Enhanced Percutaneous Penetration of Flufenamic Acid Using Lipid Disperse Systems Containing Glycosylceramides

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The usefulness of lipid disperse systems, containing soybean phosphatidylcholine (PC) and glycosylceramide (GC) as lipid components, to enhance the percutaneous penetration of flufenamic acid (FA) through rat abdominal skin was examined by both *in vitro* permeation and *in vivo* absorption studies. The penetration of FA from a simple buffer suspension (pH 3.0) containing no lipid component was poor, but was markedly enhanced when FA was incorporated in PC-dispersions. However, this enhancing effect disappeared when the PC concentration in the preparation exceeded 40 μmol/ml. Enhanced penetration of FA from PC-dispersions could also be recognized when 30% propylene glycol or 30% glycerol was used as the dispersing medium instead of the aqueous buffer solution. Addition of GC to the PC-dispersions brought further enhancement of FA penetration through the skin. The maximal effect was observed when FA was incorporated in a 10%-GC system, and the cumulative amount of FA penetrating through the skin in 24 h from this system was approximately 6-fold larger than that from the simple buffer suspension. Enhanced absorption of FA from lipid disperse systems was also confirmed by *in vivo* application of these preparations.

Keywords lipid dispersion; phosphatidylcholine; glycosylceramide; flufenamic acid; percutaneous penetration

Introduction

The skin is one of the routes of drug administration expecting a systemic effect, which can avoid hepatic first-pass elimination. However, the skin forms an impermeable barrier to most substances. Thus, to improve the therapeutic efficacy of a drug, the development of new therapeutic systems promising enhanced percutaneous penetration of the drug is desirable. It was reported recently that liposomal systems are effective and advantageous as topical transdermal drug delivery systems[1-4] and that addition of phosphatidylcholine (PC) to the dermal dosage forms enables the percutaneous absorption to be enhanced.[5-7]

In the present study, the *in vitro* penetration of flufenamic acid (FA), a nonsteroidal antiinflammatory drug, through excised rat abdominal skin from PC-dispersions was examined. We also investigated the usage of glycosylceramide (GC), a lipid component whose content is low in the stratum corneum[8] (a major barrier layer for drug penetration), in the lipid disperse systems.

Materials and Methods

Chemicals Soybean PC was supplied by Nippon Shoji Co., Ltd., Osaka. GC was supplied by Dr. H. Komatsu. Pola Kasei Co., Yokohama. FA was supplied by Sankyo Co., Tokyo. Bovine serum albumin (BSA) was obtained from Sigma Chemical Co., St. Louis, MO. Other chemicals were of reagent grade.

Preparation of Lipid Disperse Systems Chloroform solutions of the lipid mixture (PC with or without GC) and FA were transferred to a round-bottomed flask and the solvent was evaporated to dryness under nitrogen gas. Isotonic buffer solution (citric acid–Na_2HPO_4, pH 3.0) was then added to the thin film composed of the lipid mixture and FA, and the resultant material was homogeneously dispersed by mechanical vortexing and sonication with a Sonicator 5210 (Ohtake Works, Tokyo) for 15 min on ice water. The compositions of the lipid disperse systems examined in this study are listed in Table I. The condition of pH 3.0 was selected in order to make some part of the FA ($pK_a = 3.9$[9]) exist as the solid dispersion, where the maximum thermodynamic activity is constant.

***In Vitro* Penetration Study** *In vitro* penetration studies were performed using a Franz-type diffusion cell (Fig. 1). The abdominal hair of male Wistar rats (200—260 g) was removed with the depilatory, 7% calcium thioglycolate gel,[10] 2 d before the experiment. The abdominal skin was excised with care under pentobarbital anesthesia with all layers intact, and each skin preparation was mounted in the diffusion cell. The area for diffusion was 1.13 cm^2 (radius = 6 mm). Two milliliters of the test prepara-

TABLE I. Composition of Lipid Disperse Systems

a) PC-Dispersions (in 1 ml of lipid dispersions)

System	PC (μmol)	FA (μmol)
Lipid-free suspension	0	10
PC-dispersion	10	10
	20	10
	40	10
	60	10

b) PC/GC-Dispersions (in 1 ml of lipid dispersions)

System	PC (μmol)	GC (μmol)	FA (μmol)
PC-dispersion (without GC)	20	0	10
PC-GC-dispersion (1%-GC)	19.8	0.2	10
(5%-GC)	19	1	10
(10%-GC)	18	2	10
(20%-GC)	16	4	10
(30%-GC)	14	6	10

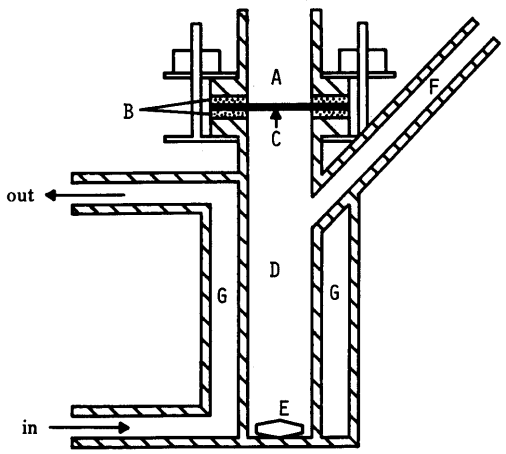

Fig. 1. Schematic Illustration of the Franz-Type Diffusion Cell

A, donor compartment; B, silicone rubber support; C, skin; D, receptor compartment; E, magnetic stirrer bar; F, sampling port; G, water jacket.

tion was applied in contact with the epidermal side. The donor cell was closed to the atmosphere with Parafilm (American Can Company, Greenwich, CT). The receptor of the cell was filled with 11.4 ml of isotonic phosphate buffer solution (pH 7.4) containing 4.2% BSA, and the receptor phase was agitated with a magnetic stirrer at 600 rpm. The receptor compartment was maintained at 37 °C using a water jacket. Samples of the receptor fluid (0.4 ml aliquots) were withdrawn periodically and replaced with the same volume of fresh buffered BSA solution. The concentration of FA in the sample was then determined by high-pressure liquid chromatography (HPLC).

***In Vivo* Absorption Study** A rat whose abdominal hair had been removed was anesthetized with pentobarbital. A test preparation (5 ml) was administered in a glass cell (inner diameter = 30 mm) fixed perpendicularly on the surface of the rat abdominal skin with Aron Alpha (Toa Chemicals Co., Ltd., Tokyo). Blood samples were then collected periodically from the jugular vein and the plasma concentrations of FA were determined by HPLC.

Pretreatment of the Skin with Drug-Free Lipid Dispersions Two milliliters of drug-free lipid dispersions (pH 3.0) was applied to the skin mounted in the diffusion cell and the skin was incubated for 12 h. After complete removal of the lipid dispersion, 2 ml of FA buffer suspensions (10 μmol/ml, pH 3.0) was applied and the percutaneous penetration of FA was measured for 24 h as described above. In the control, the skin was similarly pretreated with the lipid-free buffer solution (pH 3.0).

Determination of FA Concentration Solubilized in the Lipid Disperse Systems To determine the concentration of FA solubilized in the lipid dispersions, ultrafiltration was carried out at 20 °C using a Micropartition System (MPS-1; Amicon Co., Tokyo). Since the membrane employed in this ultrafiltration retains serum proteins completely, small liposomes or oil droplets are excluded from the filtrate. The concentration of FA in the filtrate was determined by HPLC.

Analytical Method For determining the concentration of FA by HPLC, a high-pressure liquid chromatograph (LC-5A; Shimadzu, Kyoto) equipped with a UV detector (SPD-2A, Shimadzu) operated at 290 nm was used in a reversed phase mode with a Nucleosil $5C_{18}$ column (4.6 i.d. × 150 mm). A mixture of acetonitrile and 0.025% phosphoric acid aqueous solution (7 : 3 by volume) was employed as the mobile phase at a flow rate of 1.0 ml/min.

Statistical Analysis The results were expressed as the mean ± standard error. Statistical analysis was carried out by using Student's *t* test.

Results and Discussion

To investigate the influence of PC-dispersions on the percutaneous penetration of FA, the transfer of FA across the rat abdominal skin from five kinds of disperse systems with different PC contents was examined *in vitro*. The results are summarized in Fig. 2. The penetration of FA from the lipid-free suspension, *i.e.*, the simple buffer suspension, was poor. However, when FA was applied as a PC-dispersion (10 and 20 μmol PC/ml), the cumulative amount of FA transferred into the receptor was increased approximately 3-fold compared to that of the lipid-free suspension. However, this enhancing effect was lost when the PC content in the disperse system exceeded 40 μmol/ml, and there was no statistically significant difference among the lipid-free suspension and the PC-dispersions containing 40 and 60 μmol PC/ml. These results suggest that the enhancing effect of PC-dispersions is dependent on the PC content in the disperse system.

It has been confirmed that the proper choice of vehicle is an important factor in the percutaneous penetration of drugs. Some investigators have reported that enhanced percutaneous penetration of drugs was observed when propyulene glycol, which probably increases the solubility and/or partition of drugs to the skin, was incorporated in the formulations.[11,12] In order to examine the influence of the dispersing media on the percutaneous penetration of FA from lipid disperse systems, we prepared PC-dispersions with 30% propylene glycol and 30% glycerol in the same buffer solution (pH 3.0) as the media. The penetration profiles of FA from these dispersions are shown in Fig. 3. Compared with the results in Fig. 2, a significant increase in penetration of FA through the skin was observed when buffered propylene glycol was used (Fig. 3(a)), but not when buffered glycerol was used (Fig. 3(b)) as the dispersing medium instead of the aqueous buffer solution. However, similarly to the results in Fig. 2, enhanced penetration of FA could be recognized in the PC-disperse system dispersed in these two media.

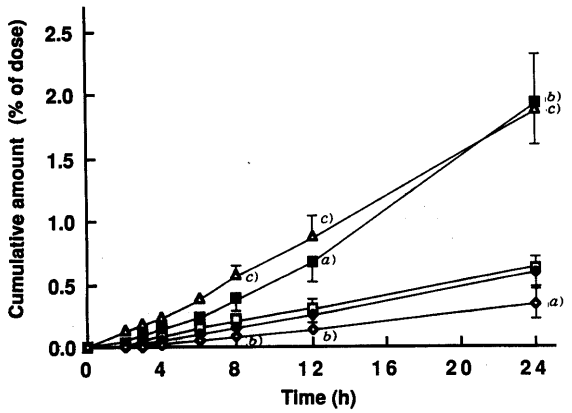

Fig. 2. Percutaneous Penetration of FA from PC-Dispersions *in Vitro*

□, lipid-free; △, 10 μmol PC/ml; ■, 20 μmol PC/ml; ◆, 40 μmol PC/ml; ◇, 60 μmol PC/ml. The results are expressed as the mean ± S.E. of 3—5 experiments. *a*) $p<0.05$; *b*) $p<0.01$; *c*) $p<0.001$, compared to the corresponding value for the lipid-free suspension.

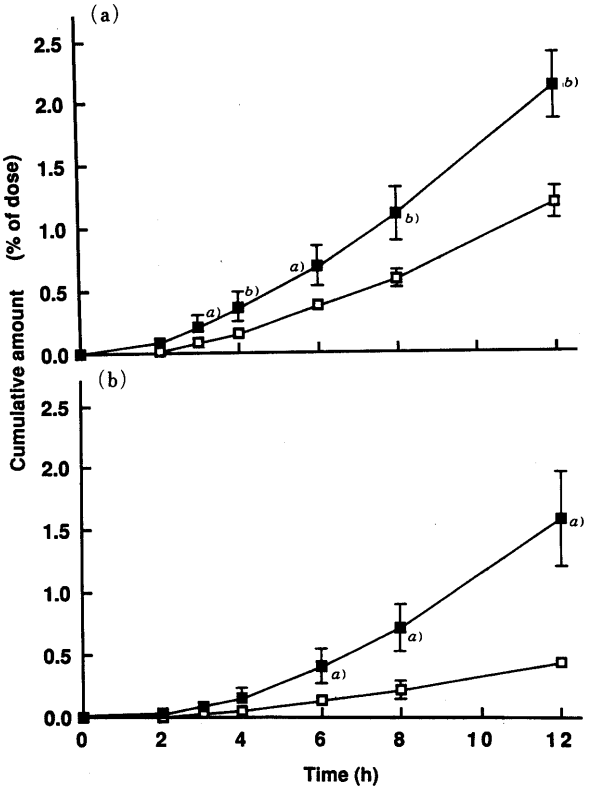

Fig. 3. Percutaneous Penetration of FA from PC-Dispersions Prepared in 30% Propylene Glycol (a) and 30% Glycerol (b)

□, lipid-free suspension; ■, PC-dispersion (20 μmol PC/ml). The results are expressed as the mean ± S.E. of 3—4 experiments. *a*) $p<0.05$; *b*) $p<0.01$, compared to the corresponding value for the lipid-free suspension.

Recently, the physiological role of epidermal lipids has been elucidated. The epidermis is differentiated into three morphologically different layers: an innermost basal layer, an intermediate granular layer and an outermost stratum corneum. The lipid compositions of these three epidermal layers vary tremendously. The stratum corneum has extremely high contents of acylglycosylceramides (AGC) and acylceramides (AC) as compared to the other two layers.[8] Wertz *et al.* reported that AGC and AC have a structurally specific function in anchoring together the adjacent intercellular lipid bilayers existing in the stratum corneum.[13,14] The intercellular lamellar sheets of the stratum corneum are regarded as the practical water barrier in the skin.[15] On the other hand, viable cells of the basal and granular layers contain none of them, but have relatively high proportions of GC.[8] We can consider therefore that addition of more hydrophilic lipids such as GC to the stratum corneum lipids will tend to make the permeation barrier more permeable. To examine the effect of GC on the percutaneous penetration of FA, lipid disperse systems containing GC at various PC/GC ratios were prepared and *in vitro* penetration studies were carried out. Figure 4 summarizes the results obtained. The addition of GC to the lipid dispersion enhanced the percutaneous penetration of FA. Maximal enhancement was observed when the PC/GC ratio in the dispersion was 9:1 (10%-GC). These findings suggest that the use of lipid disperse systems as a transdermal dosage form can enhance the percutaneous penetration of FA.

There may be two mechanisms by which lipid disperse systems enhance the transdermal penetration of FA. One is that the lipids in the lipid dispersions could exert a direct effect on the permeability characteristics of the stratum corneum, thereby enhancing the penetration of FA. The other is that the apparent solubility of FA could be increased in the lipid dispersions. To examine the action of the polar lipids on the stratum corneum, the influence of pretreatment with FA-free lipid dispersions on the percutaneous penetration of FA was investigated. Natsuki and Takabatake[7] found that pretreatment of the skin with gel-ointment base containing PC for 4 h increased the percutaneous absorption of indomethacin applied as the gel-ointment. Kato *et al.*[6] also claimed that PC in propylene glycol changed the permeability of the skin barrier, resulting in enhancement of the transdermal delivery of bunazosin. However, as shown in Fig. 5, 12-h pretreatment of the skin with FA-free lipid dispersions (PC- or PC/GC-dispersions) induced no statistically significant change in the penetration of FA from lipid-free suspensions in the present study. This agrees well with the report of Komatsu *et al.*, who found that pretreatment of the skin with liposomes induced only a small change in the penetration of butylparaben.[2] However, the direct actions of the polar

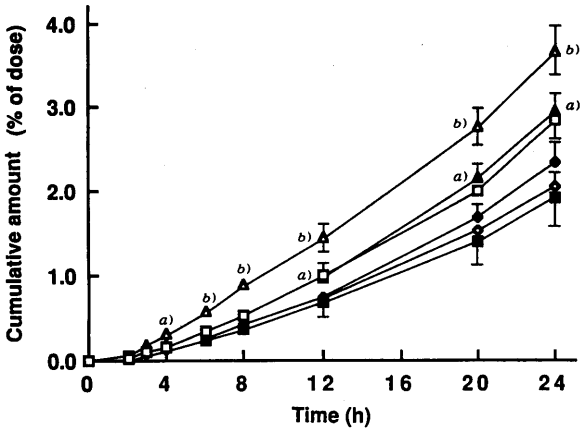

Fig. 4. Percutaneous Penetration of FA from PC/GC-Dispersions *in Vitro*

■, without GC; ◇, 1%-GC; ◆, 5%-GC; △, 10%-GC; ▲, 20%-GC; □, 30%-GC. The results are expressed as the mean ± S.E. of 3—5 experiments. *a*) $p<0.05$; *b*) $p<0.01$, compared to the corresponding value for the PC-dispersion.

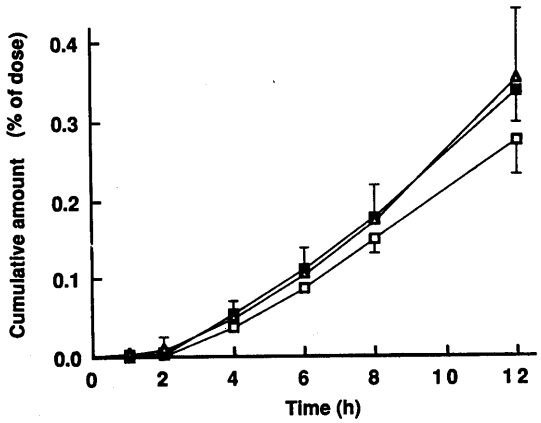

Fig. 5. Effect of Pretreatment with Drug-Free Lipid Dispersions on the Percutaneous Penetration of FA *in Vitro*

□, buffer solution; ■, FA-free PC-dispersion (without GC); △, FA-free PC/GC-dispersion (10%-GC). The results are expressed as the mean ± S.E. of 3—4 experiments.

TABLE II. FA Concentration in Filtrate of Lipid Disperse Systems

Dosage form	Concentration (μM)
Lipid-free suspension	0.87 ± 0.20
PC-dispersion (without GC)	4.78 ± 0.20[a)]
PC/GC-dispersion (10%-GC)	5.00 ± 0.26[a)]

Ultrafiltration was carried out using a Micropartition System (Amicon MPS-1). The results are expressed as the mean ± S.E. of 4 experiments. *a*) $p<0.001$, compared to the value for the lipid-free suspension.

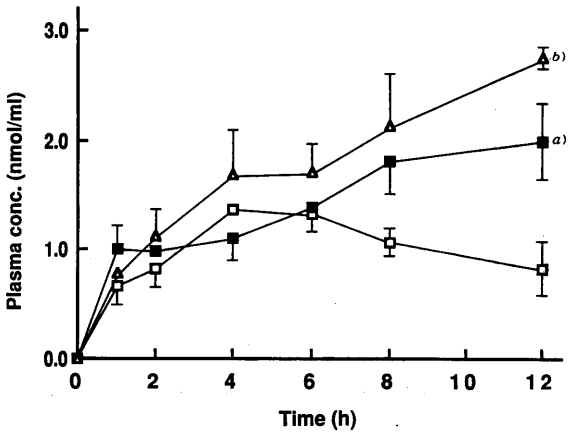

Fig. 6. Effect of Polar Lipids on the Percutaneous Absorption of FA *in Vivo*

□, lipid-free suspension; ■, PC-dispersion (without GC); △, PC/GC-dispersion (10%-GC). The results are expressed as the mean ± S.E. of 3—4 experiments. *a*) $p<0.05$; *b*) $p<0.01$, compared to the corresponding value for the lipid-free suspension.

lipids on the stratum corneum when the drug molecules were additionally suspended in such a "polar lipid-rich" environment remain unknown. To determine the apparent solubility of FA in the lipid disperse systems, ultrafiltration experiments were carried out. Table II summarizes the FA concentrations in the filtrates of the lipid-disperse systems. The FA concentration in the filtrate of either the PC- or PC/GC-dispersion was approximately 6-fold larger than that of the lipid-free suspension. Under the present conditions, some part of the FA exists as solid dispersion in all preparations, and the FA concentration in the filtrates of the preparations revealed a saturated or supersaturated state. It is suggested therefore that the apparent solubility of FA in the preparations increased when the drug was incorporated in the lipid dispersions, resulting in an increase in FA penetration. Furthermore, we examined the *in vivo* absorption of FA from three preparations: lipid-free suspension, PC-dispersion containing PC at 20 μmol/ml, and PC/GC-dispersion containing 10% GC, of which the latter two were effective for enhancing the *in vitro* penetration of FA. Figure 6 shows the plasma concentration profiles of FA following *in vivo* application of the preparations to the rat abdominal skin. Increased FA concentrations were observed when the PC- and PC/GC-dispersions were applied, and the area under the plasma concentration curves from zero to 12 h for the PC- and PC/GC-dispersions increased approximately 1.4- and 1.7-fold, respectively, as compared to that for the lipid-free suspension. These findings indicate that these lipid dispersions enhance the *in vivo* percutaneous absorption of FA. Such enhancement was more predominant in the presence of GC. These results agree with those of the *in vitro* experiments.

In conclusion, lipid disperse systems containing PC and GC represent advantageous dosage forms of FA for transdermal drug delivery, although further studies are needed to clarify both the precise mechanisms of the enhancement and the possible application to other drugs.

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