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Research paper

Skin permeation of ketotifen applied from stick-type formulation

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Abstract

A stick-typed long lasting device for both transdermal and topical drug delivery has been developed. Ketotifen fumarate (KT) was used as a model drug. The effect of a variety of permeation enhancers was investigated using hairless mouse skin in vitro. Polyoxyethylene oleyl ether (POE), among the enhancers used, most enhanced the skin permeation of KT. The permeation enhancement was mainly due to the increase in the drug solubility in the stratum corneum and the resulting increase in the partition coefficient. The rate of skin permeation of KT was approximately proportional to the loading dose of the drug.

Keywords: Ketotifen fumarate; Stick; Transdermal delivery system; Enhancer

1. Introduction

Ketotifen fumarate (KT) has been widely used to prevent allergic conjunctivitis, asthma and rhinitis by directly blocking the release of allergic intermediator from the mast cell [1]. KT is marketed in various formulations; capsules, dry syrup, syrup, eye drops and nasal solution. In addition, transdermal delivery systems for KT have also been studied by many researchers on the skin permeation mechanism, kinetics and the effects of chemical enhancers [2–7]. The transdermal delivery, however, may suffer from skin irritation [8] possibly caused by adhesive patches. In this study, we have developed a stick-type transdermal delivery system of KT which can be applied easily on the skin.

2. Materials and methods

2.1. Materials

Female Hr/Kud strain hairless mice (Kyudo Co., Ltd.,) were used. Ketotifen fumarate (KT) was purchased from Sigma Chemical Co. Materials used for the base of stick were yellow beeswax and isopropyl myristate (IPM) (Wako Pure Chemical Industries, Ltd.). Enhancers used are lauric acid, oleic acid, L-menthol, limonene, sodium lauryl sulfate (SDS), polyoxyethylene lauryl ether (PLE) (Wako Pure Chemical Industries, Ltd.) and polyoxyethylene oleyl ether (POE), glycerol mono-oleate (GO) (NOF Corp.). Other reagents used in the experiment were of special grade.

2.2. Preparation of ketotifen-stick

KT was weighed in a beaker and IPM and an enhancer were added and mixed with a stirrer. After dispersing KT, yellow beeswax was added and mixed at 70–80 °C. After the yellow beeswax was completely dissolved, the mixture was quickly poured into the stick-type container (rip tube, 5 mL H68.0 mm × D16.0 mm, PINOA) and set at room temperature. The device was then used as the ketotifen-stick. The weight fraction of KT, IPM, the enhancer and the yellow beeswax in the device were 4%, 45%, 5% and 46%, respectively.

2.3. In vitro skin permeation experiments

The abdominal skin of hairless mice excised was used for the in vitro skin permeation experiment. At first, the

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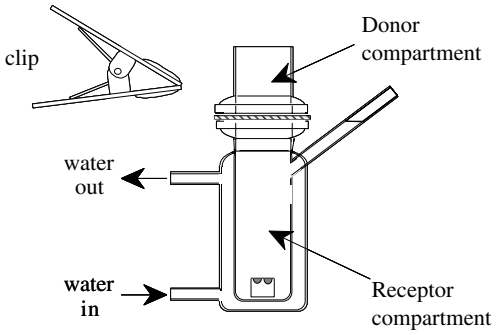

Fig. 1. Apparatus for in vitro skin permeation experiment.

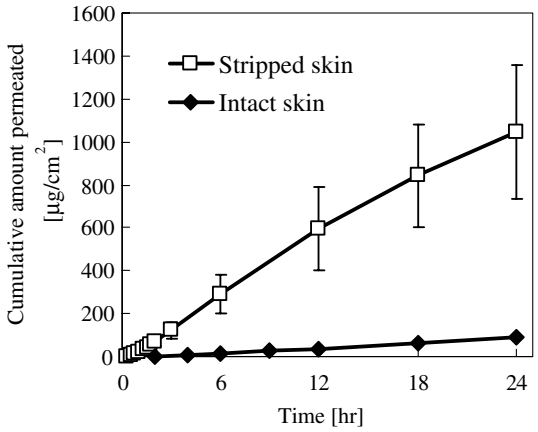

Fig. 2. Comparison of the permeability of KT through the stripped and intact skin (mean ± SD, $n = 3$). In this experiment, the side-by-side diffusion cell was used. Donor side was filled with 5 mL of KT saturated in phosphate buffer (pH 7.4) and receptor side was filled with phosphate buffer (pH 7.4). Other experiment condition was the same way as above (Section 2.3).

receptor compartment of the modified Franz diffusion cells (Fig. 1) was filled with phosphate buffer (pH 7.4). The skin was then mounted on the cell and ketotifen-stick was applied to the skin. The temperature was controlled at 37 °C. Two hundred micro litres of the receptor solution was sampled at predetermined time points. Thereafter, the same amount of fresh phosphate buffer was added to the receptor cell.

2.4. HPLC assay of KT

The concentration of KT in the in vitro experiment was determined under the following HPLC (Shimadzu Corp.) conditions. The assay system comprised a liquid chromatograph (LC-10AS), column oven (CTO-10A), UV-VIS detector (SPD-10A), system controller (SCL-10A) and autoinjector (SIL-10A). The column was Capcell pak C18 MGS 5 μm 4.5 × 250 mm (Shiseido) and its temperature was 40 °C. The measuring wavelength was 300 nm. The mobile phase is a mixture of 0.1 M tris(hydroxymethyl)aminomethane buffer (pH 9):acetonitrile = 30:70.

2.5. Skin permeation parameters

The cumulative amount of KT permeated was plotted against the time. The lag time (t_d) was determined as the intercept between the linear portion of each curve and the time axis. The steady-state rate of penetration (dQ/dt) was then calculated from the slope. The penetration profiles for the stripped and intact skin are plotted in Fig. 2. The penetration rate across the intact skin is much lower than that across the stratum corneum-removed skin. This finding indicates that the major resistance to KT penetration resides in the stratum corneum. Therefore we can neglect the effect of the viable skin for determining the skin parameters. The diffusion coefficient (D_{SC}) and the concentration on the surface of the stratum corneum (C_S) were then calculated using Eqs. (1) and (2), respectively,

$$D_{SC} = L^2/(6t_d) \quad (1)$$

$$C_S = (dQ/dt)L/D_{SC} \quad (2)$$

where L is the thickness of the stratum corneum (0.0010 cm).

3. Results and discussion

3.1. Optimum penetration enhancer

Lauric acid, oleic acid, L-menthol, limonene, sodium dodecyl sulfate (SDS), polyoxyethylene lauryl ether (PLE), polyoxyethylene oleyl ether (POE) and glycerol mono-oleate (GO) were used as the enhancer to improve the skin permeability of KT. The formulation without enhancer (51% yellow beeswax, 45% IPM, 4% KT) was used as the control. In this experiment, IPM was used as the base although it was widely used as enhancer in skin permeation experiments. The skin permeation parameters (dQ/dt, t_d, D_{SC}, C_S) were evaluated and listed in Table 1. POE showed the highest dQ/dt; it was about 5 times higher than that compared with the control. POE is the nonionic surfactant. GO, SDS and PLE which have high enhancement effect following POE are also the surfactants. This finding, therefore, suggests that these surfactants are especially effective for improving the skin partitioning for KT. The diffusion coefficients were almost independent of the penetration enhancers. The surface concentration C_S, on the other hand, increased with the penetration enhancers investigated.

3.2. Effect of POE concentration

The effects of POE concentration (1–8%) were demonstrated in Fig. 3 and the skin permeation parameters determined are listed in Table 2. dQ/dt increases with increasing in the POE concentration. The t_d, on the other hand, was nearly constant with the POE concentration under 3%. Beyond 5%, however, the time lag slightly increased. This phenomenon was also observed previously [10]. If the skin's barrier capacity decreases with time due to the concentration distribution of the enhancer in the stratum

Table 1
Effect of enhancers on skin permeation parameters of KT

	Enhancer	dQ/dt (μg/cm^2/h)	t_d (h)	D_{SC} (cm^2/s × 10^{11})	C_S (μg/mL × 10^{-5})
Control	–	0.53	4.34	1.07	0.14
1	Lauric acid	0.53	4.05	1.14	0.13
2	Oleic acid	0.67	4.42	1.05	0.18
3	L-Menthol	0.62	4.04	1.15	0.15
4	Limonene	0.53	4.70	0.99	0.15
5	SDS	1.72	5.72	0.81	0.59
6	PLE	0.84	2.42	1.91	0.12
7	POE	2.91	7.04	0.66	1.23
8	GO	2.34	5.91	0.78	0.83

5% enhancers were dissolved in each of stick containing 4% KT. Skin permeation data were analyzed using Eqs. (1) and (2). Stratum corneum thickness = 0.001 cm [9].

Table 2
Effect of POE on skin permeation parameters of KT

POE concentration (% (w/w))	dQ/dt (μg/cm^2/h)	t_d (h)	D (cm^2/s × 10^{11})	C_S (μg/mL × 10^{-5})
0	0.53 ± 0.11	4.34 ± 1.02	1.12 ± 0.29	0.14 ± 0.03
1	0.99 ± 0.14	5.11 ± 0.77	0.92 ± 0.15	0.31 ± 0.08
2	1.28 ± 0.10	4.86 ± 0.99	0.98 ± 0.21	0.37 ± 0.07
3	1.17 ± 0.32	4.94 ± 1.82	1.02 ± 0.33	0.32 ± 0.02
5	2.91 ± 1.25	7.04 ± 0.93	0.67 ± 0.09	1.23 ± 0.60
8	4.40 ± 0.69	6.87 ± 0.38	0.68 ± 0.04	1.80 ± 0.22

Each value represents the mean ± SD ($n = 3$).

corneum, the time lag may become longer than the intrinsic value because it takes appreciable hours to develop a steady-state condition of the enhancer in the skin. To avoid this overestimation of t_d and to determine the intrinsic time lag, the skin should be pretreated by the same enhancer. In this experiment, skin was pretreated by formulation with POE for 8 h. After that, ketotifen-stick was applied again. As a result, the value of t_d was similar regardless of whether pretreated skin or not (the data not shown). The diffusion coefficient and the skin surface concentration C_S were plotted as a function of the POE concentration in Fig. 4a and b, respectively. The diffusion coefficient is almost independent of the POE concentration. On the contrary, the surface concentration increased markedly with the POE

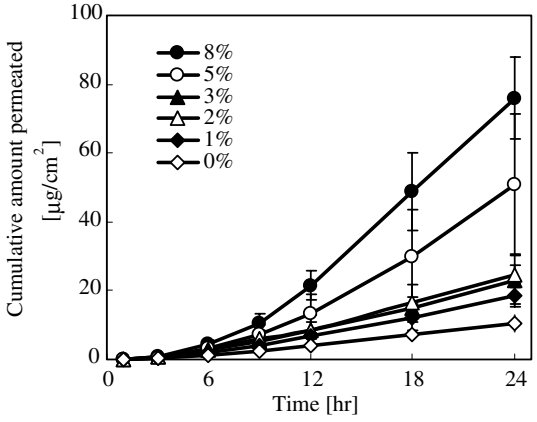

Fig. 3. Effect of POE concentration on cumulative amount of KT permeated across hairless mouse skin (mean ± SD, $n = 3$).

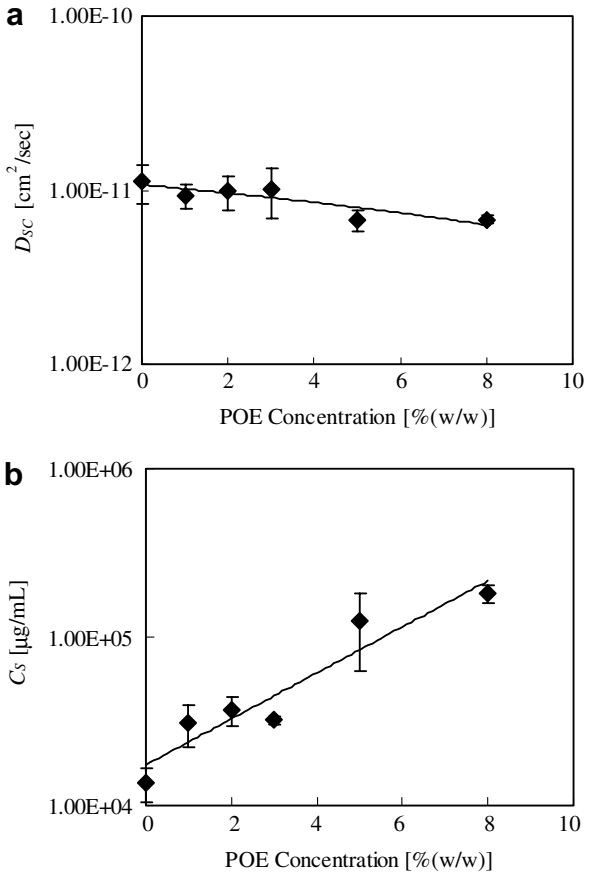

Fig. 4. Relationship between the diffusion coefficient in the stratum corneum and POE concentration (a), the concentration on the surface of the stratum corneum and POE concentration (b).

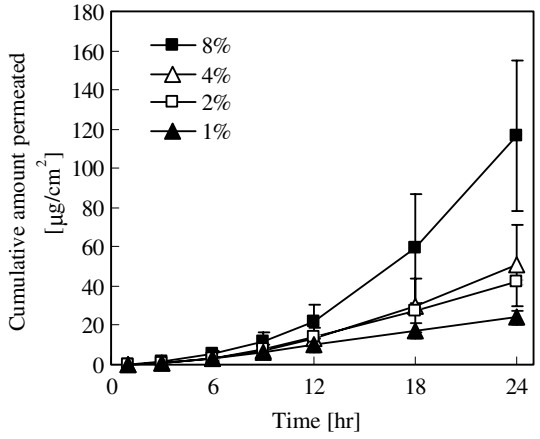

Fig. 5. Effect of KT concentration on cumulative amount of KT permeated across hairless mouse skin (mean ± SD, $n = 3$).

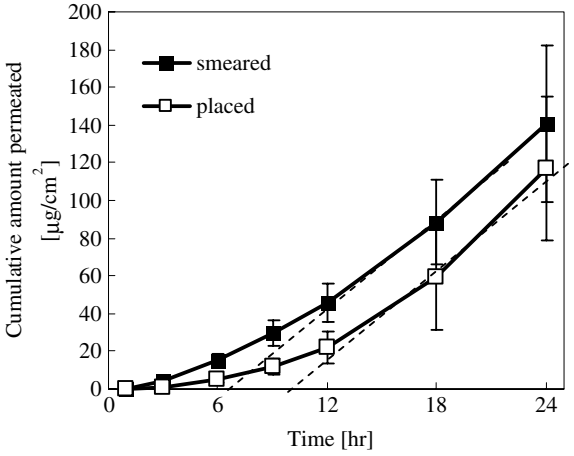

Fig. 7. Effect of the stick application method on cumulative amount of KT permeated across hairless mouse skin (mean ± SD, $n = 3$).

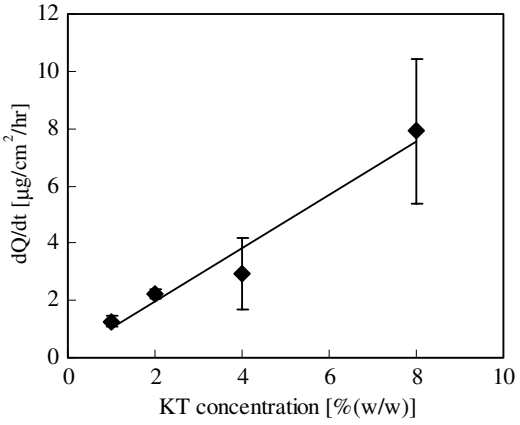

Fig. 6. Relationship between the steady-state permeation rate of KT and KT concentration.

concentration. This finding clearly indicates that the permeation enhancement is mainly due to the increase in the drug partitioning onto the surface of the stratum corneum.

3.3. Effect of KT concentration

The effects of the drug dose in ketotifen-stick on the skin penetration were shown in Fig. 5. The concentration of POE was maintained constant (5%). The steady state rates of penetration dQ/dt determined are shown in Fig. 6 as a function of KT loading dose. dQ/dt was found to be approximately proportional to the drug loading dose. This finding may indicate that the drug concentration in the device has not reached its saturation point.

3.4. Effect of the stick-applied condition

In this in vitro experiment, the ketotifen-stick (about 5 mm thick) was normally placed on the surface of the skin with the clip (placed). However ketotifen-stick can also be applied by smearing on the skin. We conducted the experiments with the stick applied on the surface of the skin

Table 3
Effect of the stick application method on skin permeation parameters of KT

Application method	dQ/dt (μg/cm²/h)	t_d (h)
Placed	7.90 ± 2.51	9.75 ± 0.53
Smeared	7.93 ± 2.60	6.29 ± 0.71

Each value represents the mean ± SD ($n = 3$).

several times by smearing (smeared). The stick composition was 42% yellow beeswax, 45% IPM, 5% POE and 8% KT. The ketotifen-stick was smeared ten times to the excised skin with a force of 2–3 N. After that, the skin was mounted on the receptor cell quickly. In this condition, the stratum corneum surface was fully coated with the drug. The penetration profiles are shown in Fig. 7 and dQ/dt and t_d determined are listed in Table 3. dQ/dt in smearing(7.93 ± 2.60 μg/cm²/h) was nearly equal to that in placing(7.90 ± 2.51 μg/cm²/h). On the other hand, the lag time was slightly reduced by smearing the stick on the skin. t_d in smearing (6.29 ± 0.71 h) was equivalent to that of other drugs with similar molecular weight ($314.5 \leqslant MW \leqslant 362.5$) [9]. In the experiment of the reference, the donor was applied in the state of the solution. Therefore, the distribution equilibrium between the donor solution and the surface of the stratum corneum must be established instantly. Similarly, since the stick device had good contact with the surface of the skin by smearing, the distribution equilibrium may be established quickly. In placing, on the other hand, it may take more time to reach the distribution equilibrium. For these reasons, t_d in smearing may be about 3.5 h shorter than that in placing.

4. Conclusion

A novel transdermal delivery system by smearing the stick type device has been developed. The once-a-day penetration profile was observed by simple several-seconds smearing operation. Among the enhancers investigated,

polyoxyethylene oleyl ether (POE) provided the highest enhancement effect for the skin penetration of KT. The permeation enhancement was mainly due to the increase in the drug solubility on the surface of the stratum corneum. Among application method investigated, placed or smeared, the steady state permeation rate was almost independent of the application method. On the other hand, the lag time was slightly reduced by smearing the stick on the skin. The stick type formulation may be useful because of easy application and improved compliance.

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