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Research Paper

Application of hydrotropy to transdermal formulations: hydrotropic solubilization of polyol fatty acid monoesters in water and enhancement effect on skin permeation of 5-FU

Koichi Takahashi[a], Megumi Komai[a], Natsumi Kinoshita[a], Emi Nakamura[a], Xiao-Long Hou[a], Tomoka Takatani-Nakase[a] and Masaya Kawase[b]

[a]Department of Pharmaceutics, School of Pharmaceutical Sciences, Mukogawa Women's University, Hyogo and [b]Department of Bioscience, Nagahama Institute of Bio-Science and Technology, Shiga, Japan

Abstract

Objectives A hydrotropic formulation containing a percutaneous enhancer was developed for the transdermal formulation of a water-soluble drug and the solubilizing mechanisms of a percutaneous enhancer in water by a hydrotropic agent were investigated. The enhancement effect was also compared with the hydrotropic formulation and the other formulations using ethanol, propylene glycol or mixed micelles.

Methods Sodium salicylate (SA) and sodium benzoate (BA) were selected as hydrotropic agents, and polyol fatty acid ester (POFE) and 5-fluorouracil (5-FU) were selected as a percutaneous enhancer and a water-soluble drug, respectively. Near-infrared (NIR) spectrophotometric and ^{1}H NMR spectroscopic studies were carried out to investigate the solubilizing mechanisms. The mean particle size in the hydrotropic formulation was measured. The in-vitro skin permeation of 5-FU and the accumulation in the skin of propylene glycol monocaprylate (PGMC), one of the monoesters of POFE, from the hydrotropic formulation or the other formulations were investigated by using Franz-type diffusion cell.

Key findings The presence of SA and BA had a visible effect on the O–H stretching band of water in the NIR region. The surface tension of SA and BA aqueous solutions was found to decrease with an increase in SA or BA concentration. Although SA interacted with PGMC in the presence of water, it did not interact with PGMC in the absence of water. Mean particle size in a solution consisting of 5% (v/v) PGMC and 30% SA aqueous solution was approximately 14 nm. ^{1}H NMR spectroscopic studies indicated that the hydrotropic salts formed aggregates with which PGMC interacted from the outside. The hydrotropic formulation prepared in this study enhanced skin permeation of 5-FU when compared with the other formulations.

Conclusions SA and BA solubilized monoesters of POFE in water, and SA interacted with PGMC in water. The hydrotropic formulation prepared in this study significantly enhanced skin permeation of 5-FU compared with the other formulations. The results suggest that a hydrotropic formulation containing PGMC may be a useful transdermal formulation for water-soluble drugs.

Keywords 5-fluorouracil; hydrotropy; penetration enhancer; percutaneous absorption; sodium salicylate

Introduction

Transdermal drug delivery offers the possibility of producing sustained plasma drug levels and of avoiding hepatic first-pass metabolism. However, the possibilities for administration by the transdermal route are limited by the barrier properties of the stratum corneum. Only a small number of materials, which tend to be lipophilic and to have low melting points, are easily transported to the underlying viable aqueous tissue.[1] To overcome the problem of skin impermeability, chemical enhancers or physical methods, such as iontophoresis and electroporation,[2–6] have been suggested. In particular, much effort has been directed towards the search for enhancers in the development of transdermal systems. Many compounds, such as fatty acids, alcohols, propylene glycol, amines and amides, are known to enhance drug permeation,[7] but many of these are not miscible with water. Pharmaceutical carriers, such

Correspondence: Koichi Takahashi, School of Pharmaceutical Sciences, Mukogawa Women's University, 11-68 Koshien Kyuban-cho Nishinomiya, Hyogo 663-8179, Japan.
E-mail: koichi@mukogawa-u.ac.jp

as mixed micelles, emulsions and co-solvent systems, are commonly employed to incorporate enhancers into transdermal formulations such as ointments, cataplasms, patches and gel formulations. Another strategy is the development of hydrotropy for transdermal formulations.

Hydrotropy is an increase in water solubility caused by the addition of a second solute.[8,9] Several hydrotropic agents, such as urea, caffeine, sodium benzoate, sodium salicylate and nicotinamide, have been identified.[10,11] The main use of this phenomenon is to increase the water solubility of insoluble or slightly soluble drugs;[11,12] there are no reports of the development of a hydrotropic formulation containing a percutaneous enhancer for the transdermal formulation of a water-soluble drug.

Fatty acid-alcohol esters are commonly used as adjuvants for cosmetics and pharmaceuticals. Some esters have been used as permeation enhancers for drugs.[13] We previously reported solubilization of polyol fatty acid monoesters in water in the presence of diclofenac sodium, resulting in enhanced permeation of diclofenac.[14] In this study, we examined two hydrotropic salts, sodium salicylate and sodium benzoate, for their ability to solubilize polyol fatty acid esters in water. Near-infrared spectrophotometry and ^{1}H NMR spectroscopy were used to investigate the physicochemical properties of the hydrotropic system. In addition, we compared the enhancement effects of polyol fatty acid esters in a hydrotropic system with those obtained in co-solvent systems and mixed micelles.

Materials and Methods

Materials

5-fluorouracil (5-FU) was purchased from Sigma-Aldrich Chemical Co. (St Louis, MO, USA). Sodium salicylate (SA) and sodium benzoate (BA) were purchased from Wako Pure Chemical Industries Co., Ltd (Osaka, Japan). The polyol fatty acid esters (POFEs) used in this study were propylene glycol monocaprylate (PGMC), glyceryl monocaprylate (GMC) and propylene glycol dicaprylate (PGDC), supplied by Nikko Chemical Co., Ltd (Tokyo, Japan). N,O-bis(trimethylsilyl)-acetamide and n-caproic acid were purchased from Tokyo Kasei Kogyo Co., Ltd (Tokyo, Japan) and Nacalai Tesque Inc. (Kyoto, Japan), respectively. Other reagents were of analytical grade and were used without further purification.

Investigation of solubilization of POFE in water by hydrotropic salts

A known concentration SA or BA solution (4.5 ml) was transferred into a screw-capped vial, POFE (0.5 ml) was added and the solution was vigorously shaken for several minutes. The concentration of SA or BA was increased until the turbidity of the solution disappeared.

^{1}H NMR measurement

The ^{1}H NMR spectra of each specimen were measured at 35.0°C on a INM-ECP500 spectrometer operating at 500 MHz (JEOL Ltd, Japan) for protons in D_2O or DMSO solution. Measurement conditions were as follows: 90° pulse width, 12.2 μs; relaxation delay, 4 s; scan, 100 times. Tetramethylsilane was used as an internal standard.

Spectrophotometric analysis

Samples were analysed by near-infrared spectrophotometry (NIRS) in a Spectrum One NTS FT-NIR spectrophotometer (PerkinElmer). Samples were placed in the NIRS sample holder (3-cm diameter round cell) until it was three-quarters full, and their spectra were registered as individual files, measured in the range 400–2500 nm at 2 nm intervals.

Surface tension measurements

Surface tension measurements were carried out at 25°C using a Surface Tensiometer ST-1 (Shimadzu, Japan).

Particle size distribution measurements

These measurements, based on scattering, were taken without dilution at 25°C by a NICOMP 380 submicron sizer (NICOMP Particle Sizing Systems Inc, Santa Barbara, CA, USA).

Transdermal formulation and solubility studies using 5-fluorouracil

The hydrotropic formulation consisted of 30% (w/v) SA or 43% (w/v) BA aqueous solution and 5% (v/v) PGMC as an enhancer. The pH values of the hydrotropic formulation of SA and BA were 6.8 and 8.0, respectively. The co-solvent formulation consisted of 50% (v/v) ethanol (EtOH) or 80% (v/v) propylene glycol (PG) solution and 5% (v/v) PGMC. The mixed micelle formulation was prepared with 2% (v/v) surfactant (Tween 80 : Span 83 = 73 : 27, HLB = 12) solution and 5% (v/v) PGMC after vigorous vortexing. Formulations without 5% (v/v) PGMC were also used.

A slight excess of 5-FU was added to each of the above formulations, and the mixtures were allowed to stand at 37°C under agitation for 12 h. The samples were then filtered through a 0.45-μm filter and the concentrations of 5-FU were measured by HPLC after appropriate dilution.

In-vitro permeation study

Male hairless rats (HWY strain, 8 weeks old) were purchased from Japan SLC (Hamamatsu, Japan). Rats were used in accordance with the Guidelines for Animal Experimentation of Mukogawa Women's University, which are based on the Guidelines for Animal Experimentation of the Japanese Association for Laboratory Animal Science. Full-thickness abdominal skin was excised from the hairless rats immediately before the experiment, and the excised skin samples were mounted in Franz-type diffusion cells. In this study, 10 ml of saline was used as the receptor medium, and 0.5 ml of the transdermal formulation solution containing approximately 1% (w/v) 5-FU was placed on the donor side. The surface area exposed for diffusion was 0.785 cm^2 (diameter = 1.0 cm). The receptor medium was kept at 37°C and stirred with a magnetic stirrer at 600 rpm. Aliquots (0.2 ml) of the receptor medium were withdrawn periodically

over 12 h. Immediately after collection of the samples, 0.2 ml of fresh saline was added. The concentration of 5-FU in the samples and the transdermal formulation solutions was determined by high-performance liquid chromatography (HPLC).

The steady state flux, J, was obtained from the initial linear portion of the penetration curve, which was based on a plot of the accumulated amount of 5-FU against time. The permeability coefficient was calculated using Eq. 1:

$$J = Pe \cdot A \cdot Cd \quad (1)$$

where Pe is the permeability coefficient, A is the surface area (0.785 cm^2 for the diffusion cells in the present study), and Cd is the concentration of 5-FU in the test solution.

Skin accumulation

The amounts of PGMC accumulating in skin tissue were measured. After the 4-h permeation experiments with the solutions containing PGMC, the skin was removed from the cell, washed with water and wiped with a cotton cloth soaked with acetone. After weighing, the skin was homogenized with 2.6 ml ethyl acetate containing n-caproic acid as an internal standard for PGMC. The homogenate was centrifuged for 10 min at 13 000 rpm, and the supernatant (1 ml) was evaporated to dryness. To determine the amount of PGMC present, the residue was redissolved in a mixture of ethyl acetate (0.1 ml) and N,O-bis(trimethylsilyl)-acetamide (0.06 ml) and, after 20 min, injected into a gas chromatograph.

Analytical methods

5-FU assay was carried out by HPLC using a system consisting of a pump (880-PU, Jasco, Tokyo, Japan), a detector (875-UV, Jasco), a 4.6 × 250 mm column packed with Nucleosil 100-5C18 (Macherey-Nagel, Germany), and an integrator (C-R3A, Shimadzu, Kyoto, Japan). The flow rate was 1.0 ml/min, and separation was performed at ambient temperature with a mobile phase of 20 mM acetic acid solution. UV detection was carried out at 260 nm.

PGMC measurement was conducted using a gas chromatograph (GC-7AG, Shimadzu) equipped with a flame ionization detector. The carrier gas was nitrogen, at a flow rate of 40 ml/min. The column was coiled glass, 1 m × 2 mm i.d., packed with Silicone OV-17 (Gasukuro Kogyo Inc., Tokyo, Japan). The column temperature program was set at 100°C for 1 min followed by a 8°C/min rise to 170°C. The temperatures of both the injector and the detector were maintained at 200°C. The limits of detection for PGMC in the skin were 0.1 μg/mg tissue. The calibration curves for PGMC were linear up to 2 μg/mg tissue.

Table 1 Minimum concentrations of hydrotropic salts to solubilize polyol fatty acid ester in water

	Concentration of POFE (% v/v)					
	PGMC		GMC		PGDC	
	5	10	5	10	5	10
SA	28	29	28	29	×[a]	×[a]
BA	41	41	41	41	×[a]	×[a]

Figures shown are % w/v. GMC, glyceryl monocaprylate; PGDC, propylene glycol dicaprylate; PGMC, propylene glycol monocaprylate. [a]The symbol × means that PGDC was not solubilized by the hydrotropic salt in water. This study was performed at 23 ± 2°C.

Statistical analysis

The results are expressed as mean ± standard deviation. Statistical significances were determined by the Bonferroni test as a post-hoc test following Kruskal–Wallis test. Differences were considered significant when the calculated P value was <0.05.

Results and Discussion

Hydrotropic solubilization of polyol fatty acid monoesters in water and its solubilizing mechanisms

In this study, SA and BA were selected as model hydrotropic salts and POFE was selected as a percutaneous enhancer. Table 1 shows the minimum concentrations of SA and BA

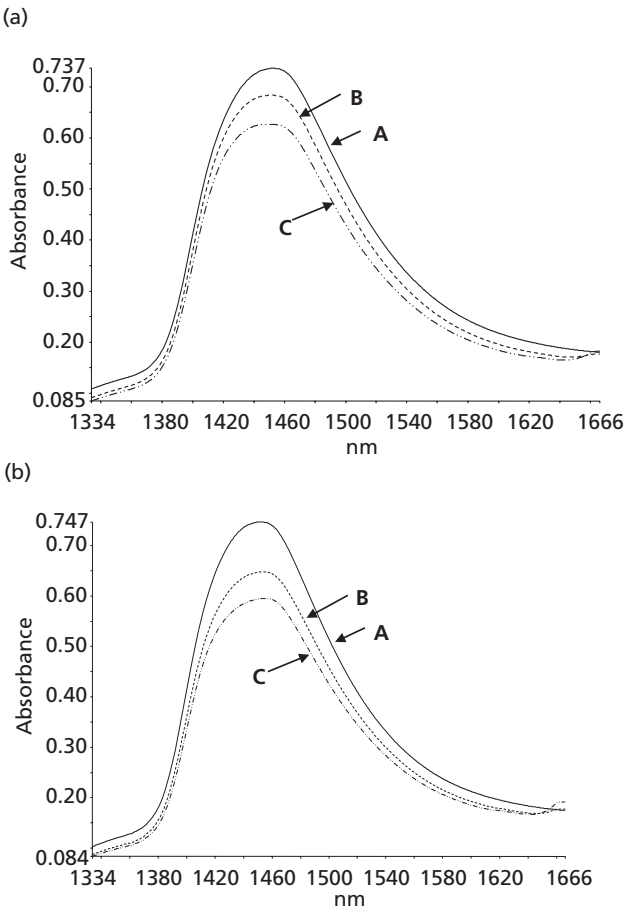

Figure 1 Effect of hydrotropic salt concentration on O–H stretching band of water in the near-infrared region for (a) sodium salicylate adn (b) sodium benzoate. A, water; B, 10% w/v hydrotropic salt solution; C, 20% w/v hydrotropic salt solution.

required to solubilize POFE in water. It was found that these hydrotropic salts were capable of solubilizing monoesters of POFE in water, but POFE diesters were not solubilized. Two possible mechanisms in the hydrotropic solubilization may be considered: one is that the hydrotropic agent exerts its effect by altering the nature of the solvent[15] and the other is that it alters the interaction between the hydrotropic agent and the solute.[10,11,16,17] To investigate the effect of the hydrotropic agent on the nature of the solvent, the stretching vibration of the O–H bond of water was determined. Changes in the water–water interaction may be reflected in changes in the characteristics of the O–H stretching band of water in the near-infrared region.[18] Figure 1 shows the effects of SA and BA on the O–H stretching band. When the concentration of the hydrotropic salt was increased, the values for wavelength of maximum absorption, V_m, and for bandwidth at mid-height, $W_{1/2}$, shifted to higher values (Table 2). From these results, the solubilization of POFE in water by SA and BA may imply that these salts affect the structure of water in favor of a more hydrophobic mode.

As NMR spectroscopy is highly sensitive to changes in the chemical environment, it has previously been used for investigations in this area.[19] The chemical shift of NMR signals is a reflection of the environment around the nuclei, and changes in chemical shift provide information on the interactions between drug molecules and other molecules. In this study, ^{1}H NMR spectroscopy was used to investigate the interaction between SA and PGMC. Figure 2 shows ^{1}H NMR spectra of SA solution and SA/PGMC solution prepared using aqueous DMSO (D_2O : DMSO = 1 : 3). The aromatic proton peaks in the SA spectrum were assigned as shown. Up-field shifts were observed for the aromatic SA protons in solutions containing PGMC in DMSO aqueous solution. However, similar shifts were not observed in the presence of PGMC in DMSO without water (data not shown). These results suggest that SA or BA interacts with PGMC in water.

Hydrotropic agents are known to self-assemble in solution.[20] In this study, the surface tension of aqueous solutions decreased with increasing concentration of SA and BA (Figure 3). The same result was previously reported by Agrawal *et al.*,[11], who also reported that these hydrotropic salts increased the specific conductance of water with increasing concentration, resulting in molecular aggregation. The

Table 2 Effects of sodium salicylate and sodium benzoate on the O–H stretching band of water

	Water	SA (% w/v)		BA (% w/v)	
	0	10	20	10	20
Vm	1449	1452	1453	1454	1455
$W_{1/2}$	156	157	160	166	170

SA, sodium salicylate; BA, sodium benzoate; Vm, wavelength of maximum absorption (nm); $W_{1/2}$, the bandwidth at mid-height.

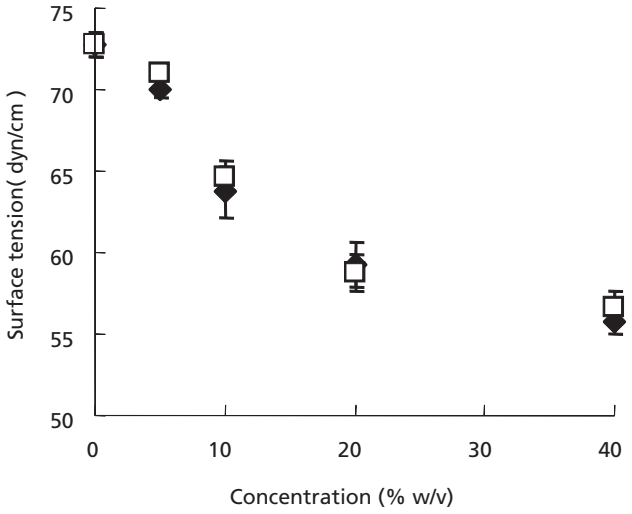

Figure 3 Effect of hydrotropic salts on surface tension of water: ◆, sodium salicylate; □, sodium benzoate. The surface tension was measured at 23 ± 2°C. Each point represents mean ± SD (n = 3–5).

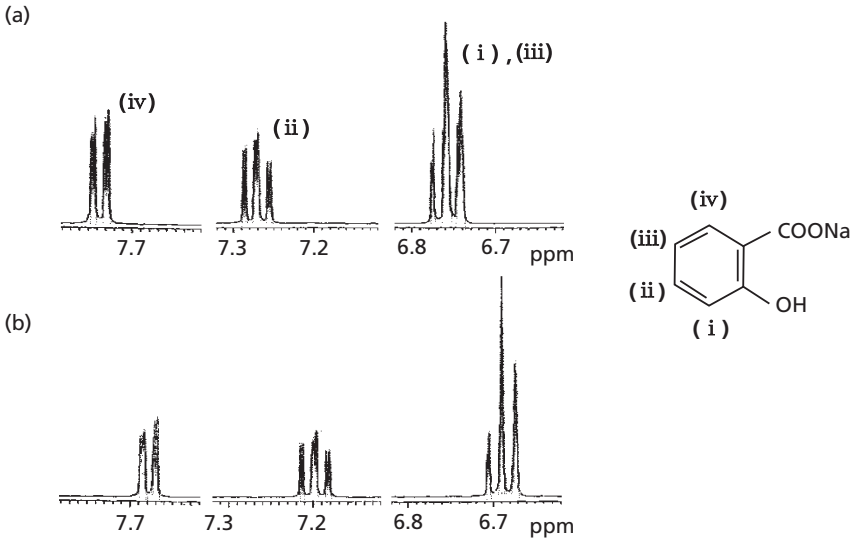

Figure 2 The ^{1}H NMR spectra of SA in a solution of D_2O/DMSO (1 : 3). (a) SA, (b) SA + PGMC.

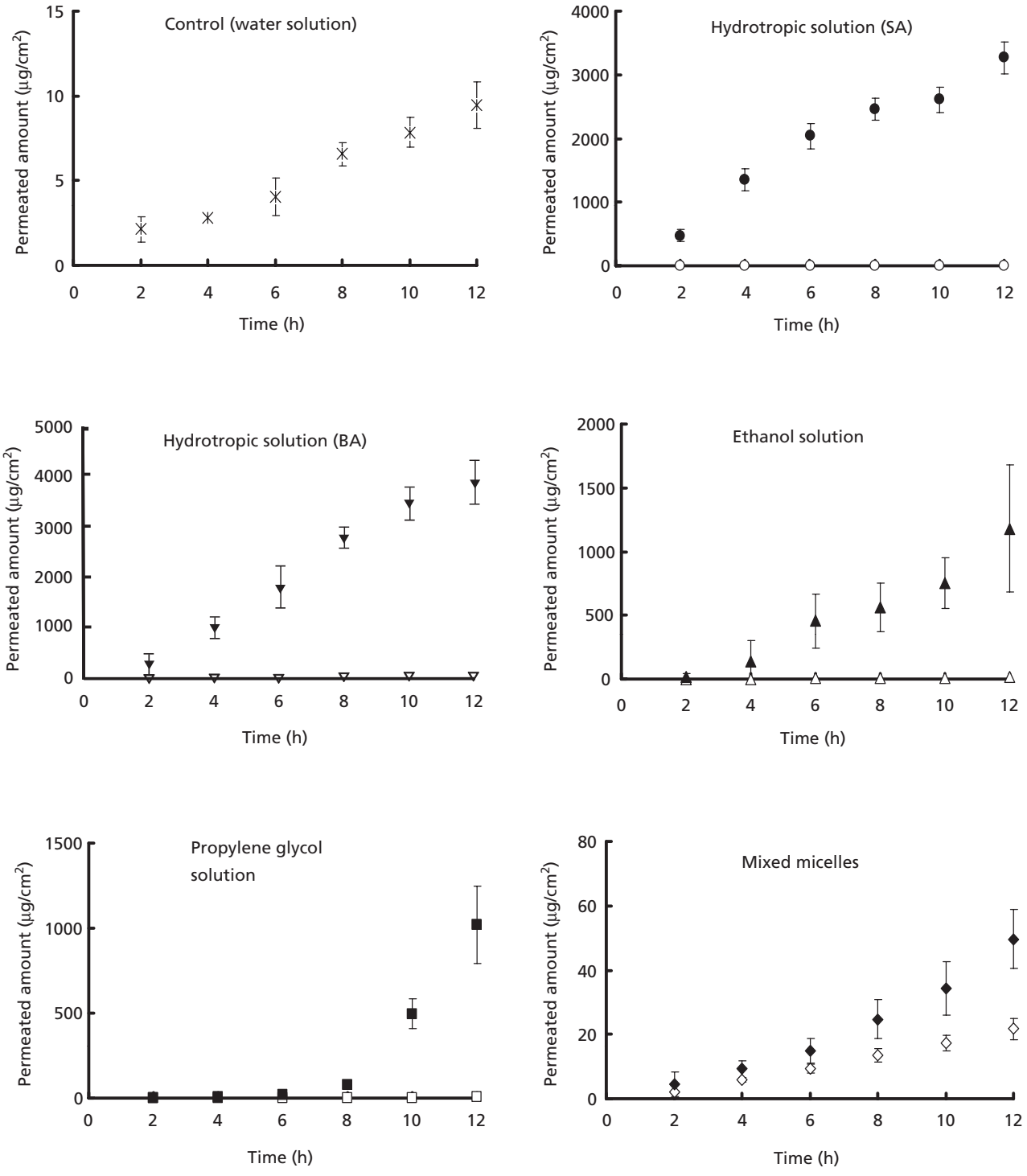

Figure 4 Effect of PGMC on permeation of 5-FU through hairless rat skin: *, control; ●, hydrotropic formulation (SA) with PGMC; ○, without PGMC; ▼, hydrotropic formulation (BA) with PGMC; ▽, without PGMC; ▲, ethanol formulation with PGMC; △, without PGMC; ■, propylene glycol formulation with PGMC; □, without PGMC; ◆, mixed micelles formulation with PGMC; ◇, without PGMC. Each point represents mean ± SD ($n = 3–5$).

planar structure of SA allows a stacking-type association. Kumar *et al.*[21] suggested that stacking of SA molecules appears at concentrations greater than 0.9 M (about 14.4% (w/v)) in aqueous solution. We found that the minimum concentration of SA required to solubilize PGMC in water was greater than this (Table 1).

The particle size distribution, which is an indication of the existence of molecular aggregation, was measured. The mean

particle size in the hydrotropic solution was approximately 14 nm (5% (v/v) PGMC and 30% (w/v) SA).

Badwan *et al*.[22] reported that the solubility mechanism of benzodiazepines in SA solution may be based on the inclusion of benzodiazepine molecules in the SA stack. If PGMC molecules were included in the SA stack, this might result in changes in the chemical shift of the NMR signals of PGMC. To investigate the effect of the presence of SA on the chemical shifts of PGMC, ^{1}H NMR spectra of PGMC and SA/PGMC solutions, prepared using DMSO/D_2O solution (D_2O : DMSO = 1 : 3), were also examined. No significant difference was observed between the chemical shifts of PGMC and those of PGMC with SA (data not shown). These results suggest that PGMC molecules are not included in the SA stack. BA also has aromatic ring as SA, therefore BA is also assumed to form a stacking-type association in aqueous phase. This suggestion is supported by the results of surface tension measurements. BA decreased the surface tension of the aqueous solution, as did SA. This fact shows that BA and SA exhibit the same behavior in aqueous solution. As BA forms a dimer via intermolecular hydrogen bonding, the particle size of BA is considered to be larger than that of SA. These results suggest that the PGMC molecule assembles on the BA particle surface, like SA. It is possible that the mechanism of SA- and BA-based solubilization may be a change in the water–water interaction caused by these species: due to this change, the molecules self-associate to form aggregates in aqueous solution, and PGMC may interact with SA or BA from outside the stack.

Enhancement effect of hydrotropic system containing percutaneous enhancer on skin permeation of 5-fluorouracil

Co-solvent systems, mixed micelles and emulsions are commonly employed to increase the effectiveness of percutaneous enhancers. PGMC was selected as a percutaneous enhancer in this study because we previously reported that PGMC enhanced the permeation of diclofenac sodium through rat skin.[23] To compare the enhancement effect of PGMC on drug permeation in co-solvent, mixed micelles and hydrotropic systems, a water-soluble drug, 5-FU, was selected. Figure 4 shows the permeation profiles of 5-FU from various formulations with or without PGMC. Permeation of 5-FU was very low when no enhancement system was used, but PGMC significantly enhanced permeation in all formulations. The steady-state flux (J) and lag time (L) were calculated from the initial linear portion plotted in Figure 4. The permeation parameters and solubilities of 5-FU are summarized in Table 3. Values of J significantly increased in the presence of PGMC. As we did not use a drug suspension as a donor solution in this study, the enhancement factor (EF) was calculated with Eq. 2 to correct the escape tendency of 5-FU from the formulation.[24]

$$EF = (Pe)/(Pe_{cont.}) \times (S)/(S_{cont.}) \quad (2)$$

where Pe and $Pe_{cont.}$ are the permeability coefficients of 5-FU in various formulations and aqueous solution (control), respectively. S and $S_{cont.}$ are the solubilities of 5-FU in various formulations and aqueous solution (control), respectively. The value of EF decreased in the following order: hydrotropic formulation (SA) > hydrotropic formulation (BA) > PG formulation > EtOH formulation > mixed micelles formulation. SA is a keratolytic substance and may enhance the permeations of 5-FU and PGMC. However, the values of the enhancement factor of SA and BA are the same order. The values of L in PG and mixed micelles were significantly greater than those of the control. We previously reported that the mechanism of the PGMC enhancement effect may be a change in the lipid-chain fluidity of the stratum corneum without lipid extraction.[23] It is therefore expected that a higher PGMC content in the skin may induce a greater enhancement effect. To judge whether this hypothesis was correct, the content of PGMC in the skin at 4 h was measured, and it was found to be higher for the hydrotropic formulation than for the other formulations (Figure 5). These results

Table 3 Permeation parameters of 5-FU from various solutions with or without PGMC through hairiless rat skin and solubility of 5-FU in those solutions

	J^a (μg/cm^2 per h)	L^a (h)	Pe^b ×10^{-4} (cm/h)	S^c (mg/ml)	EF^d
5-FU (control)	0.96 ± 0.10	1.9 ± 0.8	0.82 ± 0.08	15	1.00
+PGMC					
+SA	391 ± 25.0*	0.7 ± 0.2*	466 ± 38.7*	33	1250
+BA	388 ± 28.2*	0.7 ± 0.6*	392 ± 27.7*	35	1115
+EtOH	134 ± 10.3*	2.9 ± 1.3*	112 ± 7.79*	30	273
+PG	235 ± 55.0*	7.7 ± 0.1*	236 ± 55.2*	23	441
+M.M.	5.70 ± 1.12	3.7 ± 0.6*	10.1 ± 1.20	17	13
–PGMC					
+SA	1.26 ± 0.11	0.6 ± 0.3*	1.48 ± 0.13	32	3.85
+BA	0.96 ± 0.18	2.9 ± 0.7*	1.02 ± 0.14	33	2.74
+EtOH	1.26 ± 0.37	2.3 ± 0.5	1.12 ± 0.27	29	2.65
+PG	0.35 ± 0.21	1.4 ± 0.7	0.31 ± 0.19	23	0.58
+M.M.	1.95 ± 0.32	1.1 ± 0.2	1.94 ± 0.31	20	3.15

[a]The values of steady-state flux (J) were calculated from the initial linear portion in Figure 4. [b]The permeability coefficient (Pe) was calculated from the steady-state flux and the initial concentration of 5-FU in the donor compartment. [c]The solubility (S) of 5-FU in each solution was measured at 37°C. [d]Enhancement factor: EF = (Pe)/($Pe_{cont.}$) × (S)/($S_{cont.}$). *$P < 0.05$ vs control.

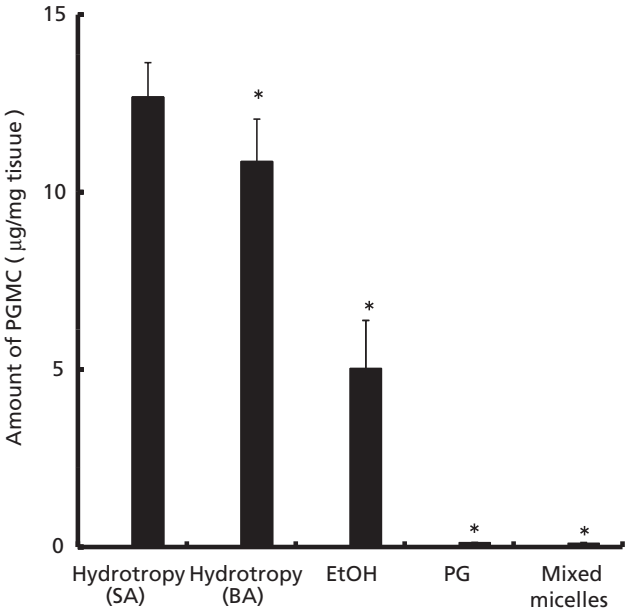

Figure 5 Amount of PGMC in skin after permeation experiment for 4 h. Hydrotropy, hydrotropic formulation; EtOH, ethanol formulation; PG, propylene glycol formulation. *$P < 0.05$ vs hydrotropic formulation. Each bar represents mean ± SD ($n = 3–5$).

suggest that PGMC is more easily partitioned to skin from the hydrotropic formulation than from the other formulations.

Conclusions

SA and BA solubilized monoesters of POFE in water, and the mean particle size in the solution was approximately 14 nm. The mechanism of solubilization may be a change in the interaction between water molecules caused by hydrotropic salts, self-association of the salt molecules in aqueous solution to form aggregates and, finally, interaction of the hydrotropic salts with PGMC. The hydrotropic formulation prepared in this study significantly enhanced skin permeation of 5-FU compared with the other formulations. Based on these results, a hydrotropic formulation containing PGMC may be a useful transdermal formulation for water-soluble drugs.

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