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Effect of Tween 20 on the Penetration of Ketoprofen through Excised Rat Skin

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트윈 20이 케토프로펜의 흰쥐 피부투과에 미치는 영향

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The effect of Tween 20 on the penetration of ketoprofen in aqueous vehicles through excised rat skin has been evaluated. The addition of Tween 20, up to 2%, in water containing ketoprofen decreased the penetration of ketoprofen through the skin compared with the reference vehicle without this surfactant. However, the addition of Tween 20 in ketoprofen aqueous vehicle increased diffusion parameter significantly which was compensated with the lowered partition parameter.

Keywords—ketoprofen, Tween 20, skin penetration

Ketoprofen, one of potent nonsteroidal anti-inflamatory drugs, has been widely used for the treatment of rheumatoid arthritis and its related conditions.[1,2] However, its oral administration has unwanted systemic side effects and gastrointestinal irritancy that may sometimes accompany ulceration.[1] To reduce such side effects of ketoprofen, transdermal dosage forms such as gels, creams and ointments were developed recently.[3-5] However, the high dose of ketoprofen makes it difficult to formulate the drug into such transdermal dosage forms. To overcome the preceding disadvantage in the transdermal formulation of ketoprofen, several attempts have been made to search for penetration enhancers for percutaneous absorption of ketoprofen.[6,7] Previously, fatty acids and urea were evaluated for the possibility to be used as the penetration enhancers for ketoprofen penetration through excised rat skins.[8] In the present study, Tween 20 which has been successfully applied in the transdermal delivery of some drugs as a penetration enhancer was evaluated to observe its role as a penetration enhancer for ketoprofen through excised rat skins.

Results and Discussion

Materials

The following reagents were used as received from the suppliers. Ketoprofen (Il Yang Pharm. Co., Seoul, Korea), Tween 20 (Sigma Chemical, St. Louis, MO, U.S.A.), ethanol, methanol, chloroform, isopropyl myristate (Junsei Chemical, Tokyo, Japan), sod. phosphate monobasic, sod. phosphate dibasic, phosphoric acid (Wako Pure Chemical, Osaka, Japan), trypsin, and acetonitrile for HPLC

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grade (Merck Co., Darmstadt, Germany). Water used for HPLC assay was distilled and deionized in house.

Ketoprofen Solutions Containing Tween 20

To study the effect of Tween 20 on the ketoprofen penetration in aqueous vehicle through rat skin, Tween 20 was added to water, followed by the addition of ketoprofen in the aqueous vehicle at the concentration of 0.7% (w/v). An aqueous vehicle which did not contain Tween 20 was used as a control. Ketoprofen penetration through the excised rate skin was determined as a function of Tween 20 concentration in water to observe the concentration effect on ketoprofen penetration. The concentration of Tween 20 in the vehicle was varied as 0, 0.5, 1 and 2% (w/v).

Skin Penetration of Ketoprofen

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

Franz-type diffusion cells were used for the 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 dermal side facing the receptor. Solutions of various compositions containing the drug were placed in the donor compartment. Twenty *ml*-volume of receptor compartment was filled with 0.05 M phosphate buffer (pH 7.4) and the temperature was kept constant at 37± 0.5℃ throughout the experiment. A half m*l* of receptor phase was withdrawn at the predetermined time intervals up to 30 hours 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 experiment 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 C_{18} column (Waters, μBondapak 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), an 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 the volume ratio of 45:55. The flow rate was 2.0 m*l*/min with an injection volume of 10 μ*l*. The standard solutions of ketoprofen were prepared with 0.05 M phosphate buffer (pH 7.4) every week.

Data Analysis

All the permeation parameters were calculated with a modified equation proposed by Okamoto[8] which described the total amount of ketoprofen, Qt, which penetrated through the skin in time, t, from the donor solution to the receptor phase as follows:

$$Qt = AK'C[D't - 1/6 - 2/\Pi^2 \sum_{1}^{\infty} (-1)^n / n^2 \exp(-D'n^2\Pi^2 t)] \quad (1)$$

where, D′; diffusion parameter (D/L), K′; partition parameter (K×L), A; area for application, L; thickness of skin, K; partition coefficient of ketoprofen between skin and donor solution and D; diffusion coefficient. D′ and K′ are not real diffusion coefficient and partition coefficient, but represent apparent diffusion parameter and partition parameter involving the skin thickness. Permeability constant (Kp) was calculated as follows:

$$Kp = D' \times K' \quad (2)$$

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 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.

Dialysis Experiment

The concentration of free ketoprofen in Tween 20 aqueous solution was measured with an equilibrium-type dialysis cell. The dialysis experiment was performed with a glass cell composed of two

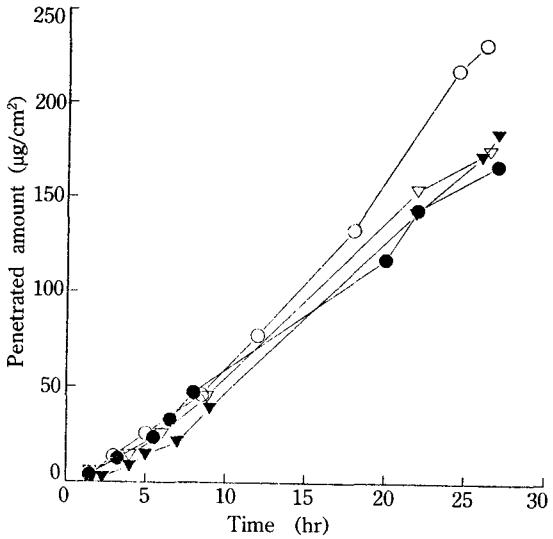

Figure 1—Effect of Tween 20 on ketoprofen penetration from aqueous solution containing 0.7% (w/v) ketoprofen through rat skin.
Key: (▼), 0%; (○), 0.5%; (●), 1.0%; (▽), 2.0%.

chambers each having a cavity of 4 m*l* capacity. The dialysis cellulose membrane (Spectrum Medical Industries, M.W. cutoff 20,000) was placed between the two chambers. One chamber contained ketoprofen and surfactant in water, while the other chamber contained water only. The solutions of the two chambers were equilibrated at 32℃ for 3 days. Then the concentration of ketoprofen in the chamber containing water only was measured spectrophotometrically at 255 nm.

Results and Discussion

It is well known that surfactants can affect the rate of skin penetration of various substances. Among them, nonionic surfactants, which have the least potential skin irritancy, are reported as potent enhancers for the penetration of several drugs through skins.[9-11] To observe the potential penetration enhancing effect of the nonionic surfactants on the ketoprofen penetration through skin, the ketoprofen penetration in water containing Tween 20 was measured using Franz-type diffusion cells with excised rat skin. Tween 20, which was reported to increase the flux of hydrocortisone and lidocaine across hairless mouse skin,[10,11] was employed as the representative for nonionic surfactant. The permeation profiles of ketoprofen from the vehicles containing Tween 20 were constructed as reported previously[8] and shown in Fig. 1. Diffusion parameter (D′), partition parameter (K′), and permeability constant (Kp) were calculated from these penetration profiles according to Equations (1), (2) and listed in Table 1. The presence of Tween 20 in ketoprofen solution at the concentration level of 0.5-2.0% increased the D′ of the drug significantly through the excised rat skin. The diffusion parameters of ke-

Table I—*Effect of Tween 20 on the Parameters for Ketoprofen Penetration from Aqueous Vehicle through Rat Skin*[a].

Tween 20 conc. (%w/v)	D′ ×10 (hr^{-1})	K′ ×10 (cm)	Kp ×10^2 (cm/hr)
0	0.44± 0.03	7.52± 0.54	3.29± 0.05
0.5	1.29± 0.12[b]	2.48± 0.25[b]	3.20± 0.06
1.0	1.75± 0.47[b]	1.56± 0.36[b]	2.61± 0.03[b]
2.0	2.06± 0.80[b]	1.30± 0.38[b]	2.51± 0.32[b]

[a] Each value represents the mean± S.D. of 3 determinations and each vehicle was saturated with ketoprofen. D′; diffusion parameter, K′; partition parameter, Kp; permeabiltiy constant.
[b] Significantly different at an alpha level of 0.05 from that of reference vehicle.

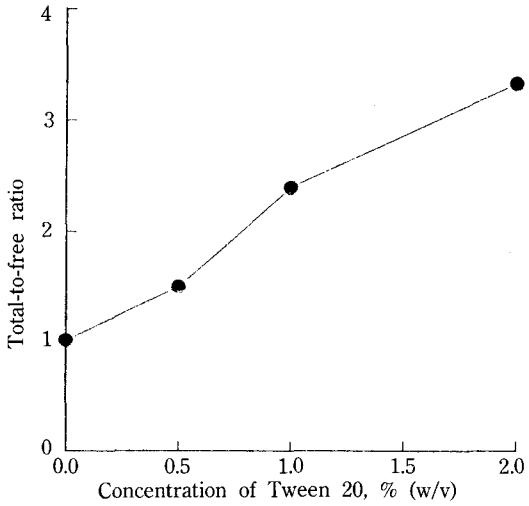

Figure 2—Total-to-free ratio of ketoprofen in aqueous Tween 20 solution containing 0.7% (w/v) ketoprofen. Each value represents the mean of five experiments.

toprofen penetration with Tween 20 were 3 to 5 times higher than the control vehicle. Those were proportional to the concentration of Tween 20. However, the increased D′ with the addition of Tween 20 in the vehicle compensated the lowered K′, which resulted in the decreased Kp for ketoprofen penetration through rat skin. The permeability of ketoprofen was decreased proportionally to the concentration of Tween 20 in water even though the trend was not pronounced. One of the drug molecules in the micelles which leads to a significant decrease in the thermodynamic activity of the drug in the vehicle. The addition of Tween 20 in ketoprofen solution actually decreased the concentration of free drug as shown in Fig. 2. The change of Tween 20 concentration in ketoprofen solution from 0 to 2% increased the total-to-free ketoprofen ratio from 1 to 3.3. It was also reported that percutaneous absorption of benzocaine from micellar solution was proportional to the concentration of the free drug.[13] However, it is interesting that D′ of the ketoprofen penetration was increased proportionally to the concentration of Tween 20. Increased D′ with the higher Tween 20 concentration in the ketoprofen solution may be explained in some cases by the impairment of the barrier function of the skin with the addition of the surfactant.

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