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 hr 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 systems1-4) 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 corneum8) (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-Na2HPO4, 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 (pKa = 3.99) 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,9,10) 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 cm² (radius = 6 mm). Two milliliters of the test prepara-

<table>
<thead>
<tr>
<th>Table I. Composition of Lipid Disperse Systems</th>
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<tr>
<td>a) PC-Dispersions (in 1 ml of lipid dispersions)</td>
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<tr>
<td>System</td>
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<tr>
<td>Lipid-free suspension</td>
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<tr>
<td>PC-dispersion</td>
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<td>b) PC/GC-Dispersions (in 1 ml of lipid dispersions)</td>
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<tr>
<td>System</td>
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<tr>
<td>PC-dispersion (without GC)</td>
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<tr>
<td>PC-GC-dispersion (10%GC)</td>
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<tr>
<td>(5%GC)</td>
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<td>(10%GC)</td>
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<td>(20%GC)</td>
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<td>(30%GC)</td>
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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 aliquot) 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
then removed was anesthetized with pentobarbital. A test preparation (5 ml)
was administered in a glass cell (inner diameter = 30 mm) fixed perpendicu-
larly on the surface of the rat abdominal skin with Aron Alpha
(Toa Chemical 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 milli-
liters of drug-free lipid dispersions (pH 3.0) were 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 Dis-
perse Systems To determine the concentration of FA solubilized in
the lipid dispersions, ultrafiltration was carried out at 20 °C using a
Micropartition System (MP5-1; 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 5C18 column
(4.6 i.d. x 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 dispersive 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 sus-
pension, 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 dispersive 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 dispersive 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
propylene 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 dispersive systems, we prepared PC-dis-
persions 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
percutaneous penetration of FA could be recognized in the PC-disperse
system dispersed in these two media.

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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.

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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.\textsuperscript{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.\textsuperscript{13,14} The intercellular lamellar sheets of the stratum corneum are regarded as the practical water barrier in the skin.\textsuperscript{15} On the other hand, viable cells of the basal and granular layers contain none of them, but have relatively high proportions of GC.\textsuperscript{9} 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\textsuperscript{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.\textsuperscript{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.\textsuperscript{23} However, the direct actions of the polar

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<th>Dosage form</th>
<th>Concentration (µm)</th>
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<tr>
<td>Lipid-free suspension</td>
<td>0.87 ± 0.20</td>
</tr>
<tr>
<td>PC-dispersion (without GC)</td>
<td>4.78 ± 0.20\textsuperscript{a}</td>
</tr>
<tr>
<td>PC/GC-dispersion (10% GC)</td>
<td>5.00 ± 0.26\textsuperscript{a}</td>
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Ultrafiltration was carried out using a Micropartition System (Amicon MPS-1). The results are expressed as the mean ± S.E. of 4 experiments. \textsuperscript{a} p<0.001, compared to the value for the lipid-free suspension.

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. \textsuperscript{a} p<0.05; \textsuperscript{b} p<0.01, compared to the corresponding value for the PC-dispersion.

Fig. 5. Effect of Pretreatment with Drug-Free Lipid Dispersion 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.

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. \textsuperscript{a} p<0.05; \textsuperscript{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.

References