 g) and large (0.24 g) amounts of Tween 60 were prepared. PVA contributes to the stabilization of dispersed droplets in the interface between the aqueous phase and organic phase.[24,25] In the present study, with the aim of increasing dispersed droplet stabilization, LL-810 (low-saponification-degree PVA) was added to morin-loaded nanoemulsions. LL-810 has a lower saponification degree than EG-25T. MCT-LL810 nanoemulsions were prepared as follows. Morin was dissolved in ethanol and added to MCT. LL-810 was dissolved in ethanol and added to the morin mixture. Ethanol was subsequently evaporated from the mixture *in vacuo* for 48 h. Tween 60 was added to the residue and stirred. Morin mixture and 1% (w/w) PVA (EG-25T) solution were separately heated to 70°C, and the PVA solution was gradually added to the morin mixture. The mixture was then stirred at 25000 rpm for 90 s, followed by sonication for 180 s.

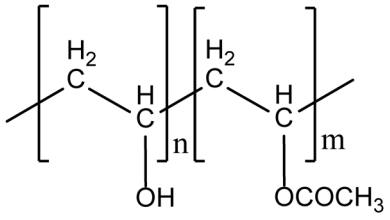

Fig. 1. Chemical Structure of PVA

Optical Microscopic Observations An optical microscope (OLYMPUS BX51, Olympus Corporation) equipped with a microscope digital camera (DP71, Olympus Corporation) was used to characterize the morphologies of the preparations. An objective lens (UplanApo, Olympus Corporation) with ×40 magnification was used.

Evaluation of Particle Size Distributions and Zeta-Potentials Morin-loaded nanoemulsions were diluted with distilled water and the particle size distribution and volume median diameter of the dispersed droplets were measured by a SALD-7100 laser diffraction particle size analyzer (Shimadzu, Kyoto, Japan). Similarly, nanoemulsions were diluted with distilled water and their zeta-potentials were assessed with a Photal ELS-Z2 zeta potential and particle size analyzer (Otsuka Electronics Co., Ltd., Osaka, Japan).

***In Vitro* Release Studies** The *in vitro* release of morin from nanoemulsions was examined using a Franz diffusion cell.[26,27] A membrane filter (Omnipore™ JVWP, Merck Millipore, pore size=0.1 μm) was mounted between the donor and receiver compartments of the Franz diffusion cell. The effective exposed area of the membrane filter was 1.77 cm^2. The receiver compartment was filled with phosphate buffer (25 mM, pH 6.8). The buffer solution in the receiver chamber was stirred with a magnetic stirrer (OCTOPUS, AS ONE Corporation, Japan) at a speed of 1500 rpm and maintained at 37°C. A morin suspension (2 mg/mL) was prepared for comparisons. In order to prepare the morin suspension, crude morin was suspended in a 1% sodium carboxymethylcellulose solution. One milliliter of morin-loaded nanoemulsions and the morin suspension were placed in the donor compartment, and at predetermined time intervals, a 0.2-mL sample of the buffer was taken and replaced with fresh buffer. The sample solution was

Table 1. Compositions of Morin-Loaded Nanoemulsions

Nanoemulsion	MCT	PVA (LL-810)	Tween 60	Morin	1% (w/w) PVA (EG-25T) Solution
	(g)				
MCT (0.18)	0.4900	—	0.1800	0.0200	9.3100
MCT (0.24)	0.4900	—	0.2400	0.0200	9.2500
MCT-LL810 (0.18)	0.4851	0.0049	0.1800	0.0200	9.3100
MCT-LL810 (0.24)	0.4851	0.0049	0.2400	0.0200	9.2500

diluted with fresh medium and absorbance at 250 nm[28] was measured using a UV spectrophotometer (UV-1800, Shimadzu Corporation, Japan) in order to evaluate the amount of the drug released.

Animal Experiments The animal protocols used in the present study were approved by the issuing committee (the Committee on the Care and Use of Laboratory Animals of Hoshi University), which is accredited by the Ministry of Education, Culture, Sports, Science, and Technology, Japan, as conforming with the Guide for the Care and Use of Laboratory Animals (Approval No. 27-047).

Male Jcl:ICR mice were obtained at 5 weeks of age. Mice were housed in temperature-controlled cages under a 12-h light-dark cycle and given free access to water and a normal chow diet for 1 week. Each mouse was fasted for 12 h before the administration of a single dose of morin-loaded nanoemulsions or the morin suspension (10 mg morin/kg) by intragastric gavage. Blood was collected from the postcava under anesthesia at predetermined times (n=3–4 for each time point) after administration, and mice were euthanized at each sampling point. Since data were collected from different mice at each sampling point, the time that the plasma concentration peak was observed (T_{max}), AUC_{0-360} and the mean residence time (MRT_{0-360}) were described using average values.

Measurement of Morin Concentrations Plasma samples were isolated from blood by centrifugation at 1000×***g*** for 15 min. The plasma concentrations of morin were measured using HPLC. The pretreatment of plasma samples was performed according to previously reported methods.[29]

HPLC was conducted using an LC-6AD pump and C-R7A chromatopac (Shimadzu, Kyoto, Japan) equipped with a Capcell Pak C_{18} MG II column (4.6×250 mm, Shiseido Co., Ltd., Tokyo, Japan) and SPD-20AV UV detector (Shimadzu). Chromatography was performed at 40°C. The injection volume was 20 μL. The mobile phase was acetonitrile: 0.2% *ortho*-phosphoric acid in water (27 : 73, v/v) at a flow rate of 1 mL/min. The detection wavelength was 250 nm. Ethylparaben was used as an internal standard. Calibration curves were obtained by a linear regression analysis of concentrations plotted against the peak area.

Statistical Analysis Tukey's test was performed for differences in median diameters and zeta-potentials. Dunnett's test was applied to compare nanoemulsions against the morin suspension in drug release profiles and plasma concentration profiles. A p-value less than 0.05 was considered to be significant.

RESULTS

Characterization of Nanoemulsions Optical micrographs of MCT nanoemulsions are shown in Fig. 2. In MCT (0.18) nanoemulsion, needle-like crystals estimated to be due to morin were included. MCT (0.24) nanoemulsion containing a large amount of Tween 60 did not include crystals. Since it was not possible to prepare MCT (0.18) nanoemulsion homogeneously, further experiments were not performed. The dispersed droplet size distribution of MCT (0.24) nanoemulsion and median diameter of dispersed droplets are shown in Fig. 3 and Table 2, respectively. MCT (0.24) nanoemulsion showed the presence of a monodisperse distribution of dispersed droplets. Regarding MCT (0.18) nanoemulsion, another preparation method was attempted, that is morin is dissolved in ethanol and added to MCT. After ethanol was subsequently evaporated from the mixture *in vacuo* for 48 h, Tween 60 was added to the residue and stirred. Thereafter, the formulation was prepared according to the MCT nanoemulsion preparing method described above. Although MCT (0.18) nanoemulsion prepared did not include crystals, dispersed droplets in MCT (0.18) nanoemulsion showed the wide size distribution, and the median diameter of dispersed droplets was 481±26 nm and significantly larger than that in other nanoemulsions.

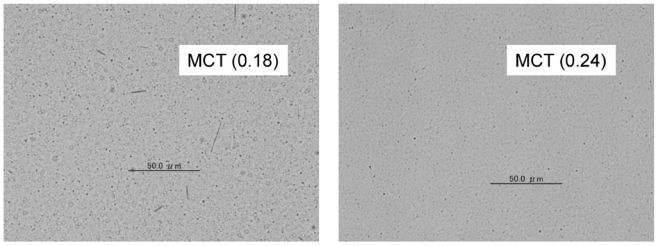

Fig. 2. Optical Micrographs of MCT Nanoemulsions (Bar=50 μm)

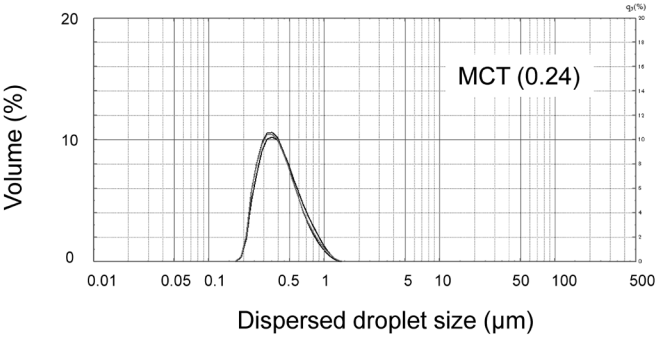

Fig. 3. Dispersed Droplet Size Distribution of MCT Nanoemulsions (n=3)

Table 2. Median Diameter and Zeta-Potential of Micelles in MCT Nanoemulsions and MCT-LL810 Nanoemulsions

Nanoemulsion	Median diameter[a] (μm)	ζ-Potential (mV)
MCT (0.24)	384±6	−31.0±1.2
MCT-LL810 (0.18)	320±9	−30.7±2.9
MCT-LL810 (0.24)	379±13	−31.9±2.1

Each value represents the mean±S.D. (n=3). *a*) Tukey's test was performed to assess differences in median diameters. MCT-LL810 (0.18) was significantly different from MCT (0.24) and MCT-LL810 (0.24) (p<0.01).

Optical micrographs of MCT-LL810 nanoemulsions are shown in Fig. 4. MCT-LL810 (0.18) and MCT-LL810 (0.24) nanoemulsions did not include crystals. The dispersed droplet size distribution of MCT-LL810 nanoemulsions and the median diameter of dispersed droplets are shown in Fig. 5 and Table 2, respectively. MCT-LL810 nanoemulsions showed the presence of a monodisperse distribution of dispersed droplets. The median diameter of dispersed droplets in MCT-LL810 (0.18) nanoemulsion was significantly smaller than those in MCT (0.24) and MCT-LL810 (0.24) nanoemulsions (p<0.01). The zeta-potential was approximately −30 mV in nanoemulsions, and did not significantly differ between the formulations (Table 2).

***In Vitro* Release of Morin from Nanoemulsions** The *in vitro* release profiles of morin from the formulations are

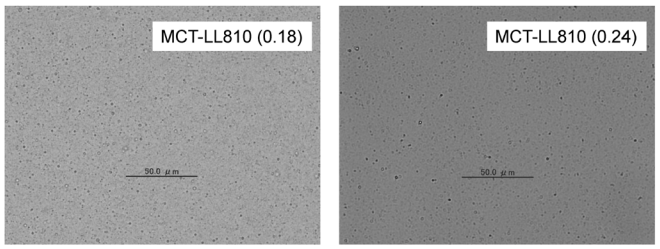

Fig. 4. Optical Micrographs of MCT-LL810 Nanoemulsions (Bar=50 μm)

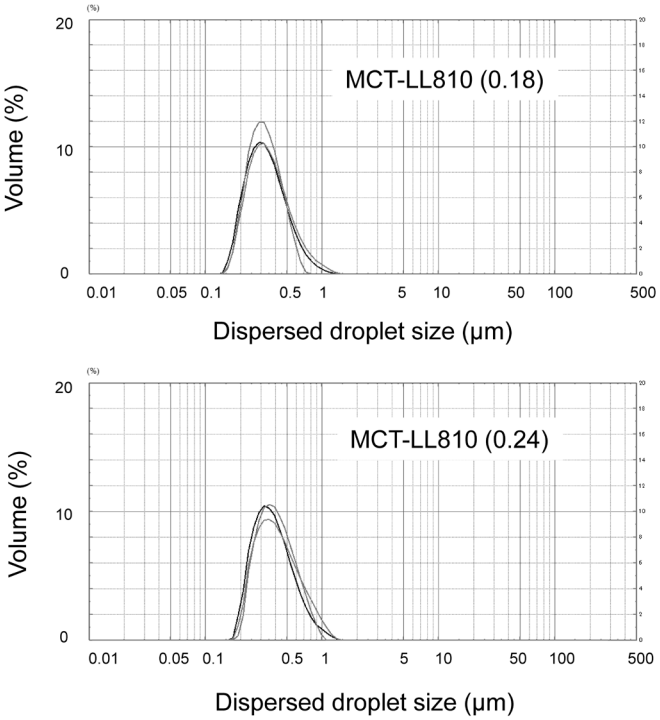

Fig. 5. Dispersed Droplet Size Distribution of MCT-LL810 Nanoemulsions (n=3)

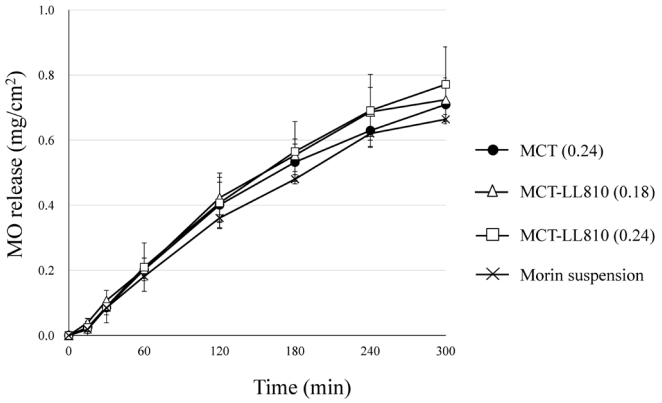

Fig. 6. Release Profiles of Morin from Nanoemulsions

Each point represents the mean±S.D. (n=3).

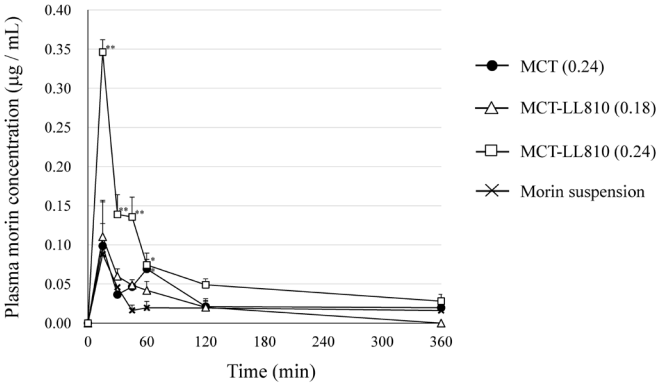

Fig. 7. Plasma Concentration–Time Curve after the Oral Administration of Morin-Loaded Nanoemulsions to Mice

Each point represents the mean±S.E. (n=3–4). Dunnett's test was performed to compare nanoemulsions>morin suspension. **p<0.01, MCT-LL810 (0.24) nanoemulsion>morin suspension. *p<0.05, MCT-LL810 (0.24) nanoemulsion and MCT (0.24) nanoemulsion>morin suspension.

shown in Fig. 6. Dunnett's test was performed to compare drug release from morin-loaded nanoemulsions against the morin suspension. No significant difference was observed in drug release between nanoemulsions and the morin suspension at any measurement point. The initial burst release was not observed in any nanoemulsions.

***In Vivo* Administration** Plasma morin concentration–time curves after the oral administration of the formulations are shown in Fig. 7. The plasma morin concentration at 60 min in MCT (0.24) nanoemulsion was significantly higher than that in the morin suspension. In MCT (0.24) nanoemulsion, plasma concentration peaks (C_{max1} and C_{max2}) were observed at 15 and 60 min (T_{max1} and T_{max2}). Although the plasma morin concentration in MCT-LL810 (0.18) nanoemulsion was not significantly higher, plasma morin concentrations at 15, 30, 45, and 60 min in MCT-LL810 (0.24) nanoemulsion were significantly higher than those in the morin suspension. The pharmacokinetic parameters measured for morin after the oral administration of the formulations are shown in Table 3. The AUC_{0-360} of MCT-LL810 (0.18) nanoemulsion was similar to that of the morin suspension. The AUC_{0-360} of MCT (0.24) nanoemulsion and MCT-LL810 (0.24) nanoemulsion were 1.4-fold and 2.9-fold higher, respectively, than those of the morin suspension. The MRT_{0-360} of MCT (0.24) nanoemulsion was greater than those of the other nanoemulsions, but was similar to that of the morin suspension. The MRT_{0-360} of MCT-LL810 (0.18) nanoemulsion was smaller than those of the other formulations, and relatively rapid drug elimination properties were observed in MCT-LL810 (0.18) nanoemulsion.

DISCUSSION

In order to improve the oral bioavailability of morin, morin-load nanoemulsions containing a large or small amount of Tween 60 were prepared, and the influence of the amount of Tween 60 added on formulation properties and bioavailability was investigated. LL-810 (low-saponification-degree PVA) was added to nanoemulsions with the aim of increasing dispersed droplet stabilization, and the influence of the addition of LL-810 on formulation properties and bioavailability was also investigated. In the preparation of nanoemulsions, the particle size of dispersed droplets was influenced by the sonication time. Particle size reductions to the micro- and often nanometer range are a widely examined option to solubilize poorly soluble drugs. Alternatively, a nanoemulsion represents one approach to achieve particle size reductions.[30] In MCT nanoemulsions, although dispersed droplet sizes decreased according to the sonication time up to 120 s, no effects were

Table 3. Pharmacokinetic Parameters of Morin after the Oral Administration of Morin-Loaded Nanoemulsions

Parameters	Morin suspension	MCT (0.24)	MCT-LL810 (0.18)	MCT-LL810 (0.24)
C_{max1} (μg/mL)*	0.088±0.069	0.099±0.029	0.110±0.045	0.346±0.016
C_{max2} (μg/mL)*	—	0.069±0.012	—	—
T_{max1} (min)**	15	15	15	15
T_{max2} (min)**	—	60	—	—
AUC_{0-360} (μg/mL·min)	7.818	10.847	7.875	22.814
MRT_{0-360} (min)	145.658	134.168	68.784	109.301

* C_{max1}: Plasma concentration of the first peak, C_{max2} : Plasma concentration of the second peak. Each value represents the mean±S.E. (n=3–4). ** T_{max1} : Time of C_{max1} observed, T_{max2} : Time of C_{max2} observed.

observed after 120 s. Hence, the sonication time of MCT nanoemulsions was set to 120 s. Since the dispersed droplet size in MCT-LL810 nanoemulsions decreased according to the sonication time up to 180 s, the sonication time of MCT-LL810 nanoemulsions was set to 180 s. Since the sonication time may be extended in MCT-LL810 nanoemulsion, LL-810 was considered to improve dispersed droplet stabilization.

In MCT nanoemulsions, it was not possible to prepare MCT (0.18) nanoemulsion homogeneously. In our previous study, morin was dissolved in Tween 60 at 115 mg/g, and dissolved in MCT at 0.43 mg/g.[23] A decrease in Tween 60 may induce a reduction in morin solubility. Since it was not possible to prepare MCT (0.18) nanoemulsion, another preparation method, which involves initially dissolving morin in ethanol, was attempted. Although MCT (0.18) nanoemulsion prepared by another method did not include crystals, dispersed droplets in MCT (0.18) nanoemulsion showed the wide size distribution, and the median diameter was significantly larger than those in other nanoemulsions. The formulation properties of MCT (0.18) nanoemulsion were inferior. Since there is a possibility that the heterogeneity in the formulation properties affects drug absorption, we abandoned to compare MCT (0.18) nanoemulsion with other formulations.

In MCT-LL810 nanoemulsion, both nanoemulsions (MCT-LL810 (0.18) and MCT-LL810 (0.24)) were prepared as homogeneous formulations. Since the nanoemulsion containing LL-810 could be prepared with a small amount of Tween60, LL-810 appears to have stabilized dispersed droplets and contributed to decreasing the amount of surfactant in the preparation of nanoemulsions. The zeta-potential of the nanoemulsions was approximately −30 mV, and charged particles exhibiting a high zeta-potential (±30 mV or more) aggregated slightly less as a result of electrostatic repulsion.[31,32]

In the release profiles of morin from nanoemulsions, a significant difference was not observed between nanoemulsions and the morin suspension. Since morin was dissolved in Tween 60 more than in MCT, existence of morin at the border between the oil phase and surfactants was presumed. Kelmann *et al.* reported that the drug release from the nanoemulsions observed *in vitro* can be explained by the fact that the drug diffusion from the oily core and interface is hindered by the aqueous medium, which acts as a barrier to drug transport due to its low solubility in water. In the same way, the free drug presents a behavior, which is typical from a dispersed system, considering a low aqueous soluble drug.[33] In the case of the nanoemulsion, both of sustained release[34,35] and rapid release[36] as compared with the drug suspension were reported. In the present study, the release profiles indicate that morin was incorporated into the border between the oil phase and surfactants and was gradually released from dispersed droplets by diffusion at a similar diffusion rate to that of the morin suspension. Since the initial burst release was not observed in nanoemulsions, the possibility that morin hardly existed in the aqueous phase was considered. The release profiles between MCT nanoemulsion and MCT-LL810 nanoemulsion did not significantly differ; therefore, LL-810 did not appear to affect the release of morin from nanoemulsions.

In the *in vivo* oral administration study, the AUC_{0-360} of MCT (0.24) nanoemulsion and MCT-LL810 (0.24) nanoemulsion were higher than that of the morin suspension. The highest AUC_{0-360} was observed in MCT-LL810 (0.24) nanoemulsion at a value that was 2.9-fold higher than that of MCT-LL810 (0.18) nanoemulsion. The difference between MCT-LL810 (0.24) nanoemulsion and MCT-LL810 (0.18) nanoemulsion was the amount of Tween 60 added. Dispersed droplet sizes were smaller in MCT-LL810 (0.18) nanoemulsion than in MCT-LL810 (0.24) nanoemulsion, and zeta-potentials and drug release properties were similar between MCT-LL810 (0.18) nanoemulsion and MCT-LL810 (0.24) nanoemulsion. Differences in formulation characteristics did not appear to affect their bioavailabilities. Hence, the suppression of P-gp-mediated efflux by Tween 60 was considered to be a reason for the higher AUC_{0-360} observed in MCT-LL810 (0.24) nanoemulsion. In intestinal transport, the interaction between a drug substance and P-gp limits transepithelial absorptive permeation, thereby limiting oral bioavailability. Several non-ionic surfactants such as Tween and poloxamers have been investigated for their ability to increase *in vitro* absorption by inhibiting efflux transporters.[37–43] Despite the large number of *in vitro* studies conducted to date, few have investigated the ability of surfactants to increase bioavailability in *in vivo* models. Nielsen *et al.* investigated the ability of Tween 20 to increase oral digoxin absorption *in vivo* after its co-administration to Sprague Dawley rats.[44] They concluded that the amount of Tween 20 required to increase the oral bioavailability of digoxin *in vivo* was 10% of the dosing volume. Since Tween 20 did not enhance digoxin absorption in Sprague Dawley mdr1a(−/−) rats, the increase observed in digoxin bioavailability *in vivo* in rats appears to be mediated by the modulation of P-gp transport activity rather than through the solubilization of digoxin. In the present study, the dosage of Tween 60 was lower than that reported by Nielsen. Since Tween 60 was incorporated into dispersed droplets in nanoemulsions, dispersed droplets containing Tween 60 may have been transported into small-intestinal epithelial cells, and the suppression of P-gp transport activity by permeated Tween 60 was considered. Although dispersed droplets in MCT-LL810 (0.18) nanoemulsion and MCT-LL810 (0.24)

nanoemulsion were transported into small-intestinal epithelial cells, permeated Tween 60 in MCT-LL810 (0.24) nanoemulsion was considered to be sufficient to suppress P-gp-mediated efflux. The AUC_{0-360} in MCT-LL810 (0.24) nanoemulsion was 2.1-fold higher than that in MCT (0.24) nanoemulsion. These results suggest that MCT (0.24) nanoemulsion was inferior to MCT-LL810 (0.24) nanoemulsion for initial permeability into small-intestinal epithelial cells. Since MCT (0.24) nanoemulsion showed a greater MRT_{0-360} value and T_{max2} at 60 min, dispersed droplets in MCT (0.24) nanoemulsion appeared to gradually permeate into small-intestinal epithelial cells as the dispersed droplets were maintained in the gastrointestinal mucus. There is a possibility that P-gp-mediated efflux was temporarily suppressed by Tween 60 incorporated in MCT (0.24) nanoemulsion. The efflux of drug might be inhibited during that time, and T_{max2} was observed after 60 min. Based on these results, dispersed droplets in MCT-LL810 (0.24) nanoemulsion may have rapidly permeated through the mucus layer and then rapidly transported into small-intestinal epithelial cells, and the suppression of P-gp-mediated efflux by permeated Tween 60 was considered.

In the present study, MCT-LL810 nanoemulsion containing LL-810 was prepared with a reduced amount of Tween 60. However, the AUC_{0-360} of MCT-LL810 (0.18) nanoemulsion did not increase because the absorption of morin is limited by P-gp-mediated efflux. The AUC_{0-360} of MCT (0.24) nanoemulsion and MCT-LL810 (0.24) nanoemulsion were higher than that of the morin suspension, and dispersed droplets containing Tween 60 may have been transported into small-intestinal epithelial cells and P-gp transport activity suppressed by permeated Tween 60. MCT-LL810 (0.24) nanoemulsion showed the highest AUC_{0-360} value, and from the plasma concentration profile, dispersed droplets in MCT-LL810 (0.24) nanoemulsion permeated more rapidly through the mucus layer and were more rapidly and strongly transported into small-intestinal epithelial cells than MCT (0.24) nanoemulsion. In conclusion, a novel feature of Tween 60 incorporated into the dispersed droplets of a nanoemulsion interacting with P-gp was demonstrated herein. Dispersed droplets in MCT-LL810 (0.24) nanoemulsion containing LL-810 rapidly permeated through the mucus layer and intestinal membrane, and Tween 60 incorporated into dispersed droplets interacted with P-gp-mediated efflux, increasing the bioavailability of morin.

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