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Diethyl ether fraction of Labrasol having a stronger absorption enhancing effect on gentamicin than Labrasol itself

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Abstract

In our previous study, we had reported that Labrasol has a good gastrointestinal (GI) absorption enhancing effect on poorly absorbable drugs. In order to improve further absorption enhancing effect of Labrasol on gentamicin (GM), which is a representative water-soluble, poorly absorbable drug, Labrasol was fractionated with hexane, diethyl ether, ethyl acetate and water. The absorption enhancing effect of each fraction of Labrasol and Labrasol alone were evaluated in vivo using rats. Each test formulation of GM was administered into the rat colon at a dose of 5.0 mg/kg and plasma GM concentrations were measured by a HPLC method. Among the four fractions of Labrasol and Labrasol, diethyl ether fraction showed the strongest absorption enhancing effect on GM. When the doses of diethyl ether fraction were 1.0, 0.5 and 0.1 ml/kg, the C_{max} values were 8.95 ± 1.46, 8.02 ± 2.14 and 7.41 ± 1.25 μg/ml, respectively. Moreover, AUC_{0-6} values were also maintained at high level, i.e. 27.28 ± 5.90, 20.32 ± 3.79 and 19.61 ± 2.09 μg h/ml. Based on the AUC_{0-6} values obtained with each fraction, the rank order of absorption enhancing effect on GM was diethyl ether > ethyl acetate ≒ hexane > aqueous fraction. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Gentamicin sulfate (GM); Absorption enhancement; Labrasol; Diethyl ether fraction of Labrasol; Extraction; Rat colon

1. Introduction

The aminoglycoside antibiotic, gentamicin (GM), is a well known unabsorbable drug from gastrointestinal (GI) tract. However, it is an important antibacterial agent used for the treatment of a wide variety of gram-negative bacilli and gram-positive cocci infections (Fantin et al., 1991; Drabu and Blakemore, 1990). As GM is a polarized water-soluble compound, its intestinal membrane permeability is extremely poor resulting in low bioavailability (BA) (Cox, 1970; Recchia et al., 1995). Because of poor absorption after oral administration, GM is clinically used as injections

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or topical dosage forms. However, parenteral administration of GM has been associated with side effects that include mainly nephrotoxicity and ototoxicity (Kaloyandres and Munoz, 1980; Lerner and Matz, 1980; Kitasato et al., 1990). To overcome the absorption problem and achieve oral therapy with GM, which will increase QOL of the patients, many studies have been carried out (Chiba et al., 2001; Berkovitch et al., 1993). Axelrod et al. (1998) found out a derivative of taurocholic acid, TC002, as an absorption enhancer for GM. Recently, we have reported that Labrasol, caprylocaproyl macrogolglycerides, has a strong absorption enhancing effect on GM (Hu et al., 2001). Labrasol is obtained from coconut oil and shows high tolerance and low toxicity for animals, LD_{50} is 22 g/kg for rats. Labrasol is a surfactant that contains saturated polyglycolysed C_6–C_{14} glycerides, where C_8 is 58.1% and C_{10} is 39.8%, and it was originally developed as a pharmaceutical additive for the solubilization of hydrophobic drugs. Labrasol had been employed mainly for parenteral formulations (Tran et al., 1999). Along with developing a new use, Labrasol has been in use as a main component of self-microemulsifying drug delivery system (SMEDDS).

Microemulsions are defined in general as thermodynamically stable, isotropically clear dispersions of two immiscible liquids stabilized by interfacial films of surface active molecules (Shah et al., 1994; Constantinides, 1995). These mixtures are able to form fine microemulsions with gentle agitation on being exposed to aqueous media. This property makes microemulsions as good vehicles for the oral delivery of non- or poor-absorption drugs, which usually are extremely hydrophobic (Xiao et al., 1999; Malcolmson et al., 1998). In our previous study, we have developed a novel self-microemulsion system with Labrasol to improve the GI absorption of extremely water-soluble drug (Shibata et al., 2001). Microemulsions of GM were prepared and administrated to rat small intestine and colon. Plasma GM levels following intestinal applications were compared to those obtained with intravenous (i.v.) administration. A 5.0 mg/kg dose of GM preparation containing Labrasol, 1.0 ml/kg, administrated into the colon resulted in improved BA of GM, (55.3%). To understand more about the effect of Labrasol, permeability experiments were carried out using a chamber method. The permeation of GM significantly increased in the direction of colonic mucosa to serosa with Labrasol. On the other hand, the transfer of GM from serosa to mucosa was decreased (Hu et al., 2001). Labrasol might have inhibited the intestinal secretory transporter, P-glycoprotein (P-gp). Therefore, the effect of Labrasol was ascribed to both: (1) the enhanced GM absorption from the GI lumen into the systemic circulation; and (2) the inhibition of efflux of GM from the enterocytes to the GI lumen.

However, it has to be noticed that the high dose of Labrasol, 1.0 ml/kg, as a pharmaceutical additive for rats may not be acceptable for big animals or humans, where the dose increase proportionately with the body weight. Low and efficient dose of Labrasol is clinically required. However, when the dose of Labrasol was decreased from 1.0 to 0.2 ml/kg, the absorption enhancing effect decreased, where the AUC_{0-6} decreased by approximately 63% (21.18 ± 4.16–7.72 ± 2.19 μg h/ml). Labrasol is a mixture consisting of 30% mono-, di- and triglycerides of C_8 and C_{10} fatty acids, 50% of mono- and di-esters of poly(ethylene glycol) (PEG) and 20% of free PEG400 (Kreilgaard et al., 2000). In order to obtain stronger absorption enhacing effect of Labrasol on GM even at low dose, fractionation of Labrasol was carried out with organic solvents having different polarity. Four fractions, hexane, diethyl ether, ethyl acetate and aqueous fraction were obtained. To determine which fraction of Labrasol has stronger absorption enhancing effect on GM than Labrasol itself, the absorption enhancing ability of each fraction has been investigated in vivo in rats.

2. Materials and methods

2.1. Materials

Gentamicin sulfate, *n*-hexane, diethyl ether, ethyl acetate, 1-pentanesulfonate, sodium sulfate, boric acid and acetic acid were obtained from Nacalai Tesque Inc. (Kyoto, Japan). Labrasol

(Gattefösse, France) was a gift from Chugai boyeki Co., Ltd. (Tokyo, Japan). *O*-phthalaldehyde (OPA) was obtained from Ishizu Seyaku, Ltd. (Osaka, Japan). Male Wistar rats (300–350 g) used in the study were obtained from Nippon SLC Company (Hamamastu, Japan). Standard solid meal of commercial food (LabDiet®) was obtained from Nippon Nousan Co., Ltd. (Yokohama, Japan). All other materials used were of reagent grade and were used as received. Animal experiments were carried out in accordance with the Guidelines for Animal Experimentation in Kyoto Pharmaceutical University.

2.2. Fractionation of Labrasol

Labrasol (10 ml) was mixed with water (10 ml) and then, hexane (80 ml) was added. After shaking for 20 min and standing for 10 min, two layers were separated. Hydrophobic components of Labrasol were extracted into upper hexane phase. To the lower layer, 120 ml of diethyl ether and 5 ml of ethanol were added. After 20 min shaking and standing for 10 min, the upper diethyl ether phase was separated. Finally, 120 ml of ethyl acetate was added to the lower phase. After 20 min shaking and standing for 10 min, the upper ethyl acetate phase was separated. The lower phase was collected as aqueous fraction. The solvents of each organic phase and aqueous fraction were condensed for 1 h by reduced pressure distillation.

2.3. Microemulsion preparations

Table 1 shows the formulations used in absorption studies. To prepare microemulsion formulations, GM was initially dissolved in saline. Then, Labrasol or Labrasol fraction was added. The ratio of saline to each additive was constant at 1:1 (v/v), which had been confirmed as the most optimum ratio (Hu et al., 2001). Upon mixing, transparent microemulsion formulations were obtained. Microemulsions containing GM were

Table 1
Test preparations used in the absorption study

Preparation no.	Site of administration	GM dose (mg/kg)	Volume of test preparation (ml/kg)	Surfactant	Surfactant dose (ml/kg)
1	i.v.	1	0.5	–	–
2		5	2	–	–
3			2		1
4		5	1	Labrasol	0.5
5			0.4		0.2
6		5	2	Hexane fraction	1
7	i.c.		1		0.5
8			2	Diethyl ether	1
9		5	1	fraction	0.5
10			0.2		0.1
11		5	2	Ethyl acetate fraction	1
12		5	2	Aqueous residue	1

GM was dissolved in saline without or with individual additive, i.e. Labrasol or fractions of Labrasol, and the final GM dose was 5.0 mg/kg. The ratio of saline to additive was fixed at 1:1.

equilibrated at an ambient temperature overnight and then used for animal experiments.

2.4. Absorption studies

In the in vivo experiments, rats fasted overnight were used for absorption studies. As the i.v. injection of high dose GM induces acute renal failure (Barza et al., 1975), a lower GM dose, 1.0 mg/kg, was chosen for i.v. administration. Test GM preparation was injected into the left femoral vein and blood samples (0.3 ml) were collected from the right jugular vein at 10, 20, 30, 60, 90, 120, 180, 210, 240 and 360 min after administration. For intra-colonic (i.c.) administration, high GM dose, 5.0 mg/kg, was used. The abdominal incisions were made and colon catheters were surgically implanted in anesthetized rats. Because GM can be absorbed from vulnus that was made by inserted cannula, the colon catheter was perforated from ileocaecal junction and across caecum inserted approximate 3.0 cm into the colon to prevent GM absorption from the catheter vulnus. Blood samples (0.3 ml) were collected from the right jugular vein at 15, 30, 60, 90, 120, 180, 240, 300 and 360 min. The blank blood sample was taken at 5 min prior to the administration of test preparations. Plasma was obtained from whole blood by centrifugation at 14,000 rpm for 5 min using Kubota 1720 centrifuge (Tokyo, Japan), and then stored at -80 °C until analysis.

2.5. Turbidity study

In order to examine the property of microemulsion, the turbidity study was carried out. Labrasol or diethyl ether fraction of Labrasol was added to deionized water. The turbidities of different concentrations of two emulsifiers were measured at 700 nm wavelengths with UV–Vis spectrophotometer (Shimadzu UV-1600).

2.6. HPLC analytical method for GM

GM in plasma was purified according to Anhalt's method (Anhalt, 1977; Tawa et al., 1998). A column was prepared from CM-Sephadex (C25) (Muromachi Kagaku Kogyo Kaisha, Ltd., Tokyo, Japan) with a bed volume of 1.0 ml. The column was washed with 1 ml of 0.2 M sodium sulphate solution (rinse buffer). A 100-μl volume of plasma was applied to the column followed by 1 ml of the rinse buffer. Thereafter, 1 ml of the rinse buffer was added to the column twice to wash out protein adulterant. After the column was drained completely, 500 μl of an alkaline buffer containing 10 mM of sodium hydroxide in 0.2 M sodium sulphate solution (elution buffer) was added and all the eluted solution was collected as HPLC injection sample.

The concentration of GM was determined by HPLC post column method that was developed by Hitachi Co. Ltd. A model LC-10AS pump (Shimadzu Corp.) was used to deliver the mobile phase (1.0 ml/min) containing 0.02 M 1-pentanesulfonate, 0.05 M sodium sulfate and 0.1% acetic acid in water–acetonitrile mixture (98:2). A Hitachi Gel #3056 column (15 cm × 4.6 mm i.d.) was used for the analysis. The OPA reagent (6.0 mM), which contains 0.35 M boric acid, 0.30 M sodium hydroxide and 0.03 M 2-mercaptoethanol, was delivered at a flow-rate of 0.5 ml/min to the column effluent via a mixing T-piece with a 10AS pump (Shimadzu Corp.). A reaction coil consisting of a Teflon tube (0.33 μm × 7 m) was placed between the mixing T-piece and a fluorescence detector (Shimadzu RF-10AXL fluorometer) set at an excitation wavelength of 360 nm and an emission wavelength of 450 nm.

The calibration of GM was linear over 0.1–20.0 μg/ml. The detection limit for GM was 0.05 μg/ml. In the other word, this analytical method is suitable for measuring samples which contain above 5.0 ng of GM.

2.7. Pharmacokinetic analysis

Pharmacokinetic (PK) parameters were calculated by a non-compartmental PK analysis method using WINHARMONY software (Yoshikawa et al., 1998). The time when GM concentration reached its maximum, T_{max}, and the maximum concentration of plasma GM,

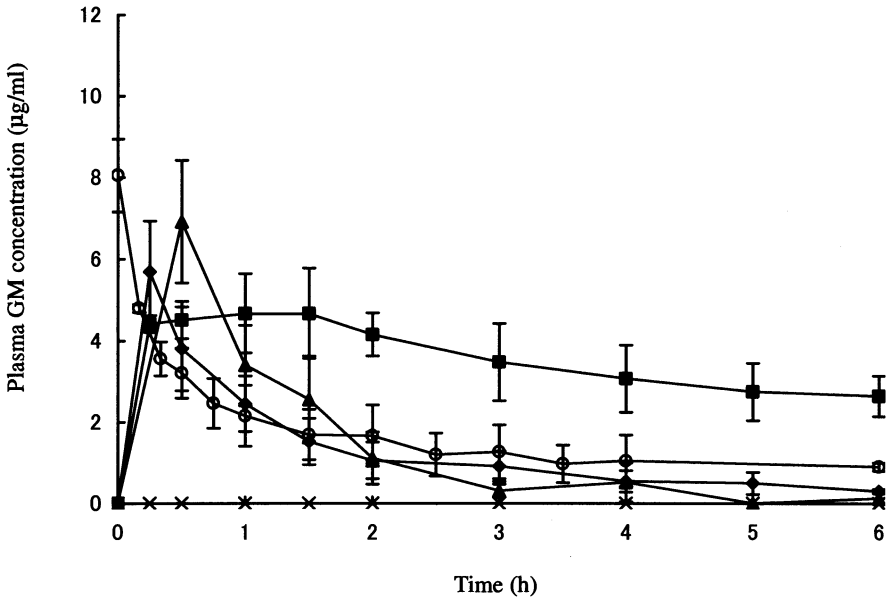

Fig. 1. Plasma GM concentration vs. time profiles following i.v. (○) and i.c. administrations in rats. In i.v. administration, GM was dissolved in saline and the dose of GM was 1.0 mg/kg. In i.c. administrations, GM was dissolved in saline with or without Labrasol and the final GM dose was 5.0 mg/kg. The ratio of saline to Labrasol was fixed at 1:1 and three doses of Labrasol were used 1.0 (■), 0.5 (▲) and 0.2 ml/kg (◆). As control, GM saline solution was administrated into rat colon without any absorption enhancer (×). Values are the mean ± SE of 3–4 animals.

C_{max}, were determined from the authentic GM concentration vs. time data. The area under the plasma GM concentration vs. time curve (AUC) and the area under the first-moment curve (AUMC) after administration of the test preparation were calculated using the linear trapezoidal rule up to the last measured GM plasma concentration. The mean residence time (MRT) was calculated by $AUMC_{0-\infty}/AUC_{0-\infty}$. The absolute BA of GM was calculated from the AUC_{0-6} following i.c. and i.v. administratons using the following equation:

$$BA = \frac{AUC_{i.c.}}{AUC_{i.v.}} \times \frac{Dose_{i.v.}}{Dose_{i.c.}} \times 100 \ (\%)$$

2.8. Statistical analysis

Means of two groups were compared using non-paired student's *t*-tests. All values are expressed as their mean ± SE. When comparing multiple groups, one-way analysis of variance (ANOVA) was applied with the Turkey multiple comparison procedure.

3. Results

One extraction batch was 10 ml Labrasol with 10 ml water. After 1 h reduced pressure evaporation of each fraction, the remaining volumes of four fractions, i.e. hexane fraction, diethyl ether fraction, ethyl acetate fraction and aqueous fraction, were 1.3 ± 0.2, 7.3 ± 0.4, 2.0 ± 0.3 and 2.4 ± 0.3 ml, respectively. Fig. 1 shows the rat plasma GM concentration vs. time profiles following either i.v. administration of GM solution or i.c. administration of GM formulations with or without Labrasol. In the case of i.v. administration, the plasma GM concentration rapidly decreased from 8.05 ± 0.89 to 2.15 ± 0.75 μg/ml within 1 h after administration. At 4 h after dosing, low GM concentration, 1.05 ± 0.63 μg/ml, was detected in the plasma, indicating that GM has high clearance and rapidly eliminates from the systemic circulation. When GM saline solution was administrated into the colon, GM was not detected in the systemic circulation. This result consists with other reports that GM cannot be absorbed from

GI without absorption enhancer (Cox, 1970; Recchia et al., 1995). The C_{max} following intracolonic administration of GM, 5.0 mg/kg, along with 1 ml/kg of Labrasol was 4.67 ± 1.11 μg/ml. Also, the plasma GM concentration was maintained at 2.63 ± 0.49 μg/ml with Labrasol even at 6 h after administration. When the dose of Labrasol was reduced from 1.0 to 0.5 to 0.2 ml/kg, the C_{max} values did not significantly differ, i.e. 4.67 ± 0.97, 6.92 ± 1.5 and 5.69 ± 1.24 μg/ml, respectively (Fig. 1). However, the mean AUC from time 0 to 6 h significantly decreased, i.e. 21.18 ± 4.16, 8.15 ± 2.80 and 7.72 ± 2.19 μg h/ml, and showed dose-dependence on Labrasol (Table 2).

In order to compare the absorption enhancing effect of each fraction of Labrasol, GM test formulations were prepared by adding individual Labrasol fraction, i.e. hexane, diethyl ether, ethyl acetate and aqueous residue. In the case of hexane fraction, two doses, 1.0 and 0.5 ml/kg, were used and the plasma GM concentration vs. time profiles after colonic administration is shown in Fig. 2. The results did not show dose-dependency between the two doses of hexane fraction. By comparing with the Labrasol formulations, the C_{max} values of hexane fractions were not high, 4.94 ± 0.65 (1.0 ml/kg) and 3.78 ± 0.73 μg/ml (0.5 ml/kg). Moreover, at 6 h after administration, the plasma GM concentrations were very low, 0.12 ± 0.09 μg/ml (1.0 ml/kg) and 0.13 ± 0.05 μg/ml (0.5 ml/kg).

Fig. 3 shows the plasma GM concentration vs. time profiles obtained after the i.c. administration of GM solution added with ethyl acetate fraction or aqueous fraction, where the administered amount of each fraction was 1.0 ml/kg. By comparing to the plasma GM level vs. time profiles of Labrasol, it was also observed that the C_{max} values were low, 2.68 ± 1.32 and 1.04 ± 0.73 μg/ml, respectively. It suggests that both fractions of ethyl acetate and aqueous residue did not have enough absorption enhancing effect on GM even at high dose of 1.0 ml/kg.

Plasma GM concentration vs. time profile following i.c. administration of GM preparations containing different doses of diethyl ether faction, i.e. 1.0, 0.5 and 0.1 ml/kg, is shown in Fig. 4. By comparing to the Labrasol itself, the plasma GM concentrations were significantly increased by the addition of diethyl ether fraction at all the doses,

Table 2
PK parameters of GM after i.v. or i.c. administration of test preparations to rats

	Preparation	C_{max} (μg/ml)	T_{max} (h)	MRT[a] (h)	$AUC_{0-6\ h}$ (μg h/ml)	BA (%)
i.v.	1	8.05 ± 0.89	–	–	7.66 ± 2.19	100
	2	ND	ND	ND	ND	ND
	3	4.67 ± 0.97	0.81 ± 0.09	8.07 ± 0.47	21.18 ± 4.16	55.3
	4	6.92 ± 1.50	0.50 ± 0.01	3.23 ± 0.21	8.15 ± 2.80	21.3
	5	5.69 ± 1.25	0.50 ± 0.01	3.07 ± 0.77	7.72 ± 2.19	20.2
i.c.	6	4.94 ± 0.65	0.50 ± 0.01	1.64 ± 0.55	6.51 ± 1.69	17.0
	7	3.78 ± 0.73	0.50 ± 0.01	1.95 ± 0.17	6.33 ± 1.26	16.5
	8	8.24 ± 1.47	1.25 ± 0.10	8.24 ± 2.17	27.28 ± 5.90	71.2
	9	8.02 ± 2.14	0.88 ± 0.09	5.47 ± 0.90	20.32 ± 3.79	53.1
	10	7.41 ± 1.25	0.63 ± 0.04	4.74 ± 1.36	19.61 ± 2.09	51.2
	11	2.68 ± 1.32	1.25 ± 0.10	2.05 ± 0.54	8.03 ± 3.89	21.0
	12	1.03 ± 0.74	0.75 ± 0.05	1.50 ± 0.56	2.16 ± 1.75	5.6

GM was dissolved in saline with or without surfactants. Preparations 1 and 2 were GM saline solution. Preparations 3–5 were GM solution with Labrasol. Preparations 6 and 7 were GM solution with hexane fraction of Labrasol. Preparations of 8–10 were GM solution with diethyl ether fraction of Labrasol. Preparation 11 was GM solution with ethyl acetate fraction of Labrasol. Preparation 12 was GM solution with aqueous residue of Labrasol. Values are the mean ± SE of 3–4 experiments.

[a] $MRT = AUMC_{0-\infty}/AUC_{0-\infty}$.

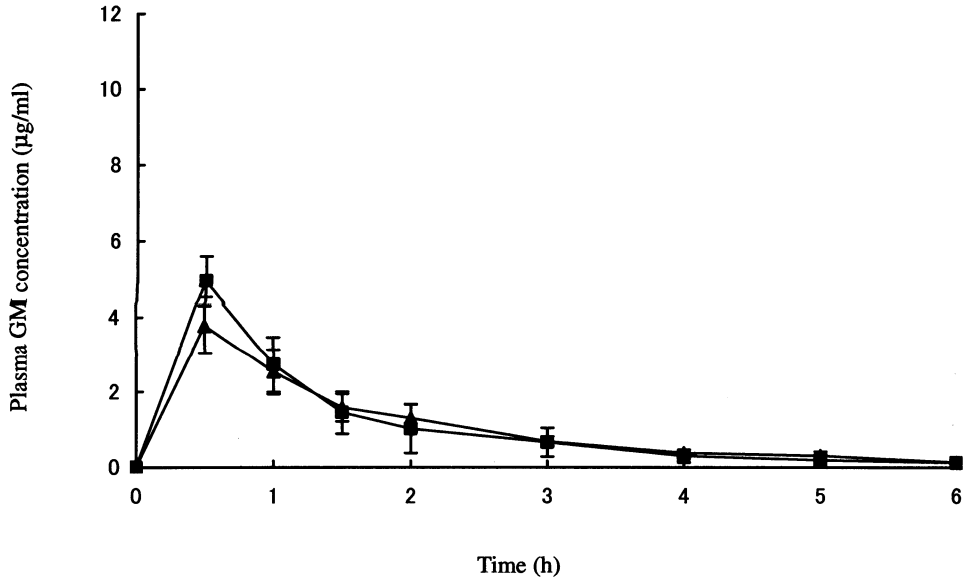

Fig. 2. Plasma GM concentration vs. time profiles following colonic administration in rats. GM was dissolved in saline with hexane fraction of Labrasol and the final GM dose was 5.0 mg/kg. The ratio of saline to hexane fraction of Labrasol was fixed at 1:1 and two doses of hexane fraction of Labrasol was used 1.0 (■) and 0.5 ml/kg (▲). Values are the mean ± SE of 3–4 animals.

where the C_{max} values were 8.24 ± 1.47, 8.02 ± 2.14 and 7.41 ± 1.25 μg/ml with the doses of 1.0, 0.5 and 0.1 ml/kg, respectively. At 6 h after administration, the plasma GM concentrations were 2.80 ± 1.17 μg/ml (1 ml/kg), 1.14 ± 0.51 μg/ml (0.5 ml/kg), and 1.91 ± 0.30 μg/ml (0.1 ml/kg).

In Fig. 5, the AUC_{0-6} values obtained from the i.c. administration of GM solution containing Labrasol or diethyl ether fraction of Labrasol were compared. At high dose of 1.0 ml/kg, both fractions showed high AUC_{0-6} values, 21.18 ± 4.16 (Labrasol) and 27.28 ± 5.90 (diethyl ether fraction of Labrasol) μg h/ml. However, when the dose was reduced to half, 0.5 ml/kg, the AUC_{0-6} decreased by 38.5% (21.18 ± 4.16–8.15 ± 2.80 μg h/ml) with Labrasol. On the other hand, decrease in dose of diethyl ether fraction from 1 to 0.1 ml/kg did not significantly decreased the AUC_{0-6}. Comparing with Labrasol, the AUC_{0-6} values obtained from diethyl ether fraction were always high, 27.28 ± 5.90 (1 ml/kg), 20.32 ± 3.79 (0.5 ml/kg) and 19.61 ± 2.09 (0.1 ml/kg) μg h/ml.

Table 2 summarizes the PK parameters obtained from the plasma GM concentration vs. time curves following the i.v. and i.c. administrations. As can be seen from the data, the microemulsion of GM with Labrasol or diethyl ether fractions resulted in a significant absorption enhancement. Based on the AUC_{0-6} values obtained following i.c. administration of GM with each fraction, the rank order of improving GM absorption from colon was found to be diethyl ether (27.28 ± 5.90 μg h/ml) > ethyl acetate (8.03 ± 3.88 μg h/ml) ≒ hexane (6.51 ± 1.69 μg h/ml) > aqueous fraction (2.16 ± 1.75 μg h/ml), at a dose of 1.0 ml/kg.

In Fig. 6, the turbidities of mixture containing different concentration of Labrasol or diethyl ether fraction were shown. In the case of Labrasol, tubidity was invisible in the concentration range of 0.1–2%. Comparably, the cloudy window of diethyl ether fraction was narrow, 0.3–1%.

4. Discussion

In order to increase the GI absorption of GM, we have focused on a novel self-microemulsion system. Conventionally, microemulsion technol-

ogy is one of the important drug delivery principles, which can improve the absorption of non- or poorly-water soluble drugs and meliorates high inter-subject variation on their absorption. To obtain a high quality therapy, plasma drug concentration vs. time profile must be obtained with high reproducibility. The reason for the poor or variable GI absorption of hydrophobic drugs is poor solubility in water, resulting in poor accessibility to the GI absorption membrane. The pre-epithelial, unstirred, aqueous layer is a barrier to hinder the poorly soluble drugs from reaching the absorption site. In other words, the low dissolution rate in the water layer caused their poor absorption. However, once transported through aqueous layer, hydrophobic drugs can easily permeate the basolateral membrane and enter into the systemic circulation. SMEDDS provides a good vehicle that accelerates dissolution of hydrophobic drugs in the intestinal fluid resulting in better absorption. SMEDDS used for hydrophobic drugs is usually composed of three components, i.e. water, surfactant and cosurfactant. Surfactants that have appropriate hydrophilic–lipophilic balance (HLB) value could dissolve hydrophobic drugs in water and enlarge their exposure area in the intestine. Cosurfactant can be considered as an important factor, because it acts as an intermediate between surfactant and water. By adding cosurfactant into the system, the interfacial tension between low HLB surfactant containing hydrophobic drug and water phase decreases, the hydrocarbon region of the interfacial film is fluidized, and the bending stress of the interface is decreased (Rubino and Yalkowsky, 1987; Lindstrom, 1979; Yalkowsky et al., 1976). Namely, cosurfactant stabilizes the low HLB value surfactants containing hydrophobic drug in hydrophilic environment. For the selection of a suitable self-emulsifying vehicle, it is important to assess the area of self-emulsifying region in the phase diagram, among the three factors, i.e. water, surfactant and cosurfactant, which percentage decides SMEDDS characteristic. A good system could increase the BA of poorly absorbable drugs, where droplets apparently spread easily into water and becomes transparent like solution. On the other hand, when a formulation immediately coalescents to oil droplets under non-stirring condition, drug absorption enhancement cannot be obtained.

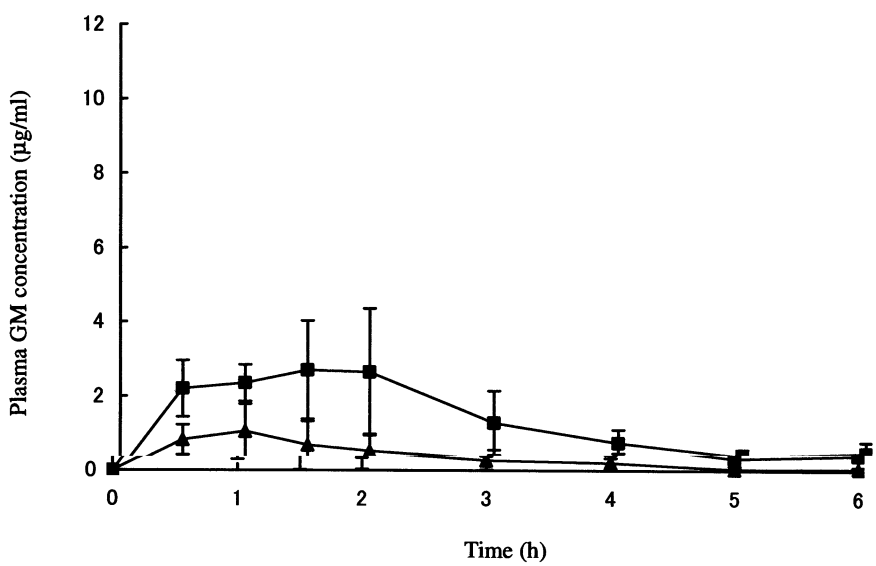

Fig. 3. Plasma GM concentration vs. time profiles following colonic administrations in rats. GM was dissolved in saline with ethyl acetate fraction of Labrasol (1.0 ml/kg) (■) or aqueous residue (1.0 ml/kg) (▲) and the final GM dose was 5.0 mg/kg. The ratio of saline to additive was fixed at 1:1. Values are the mean ± SE of 3–4 animals.

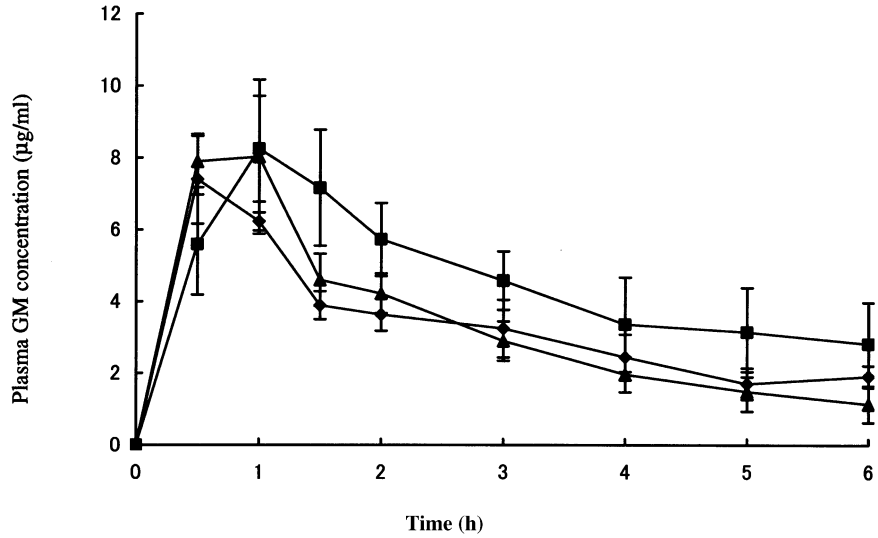

Fig. 4. Plasma GM concentration vs. time profiles following colonic administrations in rats. GM was dissolved in saline with ethyl ether fraction of Labrasol and the final GM dose was 5.0 mg/kg. The ratio of saline to ether fraction of Labrasol was fixed at 1:1 and three doses of ether fraction of Labrasol were administrated 1.0 (■), 0.5 (▲), and 0.1 ml/kg (◆). Values are the mean ± SE of 3–4 animals.

Labrasol is a representative surfactant, which is often used in SMEDDS of hydrophobic drugs. However, in this study we focused on the absorption enhancing effect of Labrasol and Labrasol fractions on the highly water-soluble, low-membrane permeable drug, GM. Hydrophilic drugs like GM have good water solubility, which do not require enlarging absorption area in the intestine. However, the main rate-limiting barrier for the absorption/diffusion of hydrophilic drugs is the lipophilic layer of intestinal epithelial cells that covers the luminal surface of the intestinal wall. Hydrophilic drugs are lacking capability to cross the layer of intestinal epithelial cells. The function of Labrasol or Labrasol fraction used for the absorption enhancement of water-soluble drugs is to assist in transport through intestinal epithelial layer, which completely differs from the use for hydrophobic drugs. Therefore, using Labrasol or Labrasol fractions for hydrophobic or hydrophilic drugs are two completely different concepts. Namely, as mentioned above, Labrasol or other surfactants provide larger area for the hydrophobic drugs by the fine emulsion droplets and subsequently lipolysis and the formation of mixed micelles occur. On the other hand, the formulations of hydrophilic drugs by imparting some lipophilicity with Labrasol could improve the diffusion of drugs across the epithelial barrier, which is the main role of Labrasol used in the DDS for hydrophilic drugs.

It can be observed that the microemulsion of GM was very simple, consisting of water and Labrasol, and there was no cosurfactant. In the case of hydrophobic drugs, low HLB surfactants are always selected, because they can easily dissolve hydrophobic drugs to provide enlarged interface in GI. However, the interfacial tension consisting between water and low HLB surfactant containing drug is generally high due to polymerization. Cosurfactant remits the interfacial tension between water and surfactant as a stabilizer. However, in our system we used high HLB value surfactant, Labrasol, having a HLB value of 14. Labrasol can be easily dissolved in water, in which large amount of hydrophilic drugs also dissolve. Because there was not much interfacial tension between water and Labrasol, cosurfactant is frequently not required. In other words, it can be considered that Labrasol acts as both absorp-

tion enhancer (surfactant) and cosurfactant in this system. Hence, it suggests that high HLB value of surfactant like Labrasol or Labrasol fractions is more useful for hydrophilic drugs than hydrophobic drugs. The absence of cosurfactant avoids the complex relations in the emulsifying phase diagram, and hence is superior to surfactant–cosurfactant system.

Labrasol showed a strong absorption enhancing effect on water-soluble drugs like GM in our previous studies (Hu et al., 2001). However, the dose of Labrasol, 1.0 ml/kg, is too high to be used in large animals or humans because the amount of Labrasol will be too huge according to body weight. At the same time, there is a potential problem of Labrasol induced intestinal irritancy. In addition, regarding tolerance and toxicological data of Labrasol it was reported that LD_{50} is 22 g/kg for oral administration in rat; 13-week oral subchronic toxicity study in dogs at 3 g/kg per day showed well toleration. To reduce the risk of epithelia irritancy and make acceptable dose for large animals or human, in this study the dose of Labrasol was decreased and the feasibility of absorption enhancing effect on GM was evaluated using rats. Although the C_{max} values were high, when the doses of Labrasol were reduced to 0.5 and 0.2 ml/kg, AUC_{0-6} values accordingly decreased. Hence, the absorption enhancing effect of Labrasol showed a clear dose-dependency. In addition, we used Labrasol for improving the absorption of peptide/protein drug, insulin. By formulating with Labrasol, extensive hypoglycemic effect was obtained in rats. However, the BA of insulin measured by ELISA assay method was 2.0% (Eaimtrakarn et al., in press). This value is too low for the development of oral insulin preparation. Therefore, we have to look for a stronger absorption enhancer that can improve drug absorption even at low dose and has comprehensive applicability.

In order to improve the absorption enhancing effect of Labrasol, there are two strategies: (a) increasing the local concentration of Labrasol in the GI tract by some pharmaceutical technologies; (b) improving absorption enhancing effect of Labrasol by extraction or purification. In this paper, the second approach was focused and the capability of absorption enhancing effect of Labrasol fractions was assessed. Meanwhile, we have reported that a novel drug delivery system, gastrointestinal mucoadhesive patch system (GI-

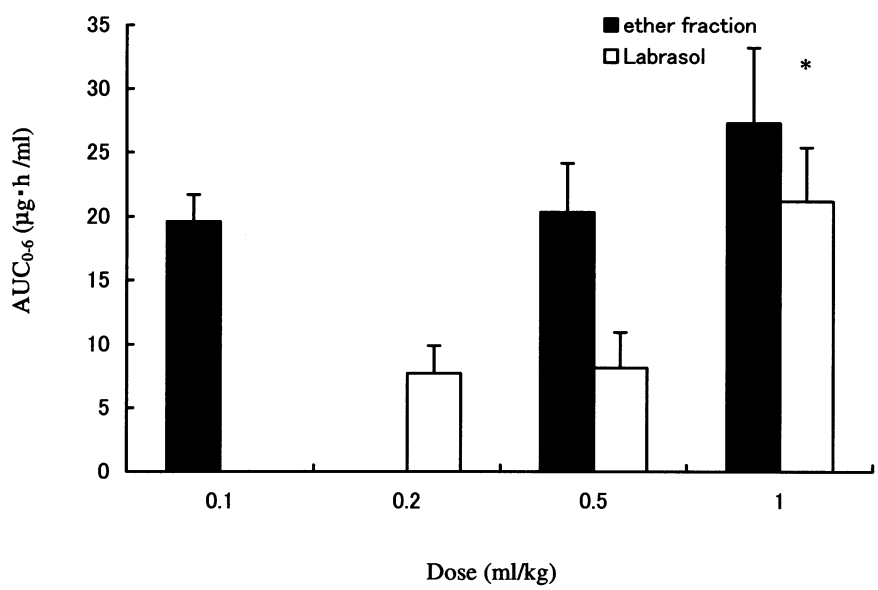

Fig. 5. Effect of Labrasol or diethyl ether fraction of Labrasol doses on AUC_{0-6} following i.c. administrations. Values are the mean ± SE of 3–4 animals. *Shows significant difference with each other dose groups at $P < 0.05$.

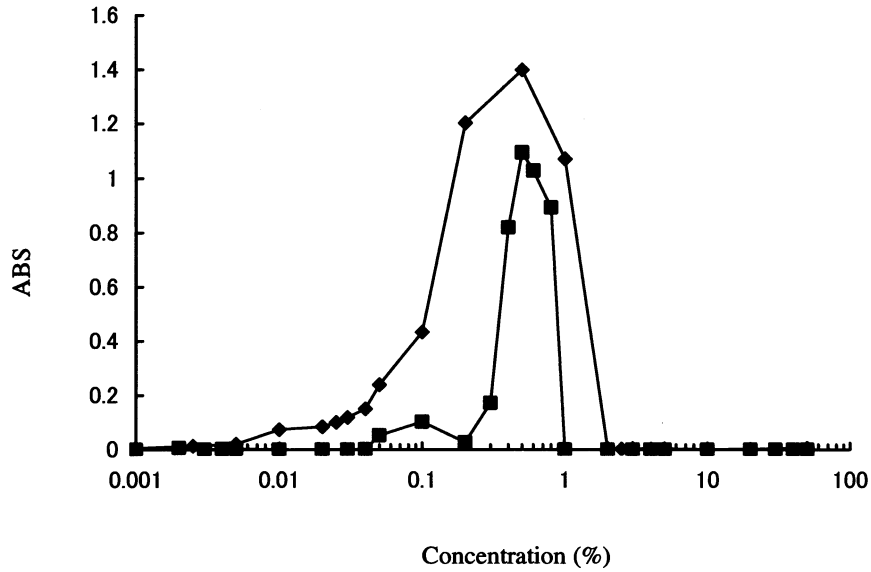

Fig. 6. Turbidities of Labrasol and diethyl ether fraction of Labrasol. Turbidities of Labrasol (◆) and diethyl ether fraction of Labrasol (■) was measured at 700 nm wavelength.

MAPS®) having muco-adhesive property, can increase the local concentration of both drug and absorption enhancer (Eaimtrakarn et al., 2001).

As Labrasol is a mixture, it can be expected that the absorption enhancing effect can be improved by fractionating the more efficacious component from Labrasol. According to the polarity index, Labrasol was consecutively extracted by hexane, diethyl ether and ethyl acetate and their fractions were obtained. Because hexane is an extremely apolar solvent, the hexane fraction extracts the low HLB components of Labrasol. Fig. 1 shows that hexane fraction did not have enough absorption enhancing effect as compared to Labrasol. Similar results were obtained with the other two fractions, i.e. ethyl acetate and aqueous residue. Interestingly, it was observed that diethyl ether fraction significantly improved the GI absorption of GM as compared to Labrasol itself. The C_{max} values of three doses, i.e. 1.0, 0.5 and 0.1 ml/kg, were high (8.24 ± 1.47, 8.02 ± 2.14 and 7.41 ± 1.25 μg/ml). Furthermore, the AUC_{0-6} was still high, 19.61 ± 2.09 μg h/ml, even with the lowest dose of 0.1 ml/kg. By comparing the C_{max} and AUC_{0-6} values, the absorption enhancing effect of diethyl ether fraction is about two times stronger than Labrasol itself. Therefore, it suggests that diethyl ether fraction of Labrasol has a stronger absorption enhancing effect on GM absorption from the GI tract.

The reason for diethyl ether fraction of Labrasol having stronger absorption enhancing effect than Labrasol is not completely clear. It is considered that both of them enhance GM absorption from GI tract by forming microemulsion. The interfacial tension between microemulsion and epithelial membrane is less compared to aqueous solution of hydrophilic drugs and epithelium. Microemulsion contributes for the improved permeability of hydrophilic drugs across epithelial membrane resulting in absorption enhancement. It is important that a good microemulsion system used to improve GI absorption of hydrophilic drugs should retain their characters even after mixing with aqueous fluids of the GI. The microemulsions of Labrasol or diethyl ether fraction of Labrasol at each dose were clear before administration into rat colon. When the formulation blends with colonic fluid, the size of microemulsion globules was changed because of the dilution. In vitro studies have indicated that the concentration range for developing turbidity was 0.1–2.0%

for Labrasol and 0.3–1.0% for diethyl ether fraction of Labrasol (Fig. 6). It implies that diethyl ether fraction of Labrasol has more tolerance in retaining the clarity even after getting diluted with intestinal fluids than Labrasol. The development of turbidity may be due to increase globule size of the emulsion. The increase in globule size would decrease the absorption enhancing effect, because the surface of emulsion contacting with the absorption membrane will be decreased. At high doses of the Labrasol or diethyl ether fraction of Labrasol can retain their clarity even after dilution with colonic fluids thereby improving the GI absorption of GM. At low doses, the diethyl ether fraction of Labrasol might have remained clear, which means the microemulsion globule size is impalpable, after dilution and enhanced the GI absorption of GM. Whereas, Labrasol might have became turbid at low doses after getting diluted by colonic fluids resulting in poor absorption enhancing effect. However, further experiments are going on to carry out to resolve the reason for improved absorption of diethyl ether fraction of Labrasol even at low doses.

In conclusion, fractionation of Labrasol has been carried out and the evaluation of each fraction for a stronger absorption enhancing effect on GM was carried out in this study. Among four fractions, i.e. hexane, diethyl ether, ethyl acetate and aqueous residue, diethyl ether extract showed a stronger absorption enhancing effect than Labrasol itself. This fraction will be useful for the oral delivery of not only low molecular weight and extremely hydrophilic drugs but also large molecules containing protein and peptide drugs.

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