

Effect of Mixed Micelle Formulations Including Terpenes on the Transdermal Delivery of Diclofenac

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The significant inhibitory action of diclofenac formulated in mixed micelles of lecithin with cholate or deoxycholate was observed on the rat hind paw edema induced by carrageenan. In the primary stage, mixed micelle formulation of deoxycholate was more effective compared with that of cholate. However, in the final term, the inhibitory action was similar in both formulations. In a previous study, the flux of diclofenac was greater in the mixed micelle formulation of deoxycholate compared with that of cholate. It was suggested that the permeation rate of diclofenac through skin was proportional to the pharmacological activity. The hind paw edema was quickly inhibited when cyclic monoterpene such as *d*-limonene or *l*-menthol was included in the formulations. All the micelle formulations significantly decreased the value of AUC estimated the hind paw thickness–time profile. This suggests that the micelle formulation of cholate in addition to deoxycholate showed significant anti-inflammatory activity to hind paw edema of rats. Incorporation of *d*-limonene or *l*-menthol was more effective on the decrease of AUC. A pharmacological study revealed that micelle formulations were able to reduce the skin irritation of chemicals.

Key words transdermal drug delivery; lecithin bile-salt mixed micelle; cyclic monoterpene; diclofenac; anti-inflammatory effect

Diclofenac is widely used for treatment of localized nonarticular rheumatism and inflammations. However, the half-life of diclofenac in human body is 1.8 h after oral administration and the hepatic first pass effect is very extensive.¹⁾ Thus, to develop the transdermal delivery system of diclofenac is important. Otherwise, the barrier ability of the stratum corneum is robust to foreign substances. Several studies attempted to increase absorption of diclofenac transdermally including the utilization of chemical enhancers.^{2–7)} The administration of chemical enhancers is recognized as an effective strategy to increase percutaneous absorption of drugs. At the same time, for hydrophobic enhancers, it is necessary to use organic solvents to dissolve enhancers into the formulation. Thus, skin irritation caused by those chemicals is not negligible. To overcome the low solubility of chemical enhancers in the formulation, the micelle system was suggested to be useful. The micelle system of bile-salt was suggested to be less irritancy to living body compared with organic solvents. To assure sufficient solubility of chemical enhancers, lecithin bile-salt mixed micelle system was selected in this study.

In a previous study, we investigated the flux of diclofenac from the micelle formulations through rat skin.⁸⁾ The lecithin bile-salt mixed micelles dissolved diclofenac in a greater amount compared with that of simple micelles composed of bile salt alone. Further, the flux was significantly increased

when *d*-limonene or *l*-menthol was incorporated in the mixed micelles. In this study, the pharmacological activity was investigated *in vivo*. In addition, the irritation of the micelle formulation to the skin was pathologically evaluated.

MATERIALS AND METHODS

Materials Sodium diclofenac (DFS) was supplied by SS Pharmaceutical Co., Ltd. (Tokyo, Japan). Diclofenac was obtained by recrystallization of DFS in acidic medium (0.1 N HCl solution). Sodium cholate, sodium deoxycholate, lecithin and carrageenan Lambda were from Sigma Chemical Co. Ltd. (St. Louis, MO, U.S.A.). *d*-Limonene and *l*-menthol were purchased from Tokyo Chemical Industries Co. (Tokyo, Japan). Purified water was produced with the Milli-Q system (Japan Millipore Co., Tokyo, Japan). The other chemicals were of reagent grade.

Preparation of Mixed Micelle Formulations The formulations of mixed micelles of diclofenac with and without cyclic monoterpene are shown in Table 1. The mixed micelle formulation was prepared as follows: Diclofenac, bile salt, lecithin, *d*-limonene or *l*-menthol and water were mixed in a vial. The vial was sealed after filling with nitrogen gas. The mixture was shaken at 65–70 °C for 15 h. The temperature of the mixture was decreased until 37 °C, and then the result-

Table 1. Formulation of Mixed Micelle System of Diclofenac (mM)

	Formulation 1 (F1)	Formulation 2 (F2)	Formulation 3 (F3)	Formulation 4 (F4)	Formulation 5 (F5)	Formulation 6 (F6)
Diclofenac	33.8	33.8	33.8	33.8	33.8	33.8
Cholate	32.5	32.5	32.5	—	—	—
Deoxycholate	—	—	—	32.5	32.5	32.5
Lecithin	5.2	5.2	5.2	5.2	5.2	5.2
<i>d</i> -Limonene	—	36.7	—	—	36.7	—
<i>l</i> -Menthol	—	—	32.0	—	—	32.0

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ing mixture was centrifuged. The supernatant was used as a mixed micelle formulation. All components were dissolved in the supernatant. Thus, the concentration of diclofenac in the mixed micelle formulation was equal to its solubility in the mixed micelle. As a control, saturated diclofenac in purified water (0.015 mg/ml) was employed.

Determination of Anti-inflammatory Effect The anti-inflammatory effect was evaluated *in vivo* using a caliper to measure carrageenan induced hind paw edema thickness. Male Wistar rats weighing 180–200 g, were used. Under anaesthetization with urethane solution (25% in saline; 3 ml/kg i.p.), the hind paw thickness was measured as a control value. After that glass cells (16 mm inner diameter, 10 mm height) containing mixed micelle formulation of diclofenac (1.0 g) were attached to the abdominal skin with cyanoacrylate-type adhesives. One hour later, 0.1 ml carrageenan solution (1% in saline) was injected into the intraplantar region of the right hind paw. At appropriate intervals the hind paw thickness was measured. Increasing percentage of hind paw thickness was calculated using the following equation,

$$\% = \{(h_1 - h_0) / h_0 \times 100\}$$

where h_0 was the hind paw thickness in the 0 h as a control values h_1 was hind paw thickness at appropriate time after injection of carrageenan. Furthermore, the anti-inflammatory effect of mixed micelle formulation was evaluated by the trapezoidal method from the area under the curve (*AUC*) of the increasing percentage of hind paw thickness–time profiles. All the experiments were sextuplet.

Histopathological Evaluation of Skin Administered with Micelle Formulations The abdominal skin of Wistar rats administered micelle formulations was excised after the experiment. The excised skin was fixed in 10% formalin for at least 24 h before routine processing then cut vertical to the skin surface at the central region in 4 mm sections. Each section was dehydrated using a graded series of ethanol solutions and embedded in paraffin wax. Tissues were divided into small pieces (about 3 μ m thick) and stained with hematoxylin and eosin. All sections were examined using optiphot light microscopy. Furthermore, all the experiments were triplicate.

Statistical Analysis The statistical analysis was performed using STATISTICA (StatSoft, Inc., Tucs, U.S.A.).

RESULTS AND DISCUSSION

Effect of Mixed Micelle Formulations on the Hind Paw Edema of Rats For anti-inflammatory drug, such as diclofenac, the inhibition of carrageenan induced edema was one of the typical modus to evaluate their pharmacological activity.⁹⁾ Carrageenan solution induced increases in thickness of the hind paw immediately. The hind paw thickness–time profile is shown in Fig. 1. Administration of diclofenac solution (0.015 mg/ml) was employed as a control. In the control, the hind paw thickness increased over 150% 4 h after the injection of carrageenan. Furthermore, this value was maintained until 12 h. The time profile of edema of control was almost similar to that in a previous report.³⁾ To increase the pharmacological activity of diclofenac, the mixed micelle formulations were evaluated. Referring to the results

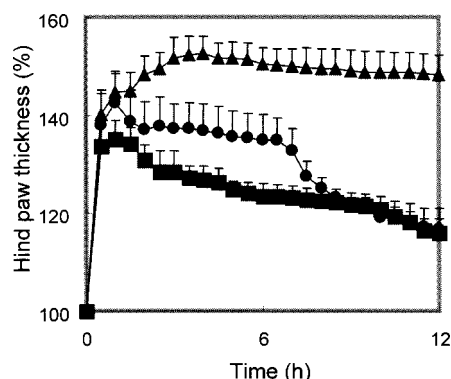


Fig. 1. Effect of Mixed Micelle Formulations on the Carrageenan Induced Hind Paw Edema

●, mixed micelles of cholate (F1); ■, mixed micelles of deoxycholate (F4); ▲, control. Each point represents the mean \pm S.D. for 6 determinations.

of a previous study, mixed ratio of bile salt to lecithin was fixed as 32.5 mm.⁸⁾ To evaluate the systemic effect of diclofenac, “remote” administration was tried in this study, that is the micelle formulation was administered on the abdominal area. The mixed micelle formulation of cholate or deoxycholate decreased the hind paw thickness markedly as shown in Fig. 1. In the primary stage, the mixed micelle formulation of deoxycholate was more effective compared with that of cholate. However, the value of hind paw thickness was almost the same in the both formulation 8 h after injection of carrageenan. In the previous study, the flux of diclofenac was greater in the mixed micelle formulation of deoxycholate compared with that of cholate.⁸⁾ It was suggested that the permeation rate of diclofenac through skin directly affected the pharmacological activity.

In Fig. 2, the time profiles of edema followed by administration of cyclic monoterpene included micelle formulation were shown. In the case of cyclic monoterpene included micelle, hind paw edema was rapidly decreased. This suggests that the formulations including *d*-limonene or *l*-menthol were more effective in anti-inflammatory activity compared with mixed micelle formulations without terpenes.

Figure 3 shows the *AUC* values calculated by the hind paw thickness–time profile. In the case of cholate, all the micelle formulations significantly decreased the *AUC* values. This suggests that the micelle formulation of cholate showed significant anti-inflammatory activity to hind paw edema of rats. Moreover, incorporation of *d*-limonene or *l*-menthol caused significant decrease of *AUC* compared with mixed micelle formulation without cyclic monoterpene. In case of deoxycholate, a similar tendency was observed. It was suggested that the micelle formulations of diclofenac were effective to decrease hind paw edema.

Relation between Pharmacological Effect and Flux of Diclofenac The relation between *AUC* of increasing hind paw edema and flux of diclofenac was demonstrated in Fig. 4. The flux of diclofenac from mixed micelle formulations through rat skin was evaluated in a previous study.⁸⁾ A negative linear relation was observed between the flux and *AUC* with statistical significance ($r=0.846$). This suggests that greater flux of diclofenac induced more effective anti-inflammatory activity. It was suggested that the permeation rate of diclofenac through skin is important for micelle formulations

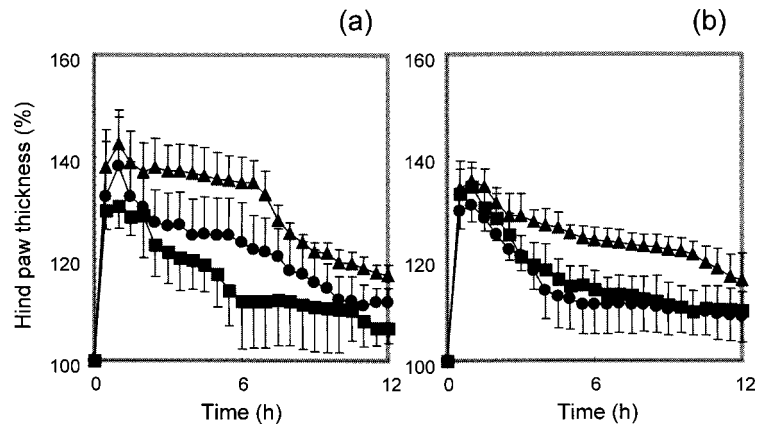


Fig. 2. Effect of *d*-Limonene or *l*-Menthol Incorporated in the Mixed Micelle Formulations on the Carrageenan Induced Hind Paw Edema

(a) Mixed micelles of cholate, (b) mixed micelles of deoxycholate. ▲, without terpenes (F1 in (a) and F4 in (b)), ●, incorporated with *d*-limonene (F2 in (a) and F5 in (b)); ■, incorporated with *l*-menthol (F3 in (a) and F6 in (b)). Each point represents the mean ± S.D. for 6 determinations.

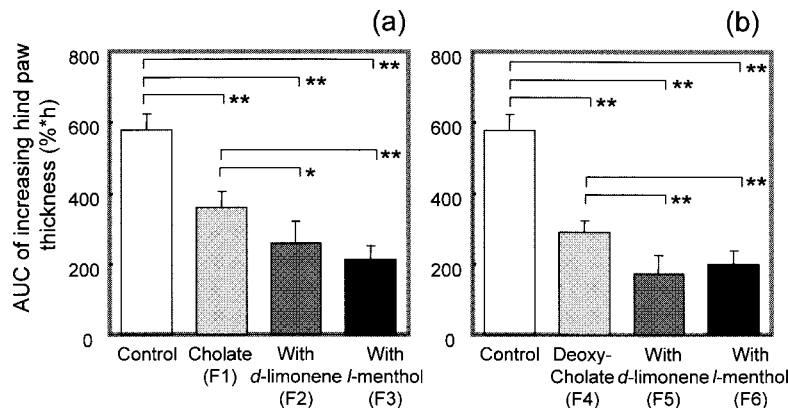


Fig. 3. Effect of Micelle Formulations on the *AUC* of Increasing Hind Paw Thickness

(a) Mixed micelles of cholate, (b) mixed micelles of deoxycholate. Each point represents the mean ± S.D. for 6 determinations. * $p < 0.05$, ** $p < 0.01$.

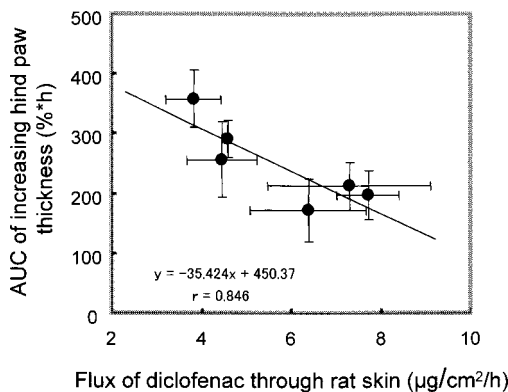


Fig. 4. Relation between Flux of Diclofenac through Rat Skin and *AUC* of Increasing Hind Paw Thickness

Each point represents the mean ± S.D. for 6 determinations.

to exhibit pharmacological activity. Predominant mechanism of enhancement of micelle systems were considered to increase in solubility of diclofenac in the formulation.⁸⁾ However, direct action of terpenes to skin surface might not be negligible.

Histopathological Evaluation of Skin Administered with Micelle Formulations The results of histopathological evaluation of skin are shown in Table 2. A slight liquefac-

tion or edema was observed in the administration of micelle formulations incorporated with cyclic monoterpenes. Some of the typical images are shown in Fig. 5. Only the photographs of the skin that had positive values of total irritation score were examined. No serious skin damage was seen in any formulations. Thus, the irritation exhibited by the administration of micelle formulations was suggested to be negligible. From the results of total irritation score, it was suggested that micelle formulations were able to reduce the skin irritation of chemicals. In the case of administration of hydrogels of *O*-alkylmenthol or *O*-acylmenthol with 40% ethanol, total irritation score was between 4 and 19.¹⁰⁾ Furthermore, synthesized cyclohexanol derivatives derived from *O*-ethylmenthol showed the total irritation score of 8 to 22 in 0.5% hydrogel containing 40% ethanol.¹¹⁾ Significant pharmacological activities as well as skin safety was accomplished by employing the lecithin and bile salts micelles formulations including terpenes such as *d*-limonene and *l*-menthol.

CONCLUSIONS

In the present study, it was clarified that the mixed micelle formulation of cholate or deoxycholate markedly inhibited the hind paw edema induced by carrageenan. In case of cyclic monoterpene included micelles, hind paw thickness

Table 2. Histopathological Evaluation of Rat Abdominal Skin at 12 h after Application of Mixed Micelle Formulations

Findings	Control	F1	F2	F3	F4	F5	F6
Epidermis							
Liquefaction	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 1 1	0 0 1
Subepidermis							
Edema	0 0 0	0 0 0	1 1 1	1 1 1	0 0 0	0 1 0	1 1 1
Dermis							
Collagen fiber swelling	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Inflammatory cell infiltration	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Hypodermis							
Collagen fiber swelling	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Inflammatory cell infiltration	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Skin appendages							
Degeneration	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0
Total irritation score	0 0 0	0 0 0	1 1 1	1 1 1	0 0 0	0 2 1	1 1 2
Mean±S.D.	0.0±0.0	0.0±0.0	1.0±0.0	1.0±0.0	0.0±0.0	1.0±1.0	1.30±0.6

Score: 0, no change; 1, very slight; 2, slight; 3, moderate; 4, marked.

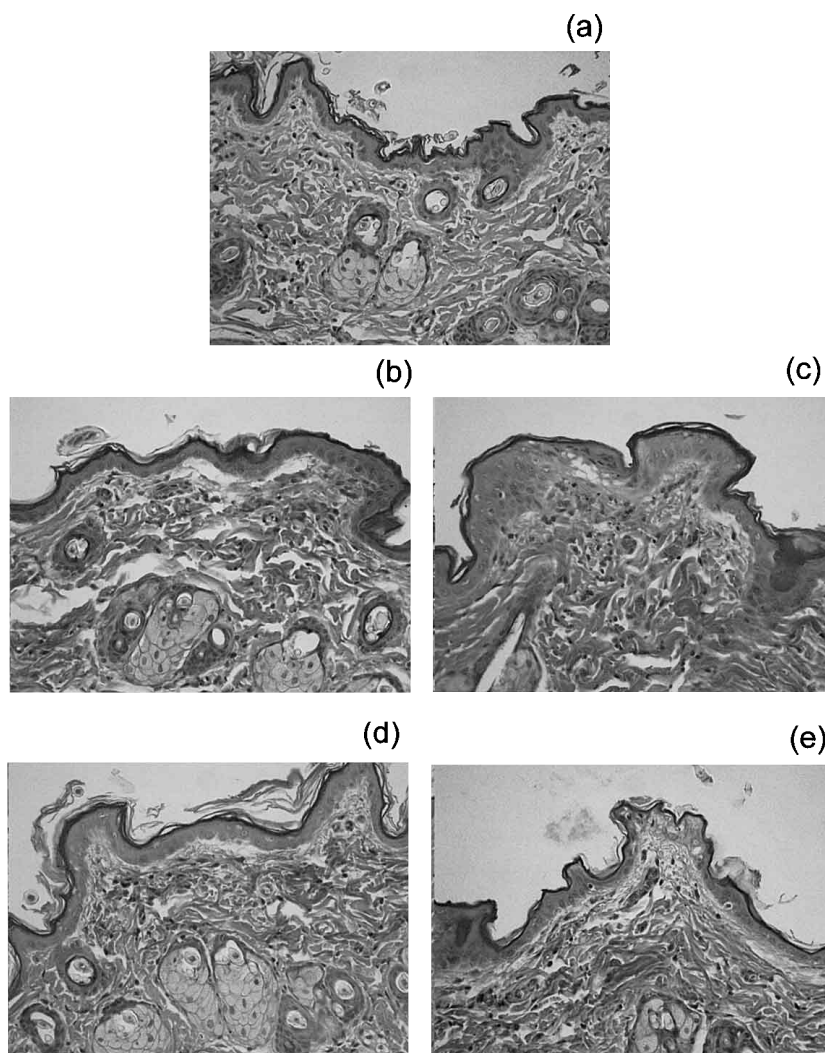


Fig. 5. Microscopic Photos of Rat Skin 12 h after Administration of Micelle Formulations (H&E Stain, ×100) (a) Control; (b) F2; (c) F3; (d) F5; (e) F6.

was more rapidly decreased. This suggests that the mixed micelle formulations including *d*-limonene or *l*-menthol were more effective in anti-inflammatory activity compared with

the formulations without cyclic monoterpene. The irritation exhibited by the administration of micelle formulations was suggested to be negligible. The mixed micelle formulations

investigated in the present study were effective and safe for transdermal delivery of diclofenac.

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