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Effect of fatty acids and urea on the penetration of ketoprofen through rat skin

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Summary

The effects of several fatty acids and urea on the penetration of ketoprofen in propylene glycol and aqueous vehicles through excised rat skins have been studied. The penetration of ketoprofen through the skins increased 8–50-fold maximum compared to the control with the addition of the fatty acids in PG and urea in water. The enhancement of skin permeability of ketoprofen with the fatty acids in propylene glycol was mainly due to the increase in ketoprofen partitioning between the skin and the vehicle. When the concentration of lauric acid in propylene glycol – responsible for maximum permeation of ketoprofen – was varied from 0 to 10% in propylene glycol, ketoprofen permeation reached maximum with 5% lauric acid. The enhancement effect of urea on the penetration of ketoprofen through excised rat skins depended on the vehicles used. Its permeation constant increased significantly when added to ketoprofen-water vehicle. On the other hand, the effect of urea was not pronounced when added to ketoprofen-PG vehicle and ketoprofen-PG : ethanol : water (1 : 3 : 6) mixture. Nevertheless, the diffusion of ketoprofen through the skin increased in all three cases. Urea seemed to form large and extensive hydrophilic diffusion channels that do not exist in fresh skin.

Introduction

Ketoprofen is a potent nonsteroidal anti-inflammatory drug which inhibits prostaglandin synthetase-cyclooxygenase. It has been widely used for the symptomatic treatment of rheumatoid arthritis, juvenile rheumatoid arthritis, osteoarthritis and other related conditions. However, its oral administration shows undesirable systemic side effects and gastrointestinal irritancy that may accompany ulceration (Harris and Vavra, 1985; Kantor, 1986). Consequently, its derivatization, complexation (Borsa et al., 1983; Chevallard et al., 1987), sustained-release dosage forms (Caruso et al., 1982; Hagan, 1982) and suppositories (Kamiya et al., 1983) have been studied to reduce such side effects. Another unique technique developed to reduce such systemic adverse actions and gastrointestinal irritation was a transdermal delivery system, for example, gels (Saita et al., 1985), creams (Kyuki et al., 1985), and ointments

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(Fujimura et al., 1985). However, a high dose of ketoprofen hinders the formulation of drug into such forms. To overcome these drawbacks, several attempts have been made to search for penetration enhancers for percutaneous absorption of ketoprofen (Yukawa et al., 1989). In the present study, fatty acids and urea which have been successfully applied in the transdermal delivery of some drugs as penetration enhancers were evaluated in order to observe their roles as penetration enhancers for ketoprofen through excised rat skins.

Materials and Methods

Materials

The following reagents were used as received from the suppliers: ketoprofen (Il Yang Pharm. Co., Seoul, Korea), propylene glycol (Shinyo Chemical Co., Osaka, Japan), caprylic acid, capric acid, lauric acid, myristic acid, oleic acid, stearic acid, urea, ethanol, methanol, chloroform, isopropyl myristate (Junsei Chemical Co., Tokyo, Japan), sodium phosphate (monobasic), sodium phosphate (dibasic), phosphoric acid (Wako Pure Chemical, Osaka, Japan), trypsin, and acetonitrile of HPLC grade (Merck Co., Darmstadt, Germany).

Rat skins were harvested from male Sprague-Dawley rats weighing 200–250 g. After removal of the dorsal hair with an electric clipper, the skin was excised from the rats and stored in a freezer at −20°C. The frozen skin was thawed before mounting on the diffusion apparatus.

Ketoprofen solutions containing fatty acids and urea

To study the effect of fatty acids on ketoprofen penetration in propylene glycol (PG) through rat skin, caprylic acid (CLA), capric acid (CRA), lauric acid (LA), myristic acid (MA), oleic acid (OA), and stearic acid (SA) were added to PG vehicle. With the exception of SA, the contents of each fatty acid in PG were 10%. SA concentration in PG was fixed at 3% due to its limited solubility in PG. To maximize the thermodynamic activity of ketoprofen in the vehicles, all vehicles were saturated with ketoprofen. A PG vehicle containing no fatty acid was used as a control. In addition, the magnitude of ketoprofen penetration through excised rat skin was determined as a function of LA concentration in PG to observe the concentration effect of fatty acids on ketoprofen penetration. The concentration of LA in PG was varied as 0, 2, 5 and 10%.

The role of urea as a ketoprofen penetration enhancer was studied employing three different vehicles: 20% urea in water, 10% urea in PG, and 6, 24, 36% urea in PG:ethanol:water (1:3:6) mixture. Each vehicle without urea was used as control.

Treatment of excised rat skin with urea

In order to gain further insight into the role of urea in ketoprofen penetration through rat skin, excised rat skin was pretreated with urea and determination of ketoprofen penetration was performed. 20% urea aqueous solution was applied on the excised rat skin mounted on the diffusion cell for 12 h. As a control, another rat skin was also pretreated with normal saline for 12 h. After the removal of urea solution or normal saline, ketoprofen aqueous solution excluding urea was applied on the excised rat skin and the penetration profile of ketoprofen through the skin was monitored.

Determination of skin penetration of ketoprofen

Franz-type diffusion cells were used for skin penetration experiments. The cells had an effective permeation area of 3.14 cm^2. The excised rat skin was set in place with the stratum corneum facing the donor compartment and the dermis side facing the receptor. Solutions of various compositions containing the drug were placed in the donor compartment. 20 ml volume of the receptor compartment was filled with 0.05 M phosphate buffer (pH 7.4) and the temperature was kept constant at 37 ± 0.5°C throughout the experiment. 0.5 ml of receptor phase was withdrawn at predetermined intervals up to 30 h and the same volume of fresh phosphate buffer was replaced immediately after each sampling. Ketoprofen in the receptor phase was determined with the following HPLC method. The same experi-

ment for each solution was repeated more than three times.

HPLC analysis of ketoprofen in receptor phase

The amount of ketoprofen in each sample was determined using a liquid chromatographic system (Hitachi, Model 638-50) with a C18 column (Waters, μ-Bondapak RP-18, 30 cm × 3.9 mm i.d., 10 μm). The system consisted of a reciprocating pump (Hitachi, Model L6200), an injector (Hitachi, Model LC-Oraganizer), a UV detector (Waters, Model 440) at 254 nm, and an integrator (Phillips, Model 4810). The mobile phase was a mixture of acetonitrile and 0.02 M phosphate buffer (pH 3) with a volume ratio of 45:55. The flow rate was 2.0 ml/min with an injection volume of 10 μl. The standard solutions of ketoprofen were prepared with 0.05 M phosphate buffer (pH 7.4) every week.

Determination of partition coefficient of ketoprofen

The degree of ketoprofen partitioning between isopropyl myristate (IPM) and PG (with or without LA) was determined as follows: 160 mg of ketoprofen was added to 1 ml of PG or PG which contained 5% LA. The solution was then mixed with an equal volume of IPM and rotated in a 20°C water bath for 2 days. The concentration of ketoprofen in IPM was determined with the HPLC method described previously.

Data analysis

Assuming that the skin was a homogeneous plane barrier sheet, the total amount of ketoprofen Q_t, which penetrated the skin in time t from the donor solution to the receptor phase is given by (Crank, 1975):

$$Q_t = A \cdot L \cdot K \cdot C \cdot \left\{ Dt/L^2 - 1/6 - 2/\pi^2 \sum_{1}^{\infty} \times \left[(-1)^n/n^2 \exp(-Dn^2\pi^2 t/L^2) \right] \right\} \quad (1)$$

where A is the area for application, L the thickness of skin, K the partition coefficient of ketoprofen between skin and donor solution and D the diffusion coefficient.

Since it is difficult to determine the thickness of the real diffusion barrier correctly, a modified equation of the above model was proposed by Okamoto et al. (1986):

$$Q_t = A \cdot K' \cdot C \cdot \left\{ D't/L^2 - 1/6 - 2/\pi^2 \sum_{1}^{\infty} \times \left[(-1)^n/n^2 \exp(-D'n^2\pi^2 t/L^2) \right] \right\} \quad (2)$$

where D' represents D/L and K' is KL.

D' and K' are not real diffusion and partition coefficients, but apparent diffusion and partition parameters, respectively, involving the skin thickness. The permeability constant (K_p) and lag time (t_L) were calculated as follows:

$$K_p = D'K' \quad (3)$$

$$t_L = 1/6D' \quad (4)$$

To calculate D' and K', skin penetration data were fitted to the modified equation and analyzed with a non-linear least squares computer program. The means of all data are presented here with their standard deviation (S.D.). Student's t-test was performed on the ketoprofen permeation parameters to determine significant differences in the various vehicles tested at an alpha level of 0.05.

DSC determination for study of lipid fluidity

For differential scanning calorimetric (DSC) determination, rat skins were treated with trypsin to obtain stratum corneum sheets. The epidermis was separated from the dermis after several hours of incubation at 37°C on filter paper saturated with 0.5% trypsin in phosphate buffered saline. Any remaining epidermis cells were removed by gentle agitation of the stratum corneum sheets. They were rinsed with distilled water and stored in a desiccator until use. Prior to DSC determination, dried stratum corneum of known mass was incubated for 5 h in a 10% solution of OA or LA in PG. At the end of incubation, the samples were washed with ethanol, dried for several hours

over a desiccant and reweighed. All samples were then placed for several days in a chamber maintained at 95% relative humidity. Stratum corneum sheets were equilibrated to a water content of about 30% (w/w) under the above conditions. As a control, dried stratum corneum or solvent-extracted stratum corneum with chloroform : methanol was incubated in PG and subjected to DSC determination in the same way.

The differential scanning calorimeter (Dupont Inst., Model 912) was used at a scan rate of 10°C/min. The temperature range was from 30 to 160°C.

Results and Discussion

Effect of fatty acids on ketoprofen penetration through rat skin

Fatty acids are known to be potent enhancers of the penetration of several drugs through skin. To observe the potential penetration enhancing effect of the fatty acids on ketoprofen penetration through skin, ketoprofen penetration in PG vehicles containing various individual fatty acids was measured using Franz-type diffusion cells with excised rat skin. The permeation profiles of ketoprofen from these vehicles containing some of the fatty acids used for this study are shown in Fig. 1. The diffusion parameter (D'), partition parameter (K'), permeability constant (K_p) and lag time (t_L) were calculated from these penetration profiles according to Eqns 2–4 and listed in Table 1. While only MA and OA increased the diffusion parameters significantly, all the fatty acids used showed significant increase in the par-

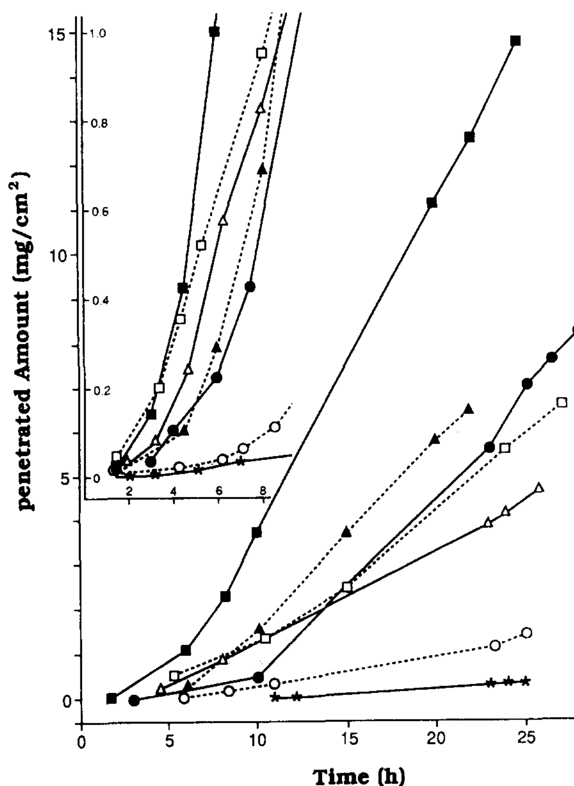

Fig. 1. Effects of saturated fatty acids on ketoprofen penetration through the skin. Each vehicle was composed of 10% fatty acid in propylene glycol and saturated with ketoprofen. (∗) Propylene glycol; (○) stearic acid; (△) myristic acid; (□) oleic acid; (●) caprylic acid; (▲) capric acid; (■) lauric acid.

TABLE 1

Effects of various fatty acids in propylene glycol on the parameters for ketoprofen penetration through rat skin [a]

Fatty acids	Concentration (%)	D' ($\times 10^2$) (h^{-1})	K' ($\times 10$) (cm)	K_p ($\times 10^3$) (cm/h)	t_L (h)
None		2.32 ± 0.42	0.15 ± 0.03	0.34 ± 0.01	7.2
Caprylic acid	10	1.72 ± 0.39	4.99 ± 1.86 [b]	8.16 ± 1.33 [b]	9.7
Capric acid	10	1.80 ± 0.23	5.00 ± 0.96 [b]	9.00 ± 2.00 [b]	9.3
Lauric aicd	10	2.75 ± 0.43	5.77 ± 1.52 [b]	15.5 ± 2.13 [b]	6.1
Myristic acid	10	4.12 ± 0.98 [b]	1.10 ± 0.39 [b]	4.55 ± 0.85 [b]	4.0
Oleic acid	10	7.84 ± 0.55 [b]	0.44 ± 0.03 [b]	3.47 ± 0.34 [b]	2.1
Stearic acid	3	1.48 ± 0.08 [b]	1.70 ± 0.07 [b]	2.50 ± 0.11 [b]	11.3

[a] Each value represents the mean ± S.D. of three determinations and each vehicle was saturated with ketoprofen. D', diffusion parameter; K', partition parameter; K_p, permeability constant; t_L, lag time.

[b] Significantly different at an alpha level of 0.05 from that of control vehicle.

tition parameter and permeability constant in the penetration of ketoprofen through excised rat skin. LA demonstrated the maximum increase in permeability constant, followed by CRA, CLA, MA, OA, and SA in that order. The permeation constants of ketoprofen penetration with these fatty acids were 8–50-fold higher than the PG control vehicle. The overall increase in ketoprofen permeability was mainly due to the increase in K' rather than D'. Among the fatty acids of equal carbon number, unsaturated C_{18} acid (OA) was a more effective enhancer than the corresponding saturated acid (SA). This may partially be due to the fact that vehicle containing SA was more viscous than that containing OA.

Since LA showed the most pronounced effect on the penetration of ketoprofen, it was selected for the study of the concentration effect of the fatty acid on ketoprofen penetration through rat skin. The penetration parameters calculated from the penetration profiles of ketoprofen from PG vehicles containing 0, 2, 5 and 10% LA are listed in Table 2. The addition of LA to PG increased ketoprofen permeability significantly from 50- to 70-fold compared to the PG control vehicle. However, the permeation constant of ketoprofen did not increase linearly with the increase in the amount of LA in PG. The maximum permeability constant was achieved with 5% LA. As explained previously, K' rather than D' contributed to the enhanced permeability constant of ketoprofen penetration from PG vehicle containing LA. To examine whether an increase in ketoprofen permeability was directly related with skin/vehicle partition coefficient (P_c), the effect of LA on ketoprofen P_c between IPM and PG (with or without LA) was determined. The P_c values of ketoprofen between IPM and PG containing 0, 2, 5 and 10% LA were 0.12, 0.14, 0.16 and 0.21, respectively. The large increase in ketoprofen permeability with LA could not simply be due to the P_c of ketoprofen.

Also, the ketoprofen permeation constant with PG which contained 10% LA was lower than that with PG containing 5% LA, even though 10% LA-PG vehicle resulted in a higher P_c value between IPM and PG vehicle than 5% LA-PG vehicle.

Study of lipid fluidity in stratum corneum using DSC

It has been proposed that the DSC peaks near 65, 75 and 105°C for human and porcine stratum corneum were due to the thermal transitions involving intercellular lipids, lipid-protein complexes and intercellular keratin, respectively (Golden et al., 1986, 1987). In order to identify the components responsible for the observed thermal transitions in this study, a stratum corneum of rat skin was solvent-extracted prior to DSC determination. Fig. 2 shows the thermograms of rat stratum corneum extracted with chloroform:methanol (upper trace) and an unextracted one (lower trace). The thermogram of the unextracted stratum corneum showed two small peaks at 53 and 75°C and one broad large peak at 107°C. When compared to this reference, the thermogram of the extracted stratum corneum

TABLE 2

Effect of lauric acid concentration in propylene glycol on the parameters for ketoprofen penetration through rat skin [a]

Lauric acid (%)	P_c^b ($\times 10^2$) (h^{-1})	D' ($\times 10$) (cm)	K' ($\times 10^3$) (cm/h)	K_p (h)	t_L
0	0.12	2.32 ± 0.42	0.15 ± 0.03	0.34 ± 0.01	7.2
2	0.14	3.11 ± 0.20 [c]	4.98 ± 0.80 [c]	15.4 ± 2.13 [c]	5.4
5	0.16	2.06 ± 0.18	10.8 ± 1.10 [c]	22.0 ± 0.51c	8.1
10	0.21	2.77 ± 0.13	5.85 ± 0.64 [c]	16.3 ± 2.50 [c]	6.0

[a] Each value represents the mean ± S.D. of three determinations and each vehicle was saturated with ketoprofen. D', K', K_p, and t_L are the same as in Table 1.
[b] Partition coefficient of isopropyl myristate/vehicle.
[c] Significantly different at an alpha level of 0.05 from that of control vehicle.

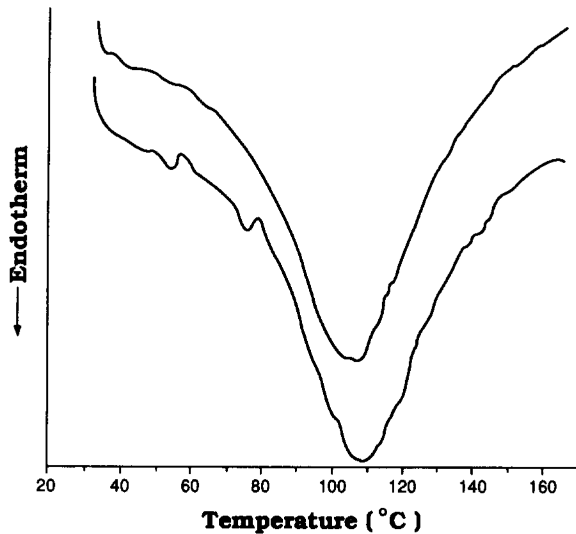

Fig. 2. DSC thermal profile obtained from rat stratum corneum hydrated to 30% (w/w) water content. (Upper trace) Thermal profile of a sample extracted with chloroform-methanol; (lower trace) profile of the control.

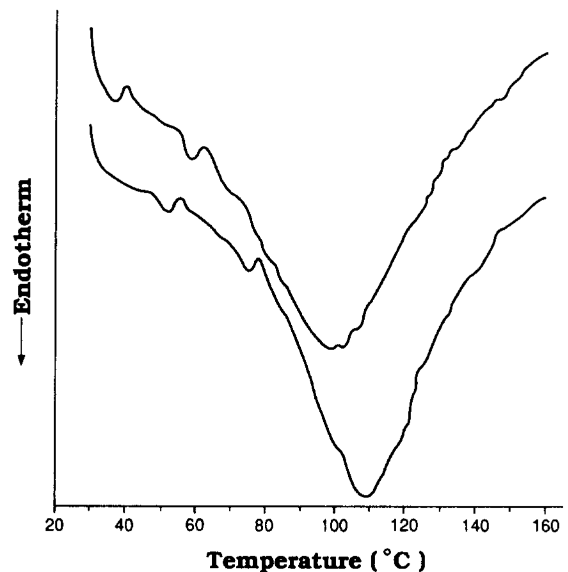

Fig. 3. DSC thermal profile obtained from rat stratum corneum hydrated to 30% (w/w) water content. (Upper trace) Thermal profile of a sample treated with 10% oleic acid in propylene glycol; (lower trace) profile of the control.

showed no peaks of transitions near 53 and 75°C, which indicates that these two peaks are probably due to the thermal transitions involving intercellular lipids and lipid-protein complexes. However, unlike the previous reports (Golden et al., 1986, 1987), the highest temperature transition occurred in both the extracted and unextracted stratum corneum. This suggests that a protein transition involving keratin leads to the highest temperature peak.

LA and OA were selected in the study of the mechanism of enhancing effects on ketoprofen permeation by fatty acids, since LA showed the maximum increase in partition coefficient while OA led to the maximum increase in the diffusion parameter for the penetration of ketoprofen among the fatty acids studied. Figs 3 and 4 show the DSC thermograms of rat stratum corneum treated with 10% LA in PG (upper traces) and stratum corneum treated with PG only (lower traces). The midpoints of the thermal transition (T_m) near 53 and 75°C were shifted to a lower temperature range in OA-treated stratum corneum while little change was observed for the peak near 107°C. Compared to the treatment with PG, LA did not change the T_m of the peak near 53 and 75°C, but a slight change in sharpness (ratio of peak height to width) of the high temperature peak was brought about. A decrease in T_m reflects a thermal transition starting from a

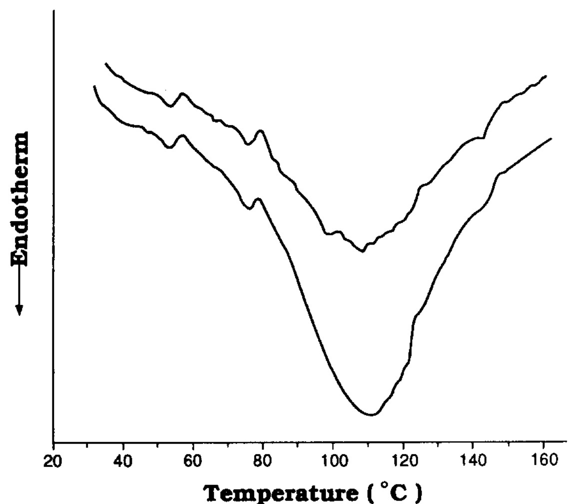

Fig. 4. DSC thermal profile obtained from rat stratum corneum hydrated to 30% (w/w) water content. (Upper trace) Thermal profile of a sample treated with 10% lauric acid in propylene glycol; (lower trace) profile of the control.

TABLE 3

Effect of urea in various hydrophilic solutions on the parameters for ketoprofen penetration through rat skin [a]

Solvents	Urea (%)	Concentration [b] (mg/ml)	D' ($\times 10^2$) (h^{-1})	K' ($\times 10$) (cm)	K_p ($\times 10^3$) (cm/h)	t_L (h)
H_2O	0	0.7	4.38 ± 0.29	7.53 ± 0.54	32.9 ± 0.57	3.8
H_2O	20	10.8	6.56 ± 0.93 [d]	0.56 ± 0.03 [d]	3.67 ± 0.40 [d]	2.5
PG	0	170	2.32 ± 0.42	0.15 ± 0.03	0.34 ± 0.01	7.2
PG	10	210	7.21 ± 0.84 [d]	0.03 ± 0.02 [d]	0.23 ± 0.08 [d]	2.3
PG : EtOH : H_2O [c]	0	8	2.75 ± 0.04	2.13 ± 0.07	5.85 ± 0.10	6.1
PG : EtOH : H_2O	6	8	2.35 ± 0.10 [d]	3.03 ± 0.17 [d]	7.61 ± 1.06 [d]	7.1
PG : EtOH : H_2O	24	8	3.02 ± 0.10 [d]	2.60 ± 0.15 [d]	7.84 ± 0.24 [d]	5.5
PG : EtOH : H_2O	36	8	9.53 ± 0.88 [d]	0.75 ± 0.08 [d]	7.13 ± 0.13 [d]	1.7

[a] Each value represents the mean ± S.D. of three determinations. D', K', K_P, and t_L are the same as in Table 1.
[b] Ketoprofen concentration.
[c] Propylene glycol : ethanol : water = 1 : 3 : 6.
[d] Significantly different at an alpha level of 0.05 from that of control vehicle.

less ordered, more heterogeneous state. The changes in the thermal profile seen with the OA-treated sample suggest that its incorporation into stratum corneum resulted in decreased lipid order. It has been reported that saturated and *cis*-monounsaturated fatty acids have different lipid packing properties due to their sharp kink at the double bond (Small, 1984). The results of this study indicate that OA and LA have different fluidizing effects on the stratum corneum. In addition, OA has been shown to be an effective penetration enhancer for several other chemicals (Akhter and Barry, 1984; Cooper, 1984). These results suggest that skin penetration of penetrants is ultimately related to their packing in the intercellular lipid domains of the stratum corneum. Thus, DSC data of this study suggest that OA functions by partitioning into the lipid regions of the stratum corneum, disrupting their structure. In other words, intercellular lipids play an important role in ketoprofen skin penetration. However, the fluidizing effect using LA on the stratum corneum was not shown in this study. The present results are in good agreement with that mentioned above that OA increased the diffusion parameter of ketoprofen penetration while LA did not.

TABLE 4

Parameters for ketoprofen penetration through rat skin pretreated with 20% aqueous solution and hydrated with normal solution [a]

System	D' ($\times 10^2$) (h^{-1})	K' ($\times 10$) (cm)	K_p ($\times 10^3$) (cm/h)	t_L (h)
Control (water)	4.38 ± 0.29	7.53 ± 0.54	32.9 ± 0.57	3.8
Pretreatment with urea	13.6 ± 5.31 [b]	4.49 ± 1.49 [b]	56.5 ± 2.30 [b]	1.2
Hydration with saline	7.77 ± 1.22 [b]	8.87 ± 1.08	68.1 ± 3.01 [b]	2.1

[a] Each value represents the mean ± S.D. of three determinations. D', K', K_P, and t_L are the same as in Table 1.
[b] Significantly different at an alpha level of 0.05 from that of control.

Effect of urea on ketoprofen penetration through rat skin

To study the penetration enhancing effect of urea in different vehicles on ketoprofen penetration through skin, the penetration of ketoprofen from vehicles containing urea through excised rat skin was measured. The relevant penetration parameters calculated from the penetration profiles of ketoprofen through rat skin are listed in Table 3. Irrespective of the kind of vehicle used, urea decreased the lag time in the penetration of ketoprofen through excised rat skin. However, the effect of urea on ketoprofen penetration through excised rat skin was dependent upon the vehicles used. When water was employed as the vehicle, the addition of urea increased D' slightly, but decreased the permeation constant of keto-

profen significantly from 32.9×10^{-3} to 3.67×10^{-3} cm/h due to the large reduction in K' of the drug from 0.75 to 0.06 cm. On the other hand, when PG and PG : ethanol : water (1 : 3 : 6) mixture were used as the vehicles, the effect of urea on ketoprofen permeation was not pronounced, although the results were statistically different. Urea in PG increased D' of ketoprofen approx. 3-fold compared to the control PG vehicle, which was compensated by a 5-fold decrease in K' of the drug, thus resulting in a slight decrease of the overall permeation constant of ketoprofen. When urea was included in the PG : ethanol : water (1 : 3 : 6) mixture, similar results were obtained to those in PG. When urea was added in the PG : ethanol : water (1 : 3 : 6) mixture at a concentration of 36%, D' of ketoprofen increased about 4-fold compared to the control PG:ethanol:water (1 : 3 : 6) mixture while K' of the drug decreased similarly, thus resulting in a slight increase in ketoprofen penetration across the dermis layer.

Urea has successfully been used as a penetration enhancer for several drugs. It is known to cause two changes in the barrier function of the skin; it increases the hydration of the stratum corneum and induces keratolysis of the skin after prolonged contact. Consequently, it might contribute to the increase in the diffusion parameter for ketoprofen skin penetration, in good agreement with the results of this study in that urea increased D' when added to the three different vehicles: water, PG and PG : ethanol : water (1 : 3 : 6) mixture.

Pretreatment of skin with urea and hydration effect

To determine whether the decrease in the partition parameter, K', for ketoprofen penetration was due to the presence of urea in ketoprofen solution, the skin was pretreated with urea and subjected to the same penetration experiment using ketoprofen aqueous solution free of urea. Fig. 5 presents the penetration profiles of ketoprofen through excised rat skins which were pretreated with urea solution or normal saline. The penetration parameters calculated from each penetration profile are listed in Table 3. Ketoprofen penetrated more rapidly through the skin hydrated with normal saline or pretreated with urea than through untreated skin. Although the penetration profiles of ketoprofen from the vehicles and the overall increase in K' of the drug through both hydrated and urea-pretreated skin were similar, the effect of urea pretreatment of the skin on the penetration parameters was different from that of hydration with normal saline based on the calculated penetration parameters. Compared to the control, pretreatment of the skin with 20% urea solution increased D' from 4.4×10^{-2} to 13.6×10^{-2} h^{-1} for ketoprofen penetration, but decreased K' of the drug from 0.75 to 10.45 cm. On the other hand, hydration with normal saline increased both parameters (D' and K') for ketoprofen penetration even though the increases were less than 2-fold. Based on these results, the decrease in the partition parameter for ketoprofen penetration on the addition of urea into the ketoprofen solution may be due solely to the effect of urea on the skin.

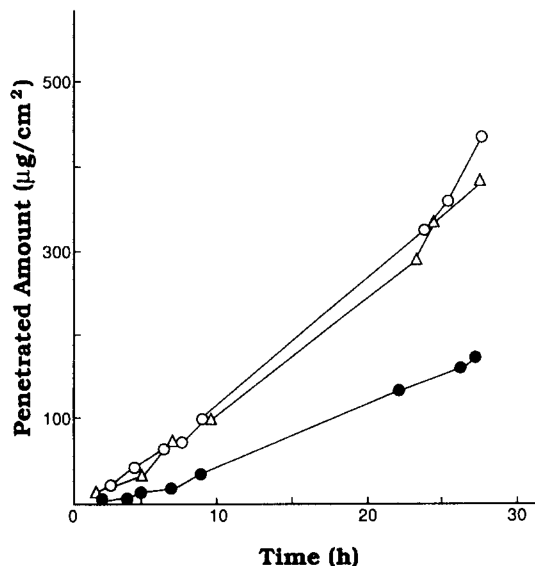

Fig. 5. Effects of hydration with normal saline and pretreatment with urea solution on ketoprofen penetration through rat skin. (○) Hydration with normal saline; (△) pretreatment with 20% urea in water; (●) control.

If the major interaction of urea with skin is not only hydration but also keratolysis of skin, protein then might have played an important role in ketoprofen partitioning into the skin. In that case,

both hydration and pretreatment with urea solution might have altered the ultrastructure of the stratum corneum, leading to the formation of large and extensive hydrophilic diffusion channels which do not exist in fresh, untreated skin based on the fact that both pretreatment with urea and hydration with normal saline increased the diffusion parameters for ketoprofen penetration through excised rat skin.

Conclusion

The penetration rate of ketoprofen from PG or water increased on the addition of fatty acids in PG and urea in water. The enhancement of the skin permeability of ketoprofen on the addition of fatty acids was mainly due to an increase in the partition parameter. Among the fatty acids studied, LA induced maximum ketoprofen permeation which showed an approx. 50-fold greater permeation constant for ketoprofen penetration. When the concentration of LA in PG was varied from 0, 2, 5 to 10%, ketoprofen permeation reached its highest value in 5% LA in PG. Although the mechanisms of the enhancing effect of fatty acids could not be clearly identified, we can assume that at least the enhancing mechanisms of LA and OA were different.

The enhancing effect of urea on the penetration of ketoprofen through rat skin depended on the three different vehicles used. It increased the permeation constant of ketoprofen through excised rat skin significantly when added to ketoprofen-water vehicle while it slightly decreased the permeation constant when added to ketoprofen-PG vehicle. The addition of urea into ketoprofen-PG : ethanol : water (1 : 3 : 6) mixture at concentrations of 6, 24 and 36% resulted in a slight increase in the permeation constant. However, the diffusion parameter for ketoprofen through rat skin increased with all three vehicles containing urea. Hydration and pretreatment with urea aqueous solution resulted in a large increase in the permeability constant for ketoprofen penetration, indicating that hydrophilic diffusion channels were important for ketoprofen penetration through rat skin.

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