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RESEARCH PAPER

Topical Oleo-Hydrogel Preparation of Ketoprofen with Enhanced Skin Permeability

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ABSTRACT

In an attempt to improve the skin penetration of ketoprofen, various transdermal formulations were prepared, and their in vitro skin permeability and in vivo percutaneous absorption were evaluated. In vitro permeation studies were performed using a modified Franz cell diffusion system in which permeation parameters such as cumulative amount at 8 hr Q_{8hr}*, steady-state flux* J_{ss}*, or lag time* t_L *were determined. In the in vivo percutaneous absorption study using the hairless mouse, maximum concentration* C_{max} *and area under the curve at 24 hr* AUC_{24h} *were measured. The optimal transdermal formulation (oleo-hydrogel formulation) of ketoprofen showed a* Q_{8hr} *value of 227.20* $\mu g/cm^2$*, a* J_{ss} *value of 29.61* $\mu g/cm^2/hr$*, and a* t_L *value of 0.46 hr. The* Q_{8hr} *and* J_{ss} *values were about 10-fold* ($p < .01$) *higher than those* ($Q_{8hr} = 19.61\ \mu g/cm^2$; $J_{ss} = 2.66\ \mu g/cm^2/hr$) *from the K-gel and about 3.5-fold* ($p < .01$) *than those* ($Q_{8hr} = 60.00\ \mu g/cm^2$; $J_{ss} = 7.99\ \mu g/cm^2/hr$) *of the K-plaster. In the in vivo percutaneous absorption, the* C_{max} *(6.82* $\mu g/ml$*) and* AUC_{24h} *(55.74* $\mu g \cdot hr/ml$*) values of the optimal formulation were significantly* ($p < .01$) *higher than those of K-gel and K-plaster. The relative bioavailability of the oleo-hydrogel following transdermal administration in reference to oral administration was about 37%, and the* C_{max} *value (4.73* $\mu g/cm^2$*) in the hypodermis following topical administration was much higher than those from the conventional products (*C_{max} *of K-gel and K-plaster were* $0.92 \pm 0.19\ \mu g/cm^2$ *and* $1.27 \pm 0.37\ \mu g/cm^2$*, respectively).*

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These data demonstrate that the oleo-hydrogel formulation of ketoprofen was more beneficial than conventional products (K-gel and K-plaster) in enhancing transdermal permeation and skin absorption of ketoprofen. Furthermore, there was a good correlation between in vitro permeation parameters and in vivo percutaneous absorption parameters.

INTRODUCTION

Ketoprofen is a potent nonsteroidal anti-inflammatory drug (NSAID) that inhibits prostaglandin synthetase-cyclooxygenase. Nonsteroidal anti-inflammatory drugs have been widely used in the treatment of rheumatoid arthritis and other related conditions (1). However, they carry the risk of undesirable systemic side effects and gastrointestinal irritation at the usual dose of oral administration (2). Since NSAIDs are used for a prolonged period, it is important that the side effects of these drugs should be reduced by any possible means.

Considering the fact that most inflammatory diseases occur locally and near the surface of the body, topical application of NSAIDs (3–10) on the inflammed site can offer the advantage of delivering the drug directly to the disease site and producing high local concentrations. This bypasses gastric irritation and also reduces adverse systemic effects. Therefore, a transdermal delivery offers several advantages over conventional routes of drug administration. However, the barrier properties of intact skin limit the permeability of a wide variety of substances, including pharmaceutical active agents.

To overcome these problems, the development of an optimal vehicle system for high skin permeation of ketoprofen is required. In developing a transdermal delivery system, two criteria are considered. One is to achieve adequate flux across the skin, and the other is to minimize the lag time in skin permeation (11). In the present study, we focused on the hydrogel system containing an emulsion (oleo-hydrogel system) for which there were no extensive reports in pharmaceutical investigations. The object of this study was to formulate a ketoprofen transdermal preparation using the oleo-hydrogel system and to evaluate the skin permeability, in comparison with other conventional products, across the skin of the hairless mouse.

MATERIALS AND METHODS

Materials

Ketoprofen was purchased from Nobel Chemicals AB (Sweden). The following reagents were kindly provided as gifts from the suppliers: *N*-methyl pyrrolidone (NMP; ISP Technologies, NJ), Miglyol 812N (Hüls AG, Germany), Labrafil M 1944CS (Gattefosse, France), Polyoxyl-35-castor oil (BASF, Germany), and carbomer 940 (BF Goodrich Co., OH). All chemicals used were reagent grade, and other solvents were high-performance liquid chromatography (HPLC) grade. Hairless mice (5–6 weeks old, 20–25 g, Charles River), fed a standard laboratory diet and allowed tap water ad libitum, were used. The mice were kept at a temperature of 20°C–27°C and a humidity of 55% ± 10% throughout the study. The composition of the Pluronic gel formulation (w/w) was as follows: 3% ketoprofen, 20% Pluronic F127, 10% NMP, 10% oil, 8% surfactant and water. The concentration of ketoprofen in conventional products (K-gel, I Company, Korea; K-plaster, P Company, Korea) was 3%.

Preparation of Oleo-Hydrogel Transdermal Formulation

The oleo-hydrogel transdermal formulation was prepared as follows. Ketoprofen was dissolved in NMP, surfactant, and oil mixture to prepare the emulsion preconcentrate. Separately, the gelling agent, such as carbomer, was dissolved in purified water with uniform stirring to prepare the hydrogel. The emulsion preconcentrate was added portionwise to the hydrogel under stirring to prepare the emulsion, which was then mixed with the alkalizing agent triethanolamine to obtain the desired topical formulation of ketoprofen in the form of hydrogel emulsion.

In Vitro Studies

In Vitro Permeation Studies

Franz cells were modified as follows. The bottom of the diffusion cell was designed to make a small crossed-stirrer (0.98 cm × 0.98 cm, spinbar, Bel-art products, NJ) rotate freely. The effective diffusional area of the cell was 3.14 cm^2 ($r = 1$ cm), and the volume of the receiver compartment was 13 ml. The cell was immersed in a water bath (37°C) on the Franz cell diffusion tester (dissolu-

tion tester DST-S30, Fine Instruments, Seoul, Korea), and the receiver compartment was filled with 13 ml of 50 mM phosphate-buffered saline (PBS) solution (pH 7.4) maintained at 37°C and mixed throughout the experiment under stirring (600 rpm).

In vitro permeation studies with intact hairless mouse skin were performed as follows. Full-thickness skin of the hairless mouse was obtained from 5–6-week-old animals (20–25 g). Mice were anesthetized with 25% urethane (1.2 g/kg i.p.) saline solution. The abdominal skin was excised, and the adhering subcutaneous fat on the dermal site was removed. The excised skin was inserted into the diffusion cell, and the permeation study was undertaken as described in the above procedure. The stratum corneum side of the skin was exposed to ambient laboratory conditions, whereas the dermal side was bathed with receptor medium. As the donor compartment was charged with 45 mg (1.35 mg as ketoprofen) of ketoprofen formulation containing 3% (w/w) ketoprofen, the permeation studies were started. Aliquots of 300 ml were withdrawn from the receiver compartment across the sampling port periodically for 8 hr and replaced with an equal volume of fresh PBS solution. Of the aliquot, 50 μl were injected into the HPLC column.

Determination of Ketoprofen

The samples of the collected solution were filtered through Millipore filters (Millex HV 0.45-μm filter unit, Millipore), and analyzed by the HPLC method as reported by Satterwhite and Boudinot (12) with some modifications. The HPLC system consisted of a solvent delivery pump (Hitachi L-7100), an ultraviolet (UV) detector (Hitachi L-7400), an autosampler (Hitachi L-7200), a personal computer system (as an integrator), and a column (3.9 mm × 300 mm, μ-Bondapak C_{18}, Waters). The mobile phase was acetonitrile:10 mM phosphate buffer (pH 7.5) (25:75), and the flow rate was 1.0 ml/min. A wavelength of 258 nm was selected, and the column was maintained at room temperature.

Calculation of Cumulative Drug Amount

The amount of ketoprofen in total receiver solution was determined from a calibration curve. The cumulative drug permeation Q_n, corresponding to the time of the *n*th sample, was calculated from the following equation (13):

$$Q_n = V_R C_n + \sum_{i=0}^{n-1} V_s C_i \quad (1)$$

where C_n and C_i are the drug concentrations of the receiver solution at the time of the *n*th sample and the *i*th sample, respectively, and V_R and V_S are the volumes of the receiver solution and the sample, respectively. C_o is defined to be equal to 0.

Calculation of Permeation Parameters

The permeation parameters of ketoprofen (lag time t_L, permeability coefficient through the skin K_p, diffusion coefficient within the skin D, and partition coefficient between the skin and the vehicle K) were calculated from the penetration data (14). The penetration profiles were constructed by plotting the total amount of ketoprofen penetrated versus time. The *x*-intercept of the extrapolated linear region of the curve gives t_L. D was calculated from t_L with known thickness (hairless mouse, 0.07 cm) of the penetration barrier L using Eq. 2. The slope of the linear portion of the profile, determined by linear regression analysis, was J_{ss}. K_p was calculated by dividing J_{ss} by the employed concentration of ketoprofen C_s (Eq. 3), and K was calculated with Eq. 4. The significance of the difference in parameter values was tested by a nonpaired Student *t* test.

$$t_L = L^2/6D \quad (2)$$

$$J_{ss} = (K \cdot D \cdot c_s)/L = K_p \cdot C_s \quad (3)$$

$$K_p = (K \cdot D)/L \quad (4)$$

In Vivo Studies

In Vivo Skin Absorption Studies

After hairless mice were fixed on their backs, each topical preparation was spread onto the 1.2 cm^2 (1.0 cm × 1.2 cm) delimited area of normal skin. The limitation was performed by placing a silicone glue cell (1 component RTV Neutral cure system, Shin-etsu silicone, Korea) rigidly on the skin to prevent leakage of the preparation over the defined area. The dose of each preparation was adjusted to 20 mg/kg. In addition, ketoprofen was administered intramuscularly or orally at a dose of 20 mg/kg. The mice were kept at a temperature of 20°C–27°C and a humidity of 55% ± 10% throughout the study. At 0.5, 1, 3, 6, 9, 12, 18, and 24 hr after the application of each preparation, the drug remaining unabsorbed on the applied site was removed by successive washing and rinsing twice with ethanol and water with the aid of a commercial cotton swab. The stratum corneum layer of abdominal skin was taken according to the reported stripping method (11). The layer was taken by stripping off the cellophane adhesive tape (3M No. 810, Scotch

brand, 3M Company) of 8 cm^2 width (2.0 cm × 4.0 cm) stuck previously on the skin surface. Repeated stripping (20 times) could take the stratum corneum layer from the skin. Then, viable tissue (epidermis plus dermis) and hypodermis were taken from the applied site, and whole blood was collected through heart puncture as much as possible. Finally, the liver was collected from the abdomen and rinsed with saline solution.

Determination of Ketoprofen in Plasma Concentration

Ketoprofen in plasma was quantified by HPLC as reported by Schmitt and Guentert (15) with some modification. An aliquot of 100 μl of plasma was mixed with 100 μl of a naproxen standard solution (10 μg naproxen/ml of internal standard in methanol) and 200 μl of 1.0 M phosphate buffer (pH 2.0). After extraction with 5 ml of diethyl ether, 3 ml of the organic phase was transferred to another fresh glass tube and evaporated to dryness under a stream of nitrogen gas at 30°C in a dry block bath (Model MG-2, Eyela, Japan). The residue was reconstituted in 200 μl of mobile phase, vortex mixed on a multitube vortex mixer (No. 58816-116, VWR Company) at a setting of 4 for 2 min, and ultrasonicated for 5 min. The reconstituted samples were transferred to autosampler vials, and 50 μl were injected for each sample into the HPLC column.

Quantitation of Ketoprofen in Viable Tissue, Hypodermis, or Liver

A sample (1.2 cm^2) of viable tissue, hypodermis, or liver (rinsed with saline) was added to 10 ml of 0.15 M Tris buffer (pH 7.6), homogenized at 10,000 rpm for 10 min, and centrifuged at 12,000 rpm for 5 min. To each supernatant (3 ml), 100 μl of a naproxen standard solution (internal standard, 10 μl/ml in methanol) and 200 μl of 1.0 M phosphate buffer (pH 2.0) were added. The determination of ketoprofen concentration was carried out as described above.

RESULTS AND DISCUSSION

In Vitro Studies

Effect of Gel pH on Drug Permeation

The effect of pH of the gel (3% ketoprofen in 1% carbomer oleo-hydrogel) on the release of ketoprofen (pK_a 5.02) was examined at a gel pH of 3.5, 4.0, 4.6, 5.0, and 6.0, which were adjusted using triethanolamine. The steady-state flux from the gel of pH 3.5, 4.0, 4.6, 5.0, and 6.0 is 27.12 ± 3.02, 28.48 ± 2.95, 29.61 ± 2.69, 27.82 ± 1.94, and 28.78 ± 3.22 μg/cm^2/hr, respectively (Table 1). The pH dependence for the permeation of ketoprofen was not significant ($p < .05$). Since ketoprofen is in emulsion droplets containing an oil component, the diffusable unionized form of ketoprofen in the aqueous gel may not be responsible for the skin permeation of drug.

Effect of Carbomer Concentration on Drug Permeation

The permeation rates of ketoprofen from oleo-hydrogels containing various carbomer concentrations were determined. The amount of ketoprofen in the oleo-hydrogel was kept at 3%, and the carbomer concentration was varied (1.0%, 1.5%, 2.0%, and 3.0%). The steady-state flux J_{ss}, calculated from the linear portion of the curve, decreased exponentially as a function of carbomer concentration (%w/w) in the oleo-hydrogel, which might be due to a reduction in the amount of released drug molecules available for the permeation through the skin. The relationship between the steady-state flux and carbomer concentration is shown in Fig. 1a. The calculated regression line was $\log (J_{ss}) = -0.1256\ (\pm 9.682 \times 10^{-3}) \times (\text{carbomer } \%) + \log 39.273(\pm \log 1.042)$ ($r = .9941$, $p < .006$). A good correlation is suggested by a high coefficient of determination ($r > .99$).

Effect of Drug Concentration on Drug Permeation

To evaluate the effect of initial drug loading in the gel on ketoprofen permeation through the skin, the ketoprofen concentration was varied (1%, 2%, 3%, 4%, and 5%) in 1% carbomer gel. The diffusion coefficients of ketoprofen in the oleo-hydrogels containing different ketoprofen concentrations were calculated and are expressed in Fig. 1b. The diffusion constant of ketoprofen decreased exponentially as the drug concentration increased in the oleo-hydrogel. The regression line was $\log (D) = -0.0441\ (\pm 4.7565 \times 10^{-3}) \times (\text{ketoprofen } \%) + \log 23.909\ (\pm \log 1.037)$ ($r = 0.9830$, $p < .003$).

Effect of *N*-Methyl Pyrrolidone Concentraion on Drug Skin Permeation

In this study, NMP was added as an enhancer to increase the steady-state flux of ketoprofen selectively. The effect of NMP on the permeation of ketoprofen was studied using the oleo-hydrogel with 3% ketoprofen in 1% carbomer and varying the NMP concentration (0%, 5%, 10%, and 15%). The permeation rate and the lag time of ketoprofen at various NMP concentrations were deter-

Table 1

Percutaneous Penetration Parameters of Ketoprofen Through Full-Thickness Hairless Mouse Skin with Various Vehicles (Mean ± SE, n = 3)

	Cumulative Amount at 8 hr (Q_{8h}, μg/cm^2)	Flux (J_{ss}, μg/cm^2/hr)	Lag Time (t_L, hr)	Permeability Coefficient (K_p, cm/hr × 10^5)	Diffusion Coefficient (D, cm^2/hr × 10^4)	Partition Coefficient (K × 10^2)
pH						
3.5	210.30 ± 23.34	27.12 ± 3.02	0.47 ± 0.10	90.40 ± 10.07	17.38 ± 3.79	3.641 ± 1.20
4.0	220.13 ± 22.80	28.48 ± 2.95	0.52 ± 0.12	94.93 ± 9.83	15.71 ± 3.13	4.230 ± 1.31
4.6	227.20 ± 19.70	29.61 ± 2.69	0.46 ± 0.10	98.71 ± 8.97	18.50 ± 3.46	3.929 ± 1.07
5.0	215.42 ± 15.02	27.82 ± 1.94	0.48 ± 0.08	92.73 ± 6.46	17.01 ± 2.72	3.816 ± 0.80
6.0	220.91 ± 24.72	28.78 ± 3.22	0.57 ± 0.15	95.93 ± 10.73	14.33 ± 3.21	4.686 ± 1.57
Carbomer (%)						
1.0	227.20 ± 19.70	29.61 ± 2.69	0.46 ± 0.10	98.71 ± 8.97	18.50 ± 3.46	3.929 ± 1.07
1.5	191.13 ± 21.40	24.89 ± 2.79	0.33 ± 0.09	82.97 ± 9.29	24.75 ± 5.51	2.347 ± 0.79
2.0	169.21 ± 23.35	22.58 ± 3.11	0.59 ± 0.16	75.27 ± 10.39	13.84 ± 3.82	3.807 ± 1.46
3.0	142.58 ± 18.67	18.89 ± 2.48	0.66 ± 0.18	62.97 ± 8.25	12.37 ± 3.24	3,563 ± 1.40
Pluronic (%)						
20.0	110.92 ± 10.35	15.00 ± 1.40	0.72 ± 0.15	50.00 ± 4.67	11.34 ± 2.12	3.086 ± 0.86
Ketoprofen (%)						
1.0	80.44 ± 6.44	9.86 ± 0.79	0.38 ± 0.07	98.60 ± 7.90	21.49 ± 3.44	3.212 ± 0.77
2.0	162.11 ± 14.39	20.71 ± 1.84	0.43 ± 0.08	103.55 ± 9.20	18.99 ± 3.30	3.817 ± 1.02
3.0	227.20 ± 19.70	29.61 ± 2.69	0.46 ± 0.10	98.71 ± 8.97	18.50 ± 3.46	3.929 ± 1.07
4.0	276.52 ± 23.53	36.56 ± 3.11	0.51 ± 0.10	91.40 ± 7.78	16.01 ± 2.72	3.996 ± 1.02
5.0	307.30 ± 37.67	41.10 ± 5.04	0.58 ± 0.17	82.20 ± 10.08	14.08 ± 3.46	4.087 ± 1.48
NMP (%)						
0.0	72.01 ± 10.30	9.94 ± 1.42	0.74 ± 0.25	33.13 ± 4.74	11.04 ± 3.16	2.101 ± 0.80
5.0	163.14 ± 16.10	21.38 ± 2.11	0.63 ± 0.14	71.27 ± 7.03	12.96 ± 2.56	3.849 ± 1.14
10.0	227.20 ± 19.70	29.61 ± 2.69	0.46 ± 0.10	98.71 ± 8.97	18.50 ± 3.46	3.929 ± 1.07
15.0	215.92 ± 19.26	27.69 ± 2.47	0.32 ± 0.07	92.30 ± 8.23	25.52 ± 4.55	2.532 ± 0.68
Conventional products (3% Ketoprofen)						
Oleo-hydrogel	227.20 ± 19.70	29.61 ± 2.69	0.46 ± 0.10	98.71 ± 8.97	18.50 ± 3.46	3.929 ± 1.07
K-gel	19.61 ± 1.55	2.66 ± 0.21	0.63 ± 0.11	8.87 ± 0.70	12.96 ± 2.05	0.479 ± 0.11
K-plaster	60.00 ± 3.60	7.99 ± 0.48	0.48 ± 0.07	26.63 ± 1.60	17.01 ± 2.04	1.096 ± 0.20

mined (Fig. 1c). The presence of NMP in the gel increased the steady-state flux significantly, although the increase in the steady-state flux of ketoprofen was not linear with the increase in the amount of NMP. The maximum permeability was achieved with 10% NMP. Meanwhile, the decrease in the lag time was linear with the increase of NMP (Fig. 1c). The linear regression was lag time = -0.0286 ($\pm 1.5875 \times 10^{-3}$) × (NMP %) + 0.752 ($\pm 0.015$) ($r = .9969$, $p < .004$).

Relative Permeation of Ketoprofen from Pluronic F127 Gel and Carbomer Gel

The skin permeation of ketoprofen from carbomer gel was compared with that from a Pluronic gel. The steady-state flux of ketoprofen from carbomer gel was approximately two times higher than that from the Pluronic gel (Table 1). The increased skin permeation may suggest the higher emulsion droplet stability of the carbomer gel, as well as the better drug-releasing property of carbomer. Based on these results, the formulation of a ketoprofen oleo-hydrogel that showed a maximum percutaneous absorption was 3% ketoprofen, 1% carbomer, 10% NMP, 10% oils, 8% surfactants and water, adjusted to pH 4.6 by triethanolamine.

Relative Skin Permeation of Ketoprofen from Oleo-Hydrogel and Conventional Products

Table 1 represents the cumulative amount over time in the hairless mouse skin following application of oleo-hydrogel, K-gel or K-plaster. The cumulative amount and

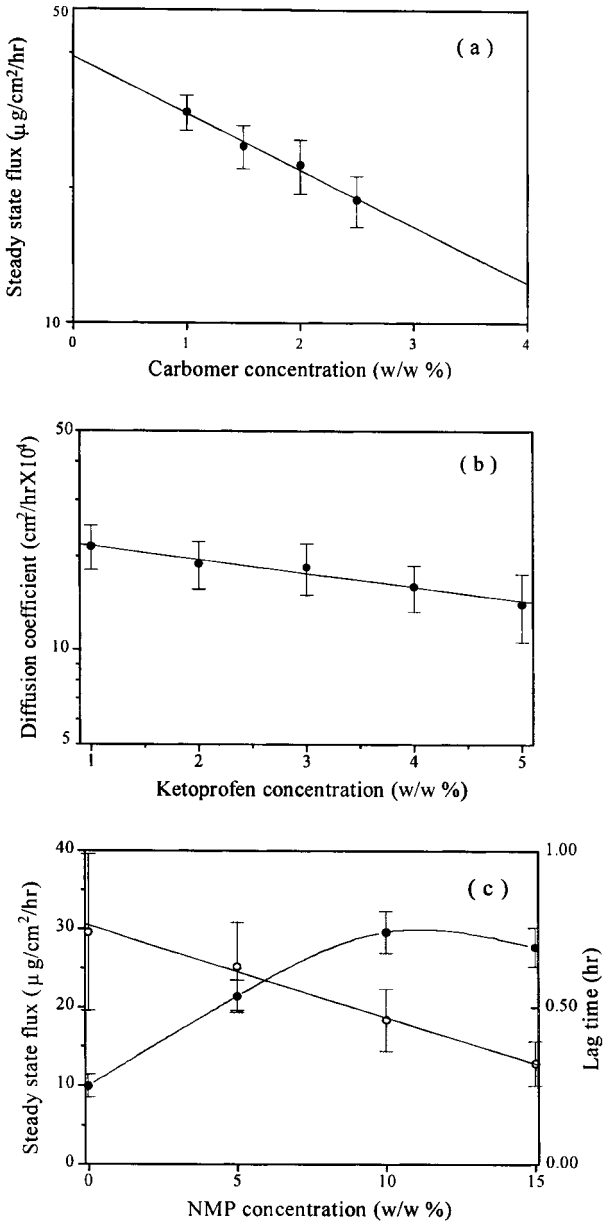

Figure 1. Effect of (a) carbomer, (b) initial concentration, and (c) NMP concentration on the skin permeation parameters of ketoprofen from the oleo-hydrogel through excised hairless mouse skin (mean ± SE, $n = 3$). For Fig. 1c, ● is steady-state flux, and ○ is lag time.

the steady-state flux of the oleo-hydrogel formulation were much higher than those of K-gel or K-plaster (Table 1). The cumulative amount (227.20 ± 19.70 μg/cm^2) and the steady-state flux (29.61 ± 2.69 μg/cm^2/hr) from the oleo-hydrogel formulation became about 10-fold ($p < .01$) higher than those from the K-gel (Q_{8hr} = 19.61 ± 1.55 μg/cm^2, J_{ss} = 2.66 ± 0.21 μg/cm^2/hr) and about 3.5-fold ($p < .01$) higher than those from the K-plaster (Q_{8hr} = 60.00 ± 3.60 μg/cm^2, J_{ss} = 7.99 ± 0.48 μg/cm^2/hr).

In Vivo Studies: Bioavailability

Ketoprofen was injected intramuscularly into hairless mice at a dose of 20 mg/kg. For oral administration, hairless mice received the same dose of ketoprofen suspended in physiological saline solution. In the topical application, the 3% ketoprofen gel was applied over the abdominal skin at a dose of 20 mg/kg. Blood samples were collected at the predetermined time intervals, and ketoprofen in plasma was quantified using an HPLC method. Figures 2 and 3 show the time-dependent absorption in plasma following intramuscular injection, oral and topical administrations of ketoprofen. Systemic absorption of ketoprofen was evaluated by measuring drug concentration in plasma, and the profiles of plasma level versus time were analyzed individually using the area/moment analysis.

The pharmacokinetic parameters determined from each route of administration are listed in Table 2. The

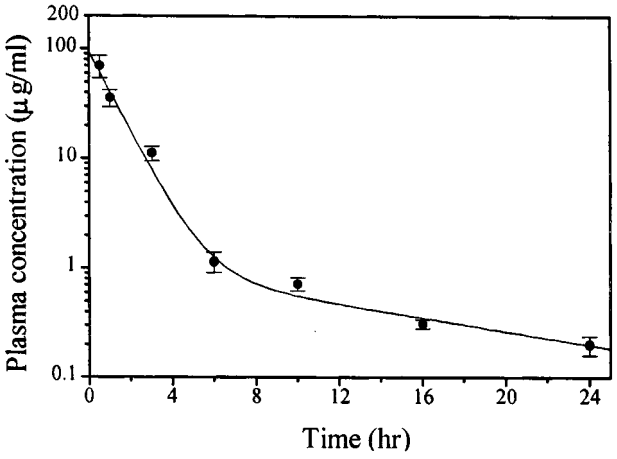

Figure 2. Plasma concentration-time profiles following intramuscular administration of ketoprofen in hairless mice (mean ± SD, $n = 3$). The regression equation is $Y = 93.10(\pm 8.37)e^{-0.8581(\pm 0.0357)t} + 1.11(\pm 0.13)e^{-0.0721(\pm 0.0115)t}$ ($r = 0.9722$).

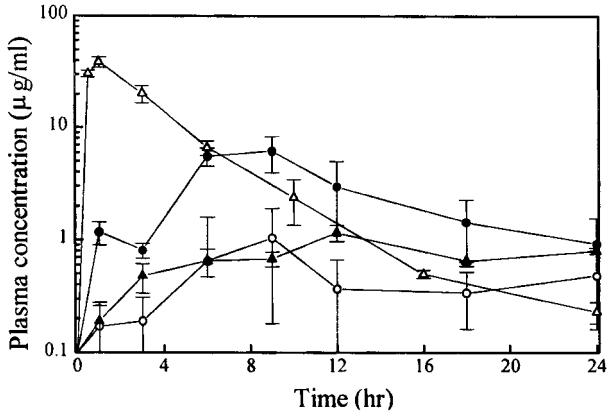

Figure 3. Plasma concentration-time profiles following oral and topical administration of ketoprofen in hairless mice (mean ± SD, $n = 3$); ●, oleo-hydrogel; ○, K-gel; ▲, K-plaster; △, by mouth.

C_{max} after the topical application was about 3–17% of that after oral dosing, suggesting that the topical gel has an advantage in reducing the systemic side effects due to the low blood concentration of ketoprofen after topical administration. The plasma concentration of the drug after topical application was considerably sustained, with a delayed T_{max}. Especially, the maximum concentration of ketoprofen in oleo-hydrogel was significantly higher than those of the conventional products ($p < .01$) (Table 2). Based on the AUCs of each administration route, the relative bioavailability of topical oleo-hydrogel application in reference to oral administration was estimated to be about 37%, while the reported bioavailability of other NSAID topical preparations ranged between 1% and 20%. The greater bioavailability of the ketoprofen oleo-hydrogel may be due to the increased skin permeability of ketoprofen, which is ascribed to the good drug release property of oleo-hydrogel and a penetration-enhancing effect of *N*-methyl-2-pyrrolidone.

The plasma concentration of ketoprofen after intramuscular administration declined in a biexponential manner (Fig. 2) with the following regression equation: $Y = 93.10\ (\pm 8.37)\ e^{-0.8581(\pm 0.0357)t} + 1.11(\pm 0.13)\ e^{-0.0721(\pm 0.0115)t}$ ($r = .9722$). Therefore, the pharmacokinetic parameters of ketoprofen were determined according to the two-compartment open model: α (the first-order rate constants at α phase) equal to 0.86 ± 0.05 hr^{-1}; β (first-order rate constants at β phase) equal to 0.07 ± 0.01 hr^{-1}; $t_{1/2}$ (elimination half-life at β phase) equal to 6.20 ± 4.32 hr; k_{12} (rate constant from the central compartment to tissue) equal to $8.99 \times 10^{-2} \pm 5.45 \times 10^{-3}$ hr^{-1}; k_{21} (rate constant from the tissue to central compartment) equal to $8.16 \times 10^{-2} \pm 1.65 \times 10^{-2}$ hr^{-1}; k_{10} (elimination rate constant from the central compartment) equal to $7.59 \times 10^{-1} \pm 3.88 \times 10^{-2}$ hr^{-1}; V_1 (distribution volume of central compartment) equal to 5.35 ± 0.60 ml; and V_2 (distribution volume of tissue compartment) equal to 56.90 ± 4.57 ml. The pharmacokinetics of percutaneous absorption has been widely discussed, and several effective models have been developed for the explanation of the absorption behavior of drugs through the skin (16–25). However, many of these mathematical models are rather complicated, and relatively many parameters are required to predict the plasma concentrations. In this study, we employed a simple model (8), illustrated in Fig. 4, based on the assumption of a constant penetration rate through the skin after the initial induction period (lag time). The plasma concentration of ketoprofen can be given as follows:

$$C = \frac{R_p}{V_1 k_{10}} \left\{ 1 + \frac{\beta - k_{10}}{\alpha - \beta} e^{-\alpha(t - t_L)} + \frac{k_{10} - \alpha}{\alpha - \beta} e^{-\beta(t - t_L)} \right\} \quad (5)$$

Table 2

Pharmacokinetic Parameters for Absorption of Ketoprofen After Various Administrations

Administration Route	AUC_{24hr} (μg · hr/mℓ)	C_{max} (μg/mℓ)	T_{max} (hr)	Relative BA %[a]
Intramuscular	116.80 ± 9.00	70.32 ± 16.48	0.50 ± 0.00	77
By mouth	152.10 ± 5.13	38.68 ± 4.01	1.00 ± 0.00	100
Topical				
Oleo-hydrogel	55.74 ± 11.17	6.82 ± 1.83	7.00 ± 1.73	37
K-gel	10.95 ± 4.05	1.39 ± 0.78	13.00 ± 9.64	7
K-plaster	17.00 ± 3.91	1.03 ± 0.26	11.00 ± 1.73	11

[a] Relative bioavailabiltiy (BA) in reference to oral administration.

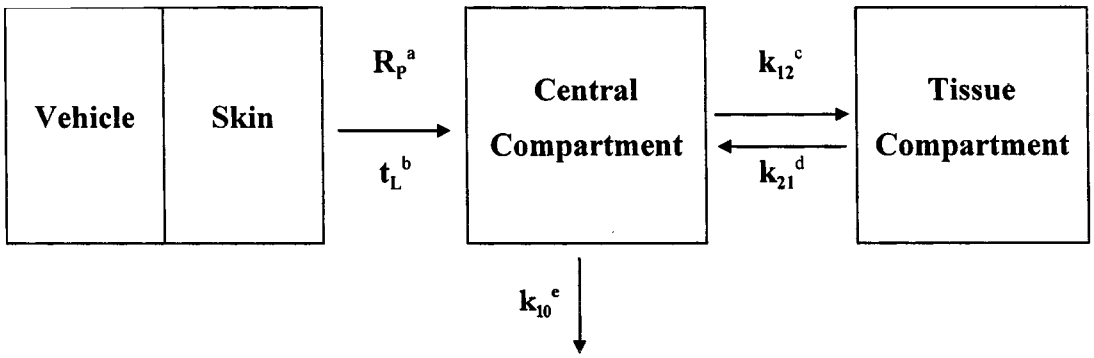

Figure 4. Schematic representation of percutaneous absorption and elimination of drugs; [a]R_p, penetration rate; [b]t_L, lag time; [c]k_{12}, rate constant from the central compartment to tissue; [d]k_{21}, rate constant from the tissue to central compartment; [e]k_{10}, elimination rate constant from the central compartment.

where C is the plasma concentration, R_p is the apparent penetration rate, t is a time, and t_L is the lag time. The details of other parameters, such as α, β, V_1, and k_{10}, are the same as those given in the discussion of Fig. 2.

Based on the simple model, the skin penetration rate and the lag time of ketoprofen from oleo-hydrogel and conventional products were calculated. The skin permeation rates R_p from the oleo-hydrogel, K-gel, and K-plaster are 13.27 ± 2.65, 2.31 ± 0.62, and 3.30 ± 0.76 μg/hr, respectively, and the lag times t_L are 0.56 ± 0.10, 0.88 ± 0.24, and 0.68 ± 0.17 hr, respectively.

Next, the drug concentration in the viable epidermis, dermis, and hypodermis tissues after administration was determined to see the actual amount of ketoprofen absorbed from the gel into the respective tissue. The 3% ketoprofen gel was applied on abdominal skin (20 mg/kg), and 0.5, 1.0, 3.0, 6.0, 9.0, 12.0, 18.0, and 24.0 hr later, the viable tissue and hypodermis under the applied area were taken for the quantification of ketoprofen concentration. Figures 5a and 5b show the change of ketoprofen concentration in the viable tissue and the hypodermis as a function of time after the topical application. The maximum concentration ($C_{max} = 4.73 \pm 0.76$ μg/cm^2) in the hypodermis following topical administration was much higher than that after intramuscular injection (1.60 ± 0.17 μg/cm^2) or that after oral administration (0.72 ± 0.06 μg/cm^2). The high local drug concentration in the hypodermis ($C_{max} = 4.73 \pm 0.76$ μg/cm^2) suggests good percutaneous absorption of ketoprofen from the oleo-hydrogel (C_{max} of K-gel and K-plaster were 0.92 μg/cm^2 and 1.27 μg/cm^2). Figure 5c shows the amount-time profile in the liver following intramuscular injection, oral and topical administrations of ketoprofen. The maximum concentration in the liver (1.19 ± 0.63 μg/g) after oral administration was significantly higher than that of each topical application (C_{max} of oleo-hydrogel, K-gel, and K-plaster were 0.20 ± 0.03, 0.09 ± 0.02, and 0.07 ± 0.09 μg/g, respectively; $p < .01$). As regards toxicity, a lower liver concentration of ketoprofen may be more beneficial to minimize its systemic side effect.

Correlations Between In Vitro Permeation and In Vivo Skin Permeability of Ketoprofen Topical Formulations

The relationship between in vitro permeation and in vivo skin permeability of ketoprofen topical formulations was investigated by comparing in vivo permeability parameters such as penetration rate R_p and in vitro permeation parameters such as steady-state flux J_{ss}. There was a very high correlation between the log of in vivo penetration rate R_p and the in vitro steady-state flux J_{ss}: $\log Y = 0.0281 \quad (\pm 1.807 \times 10^{-4})X + 0.2911(\pm 3.2112 \times 10^{-3})$, $r = .9999$, $p < .005$ (Fig. 6a). Separately, the relation between bioavailability and cumulative amount of ketoprofen was examined. The bioavailability AUC_{24hr} exhibited a good correlation with the in vitro cumulative amount at 8 hr Q_{8hr}: $Y = 0.2204\ (\pm 0.0132)X + 5.3552\ (\pm 1.7946)$, $r = .9982$, $p < .04$ (Fig. 6b).

Based on these results, it is suggested that, in ketoprofen topical formulations, the data of in vivo permeability parameters such as bioavailability AUC_{24hr} or skin penetration rate R_p may be predicted from the data of in vitro permeation parameters such as cumulative amount at 8 hr Q_{8hr} or steady-state flux J_{ss}. Thus, it seems convenient and advantageous to use in vitro permeation data instead of plasma data, which requires frequent venipuncture for sampling, in the development, evaluation, and quality control of ketoprofen transdermal formulations.

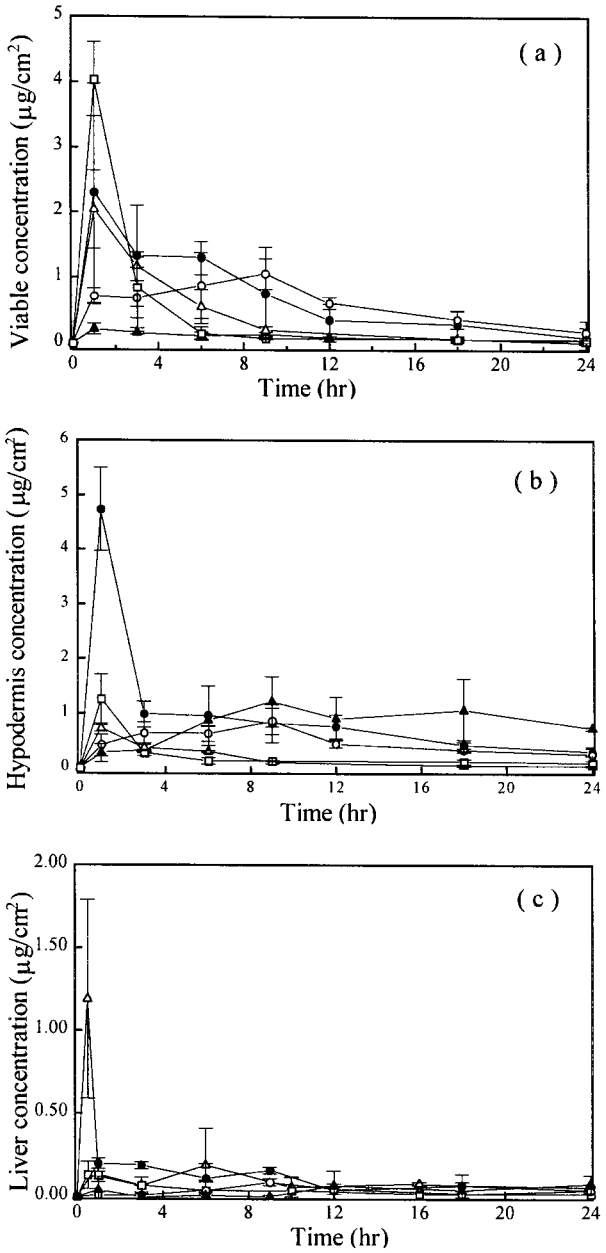

Figure 5. Drug concentration profile in (a) viable tissue, (b) hypodermis under the applied site, and (c) liver concentration as a function of time after oral, intramuscular injection, and topical applications of 3% ketoprofen oleo-hydrogel (mean ± SD, $n = 3$); ●, oleo-hydrogel; ○, K-gel; ▲, K-plaster; △, by mouth; □, intramuscular.

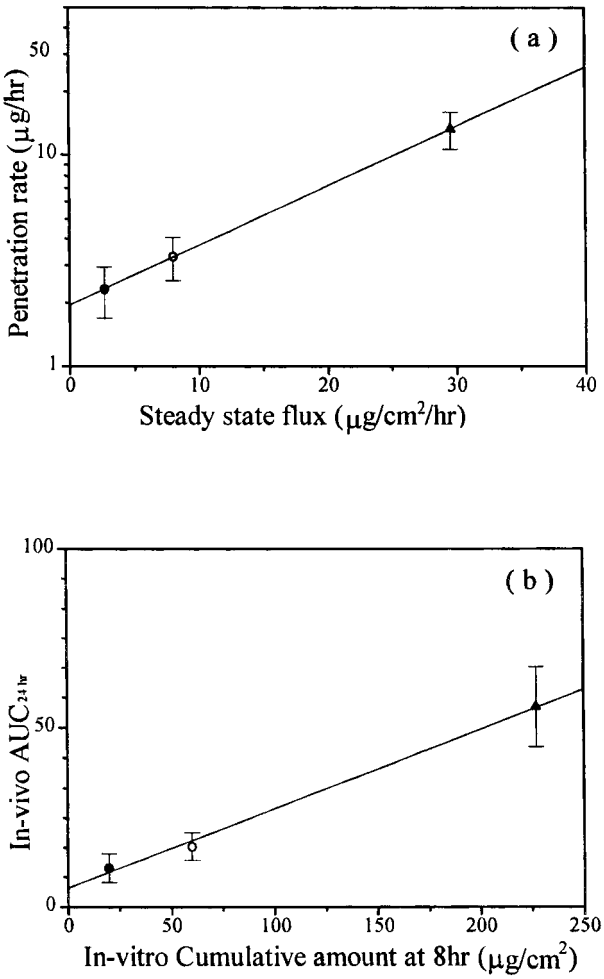

Figure 6. Correlation between in vivo permeability parameters (R_p, AUC_{24hr}) and in vitro permeation parameters (J_{ss}, Q_{8hr}) in hairless mice after topical administration (mean ± SD, $n = 3$); ●, K-gel; ○, K-plaster; ▲, oleo-hydrogel.

CONCLUSION

In an attempt to obtain an optimal composition of a tropical ketoprofen preparation, we evaluated ketoprofen formulations in a variety of oleo-hydrogel vehicles using a modified Franz diffusion cell, which is a standard in vitro technique for evalutaion of percutaneous drug delivery.

In the in vitro skin penetration test, the optimization of ketoprofen oleo-hydrogel formulation was assessed by the consideration of main factors such as oils, pH, enhancers, gelling agents, and drug concentrations. In pH dependence, the permeation of ketoprofen was not significantly different between pH 3.5 and pH 6.0. While

the steady-state flux J_{ss} decreased exponentially as a function of carbomer concentration in the gel, the diffusion coefficient decreased exponentially as the drug concentration increased in the gel. It is noteworthy that NMP as an enhancer selectively increased the steady-state flux and decreased the lag time. The optimal oleo-hydrogel topical formulation showed a Q_{8hr} value of 227.20 ± 19.70 μg/cm^2, J_{ss} value of 29.61 ± 2.69 μg/cm^2/hr, and lag time of 0.46 ± 0.10 hr, and the Q_{8hr} and J_{ss} values are about 10-fold ($p < .01$) higher than those for the K-gel ($Q_{8hr} = 19.61 \pm 1.55$ μg/cm^2, $J_{ss} = 2.66 \pm 0.21$ μg/cm^2/hr) and about 3.5-fold ($p < .01$) higher than those for the K-plaster ($Q_{8hr} = 60.00 \pm 3.60$ μg/cm^2, $J_{ss} = 7.99 \pm 0.48$ μg/cm^2/hr).

In the in vivo percutaneous absorption study, the C_{max} (6.82 ± 1.83 μg/ml) and AUC_{24hr} (55.74 ± 11.17 μg · hr/ml) of ketoprofen oleo-hydrogel in plasma were significantly higher than those of conventional products ($p < .01$). The relative bioavailability of oleo-hydrogel in reference to oral administration was about 37%, and the maximum concentration in the hypodermis after topical administration ($C_{max} = 4.73 \pm 0.76$ μg/cm^2, skin) was much higher than those of the conventional products (C_{max} of K-gel and K-plaster were 0.92 ± 0.19 μg/cm^2 and 1.27 ± 0.37 μg/cm^2, respectively). In addition, the maximum ketoprofen concentration in plasma (36.68 ± 4.01 μg/ml) and liver (1.19 ± 0.63 μg/g, liver) after oral administration was significantly higher than C_{max} in plasma (6.82 ± 1.83 μg/ml) and liver (0.20 ± 0.03 μg/g) after the oleo-hydrogel transdermal application. Thus, increased transdermal delivery of ketoprofen to the normal skin, as well as decreased delivery to the systemic circulation, could be achieved by incorporating ketoprofen in the oleo-hydrogel transdermal drug delivery system. Finally, there was good correlation between in vitro permeation data and in vivo data, suggesting that, in the ketoprofen formulation, in vivo permeability parameter data may be predicted from the in vitro permeation parameter data.

Further study is needed to evaluate the anti-inflammatory effect of ketoprofen in an oleo-hydrogel formulation by the carrageenan-induced edema method and clinical trial.

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