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The novel formulation design of O/W microemulsion for improving the gastrointestinal absorption of poorly water soluble compounds

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Abstract

The design of the novel O/W microemulsion formulation, which enhances the oral bioavailability by raising the solubility of poorly water soluble compounds was examined. Using medium chain fatty acid triglyceride (MCT), diglyceryl monooleate (DGMO-C), polyoxyethylene hydrogenated castor oil 40 (HCO-40), ethanol and PBS (pH 6.8) as an oil phase, a lipophilic surfactant, a hydrophilic surfactant, a solubilizer and an aqueous phase, at the mixture ratio of 5%/1%/9%/5%/80% (w/w), respectively, the O/W microemulsion with an average particle diameter of 20 nm or less was prepared. Moreover, for nine kinds of poorly water soluble compounds, such as Ibuprofen, Ketoprofen, Tamoxifen, Testosterone, Tolbutamide and other new compounds, the solubility to water was increased from 60 to 20,000 times by this O/W microemulsion formulation. The AUCs in plasma concentration of Ibuprofen and a new compound, ER-1039, following single oral administration of these compounds as the O/W microemulsion to fasted rats were equivalent to that of solution administration or increased by nine and two times that of suspension administration, respectively. Accordingly, this novel O/W microemulsion is a useful formulation, which enhances the oral bioavailability by raising the solubility of poorly water soluble compounds.

Keywords: O/W microemulsion; Poorly water soluble compounds; Solubility; Gastrointestinal absorption; Enhancing effect; Rat

1. Introduction

The compounds with powerful pharmacological activity have been screened by remarkable progress of modern technology, such as structure-based drug

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design (SBDD), combinatorial chemistry and high throughput screening (HTS) in recent years. These compounds often show high lipophilicity and slight water solubility as the fundamental physicochemical characteristics. In the case of the orally administered formulation, the lipophilic drugs show the poor gastrointestinal absorption because of the low solubility or dissolution rate to water, so the oral bioavailability is low and its variation large. Therefore, even if it has powerful pharmacological activity, the clinical efficacy which is expected is sometimes not realized. In order to increase the clinical efficacy of the lipophilic compounds at the time of oral administration, many trials which improve the gastrointestinal absorption and raise bioavailability have been made. As the technologies for improvement, there is the compound independent system, pulverization (Atkinson et al., 1962), crystal polymorphism selection (Miyazaki et al., 1975), salt formation (Berge et al., 1977) and the compound/formulation addition system, solid dispersion (Hasegawa et al., 1985), mixed pulverization (Yamamoto et al., 1976), complex formation agent (Uekama et al., 1992), wet agent (Chiou et al., 1976) and fine particle formulation system, emulsion (Kararli et al., 1992; Myers and Stella, 1992; Palin et al., 1986; Stella et al., 1978; Toguchi et al., 1990), microemulsion (Panayiotis, 1995) and liposome (Schwendener and Schott, 1996). The technologies of pulverization and solid dispersion often produce the aggregation of the compound (Lin et al., 1968) and it is difficult for these technologies to maintain physicochemical stability of the compound (El-Banna et al., 1978), so we focused our attention on the O/W microemulsion. This formulation is the mixture of an oil, surfactants (lipophilic and hydrophilic agents) and an aqueous phase, and if needed, a solubilizer is added and it is oil in water type dispersion system which is thermodynamically stabilized by the interface phase of the surfactants. O/W microemulsion is the formulation which is expected to increase the solubility by dissolving poorly water soluble compounds into an oil phase and to enhance oral bioavailability, and it is also possible for this formulation to raise lymph directivity and to avoid hepatic first pass metabolism depending on the kind of oil. Enhancement of the gastrointestinal absorption by O/W microemulsion is actually demonstrated with various lipophilic drugs, such as a potent immuno-suppressive drug, Cyclosporin A (Gao et al., 1998; Ritschel, 1991) and an anti-malaria drug, Halofantrine (Porter et al., 1995, 1996).

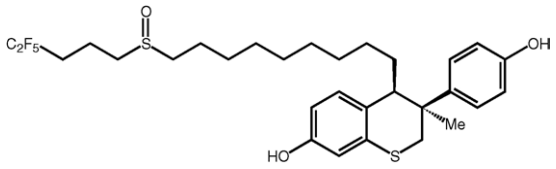

Fig. 1. Chemical structure of ER-1039.

Thus, in this research, we tried to design the novel O/W microemulsion formulation which enhances the oral bioavailability by raising the solubility of poorly water soluble compounds. First, diglyceryl monooleate (DGMO-C), polyoxyethylene hydrogenated castor oil 40 (HCO-40) and ethanol as a lipophilic surfactant, a hydrophilic surfactant and a solubilizer, respectively, were used to examine the kind of oil and the mixture ratio of surfactants which form a good O/W microemulsion according to an index for the turbidity and the particle diameter. Next, the improving effect of the solubility by this O/W microemulsion was examined for about 11 kinds of poorly water soluble compounds, such as Ibuprofen, Ketoprofen, Chloramphenicol, Testosterone, Tolbutamide, Tamoxifen, Disopyramide and other new compounds. And in these compounds, using Ibuprofen and a new compound, ER-1039 (Fig. 1) as model compounds, the plasma concentration profiles following single oral administration of each compound to rats were compared among a solution, a suspension and an O/W microemulsion, and the enhancing effect on the gastrointestinal absorption of poorly water soluble compounds by O/W microemulsion was evaluated.

2. Materials and methods

2.1. Materials

As the examined compounds, AG-041R, BO-653, ER-1039 and ER-1258 were synthesized by Chugai Pharmaceutical Co. Ltd. (Tokyo, Japan). Chloramphenicol, Disopyramide, Ibuprofen, Ketoprofen, Tamoxifen and Tolbutamide were purchased from Sigma Chemicals Company (MO, USA). Testosterone

was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan).

As the internal standard compounds, ICI182,780 were synthesized by Chugai Pharmaceutical Co. Ltd. (Tokyo, Japan).

As reagents, ethyl olivate (EOO), olive oil, DGMO-C and HCO-40 were obtained from NIKKO Chemicals Co. Ltd. (Tokyo, Japan). MCT was obtained from NOF Corp. (Tokyo, Japan). Tributyrin was purchased from Tokyo Kasei Kogyo Co. Ltd. Mineral oil and gum arabic were purchased from Sigma Chemicals Company. PEG 200, Anhydrous ethanol, ethanol and hydrochloric acid were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Sodium carboxymethyl cellulose (CMC-Na) was purchased from JUNSEI Chemical Co. Ltd. (Tokyo, Japan). Heparin sodium was purchased from Aventis Pharma Ltd. (Tokyo, Japan). Saline was purchased from Otsuka Pharmaceutical Factory Inc. (Tokushima, Japan). Dulbecco's PBS tablet was purchased from Takara Bio Inc. (Siga, Japan). PIC-A was purchased from Waters Corp. (MA, USA).

2.2. Preparation of O/W microemulsions for formulation examination (Experiment-1)

A series of O/W microemulsions were prepared in each of 25 formulations. Namely, 5% (w/w) of an oil (EOO, MCT, olive oil, tributyrin or mineral oil), 1–5% (w/w) of a lipophilic surfactant (DGMO-C), 5–1% (w/w) of a hydrophilic surfactant (HCO-40) warmed and dissolved at 50 °C and 5% (w/w) of an anhydrous ethanol were accurately weighed in individual glass vials. The components were mixed by agitating at room temperature for 10 min. Then, 80% (w/w) of PBS (pH 6.8) was added to each vial and it was agitated at room temperature for 2 h. O/W microemulsion was prepared, and the measurements of the turbidity and the particle diameter were performed.

2.3. Preparation of the sample solutions for the solubility measurement to ethanol and water of the various model compounds (Experiment-2)

The various model compounds shown in Table 1, were used. ER-1039 and ER-1258 used as the new compounds that have a steroidal structure and is an anti-estrogen receptor pure antagonist, and BO-653 and AG-041R was a new LDL oxidization control agent and a new anti-gastrin agent, respectively. These compounds were weighed 10–100 mg, respectively. The proper volume of ethanol or water was added, respectively, and the solution was agitated at room temperature one whole day and night. Then, ultrasonic processing was further performed for 10 min. After filtering the sample solution with a 0.1 μm filter, filtrate was diluted with ethanol and its absorbance was measured.

Table 1
Measuring wavelength and solubility in ethanol or distilled water at room temperature, of model compounds

Compound	Wavelength (nm)	Solubility (mg/mL)	
		In ethanol	In water
ER-1258	220	337	N.D.
BO-653	304	273	N.D.
Chloramphenicol	279	237	6.75
Testosterone	245	210	0.02
Ibuprofen	225	209	N.D.
Ketoprofen	258	207	N.D.
AG-041R	250	201	N.D.
ER-1039	230	194	N.D.
Disopyramide	264	191	1.58
Tolbutamide	232	120	0.08
Tamoxifen	243	94.6	N.D.

2.4. Preparation of model compound entrapped O/W microemulsions for solubility examination (Experiment-3)

An oil (MCT), a lipophilic surfactant (DGMO-C), a hydrophilic surfactant (HCO-40) warmed and dissolved at 50 °C, and the anhydrous ethanol solution contained the 10 kinds of each model compound except ER-1039, shown in Table 4, (compound concentration: from 100 to 900 mg/mL) were accurately weighed in glass vials at the mixture ratio of 5%/1%/9%/5% (w/w), respectively. The components were mixed by agitating at room temperature for 10 min. Then, 80% (w/w) of PBS (pH 6.8) was added to each vial and it was agitated at room temperature for 2 h, each of the model compounds entrapped O/W microemulsions were prepared, and the measurement of the compound concentration was performed. In addition, for ER-1039, the following O/W microemulsions for rat administration were used.

2.5. Gastrointestinal absorption examination of model compound entrapped formulations in rats and beagle dogs (Experiment-4)

2.5.1. Animals

Male rats of Sprague–Dawley (SD) strain (7-week-old) were purchased from SLC Japan Inc. (Shizuoka, Japan) and used for experiments at age 8 weeks after breeding for 1 week. Animals were fasted overnight prior to administration, but freely ingested drinking water. All animal experiments complied with the standards set out in the guidelines of Chugai Pharmaceutical Co. Ltd.

2.5.2. Preparation of compound entrapped formulations for administration

The formulations for administration, a solution, a suspension and O/W microemulsions, were prepared for ER-1039 and Ibuprofen, a commercial anti-inflammation medicine.

2.5.2.1. Solution. After adding ethanol to each compound and dissolving it, four volumes of 66.7% (w/w) PEG200 solution were added to one volume of the ethanol solution containing each compound and mixed. A 5 mg/mL of the ER-1039 solution and 10 mg/3 mL of the Ibuprofen solution were prepared.

2.5.2.2. Suspension. Each compound was ground and filtrated by 45–75 μm of mesh. In an agate mortar, 5% (w/v) gum arabic solution and 0.5% (w/v) CMC-Na solution was added little by little to ER-1039 and Ibuprofen, respectively. It was mixed until it became uniform, and 5 mg/mL of ER-1039 suspension and 10 mg/3 mL of Ibuprofen suspension were prepared.

2.5.2.3. O/W microemulsion. For ER-1039, an oil (EOO, MCT or mineral oil), a lipophilic surfactant (DGMO-C) and a hydrophilic surfactant (HCO-40) warmed and dissolved at 50 °C, and the anhydrous ethanol solution containing ER-1039 (concentration: 100 mg/mL) was accurately weighed in a glass vial at the mixture ratio of 5%/5%/5%/5% (w/w), respectively. Moreover, for Ibuprofen, an oil (MCT), a lipophilic surfactant (DGMO-C) and a hydrophilic surfactant (HCO-40) warmed and dissolved at 50 °C, and the anhydrous ethanol solution containing Ibuprofen (concentration: 200 mg/3 mL) was accurately weighed in a glass vial at the mixture ratio of 5%/1%/9%/5% (w/w), respectively. The components were mixed by agitating at room temperature for 10 min. Then, 80% (w/w) of PBS (pH 6.8) was added to each vial and it was agitated at room temperature for 2 h, and 5 mg/mL of ER-1039 and 10 mg/3 mL of Ibuprofen entrapped O/W microemulsions were prepared.

2.5.3. Administration and Samples collection

After anesthesia with diethylether during surgery, the femoral artery of the rat was cannulated with a polyethylene tube-50 filled with 50 IU/mL of heparin in saline. After rats were fixed in the experimental cage for blood collection and recovered from anesthesia by neglect of 2 h, the single administration of each compound into the stomach was carried out using oral sonde as a solution, a suspension and an O/W microemulsion at a dose of 20 mg/kg for ER-1039 or 10 mg/kg for Ibuprofen. The volume in the case of administration was set up so that it could become the designed dose by correcting from the measurement of each concentration of the compounds in the formulation.

Blood samples of 0.3 mL were withdrawn into non-heparinized tubes through the polyethylene tube-50 filled with sodium heparin and inserted into the femoral artery at designated time intervals (before administration and 5, 15, 30 min, 1, 2, 4, 6, 8, 24 h after administration). The collected blood sample was centrifuged at 4 °C, 15,000 rpm for 3 min, and the plasma sample was taken and transferred into capped tubes. Plasma samples were frozen and stored at −80 °C until measurement.

2.6. Stability examination of ER-1039 entrapped O/W microemulsion (Experiment-5)

2.6.1. Preservation stability

ER-1039 entrapped O/W microemulsion (formulation-1, 9 mg/mL) was prepared by the same method as shown in Section 2.5.2.3, and stored for 10 days and 2 weeks at room temperature and 4 °C, respectively. Samples were obtained on the 4th and 10th day for the room temperature and the 7th and 14th day for 4 °C after the storage, and the measurements of the ER-1039 concentration and the particle diameter were performed.

2.6.2. Stability in an acidic solution

One volume of ER-1039 entrapped O/W microemulsion (formulation-1, 9 mg/mL) prepared by the same method as shown in Section 2.5.2.3 was added to four volumes of the solution of pH 1.2 prepared by 5 M hydrochloric acid and PBS (pH 6.8) and mixed. Samples were obtained at 4 and 24 h after storage at 37 °C, and the measurements of the ER-1039 concentration and the particle diameter were performed.

2.6.3. Stability in the existence of a rat bile

One volume of ER-1039 entrapped O/W microemulsion (formulation-1, 9 mg/mL) prepared by the same method as shown in Section 2.5.2.3 was added to one volume of a fresh bile collected from rat and mixed. Samples were obtained at 4 and 24 h after storage at 37 °C, and the measurements of the ER-1039 concentration and the particle diameter were performed.

2.7. The evaluation of physical properties of O/W microemulsions

The turbidity of each O/W microemulsion was measured at 650 nm by a spectrum photometer. Each O/W microemulsion was diluted with PBS (pH 6.8) moderately and the particle diameter was measured using a dynamic light scattering particle sizer (NICOMP370).

2.8. The measurement of the solubility of various model compounds in the ethanol and water, and the model compound concentration in O/W microemulsions

The absorbance of the sample solution and that of the standard solution at the various concentrations for the compounds shown in Table 1 were measured and the solubility of each compound was calculated from the absorbance of the sample solution using the created standard curve.

Moreover, ethanol was added to each O/W microemulsion for the solubility examination and each formulation for in vivo administration and was mixed and dissolved. After diluting using the mobile phase of HPLC, the model compound concentration was measured by the absolute standard curve method or the internal standard method using HPLC system (Waters Corp.). HPLC conditions are shown in Table 2.

Table 2
(A) HPLC and (B) LC/MS/MS conditions for measurement of compound solubility in O/W microemulsion at Experiment-3, compound concentration in dosage formulation and plasma

(A) HPLC

Compound	Column	Eluent	Detected wavelength (nm)
AG-041R	YMC A-312 C18 (150 mm × 4.6 mm i.d.)	MeCN:H_2O = 6:4	UV245
BO-653	YMC A-203 C8 (250 mm × 4.6 mm i.d.)	MeCN:MeOH:H_2O = 6:30:5	UV294
ER-1039	Develosil ODS HG-5 (150 mm × 4.6 mm i.d.)	MeCN:H_2O:TFA[a] = 600:400:1	UV230
ER-1258	Develosil ODS HG-5 (150 mm × 4.6 mm i.d.)	MeCN:H_2O:TFA[a] = 750:250:1	UV220
Chloramphenicol	YMC A-312 C18 (150 mm × 4.6 mm i.d.)	50 mM AcOH:50 mM $AcONH_4$:H_2O:MeCN = 9.0:13.5:22.5:55.0	UV254
Disopyramide	YMC A-312 C18 (150 mm × 4.6 mm i.d.)	50 mM AcOH:50 mM $AcONH_4$:H_2O:MeCN = 9.0:13.5:22.5:55.0	UV254
Ibuprofen	YMC A-312 C18 (150 mm × 4.6 mm i.d.)	MeOH:H_2O:H_3PO_4 = 700:300:1	UV225
Ketoprofen	YMC A-312 C18 (150 mm × 4.6 mm i.d.)	MeCN:MeOH:H_2O = 36:54:10	UV265
Tamoxifen	YMC A-312 C18 (150 mm × 4.6 mm i.d.)	MeCN:10 mM PB[b] (pH 2.0) = 4:6	UV243
Testosterone	YMC A-312 C18 (150 mm × 4.6 mm i.d.)	MeCN:H_2O:TFA[a] = 430:570:1	UV254
Tolbutamide	YMC A-312 C18 (150 mm × 4.6 mm i.d.)	MeCN:1/15 M PB[b] (pH 7.0):PIC-A = 225:775:3	UV254

(B) LC/MS/MS

Compound	Column	Eluent	Ionization method
ER-1039	Develosil ODS UG-5 (10 mm × 1.5 mm i.d.) Develosil ODS UG-3 (100 mm × 1.5 mm i.d.)	A:MeCN:H_2O:TFA[a] = 500:500:1 B:MeCN:H_2O:TFA[a] = 900:100:1 (gradient method)	ApcI+

[a] Trifluoroacetic acid.
[b] Phosphate buffer.

Table 3
Physicochemical properties of O/W microemulsion

(1) Oil—EOO

Oil:surfactant:co-surfactant (EOO:DGMO-C:HCO-40)	Emulsification	Turbidity at 650 nm	Particle size (nm)		
			Peak-1	Peak-2	Peak-3
5:1:9	Emulsified	0.064	13		
5:2:8	Emulsified	0.083	13		
5:3:7	Emulsified	0.95		23 (96.4)	100 (3.6)
5:4:6	Emulsified	7.1		29 (95.2)	115 (4.8)
5:5:5	Emulsified	27		46 (80.0)	117 (20.0)

(2) Oil—MCT

Oil:surfactant:co-surfactant (MCT:DGMO-C:HCO-40)	Emulsification	Turbidity at 650 nm	Particle size (nm)		
			Peak-1	Peak-2	Peak-3
5:1:9	Emulsified	0.019	19		
5:2:8	Emulsified	0.024	18		
5:3:7	Emulsified	0.072		26 (98.2)	109 (1.8)
5:4:6	Emulsified	0.068		27 (99.1)	112 (0.9)
5:5:5	Emulsified	7.3		25 (98.2)	127 (1.8)

(3) Oil—tributyrin

Oil:surfactant:co-surfactant (tributyrin:DGMO-C:HCO-40)	Emulsification	Turbidity at 650 nm	Particle size (nm)		
			Peak-1	Peak-2	Peak-3
5:1:9	Emulsified	12	16 (99.7)		107 (0.3)
5:2:8	Emulsified	0.26	16 (99.8)		147 (0.2)
5:3:7	Emulsified	0.11	14 (97.7)		114 (2.4)
5:4:6	Emulsified	0.065	19 (96.8)	68 (3.2)	
5:5:5	Emulsified	0.16	8 (58.4)	28 (41.4)	140 (0.3)

(4) Oil—olive oil

Oil:surfactant:co-surfactant (olive oil:DGMO-C:HCO-40)	Emulsification	Turbidity at 650 nm	Particle size (nm)		
			Peak-1	Peak-2	Peak-3
5:1:9	Not emulsified				
5:2:8	Not emulsified				
5:3:7	Emulsified	105	12 (83.2)	63 (14.1)	239 (2.6)
5:4:6	Emulsified	97	18 (87.2)	72 (10.5)	233 (2.3)
5:5:5	Emulsified	38		20 (98.9)	223 (1.1)

(5) Oil—mineral oil

Oil:surfactant:co-surfactant (mineral oil:DGMO-C:HCO-40)	Emulsification	Turbidity at 650 nm	Particle size (nm)		
			Peak-1	Peak-2	Peak-3
5:1:9	Not emulsified				
5:2:8	Not emulsified				
5:3:7	Not emulsified				
5:4:6	Not emulsified				
5:5:5	Emulsified	45		43 (98.1)	148 (1.9)

Values in parentheses are percentages.

2.9. Assay of plasma samples

For ER-1039, after the organic solvent containing the internal standard (ICI182,780) was added and mixed with the standard plasma containing the various concentrations of ER-1039 or the sample plasma, centrifugation was carried out at 4 °C, 15,000 rpm for 5 min. After the layer of the organic solvent was removed as completely as possible and transferred into glass tubes, this was evaporated with nitrogen gas at 40 °C. The samples were reconstituted with the mobile phase of LC. The centrifugation was carried out at 4 °C, 15,000 rpm for 5 min and the supernatant was measured using LC/MS/MS (LC: UMA, Michrom BioResources Inc., Auburn, CA, MS: QUATTRO, UG Biotech., Manchester, UK) on the conditions shown in Table 2.

For Ibuprofen, after an organic solvent was added and mixed with the standard plasma containing the various concentrations of Ibuprofen or the sample plasma, the mixture was shaken at room temperature for 10 min. After centrifugation was carried out at 4 °C, 3000 rpm for 5 min, the layer of the organic solvent was removed as completely as possible and transferred into glass tubes. This was evaporated with nitrogen gas at 40 °C and reconstituted with the mobile phase of HPLC and measured using HPLC system (Waters Corp.) on the conditions shown in Table 2.

2.10. Data analysis and statistical analysis

The plasma concentration profiles of each compound were analyzed using WinNonlin (Ver.2.1, Pharsight Corporation, USA) and the pharmacokinetic parameters were obtained. The average value and the standard deviation were computed using Microsoft Excel 2000 (Microsoft Co., USA).

Moreover, the pharmacokinetic parameters of in vivo studies were expressed as the mean ± S.D. of four or five animals. The differences for each parameter to the time of suspension administration were assessed using Student's *t*-test. $p < 0.05$ was defined as statistically significant.

3. Results

3.1. The turbidity and the particle diameter of O/W microemulsion for formulation examination (Experiment-1)

The turbidity and the particle diameter of O/W microemulsions which were prepared by changing the kind of oil (EOO, MCT, olive oil, tributyrin or mineral oil) and the mixture ratio of surfactants (lipophilic:hydrophilic = from 1:9 to 5:5, w/w) are shown in Table 3. When EOO and MCT were used as oils, the formulations were well emulsified in all mixture ratios of surfactants, but as the mixture ratio of DGMO-C increased, there was a tendency for the turbidity and the particle diameter to become larger. Moreover, when tributyrin was used as an oil, the formulations were emulsified in all mixture ratios of surfactants, but the particle diameter was less than 100 nm only in DGMO-C:HCO-40 = 4:6 (w/w).

On the other hand, in the olive oil entrapped formulation, the oil and an aqueous phase was separated

Table 4
The solubility of various compounds in O/W microemulsion

Drug	Drug content in O/W microemulsion (mg/mL)	Drug solubility in water (mg/mL)	Ratio of solubility to water
BO-653	59.2	<0.003	19733<
ER-1258	16.9	<0.001	16900<
AG-041R	48.1	<0.003	16033<
Ibuprofen	21.4	<0.003	7133<
ER-1039	6.38	<0.001	6380<
Ketoprofen	15.4	<0.003	5133<
Tamoxifen	6.57	<0.003	2190<
Testosterone	6.15	0.02	308
Tolbutamide	5.47	0.08	68
Disopyramide	15.5	1.58	10
Chloramphenicol	10.6	6.75	1.6

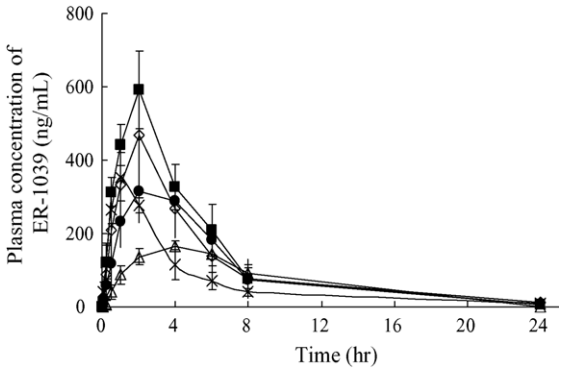

Fig. 2. Plasma concentration profile of ER-1039 following single oral administration of ER-1039 to fasted male rats as solution (●), suspension (△) and three kinds of O/W microemulsion ((■) MCT entrapped; (□) EOO entrapped; (×) mineral oil entrapped) at a dose of 20 mg/kg. Data are presented as mean ± S.D. of four or five animals.

in more than 8% of HCO-40 and a uniform emulsion was not formed. In every emulsion, the turbidity was large, and the particle diameter was not 100 nm or less. In the mineral oil entrapped formulation, the oil and an aqueous phase was separated in more than 6% of HCO-40 and a uniform emulsion was not prepared, but an emulsion was uniformly formed only in 5% of the concentration.

3.2. The solubility of various model compounds in the ethanol and water (Experiment-2)

The solubility of the examined compounds in ethanol, the solubilizer in an O/W microemulsion formulation and water is shown in Table 1. Eleven kinds of every compound dissolved well in ethanol and had a solubility of about 100 mg/mL or more. Chloramphenicol and Disopyramide dissolved also in water.

3.3. The solubility of each model compound in O/W microemulsion (Experiment-3)

The solubility of 11 kinds of each model compound at the time of making an O/W microemulsion is shown in Table 4. Although the solubility of Chloramphenicol and Disopyramide only increased 1.6 and 10 times, respectively, for nine kinds of other compounds, the solubility that was from 60 to 20,000 times that in water was obtained.

3.4. Gastrointestinal absorption of model compound (ER-1039 or Ibuprofen) entrapped formulations in rats (Experiment-4)

3.4.1. ER-1039

The plasma concentration profile and pharmacokinetic parameters of ER-1039 following single oral administration of ER-1039 to fasted rats as a suspension, a solution and three kinds of O/W microemulsions at a dose of 20 mg/kg are shown in Fig. 2 and Table 5, respectively. Moreover, the physical properties (ER-1039 concentration in each formulation and the particle diameter) are shown in Table 6. When MCT was used as an oil, the Gaussian distribution of the particle diameter of an O/W microemulsion was obtained, and the diameter was small and about 10 nm. Moreover, when EOO and mineral oil were used as oils, a Gaussian dis-

Table 5
Pharmacokinetic parameters of ER-1039 following oral administration of ER-1039 formulation to fasted male rats

Dosage form	Component	T_{max} (h)		C_{max} (ng/mL)		AUC ((ng h)/mL)		AUC ratio	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	To suspension	To solution
Suspension	5% Gum arabic solution	4.5	1.0	165.5	15.0	1720.4	292.7	1.0	0.73
Solution	PEG200/EtOH/D.W.	3.2	1.1	314.2**	84.7	2351.3*	363.3	1.4	1.0
O/W ME-1	MCT/DGMO-C/HCO-40	2.0**	0.0	590.9***	105.0	3173.5***	273.5	1.8	1.3
O/W ME-2	EOO/DGMO-C/HCO-40	2.0**	0.0	468.3***	68.4	2645.0	809.6	1.5	1.1
O/W ME-3	Mineral oil/DGMO-C/HCO-40	1.2***	0.5	354.2***	67.2	1615.3	251.3	0.94	0.69

ER-1039 was orally administered as suspension, solution and three kinds of O/W microemulsion (ME) at a dose of 20 mg/kg. Data are presented as mean ± S.D. of four or five animals.

* Significant differences was observed against administration of suspension at $p < 0.05$.

** Significant differences was observed against administration of suspension at $p < 0.01$.

*** Significant differences was observed against administration of suspension at $p < 0.001$.

Table 6
Physicochemical properties of ER-1039 contained suspension, solution and O/W microemulsion (ME)

Dosage form	Component	ER-1039 content (mg/mL)	Ratio of solubility to water	Turbidity at 650 nm	Particle size (nm)		
					Peak-1	Peak-2	Peak-3
Suspension	5% Gum arabic solution	4.88					
Solution	PEG200/EtOH/D.W.	5.46					
O/W ME-1	MCT/DGMO-C/HCO-40	6.38	6380<	0.088	10		
O/W ME-2	EOO/DGMO-C/HCO-40	7.28	7280<	12		54 (88)	304 (12)
O/W ME-3	Mineral oil/DGMO-C/HCO-40	6.38		23		63 (88)	287 (12)

Values in parentheses are percentages.

tribution was not obtained, but two peaks were found. Although 88% of the particles had a diameter of around 60 nm, 12% of the large particles had a diameter of around 300 nm.

On the other hand, the AUC ratios following administration as a solution, a MCT entrapped O/W microemulsion, a EOO entrapped O/W microemulsion and a mineral oil entrapped O/W microemulsion to that of suspension administration were 1.4, 1.8, 1.5 and 0.94, respectively. A significant increase of AUC was observed after the administration of ER-1039 as a solution or a MCT entrapped O/W microemulsion ($p < 0.05$ at a solution and $p < 0.001$ at a MCT entrapped O/W microemulsion).

3.4.2. Ibuprofen

The plasma concentration profile and pharmacokinetic parameters of Ibuprofen following oral administration of Ibuprofen to fasted rats as a suspension, a solution and an O/W microemulsion at a dose of 10 mg/kg are shown in Fig. 3 and Table 8, respectively.

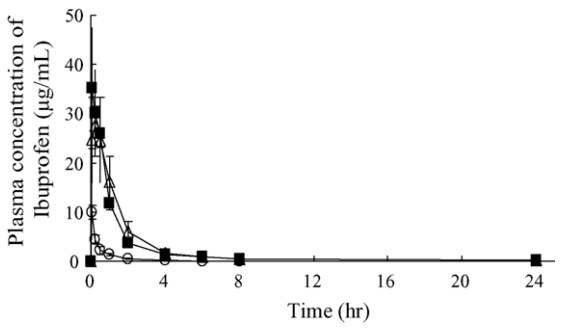

Fig. 3. Plasma concentration profile of Ibuprofen following single oral administration of Ibuprofen to fasted male rats as suspension (◯), solution (△) and O/W microemulsion (■) at a dose of 10 mg/kg. Data are presented as mean ± S.D. of four animals.

Moreover, the physical properties (Ibuprofen concentration in each formulation and the particle diameter) are shown in Table 7. The AUC ratios following oral administration as a solution and an O/W microemulsion to that of suspension administration were 9.3 and 8.7, respectively. AUC and C_{max} increased significantly in each formulation, respectively ($p < 0.001$ or 0.01).

3.5. Stability of ER-1039 entrapped O/W microemulsion (Experiment-5)

The compound concentration and the particle diameter of ER-1039 entrapped O/W microemulsion which was stored for 10 days and 2 weeks at room temperature and 4 °C, respectively, are shown in Table 9. Moreover, these parameters of this formulation, which was stored for 24 h at 37 °C in a solution of pH 1.2 or rat bile, are shown together in Table 9. The particle diameter became larger with time under the existence of a rat bile, but it was from 15 to 18 nm under the storage at the room temperature, 4 °C and in the acidic solution of pH 1.2, and hardly changed. The concentration of ER-1039 showed an almost fixed value under each condition.

4. Discussion

The influence of the kind of oil and the mixture ratio of surfactants on the region which can form an O/W microemulsion was examined. It was shown that the suitable mixture ratio of a lipophilic and a hydrophilic surfactant added, differed according to the kind of oil in order to make the O/W microemulsion which emulsifies well to be clear and with a small particle diameter, and the kind of oil suitable for the surfactants used became clear (Table 3). That is, when tributyrin and olive oil were used as oils, the oil and an aqueous phase

Table 7
Pharmacokinetic parameters of Ibuprofen following oral administration of Ibuprofen formulation to fasted male rats

Dosage form	Component	T_{max} (h)		C_{max} (μg/mL)		AUC ((μg h)/mL)		AUC ratio	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	To suspension	To solution
Suspension	0.5% CMC	0.083	0.00	9.9	1.4	5.4	0.75	1.0	0.11
Solution	PEG200/EtOH/D.W.	0.27	0.17	28.6***	5.3	50.0***	11.2	9.3	1.0
O/W ME	MCT/DGMO-C/HCO-40	0.23	0.20	35.6**	12.2	47.0***	6.4	8.7	0.94

Ibuprofen was orally administered as suspension, solution and O/W microemulsion (ME) at a dose of 10 mg/kg. Data are presented as mean ± S.D. of four animals.
** Significant differences was observed against administration of suspension at $p < 0.01$.
*** Significant differences was observed against administration of suspension at $p < 0.001$.

was separated and a uniform emulsion was not formed, or there was the mixture ratio for which the turbidity was larger and the particle diameter was not 100 nm or less. From these results, tributyrin and olive oil were found to be not the suitable oil to be used with the lipophilic/hydrophilic surfactant used in this study. On the other hand, when EOO and MCT are used as oils, in the range of from 1:9 to 5:5 as the mixture ratio of lipophilic/hydrophilic surfactant, each was emulsified well. In particular, setting the mixture ratio of lipophilic/hydrophilic surfactant to 1/9 or 2/8 (w/w), it was able to form the O/W microemulsion which had the Tyndall light and its average particle diameter was 20 nm or less and the turbidity was slight. Then, it was suggested that EOO and MCT were suitable oils to the lipophilic/hydrophilic surfactant.

Using ER-1039 as a model compound, the enhancing effect of the O/W microemulsion on gastrointestinal absorption of ER-1039 in a rat was examined. In this experiment, EOO and MCT which were able to form the good O/W microemulsions are chosen as oils, and mineral oil which is not metabolized and absorbed in a gastrointestine were selected as the negative control oil (Myers and Stella, 1992). Moreover, since in the mineral oil entrapped formulation, separation of the oil and an aqueous phase was performed in 6% (w/w) or more of concentration of HCO-40, and it did not become a uniform emulsion but it was uniformly emulsified only in 5% (w/w) of concentration (Table 3), a comparison examination was carried out having used the mixture ratio of oil/lipophilic surfactant/hydrophilic surfactant as 5/5/5 (w/w).

The significant increase of absorption rate that T_{max} decreased 1/2 times ($p < 0.01$) and C_{max} increased 3 times ($p < 0.001$) was found. AUC became twice as large and the enhancing effect over the amount of absorption was demonstrated by oral administration of ER-1039 as the MCT or EOO entrapped O/W microemulsion (formulation-1 or formulation-2, respectively), compared with the case where ER-1039 was orally administered as a suspension (Table 5; Fig. 2). In particular, since the significant increase of AUC was seen in the MCT entrapped O/W microemulsion (formulation-1) ($p < 0.001$) and this formulation was excellent in the enhancing effect compared with the EOO entrapped O/W microemulsion (formulation-2), it was shown that a result sufficient to enhance absorption using MCT as an oil was brought. In the formulation-1, it was considered that the O/W microemulsion composed of the small particles contributed to the enhancing effect on the absorption. On the other hand, C_{max} increased significantly in the mineral oil entrapped O/W microemulsion (formulation-3) compared with suspension administration ($p < 0.001$), but compared with the other O/W microemulsions (formulation-1 and formulation-2), in the formulation-3, T_{max} and C_{max} became small, AUC also fell by 50%, and it was of the same level as the suspension (Table 5; Fig. 2). Since mineral oil is not metabolized and absorbed in the gastrointestinal tract, it was considered that ER-1039 released from the O/W microemulsion was absorbed promptly but ER-1039 which remained in the oil phase did not contribute to absorption because it moved in the state where it was enclosed as it was in the inside of the gastrointestinal tract. The solubility of ER-1039 is below 1 μg/mL in the first solution (pH 1.2) and the second solution (pH 6.8) of a Japanese pharmacopoeia collapse examination and it is poorly water soluble compound classified into Class 2 of biopharmaceutical classifi-

Table 8
Physicochemical properties of Ibuprofen contained suspension, solution and O/W microemulsion (ME)

Dosage form	Component	Drug content (mg/mL)	Ratio of solubility to water	Particle size (nm)
Suspension	0.5% CMC	2.14		–[a]
Solution	PEG200/EtOH/D.W.	3.34		
O/W ME	MCT/DGMO-C/HCO-40	3.75	1250<	61

[a] Not measured (less than 45 μm).

cation system (BCS). The gastrointestinal absorption improvement of poorly water soluble compounds by O/W microemulsion is achieved by improving solubility by dissolving compounds completely into the oil phase and it was shown that in this way also for ER-1039, the small and stable O/W microemulsion was able to form using the suitable oil and the suitable surfactants, and also the solubilizer, ethanol and the gastrointestinal absorption was able to be increased by reaching the solubility that was 6000 times or more than that to water, about 6 mg/mL (Table 6). This result was in agreement with the increase of the gastrointestinal absorption based on improving solubility of the compounds demonstrated with various lipophilic drugs, such as Cyclosporin A (Gao et al., 1998; Ritschel, 1991) and Halofantrine (Porter et al., 1995, 1996). In addition, since there was not a significant difference between the pharmacokinetic parameters (C_{max} and AUC) following oral administration of solution, a full dissolution type formulation and the O/W microemulsions (formulation-1 and formulation-2), it was thought that there was no enhancing effect of the surfactants (DGMO-C and HCO-40) in these formulations on the permeability of ER-1039 in a gastrointestinal membrane (Tables 5 and 8; Figs. 2 and 3). Moreover, for the stability of O/W microemulsion in a gastrointestinal tract, the particle diameter became larger with time under the existence of rat bile and we are concerned about an interaction between the bile and the O/W microemulsion, but it hardly changed in the acidic solution of pH 1.2 and the concentration of ER-1039 showed an almost fixed value under each condition. Therefore, it was shown that an ER-1039 entrapped O/W microemulsion was a stable formulation in a stomach and a small intestine (Table 9).

The first condition for applying poorly water soluble compounds to an O/W microemulsion is dissolving and enclosing the compounds into the oil phase. In the O/W microemulsion formulation at this time, we used ethanol as the solubilizer inside of the oil phase and thought that the compound which dissolves in ethanol was applicable. Then, for the purpose of verifying the versatility of this O/W microemulsion to poorly water soluble compounds, improvement for solubility of poorly water soluble compounds by the O/W microemulsion was examined, using various poorly water soluble compounds, such as the commercial drugs and the new compounds selected from the solubility in ethanol, the solubilizer, as the model compound. Now, the O/W microemulsion currently

Table 9
Stability of ER-1039 entrapped O/W microemulsion

Dosage form	Storage condition	ER-1039 content (mg/mL)	Particle size (nm)
Preservation stability	Initial	8.94	18
	7 Days at 4 °C	11.3	15
	14 Days at 4 °C	10.8	16
	4 Days at 25 °C	9.26	16
	10 Days at 25 °C	8.34	17
Prediction of stability in intestine	Initial	8.79	18
	4 h at pH 1.2, 37 °C	9.94	18
	24 h at pH 1.2, 37 °C	9.40	17
	Initial	9.68	15
	4 h at 37 °C in bile	7.98	198
	24 h at 37 °C in bile	9.18	449

assumed is the formulation which consists of the 3 volumes of the mixtures of an oil and surfactants and 16 volumes of an aqueous phase added to 1 volume of the ethanol solution containing the compound, and is the 20 times diluted formulation of the undiluted solution of the compound. When the dosage of 10 mg/kg was set up as the type which reveals the pharmacological effect by the high dose, the concentration of the compound in the ethanol solution which can prepare an O/W microemulsion serves as 100 mg/mL from dilution magnification. On the basis of such a concentration, the eleven compounds, such as BO-653, Chloramphenicol, Ibuprofen, Ketoprofen, AG-041R, Disopyramide, Tolbutamide, Tamoxifen, Testosterone and ER-1258 including ER-1039 were selected as the compounds which dissolved well in ethanol with the solubility of about 100 mg/mL or more and it was possible to administer at a high dose of 10 mg/kg or more (Table 1), and examined the solubility in an O/W microemulsion. By using MCT, DGMO-C, HCO-40 and ethanol as an oil, a lipophilic surfactant, a hydrophilic surfactant and a solubilizer, respectively, and setting the mixture ratio of oil/lipophilic surfactant/hydrophilic surfactant/ethanol/aqueous phase to 5%/1%/9%/5%/80% (w/w), the solubility that was from 60 to 20,000 times that to water was obtained to nine kinds of poorly water soluble compounds (commercial drugs: Ibuprofen, Ketoprofen, Tamoxifen, Testosterone, Tolbutamide, new compounds: BO-653, ER-1258, AG-041R and ER-1039), and the improvement of solubility was demonstrated (Table 4).

Finally, the commercial drug, Ibuprofen was chosen from these compounds, and by using the same O/W microemulsion composition and setting the mixture ratio of oil/lipophilic surfactant/hydrophilic surfactant/ethanol/aqueous phase to 5%/1%/9%/5%/80% (w/w), the enhancing effect of the O/W microemulsion to the gastrointestinal absorption of Ibuprofen in the rat was examined.

When Ibuprofen was orally administered as an O/W microemulsion, the absorption was equivalent to that of solution administration and C_{max} increased by about four times ($p < 0.01$), AUC became about nine times ($p < 0.001$) those of suspension administration, and the enhancing effect of absorption was demonstrated (Table 8; Fig. 3). The solubility became 3.8 mg/mL by O/W microemulsion, the solubility of 1250 times or more than that to water was obtained (Table 7), and it was shown that the gastrointestinal absorption can be increased by this formulation. In addition, the enhancing effect of the gastrointestinal absorption by the improvement of solubility based on the dissolution into an oil phase is just going to be expected also for poorly water soluble compounds, Tolbutamide, Testosterone, Tamoxifen, Ketoprofen, AG-041R, ER-1258 and BO-653.

In this research, we designed the novel O/W microemulsion formulation which enhances the oral bioavailability by raising the solubility of poorly water soluble compounds. When arguing about the usefulness of an O/W microemulsion, the preservation stability is also an important item. Therefore, the preservation stabilities at room temperature and in 4 °C for the ER-1039 entrapped O/W microemulsion were examined to show the stability until 2 weeks of a short period of time (Table 9). However, since the outer phase of an O/W microemulsion is an aqueous phase, if the stability of the microemulsion is taken into consideration, on the occasion of clinical application, development of the self-emulsifying drug delivery system (SEDDS) type O/W microemulsion which does not contain an aqueous phase is desired. This system is the formulation which carries out self-emulsification under gentle agitation following contact with an aqueous phase within stomach or upper small intestine and forms a stable O/W microemulsion thermodynamically with a particle diameter of 100 nm or less (Charman et al., 1992; Craig et al., 1993; Shah et al., 1994; Wakerly et al., 1986; Ashford and Craig, 2004). Research for poorly water soluble drugs like Cyclosporin A (Erkko et al., 1997) and the cholesterol-lowering agent, Simvastatin (Kang et al., 2004), etc., is actively performed and for Cyclosporin A, the SEDDS type O/W microemulsion was put on the market using the brand name Neoral as the formulation, which enabled stable gastrointestinal absorption. The versatile novel SEDDS type O/W microemulsion formulation which enhances the oral bioavailability by raising the solubility of poorly water soluble compounds is scheduled to be designed from now on.

5. Conclusion

As the component of the O/W microemulsion, medium chain fatty acid triglyceride (MCT),

diglyceril monooleate (DGMO-C), polyoxyethylene hydrogenated castor oil 40 (HCO-40), ethanol and PBS (pH 6.8) were used as an oil phase, a lipophilic surfactant, a hydrophilic surfactant, a solubilizer and an aqueous phase, respectively, at the mixture ratio of 5%/1%/9%/5%/80% (w/w). Thereby, the O/W microemulsion with an average particle diameter of 20 nm or less was prepared. Moreover, to nine kinds of poorly water soluble compounds, such as Ibuprofen, Ketoprofen, Tamoxifen, Testosterone, Tolbutamide and other new compounds, the solubility that was from 60 to 20,000 times that to water was obtained by this O/W microemulsion formulation, and the AUCs in plasma concentration of Ibuprofen and ER-1039 following single oral administration of these compounds as an O/W microemulsion to fasted rats were equivalent to that of solution administration or increased by nine and two times that of suspension administration, respectively, that is, an enhancing effect of gastrointestinal absorption by improving its solubility was demonstrated. Accordingly, we were able to design the novel O/W microemulsion formulation which enhances the oral bioavailability by raising the solubility of poorly water soluble compounds.

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