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Effect of enhancers and retarders on percutaneous absorption of flurbiprofen from hydrogels

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Abstract

The effect of enhancers/retarders on the transdermal absorption of flurbiprofen from cellulose hydrogels was studied in vitro. The release rate of flurbiprofen and the viscosity of hydrogel matrices were also examined. The flux of flurbiprofen from cellulose hydrogels approximated that from aqueous buffers, whereas the skin reservoir of flurbiprofen was lower with hydrogels. Incorporation of the cosolvents, propylene glycol (PG) and ethanol, did not significantly increase skin absorption of flurbiprofen. Ethanol even reduced the skin reservoir of the drug. Oleic acid, an unsaturated fatty acid, produced the largest skin reservoir of the drug when incorporated into the hydrogels. D-Limonene, a cyclic monoterpene, showed the greatest ability to enhance the flux of flurbiprofen. However, phospholipids as retarders markedly reduced the skin absorption of flurbiprofen. The mechanisms by which enhancers/retarders govern flurbiprofen permeation were elucidated by in vitro permeation studies using various skin types (enhancers/retarders-pretreated skin, stratum corneum (SC)-stripped skin, and delipidized skin) and histological examination. The results suggest different mechanisms and skin structural modifications caused by different enhancers/retarders.
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Keywords: Percutaneous absorption; Flurbiprofen; Enhancer; Retarder; Hydrogel

1. Introduction

Flurbiprofen is a chiral non-steroidal anti-inflammatory drug (NSAID) of the 2-arylpropionic acid class. Flurbiprofen, one of the most potent inhibitors of platelet aggregation currently available, is used to treat gout, osteoarthritis, rheumatoid arthritis, and sunburn (Lee et al., 1992; Poul et al., 1993). Upon oral administration, the most frequently reported side effects of flurbiprofen are abdominal discomfort along with other gastrointestinal effects. Also, it has a short elimination half-life of 3.9 h and requires frequent dosing (Heyneman et al., 2000). Long-term percutaneous absorption of flurbiprofen at a controlled rate is needed. Minnesota Mining and Manufacturing (3M, USA) has patented an adhesive transdermal delivery device for the topical application of flurbiprofen (Effing et al., 1997). The device was

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found to demonstrate good skin adhesion and to penetrate moderately as determined using mouse and human skin (Chen and Fang, 2000).

The fact that the transdermal route is not more widely used is because of the inherent barrier properties of the skin. Moreover, flurbiprofen was one of the least-permeable drugs across skin among a series of lipophilic drugs (Morimoto et al., 1992). Many different approaches have been taken to overcome the barrier presented by the skin, including mechanical disruption, electrical disruption, and chemical modification (Finnin and Morgan, 1999). This study focuses on the use of chemicals to modulate the skin permeation by flurbiprofen and the drug reservoir within the skin.

One particular problem common to many drug delivery systems designed for use on skin is poor retention at the site of application. This problem may be resolved by the incorporation of bioadhesive polymers within the system. Cellulose polymers with or without charges were used to form hydrogels as the vehicle for flurbiprofen in this study. The present study also utilizes various skin membranes as permeation barriers, including nude mouse skin, stratum corneum (SC)-stripped skin, delipidized skin, and cellulose membranes, to explore the influence of chemical enhancers/retarders on skin permeation by flurbiprofen. The amount of drug retained within the skin reservoir was also determined since flurbiprofen is used to treat not only systemic diseases but also local diseases. Histological examinations with light microscopy and scanning electric microscopy (SEM) were used to assess the effect and mechanism of these enhancers/retarders on skin.

2. Materials and methods

2.1. Materials

Flurbiprofen, carboxymethyl cellulose sodium salt (CMCNa), carboxymethyl cellulose ammonium salt ($CMCNH_4$), hydroxypropyl cellulose (HPC, 1000–4000 cps), oleic acid, linoleic acid, and linolenic acid were purchased from Wako Chemical Co. (Japan). D-Limonene, L-α-lecithin, and dimyristoyl-L-α-phosphatidylcholine (DMPC) were obtained from Sigma Chemical Co. (USA). The cellulose membrane (Spectra-Por 2®, molecular weight cut-off: 12,000–14,000) was supplied by Spectrum Co. (USA). All other chemicals and solvents were of analytical grade.

2.2. Preparation of hydrogels

For the preparation of cellulose hydrogels, a 5% (w/v) concentration of polymer was added into pH 7 citrate–phosphate buffer (0.06 M) with half of the total amount (47%), after which the mixture was stirred continuously for 1 h. After 24 h, the residual pH 7 buffer (47%) and 1% (w/v) flurbiprofen were added into the mixture with continuous stirring for 1 h. A cosolvent or enhancer/retarder was also incorporated into the mixture if required.

2.3. Viscosity determination

The viscosity of the hydrogels was measured before the performance of in vitro permeation experiments. Viscosity was determined using a programmable viscometer (Model DV-II+, Brookfield Co., USA). Readings were recorded 30 s after the measurement was made, when the level had stabilized.

2.4. Preparation of skin membranes

Female nude mice (Balb/c-nu strain, ~6 weeks old) were sacrificed using ether, and full-thickness skin was excised from the dorsal region. To obtain SC-stripped skin, adhesive tape was applied to hairless mouse skin with uniform pressure and then removed. This procedure was repeated 20 times. Delipidized skin was prepared by pretreating hairless mouse skin with 1 ml of chloroform–methanol (2:1, v/v) for 60 min in order to extract the lipids from the skin. The delipid process extracted the lipid content of the whole skin.

2.5. In vitro skin permeation

In vitro skin permeation experiments were carried out using a Franz diffusion cell. The skin was mounted on the receptor compartment with

the SC-side facing upwards into the donor compartment. The donor medium was 1 g of vehicle containing flurbiprofen. The receptor medium was 10 ml of pH 7.4 citrate–phosphate buffer. The available diffusion area between cells was 0.785 cm^2. The stirring rate and temperature were kept at 600 rpm and 37 °C, respectively. At appropriate intervals, 300-μl aliquots of the receptor medium were withdrawn and immediately replaced with an equal volume of fresh buffer. The amount of flurbiprofen was determined by the HPLC method.

The amount of flurbiprofen retained in the skin was determined at the end of the in vitro permeation experiment (12 h). The skin was washed 10 times using a cotton cloth immersed in methanol. A sample of skin was weighed, cut with scissors, positioned in a glass homogenizer containing 1 ml of methanol, and ground for 5 min with an electric stirrer. The resulting solution was centrifuged for 10 min at 7000 rpm. The supernatant was analyzed by HPLC.

2.6. Analytical method for flurbiprofen

The drug content of flurbiprofen was analyzed using a HPLC system consisting of a Hitachi L-7110 pump, a Hitachi L-7200 sample processor, and a Hitachi L-7400 UV detector. A 25-cm-long, 4-mm inner diameter C18 column (LichroCart 250-4, Merck) was used. The mobile phase consisted of a 40% aqueous phase adjusted to pH 2.6 with acetic acid; and 60% acetonitrile at a flow rate of 1 ml/min. The wavelength of the UV detector was set at 245 nm.

2.7. Histological examination by light microscopy

Histological changes in nude mouse skin were examined after pretreatment with 5% enhancer/retarder for 2 h. Immediately after pretreatment, the specimen of the exposed area was examined histologically. The adjacent untreated skin area was also assessed as the control group. Each specimen was fixed in a 10% pH 7.4 buffered formaldehyde solution for at least 48 h. The specimen was cut vertically against the skin surface. Each section was dehydrated using ethanol, embedded in paraffin wax, and stained with hematoxylin and eosin. In each skin sample, three different sites were examined and evaluated under light microscopy (Nikon Eclipse 4000, Japan).

2.8. Ultrastructural examination by SEM

Excised skin samples were cut into appropriate-sized cubes and immediately fixed at 4 °C in 2% paraformaldehyde and 2.5% glutaldehye in 0.2 M cacodylate buffer (pH 7.4) overnight, washed three times with 0.2 M cacodylate and 7% sucrose buffer for 15 min post-fixed with 2% osmium tetroxide for 1 h, washed three times as above, and immersed in 0.5% aqueous uranyl acetate for 30 min. Specimens were then dehydrated in graded concentrations of ethanol, transferred to isoamyl acetate, and critical-point dried using liquid CO_2. The dried specimens were affixed with gold–palladium in an ion coater and examined with a scanning electron microscope (Hitachi S-2400, Japan). All histopathologic analyses were performed in a blinded fashion.

3. Results and discussion

3.1. In vitro percutaneous absorption of flurbiprofen from hydrogels

The percutaneous absorption of flurbiprofen from pH 7 buffer with or without cellulose polymers was investigated. The cumulative amount–time profiles for various formulations were plotted. The slopes of the resulting linear plots were calculated, and the flux (μg/cm^2/h) was determined from the slope. A pseudo zero-order equation was suitable to use with the curves of all these vehicles. The flux, drug amount within the skin, release rate across the artificial membrane, and viscosity of hydrogels are shown in Table 1. There were no statistically significant differences among the flux of flurbiprofen from the three hydrogels (ANOVA test, $P > 0.05$). Moreover, no significant difference was observed between the flux from the donor vehicles of the pH 7 buffered

Table 1
Flurbiprofen flux, amount in the skin, release rate, and viscosity after topical application of various hydrogels in vitro

Formulation	Flux (μg/cm^2/h)	Amount in skin at 12 h (μg/mg) × 10^2	Release rate (μg/cm^2/h)	Viscosity (cps × 10^{-3})
pH 7 solution	116.74±26.77	274.55±31.29	187.81±4.29	–
CMCNa	113.35±22.23	137.38±13.95	231.61±23.74	61.00±0.50
$CMCNH_2$	97.06±19.06	91.35±57.39	175.29±10.83	116.33±2.08
HPC	106.12±10.29	122.77±46.52	206.98±11.64	73.50±0.87

The polymer concentration in the hydrogel formulation was 5%. Each value represents the mean ± S.D. ($n=4$).

solution and hydrogels. When comparing the flurbiprofen amount retained in the skin, the pH 7 buffered solution showed greater ability to offer the drug a large reservoir within skin than did the hydrogels (Table 1). With respect to drug permeation across the skin from the aqueous vehicle, a drug should first diffuse out from the vehicle to the skin surface. To clarify the mechanism of the skin absorption of flurbiprofen, the release rate of drug from the vehicle across a cellulose membrane was studied. There were no significant difference (ANOVA, $P>0.05$) among the release rates of drug from the pH 7 solution and hydrogels (Table 1). This suggests that the cross-linkage structure formed by the polymers after hydration did not interact with flurbiprofen molecules.

The ability of a hydrogel system to serve as a reservoir for drug delivery is influenced by the macro- and micro-rheological properties of the matrix. Viscosity is the most widely utilized reference for the characterization of polymer structure, although it is not sufficiently comprehensive for the full determination of hydrogel strength. The viscosities of CMCNa and HPC were lower (t-test, $P<0.05$) than that of $CMCNH_4$. This demonstrates that the viscosity of the polymer matrices showed no relationship with the drug flux. This result indicates that the permeation process of flurbiprofen may be consistent with a skin-controlled mechanism, since the viscosity of hydrogels plays an important role in controlling the release of the drug if the diffusion of drug through the polymer matrix is a rate-determining step (Ho et al., 1994; Bentley et al., 1999).

3.2. Effect of cosolvents on percutaneous absorption of flurbiprofen from hydrogels

There was no significant difference in the flurbiprofen flux of CMCNa, $CMCNH_4$, and HPC hydrogels (Table 1). A product designed for topical application to a biological substrate, e.g. a mucous membrane or skin, should preferably possess adhesive properties, as these will enhance the time of location at the site of application and hence improved clinical efficacy (Jones et al., 1997). Since CMCNa exhibits greater mucoadhesive properties than do the other cellulose polymers (Doelker, 1987), CMCNa was chosen as a model vehicle to further investigate the influence of cosolvents and enhancers on flurbiprofen permeation. It can be seen in Table 2 that the flux of flurbiprofen was not affected to any significant extent (t-test, $P<0.05$) in hydrogels containing propylene glycol (PG) or ethanol. Although cosolvents did not influence the flux of flurbiprofen, this does not mean that they had no effect on skin permeation of the drug. As a function of drug solubility, the partition behavior between vehicle and skin, and skin structural modification, many mechanisms have been found by which cosolvents affect the skin permeation of drugs (Ho et al., 1994; Bendas et al., 1995; Zhao and Singh, 2000).

The presence of PG did not significantly change the mean flux, whereas it retarded flurbiprofen uptake into skin (t-test, $P<0.05$). Table 2 shows that increasing the drug solubility in the vehicle by adding PG did not influence the release rate of the drug. Thus the skin itself may play an important role in this process. The skin was pretreated with a

Table 2
Effect of cosolvent on flurbiprofen flux, amount in the skin, release rate, and viscosity after topical application of CMCNa hydrogels in vitro

Formulation	Flux (μg/cm^2/h)	Amount in skin at 12 h (μg/mg) × 10^2	Release rate (μg/cm^2/h)	Viscosity (cps × 10^{-3})
No cosolvents	113.35 ± 22.23	137.38 ± 13.95	231.61 ± 23.74	61.00 ± 0.50
+PG	101.58 ± 17.99	77.51 ± 15.59*	200.92 ± 27.34	103.67 ± 1.15*
+EtOH	140.35 ± 26.80	118.87 ± 29.53	285.93 ± 26.80*	109.33 ± 11.93*

The polymer concentration in the hydrogel formulation was 5%. The cosolvent concentration in the hydrogel formulation was 20%. Each value represents the mean ± S.D. ($n = 4$).
* t-Test, $P < 0.05$.

20% PG/pH 7 buffer mixture for 2 h followed by in vitro permeation experiments with CMCNa hydrogels. This pretreatment method avoided cosolvent effects on thermodynamic activities of the model drug. As shown in Fig. 1, neither flux nor drug reservoir in the skin were changed by PG pretreatment as compared to the non-treated group (control) and pretreatment with pH 7 buffer. This indicates that PG might cause no alteration in skin structure. Hence partitioning of the drug between the skin barrier and the hydrogel matrix seems to be the predominant cause of the reduction of the drug reservoir within the skin. The addition of cosolvents reduced the polarity of the hydrogels. Lipophilic flurbiprofen would be better incorporated into the hydrogels with PG and would produce a stronger affinity than that without PG. The partitioning of flurbiprofen to the SC was less from PG-supplemented hydrogels than from non-PG hydrogels. These findings may explain why PG-supplemented hydrogels produced a lower drug amount in the skin.

Neither flux nor drug amount in the skin were affected by the addition of ethanol to CMCNa hydrogels, however, the release rate significantly increased (t-test, $P < 0.05$) as shown in Table 2. This suggests that different mechanisms controlled by ethanol and PG influence flurbiprofen permeation. Surprisingly, pretreatment with the 20% ethanol/pH 7 buffer mixture resulted in a great retardation in the flurbiprofen flux and a decrease in the reservoir in the skin as shown in Fig. 1. Changes in skin structure induced by ethanol can reduce the permeation of some drugs (Inagi et al., 1981; Fang et al., 1999). Protein denaturation in the SC layer may be involved in this reduction of permeation.

Direct ethanol treatment of the skin structure produced a negative effect on flurbiprofen permeation. However, no significant difference (t-test, $P > 0.05$) was observed in permeation data of CMCNa hydrogels with or without 20% ethanol. This indicates that the influence of other factors predominated skin permeation of flurbiprofen from ethanol-supplemented hydrogels. The increment in the release rate may be due to increased drug solubility in the hydrogels. Thus two competing processes including skin structure alteration and drug solubility may have affected the skin permeation of flurbiprofen, resulting in offseting changes in flux and the drug reservoir within skin after incorporation of ethanol into the hydrogels.

3.3. Effect of enhancers/retarders on the percutaneous absorption of flurbiprofen from hydrogels

A series of enhancers, including fatty acids, cyclic monoterpenes, and phospholipids, were used to promote the percutaneous absorption of flurbiprofen. The concentration of enhancers in CMCNa hydrogels was set at 5%. Unsaturated fatty acids have been used as potent enhancers for flurbiprofen (Chi et al., 1995). The enhancing effect of unsaturated fatty acids on flurbiprofen permeation was also determined in this study. Oleic, linoleic, and linolenic acids which possess the same alkyl chain but different double bonds were utilized. As shown in Table 3, the three fatty acids all enhanced the flux of flurbiprofen, and the

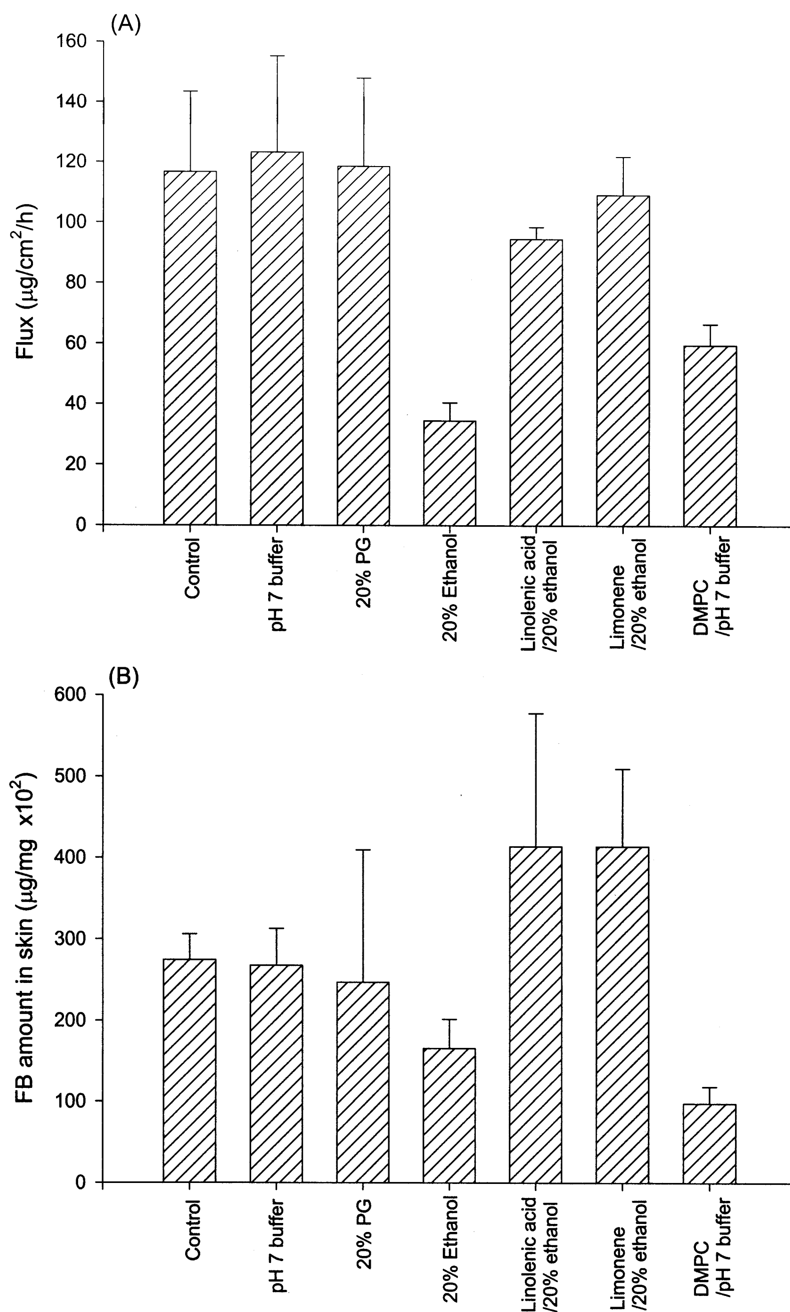

Fig. 1. Flux (A) and drug uptake in skin (B) of flurbibrofen across enhancer/retarder-treated skin from CMCNa hydrogels. Each value represents the mean ± S.D. ($n = 4$).

enhancement levels were approximately similar for the three formulations. Generally, unsaturated fatty acids which produce large increase in the partition coefficient of flurbiprofen are powerful enhancers (Chi et al., 1995). As to the drug amount trapped within the skin, oleic acid showed

Table 3
Effect of enhancers on flurbiprofen flux, amount in the skin, release rate, and viscosity after topical application of CMCNa hydrogels in vitro

Formulation	Flux (μg/cm^2/h)	Amount in skin at 12 h (μg/mg) × 10^2	Release rate (μg/cm^2/h)	Viscosity (cps × 10^{-3})
CMCNa	113.35±22.23	137.38±13.95	231.61±23.74	61.00±0.50
+Oleic acid	176.25±22.33*	283.06±67.16*	190.12±41.58	55.17±0.76
+Linoleic acid	188.97±13.86*	128.40±85.25	229.40±33.14	61.00±0.87
+Linolenic acid	207.38±10.50*	171.01±22.28*	155.73±14.51*	72.33±1.89
+Azone	101.54±26.50	100.20±8.42*	110.49±10.00*	46.33±0.29
+Limonene	298.63±34.06*	143.92±12.79	238.75±35.09	58.83±1.04
+L-α-Lecithin	110.41±10.30	100.34±21.00*	241.44±17.52	67.67±1.89
+DMPC	57.77±15.66*	30.93±18.33*	180.79±30.04*	63.83±0.58
+PG+linolenic acid	143.14±12.22	67.99±22.92*	178.91±7.95*	101.67±0.58
+EtOH+linolenic acid	161.17±26.64*	101.48±13.24*	191.97±25.67	116.00±7.94

The polymer concentration in the hydrogel formulation was 5%. The cosolvent concentration in the hydrogel formulation was 20%. The enhancer concentration in the hydrogel formulation was 5%. Each value represents the mean±S.D. ($n = 4$).
* t-Test, $P < 0.05$.

the most-potent enhancement, followed by linolenic and linoleic acids. The numbers of double bonds in the chemical structure of oleic, linoleic, and linolenic acids are one, two, and three, respectively. The more-kinked shape of fatty acids, with an increase in cis-double bond number, makes it difficult for them to insert themselves into the lipid matrices of skin, which results in a higher accumulation of flurbiprofen in the skin with oleic acid than with the other acids (Table 3). However, once they are packed into the skin, a higher skin flux of flurbiprofen could be obtained due to the wider channel produced, resulting in a similar flux for all three formulations.

The 5% linolenic acid in 20% ethanol/pH 7 buffer solution was used to treat skin for 2 h to examine the effect of fatty acids on the structure of the skin. The flux and skin reservoir of flurbiprofen were significantly higher (t-test, $P < 0.05$) for linolenic acid-treated skin than for 20% ethanol-treated skin (Fig. 1). The effect of fatty acids on skin structure modification may contribute to the enhancement of flux and the skin reservoir of flurbiprofen in CMCNa hydrogels (Table 3). There was no significant difference (t-test, $P > 0.05$) between the drug flux across non-treated and linolenic acid-treated skin (Fig. 1). The reason that linolenic acid did not show a higher flux as compared to the control group may be the hydrophobic nature of the 20% ethanol/pH 7 buffer mixture which showed a higher affinity to linolenic acid, resulting in linolenic acid partitioning in the skin being hindered.

In order to elucidate the effect of linolenic acid on the skin, various types of skin were used as barriers to obtain mechanical information on the percutaneous absorption of flurbiprofen. As shown in Fig. 2, the flux of flurbiprofen from CMCNa hydrogels across SC-stripped skin was only 1.77 times greater than that across intact skin. This can be explained by the fact that because of its lipophilicity, flurbiprofen partitions easily in SC. In the in vitro permeation experiment, the resistance of the epidermis/dermis to the overall skin permeation of the drug cannot be ignored (Morimoto et al., 1992; Sung et al., 2000). One must consider that the drug has to partition not only out of the vehicle into the SC, but subsequently from the SC into the barrier of the viable epidermis and dermis (Wenkers and Lippold, 1999). Similar fluxes of drug permeating across delipidized skin and SC-stripped skin suggest that the predominant route for percutaneous absorption of flurbiprofen is the intercellular pathway.

No significant difference (ANOVA test, $P > 0.05$) was observed among the fluxes of flurbiprofen across intact, SC-stripped, and delipidized skin after application of linolenic acid-containing hy-

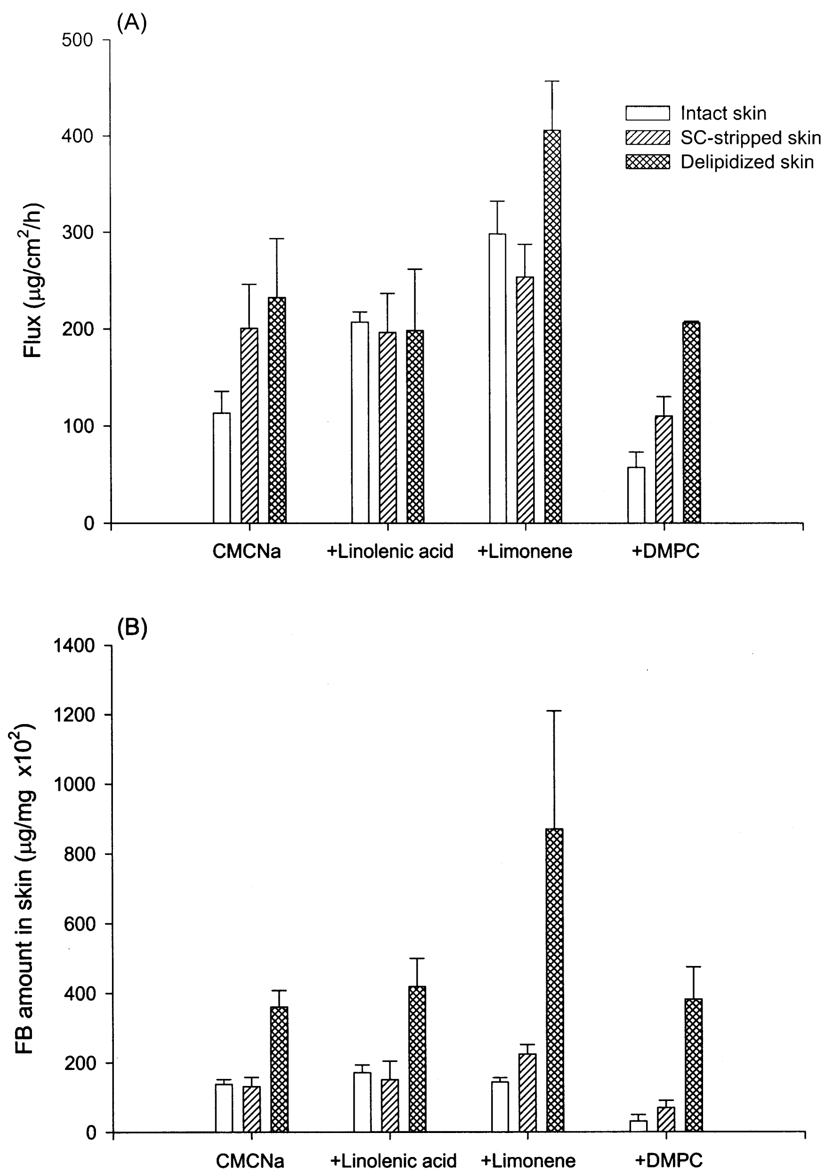

Fig. 2. Flux (A) and drug uptake in skin (B) of flurbiprofen across various types of skin from enhancer/retarder-containing hydrogels. Each value represents the mean ± S.D. ($n = 4$).

drogels (Fig. 2). These data indicate that the rate-limiting characteristics of the SC layer were overcome by application of linolenic acid to the skin. The effect of linolenic acid on skin structure occurs predominantly through the intercellular matrix.

As observed in Table 3, the cyclic monoterpene, D-limonene, produced the greatest enhancement in the flux of flurbiprofen among the enhancers studied here. D-Limonene enhanced the flux to the greatest extent without affecting tissue uptake or partitioning into the skin. In a similar manner as linolenic acid, D-limonene altered the skin structure to produce a higher flux of flurbiprofen as shown in Fig. 1. D-Limonene increases the permeation of drugs by disrupting the highly ordered structure of intercellular lipids and im-

proving the partitioning of drugs in the SC (Zhao and Singh, 2000). The flux across delipidized skin when applying D-limonene-containing hydrogels was higher (t-test, $P < 0.05$) than that with CMCNa hydrogels (control group) (Fig. 2). This suggests that D-limonene acts on the lipid-rich pathways, but also on the water-rich (non-lipoidal) pathways.

Phospholipids such as L-α-lecithin and DMPC were used as constituents to modulate the percutaneous absorption of flurbiprofen from hydrogels. Phospholipids are reported to enhance the skin permeation of many drugs (Bhattachar et al., 1992; Yokomizo, 1996; Kim et al., 1997). However, no or a negative influence on the skin permeation of flurbiprofen by phospholipids was observed in this study (Table 3). More-significant retardation on flurbiprofen permeation was observed for DMPC than for L-α-lecithin. The 5% DMPC dispersed in pH 7 buffer was used to pretreat the skin followed by application of flurbiprofen hydrogels. The reason for use of the pH 7 buffer as the medium was the hydrophilicity of DMPC. Both the flux and skin reservoir of the drug decreased (t-test, $P < 0.05$) after treatment with DMPC, indicating the ability of DMPC to modulate skin morphology. This reduction in drug permeation may be attributed to the high value of the gel–liquid crystalline phase transition temperature (T_c) of DMPC. DMPC with a high T_c (23 °C) has a more-rigid structure than do other lipids (Kirjavainen et al., 1999). Gel-state phospholipids do not affect, or even increase, the skin lipid barrier, resulting in the a reduction of drug permeation. As compared to unsaturated fatty acids, the T_c of L-α-lecithin or DMPC is much higher than those of fatty acids (−50–4 °C). L-α-Lecithin and DMPC are expected to have lower lipid solubilities than do fatty acids (Chi et al., 1995). Phospholipids with low lipid solubility have less capability to disrupt the lipid packing of SC and to insert themselves into the lipid bilayers than do kinked unsaturated fatty acids of high solubility.

The flux of drug was reduced in DMPC-containing hydrogels irregardless of the type of skin used as the barrier as shown in Fig. 2. It is plausible that many phospholipids are unable to permeate into the skin from aqueous hydrogels, and thus an extra barrier is found on the skin surface (Valjakka-Koskela et al., 1998). This may also explain the retardation in release rates across cellulose membranes from DMPC-containing hydrogels. Another explanation is that the high concentration of phospholipids (5%) used in the hydrogels may have produced micelles. It is likely that the drug is released by diffusion through the extramicellar water channels of the gel matrix (Bentley et al., 1999). The additional micelles may decrease the release rate of flurbiprofen from hydrogels, and subsequently the flux and uptake in the skin. As shown in Table 3, the incorporation of enhancers can generally change the viscosity of CMCNa hydrogels. However, this change in viscosity was negligible and might not influence the applicability of enhancer-containing hydrogels to in vivo or clinical situations.

Ethanol or PG was incorporated into the CMCNa hydrogels with 5% enhancer. The enhancer utilized in this investigation was linolenic acid. The flux of flurbiprofen was slightly but significantly increased (t-test, $P < 0.05$) over that from CMCNa hydrogels (control group) as shown in Table 3. However, the skin reservoir of the drug showed the opposite result. Partitioning of lipophilic enhancers into the skin may decrease with increased affinity of this substance to the hydrogels (Okabe et al., 1992), thus reducing the enhancement effect of linolenic acid. In this study, the phenomenon of reduced partitioning may have only influenced the uptake of drug by the skin but not the transdermal flux.

3.4. Histological examination of the skin

The skin is a multilayered organ and anatomically has many histological layers. It is generally described in terms of three tissue layers: the stratified, avascular, cellular epidermis, the underlying dermis of connective tissue, and the subcutaneous fat layer. Moreover, the highly vascularized dermis and the epidermis support several skin appendages. In this present study, the influence of enhancers/retarders on the anatomical structure is discussed with the aid of light microscopic and SEM findings.

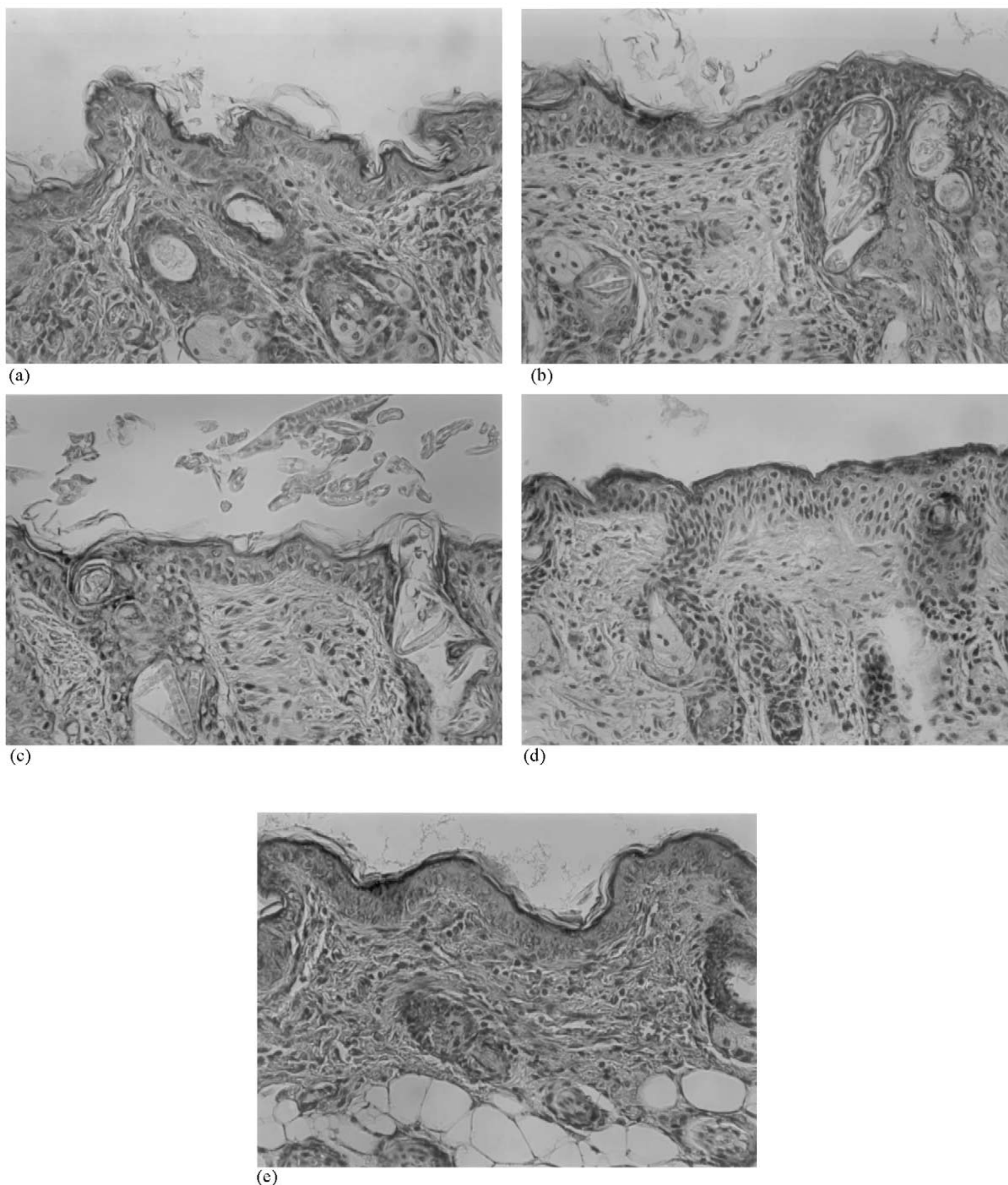

Fig. 3. Light microscopic photographs of nude mouse skin after treatment of: (a) control group (non-treatment); (b) 20% ethanol:pH 7 buffer; (c) 5% linolenic acid in 20% ethanol solution; (d) 5% D-limonene in 20% ethanol solution; and (e) 5% DMPC in pH 7 buffer.

Fig. 3 shows the microscopic appearance of nude mouse skin treated with a 20% ethanol solution, a 5% linolenic acid in 20% ethanol solution, a 5% D-limonene in 20% ethanol solu-

tion, and 5% DMPC in pH 7 buffer. As compared to Fig. 3A of the control group (non-treated skin), 20% ethanol-treated skin shows scattered, loose SC and mild dermal edema. There were more inflamed cells observed for the 20% ethanol-treated skin than for non-treated skin (Fig. 3B). The SEM images also show a flaky appearance of keratin, which may indicate the denaturation of keratinocytes in the SC layers (Fig. 4A). It is possible that these disruptions of the skin morphology may have contributed to the inhibitory effect on flurbiprofen permeation. Fig. 3C illustrates the microscopic appearance of skin after application of 5% linolenic acid. Moderate superficial and deep inflammatory cell infiltration (hyperemia) was found for linolenic acid-treated skin. Disruption and fragmentation of hair shafts were also observed, indicating the degeneration of skin appendages by linolenic acid. The SEM images of linolenic acid-treated skin showed significant modification of the skin surface (Fig. 4B). There were many aggregates of globules caused by fused, degenerated SC lipids, suggesting the extraction of intercellular lipid bilayers by linolenic acid. This phenomenon is consistent with the result of flurbiprofen permeation across various skin types. The numerous cavities presented on the surface of skin after linolenic acid treatment may contribute to pathways through which the drug molecules can pass. Further study is needed and is in progress in our laboratory to elucidate the possible mechanisms governing drug permeation across linolenic acid-treated skin.

Fig. 3D demonstrates microscopic results after treatment with D-limonene. The SC was largely lost and appears as a thin compact layer. Minimal lymphocytic infiltration was also observed. These findings may reflect the great enhancement of flurbiprofen permeation across D-limonene-treated skin. As seen in Fig. 4C, the surface of D-

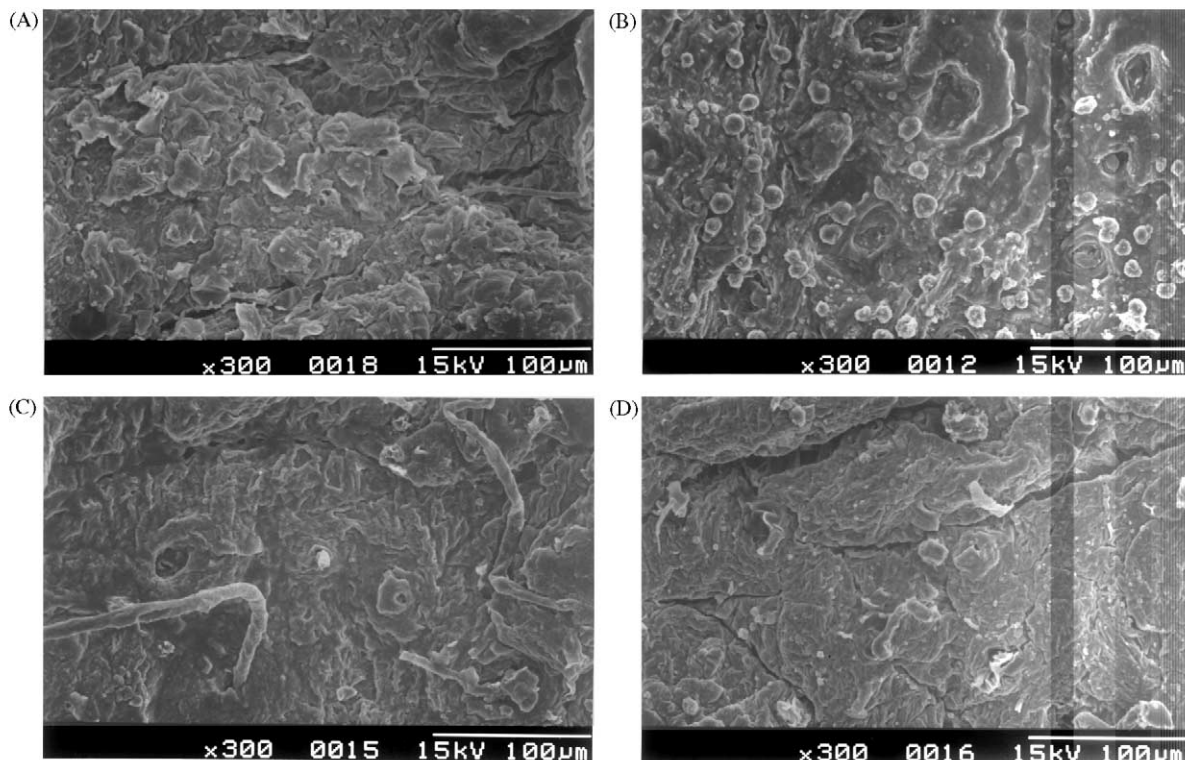

Fig. 4. SEM images of nude mouse skin after treatment with: (A) 20% ethanol:pH 7 buffer; (B) 5% linolenic acid in 20% ethanol solution; (C) the 5% D-limonene in 20% ethanol solution; and (D) 5% DMPC in pH 7 buffer.

limonene-treated skin is eroded and appears rougher than that of the 20% ethanol-treated group. There were wool-like substances on the skin surface of DMPC-treated skin. These substances seem not to be intrinsic materials of skin (Fig. 3E). It is possible that the micelles formed by DMPC contribute to the wool-like substances. A similar observation was observed in SEM images in which some globules of several micrometers in diameter are studded on the surface of the SC. The additional barrier of these micelles on the skin surface may result in reduction of flurbiprofen permeation. In general, morphological alterations of the skin structure increased in the order of the control group < DMPC < 20% ethanol < D-limonene < linolenic acid.

4. Conclusions

The transport of flurbiprofen through skin treated with enhancers from hydrogels was examined. The skin permeation of flurbiprofen from CMCNa hydrogels approximated that from an aqueous solution, suggesting the feasibility of CMCNa hydrogels in further in vivo or clinical situations because of its excellent release of the drug and moderate viscosity and bioadhesion properties. Two cosolvents, PG and ethanol, incorporated into the hydrogels produced different mechanisms governing flurbiprofen permeation. All enhancers used in this present study effectively modulated the permeated amount and/or skin reservoir of flurbiprofen. Some enhancers promoted the skin permeation of the drug; however, others significantly reduced it and these may be called ‘retarders’. These enhancers/retarders can modulate the skin absorption of flurbiprofen to attain local or systemic use of the drug when necessary.

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