Drug Development and Industrial Pharmacy, 26(1), 45–53 (2000)

RESEARCH PAPER

The Skin Permeation Mechanism of Ketotifen: Evaluation of Permeation Pathways and Barrier Components in the Stratum Corneum

Kazuhiro Inoue,[1,*] Kengo Ogawa,[1] Yukie Suzuki,[1] Junichi Okada,[1] Akira Kusai,[1] Masaru Ikeda,[1] and Kenji Nishimura[2]

[1]*DDS Research, Product Development Laboratories, Sankyo Company, Limited, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan**
[2]*Drug Metabolic and Pharmacokinetic Research Laboratories, Sankyo Company, Limited, 1-2-58, Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan*

ABSTRACT

To evaluate the pathways and barrier components in the stratum corneum (SC) for the permeation of ketotifen, the effect of delipidization on the permeation and partition was examined under several donor pHs. Assuming that ionized ketotifen (KTH+) and un-ionized ketotifen (KT) contribute independently in both permeation and partition, the intrinsic permeability coefficients and SC/water partition coefficients of both species were estimated. Delipidization enlarged the permeability of KTH+ 100 times. This suggested that the lipid phase functions as the barrier against KTH+. KT has an intrinsic permeability 100 times larger than that of KTH+. Delipidization did not result in a significant change in permeability of KT. This suggested that the permeability of KT through the lipid phase is comparable to that through the aqueous phase in delipidized SC; that is, the lipid phase functions as a highly permeable pathway for KT. On the other hand, the permeability coefficient of KT through delipidized SC was 1/34 of that through the pure aqueous layer, which had a thickness equivalent to SC. Since this suggests that the permeability of KT through the proteinaceous phase is much lower than that through the aqueous

*To whom correspondence should be addressed. Telephone: +81-3-3492-3131, ext. 4650. Fax: +81-3-5436-8568.

Copyright © 2000 by Marcel Dekker, Inc. www.dekker.com

phase, the proteinaceous phase can be assumed to function as a barrier against the permeation of KT. From these results, it is concluded that the predominant permeation pathway for KT is through the lipid phase. The SC/water partition coefficient of KT was cut in half by delipidization, but the value was still more than 100. These results show that the proteinaceous phase functions not only as the barrier, but also as the depot for KT. The knowledge obtained here will be useful for formulation design and for the selection of enhancers in a transdermal therapeutic system of ketotifen.

Key Words: *Delipidization; Ketotifen; Lipid; Partition coefficient; Permeability coefficient; Protein; Stratum corneum; Uptake.*

INTRODUCTION

Skin is a complex of several components with physicochemical characteristics that are different from each other. The permeation process of a drug through the skin involves two aspects: how the drug distributes in the components of the skin and through which components the drug permeates.

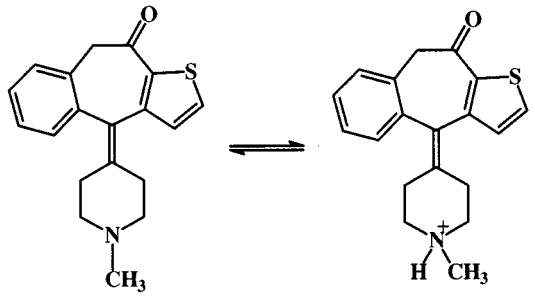
Skin consists of three layers: epidermis, corium, and subcutaneous. The epidermis is further composed of two layers: stratum corneum (SC) and viable epidermis (1). For transdermal drug delivery, the drug applied to the skin must permeate the uppermost layer (i.e., SC), and this permeation process is considered to be the major rate-determining step. The SC mainly consists of stratified flat corneocytes, which have a keratin-rich interior (2,3). It is proposed that the intercellular space between corneocytes is filled by lamella of lipid bilayers (4,5), and that the intracellular matrix is a mosaic of keratin filaments and interstitial lipid (6).

Due to the heterogeneous structure in the SC, several pathways can be considered for drug permeation, and it is difficult to specify the extent to which drug utilizes each pathway. In earlier studies, the internal structure of the SC was represented by several different types of models (7–10). They are all based on a common assumption that the SC consists of two phases (lipid and proteinaceous), and the drug permeability is explained in terms of the diffusion and uptake in these phases. More recently, the existence of the lipid pathway and the proteinaceous pathway was confirmed by several techniques using electron microscopy and laser scanning confocal microscopy (11).

The lipid phase can be removed by a simple solvent extraction technique (12), and the delipidized SC still maintains the original membranous form at a wide pH range. When delipidized SC is in contact with aqueous solution, water penetrates in the void where lipid phase existed before delipidization. Under this condition, the delipidized SC consists of the proteinaceous phase and aqueous pores that penetrate across the SC. Therefore, detailed information is expected to be obtained about the mechanism of drug permeation by comparing the permeability and uptake of the drug in intact SC with those values in delipidized SC.

Based on that idea, many permeation and partition experiments have been conducted with delipidized SC. For example, Kuo et al. (13) evaluated the effect of pH and delipidization on the transdermal permeation of oxycodone in the hairless mouse, hairless rat, and human cadaver skin in a pH range from 4 to 10. Harada et al. (14) conducted similar experiments with human SC and shed snake skin using salicylic acid as a permeant. As for partition, Raykar, Fung, and Anderson (15) established a method to evaluate the relative contribution of the lipid phase and the proteinaceous phase to drug partition with intact and delipidized human cadaver SC. In the previous reports, the role of the lipid phase on the permeability was well evaluated, but that of the proteinaceous phase was rarely discussed. One of the reasons may be that a model analysis was necessary to evaluate the contribution of the lipid phase and proteinaceous phase on drug permeability separately, and the model analysis is sensitive to the model-dependent parameters (7–10).

Here, the effect of pH and delipidization on the in vitro transdermal permeation and partition of a weak basic antiasthmatic, ketotifen (KT) (pK_a = 8.5; Fig. 1), was studied first with hairless mouse SC, as in the previous studies. As an additional study, the relative contributions of the two phases to the permeation of ketotifen were estimated by comparing the permeability through delipidized SC with that through homogeneous aqueous layers with thicknesses the same as that of SC. To obtain the latter value, the diffusion coefficient of ketotifen in homogeneous aqueous solution was measured. As for the partition, the uptake of ketotifen in the lipid and protein-



KT KTH+

Figure 1. Chemical structure of KTH+ and KT.

aceous phase could be evaluated separately according to the method of Raykar et al. (15).

Based on the results, we tried to clarify the mechanism of skin permeation of ketotifen. Furthermore, which kind of formulation and which type of enhancers are optimal for obtaining maximal skin permeability is discussed here.

EXPERIMENTAL

Materials

Ketotifen (fumarate salt) was purchased from Sigma Chemical Company (St. Louis, MO). Chloroform and methanol were obtained from Wako Pure Chemical Industries, Limited (Osaka, Japan). Sodium azide was purchased from Kanto Chemical Company, Incorporated (Tokyo, Japan). Silicon medical fluid (MF360) was obtained from Dow Corning Asia K. K. (Kanagawa, Japan). All the chemicals were reagent grade and were used as received.

Drug Assay

Ketotifen was assayed using reverse-phase high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection at 312 nm. A YMC-Pack ODS-A A-312 (YMC Co., Ltd., Kyoto, Japan) column (15 mm id) was used. Acetonitrile : water : triethylamine : acetic acid in the ratio 500 : 500 : 2 : 1 was used as the mobile phase. The flow rate was 1.5 ml/min.

Skin Preparation

Intact Full-Thickness Skin

Hairless mouse skin was used for both permeation and partition experiments. Male hairless mice, 6 weeks of age, were sacrificed by cutting the cervical vertebrae. Rectangular sections of the dorsal skin were excised from the animals, and adhering fat was removed. Each section was washed with saline, wrapped with plastic film, and stored for up to a maximum of 1 month at −60°C prior to use. Before the experiments, the sections were defrosted at room temperature.

It was confirmed in a preliminary study (data not shown) that the barrier function of the excised hairless mouse skin against the permeation of ketotifen is not altered by either freezing the skin or storing it at −60°C for up to 6 months.

Tape-Stripped Skin

Tape-stripped skin samples were prepared by stripping SC from intact full-thickness skin 20 times with adhesive tape. Tape stripping usually was performed from 10 to 15 times in previous experiments to evaluate SC-free skin (16,17), but it has been confirmed that the permeability of the skin tissues underlying the SC remains unaltered by tape stripping provided that it is not performed more than 25 times (18).

Delipidized Skin

Delipidized skin was prepared from full-thickness skin using a chloroform and methanol mixture (2/1, v/v) as an extracting solvent according to Kuo et al. (13). A piece of hairless mouse skin was mounted on a diffusion cell with the SC side facing the donor compartment. The extracting solvent filled the donor compartment to extract lipid, while phosphate-buffered saline (33 mM phosphate buffer with 0.74% sodium chloride, pH 7.2) filled the receptor compartment to maintain moisture in the skin. After extraction for 3 hr, the extracting solvent and phosphate-buffered saline were withdrawn and discarded. Traces of the extracting solvent on the surface of skin were evaporated by a stream of nitrogen gas. It was confirmed in a preliminary study (data not shown) comparing permeation of ketotifen through tape-stripped skin with and without preceding delipidization that delipidization treatment did not alter the barrier function of viable skin tissues underlying the SC.

Werts and Downing (19) reported that more than 80–90% of the lipids can be removed by extraction with this solvent, leaving lipid only 2% of the total weight of the SC. In addition, Raykar et al. (15) reported that the delipidized SC/water partition coefficient of drugs is not affected by the presence of the remaining lipid when log $P_{\text{octanol/water}}$ of the drug is less than 5. Since log $P_{\text{octanol/water}}$ of ketotifen free base is 3.7, the effect of the remaining

lipid on the delipidized SC/water partition coefficient of ketotifen can be neglected.

Raykar et al. (15) and Knutson et al. (20) demonstrated that the treatment of SC with a chloroform/methanol mixture does not denature the proteinaceous phase of SC or alter the partitioning of drugs in that phase.

Stratum Corneum Samples

Isolated SC was used in the partition experiments. A full-thickness skin sample was heated in phosphate-buffered saline (33 mM phosphate buffer with 0.74% sodium chloride, pH 7.2) at 60°C for 1 min, and SC was gently peeled off with a pincette. The phosphate-buffered saline used throughout the experiments discussed in this paper was of the same formulation as that described here. Viable tissue on SC was removed by digesting it with 0.5% trypsin in phosphate-buffered saline (33 mM phosphate buffer with 0.74% sodium chloride, pH 7.2) (12). The isolated SC was spread on poly(ethylene terephthalate) film as a support and dried in vacuo overnight (intact SC). For preparation of delipidized SC, the isolated SC was immersed in the extracting solvent (chloroform/methanol 2/1, v/v) overnight, spread again on poly(ethylene terephthalate) film, and dried in vacuo. After these treatments, the SC was kept free from fragmentation.

Comparison of the Permeability Through Full-Thickness and Tape-Stripped Skin

Intact full-thickness skin or tape-stripped skin was put on a Franz-type diffusion cell (static type donor chamber; 1-ml receptor chamber; 10 ml diffusion area; 1.77 cm^2). The receptor chamber was filled with the phosphate-buffered saline with 1% sodium azide as a preservative, and the temperature was maintained at 37°C. Ketotifen suspension (1 ml) in silicone medical fluid (0.18 mmol of free base in 1 ml) was placed on the epidermal side of the skin. Aliquots of receptor solution were withdrawn from the receptor chamber at predetermined times, and the same volume of fresh buffer was immediately added to the receptor chamber to keep the volume. The concentration of ketotifen in each sample was measured by HPLC to obtain the cumulative amount of ketotifen that permeated the skin. The steady-state permeation flux was calculated as the slope of the line after plotting the cumulative amount of permeated ketotifen per unit area versus time (see Results section).

Permeation Across Intact and Delipidized Skin as a Function of Donor pH

Two-chamber type glass diffusion cell (the volume of both donor and receptor chambers was 50 ml, and the diffusion area was 3.14 cm^2) was used. The pH of the donor phase was varied from weakly acidic to basic. The basic donor (pH above 9) was prepared by mixing 20 mM of sodium carbonate and 20 mM of sodium hydrogen carbonate. Neutral or acidic donor (pH below 9) was prepared by mixing 20 mM of sodium phosphate and 20 mM of disodium hydrogen phosphate. In the acidic or neutral donor, 3.2 mmol/L of ketotifen fumarate was completely dissolved, and in the basic donor, 1.3 mmol/L of crystalline ketotifen free base was dispersed. The receptor solution was the phosphate-buffered saline with 1% sodium azide as a preservative.

During permeation experiments, both the donor and receptor solutions were stirred by magnetic stirrers, and the whole system of the diffusion cell was immersed in water maintained at 37°C. The receptor solution was withdrawn every 2 or 3 hr up to 40 hr, and the same volume of fresh buffer was immediately added to the receptor chamber. The concentration of ketotifen in each sample was measured by HPLC to obtain the cumulative amount of ketotifen that permeated the skin. The concentration of ketotifen in the donor phase at 40 hr was also measured after removal of ketotifen crystals by filtration, if they existed, and this value was used for the calculation of the permeability coefficient.

The steady-state permeation flux was calculated as the slope of the linear segment of the curve after plotting the cumulative amount of permeated ketotifen per unit area versus time (see Results section). The permeation flux was divided by the ketotifen concentration of the donor phase at 40 hr to give the permeability coefficient.

The permeability coefficient P thus obtained was analyzed using Eq. 1, based on the pH-partition principle.

$$P = \frac{P_{(KT)}}{1 + 10^{(pK_a - pH)}} + \frac{10^{(pK_a - pH)} \times P_{(KTH+)}}{1 + 10^{(pK_a - pH)}} \qquad (1)$$

where $P_{(KT)}$ is the intrinsic permeability coefficient of KT, and $P_{(KTH+)}$ is the intrinsic permeability coefficient of KTH+. The intrinsic permeability coefficients across intact skin $P_{(KT)intact}$ and $P_{(KTH+)intact}$ and those across delipidized skin $P_{(KT)delip}$ and $P_{(KTH+)delip}$ were determined by nonlinear least-square regression analysis so that Eq. 1 best fits the experimental data.

Partition Between Either Intact or Delipidized Stratum Corneum and Aqueous Solution

A piece of intact or delipidized SC was incubated in a ketotifen aqueous solution at 37°C overnight. The pH of the solution was varied widely from weakly acidic to basic. Buffer solution of each pH was the same as that

used for the permeation studies described above. After incubation, pH and the concentration of ketotifen in the aqueous solution and the content of ketotifen in the SC were measured. The last was performed by extraction with hexane after wiping the aqueous phase off the SC with filter paper. The amount of ketotifen taken up in unit volume of SC (nmol/cm^3) was determined using an SC thickness of 0.001 cm (18,21). It was divided by the concentration of ketotifen in aqueous phase (nmol/cm^3) to give the SC/water partition coefficient of ketotifen K.

Similar to the permeation coefficient, the partition coefficient thus obtained was analyzed using Eq. 2 based on the pH-partition principle:

$$K = \frac{K_{(KT)}}{1 + 10^{(pK_a - pH)}} + \frac{10^{(pK_a - pH)} \times K_{(KTH+)}}{1 + 10^{(pK_a - pH)}} \qquad (2)$$

where $K_{(KT)}$ is the intrinsic partition coefficient of KT, and $K_{(KTH+)}$ is the intrinsic partition coefficient of KTH+. The intrinsic partition coefficients of KT and KTH+ between intact SC and aqueous solution $K_{(KT)intact}$ and $K_{(KTH+)intact}$ and those between delipidized SC and aqueous solution $K_{(KT)delip}$ and $K_{(KTH+)delip}$ were determined by nonlinear least-square regression analysis so that Eq. 2 best fits the experimental data.

Measurement of the Diffusion Coefficient of Ketotifen in Aqueous Solution

To estimate the permeability of KT through the pure aqueous layer with a thickness equivalent to the stratum corneum, the diffusion coefficient in water was measured in another set of permeation experiments. A stainless steel ring was inserted between two porous membranes with a pore size of 0.4 μm (FR-40, Fuji Photo Film Co., Ltd., Kanagawa, Japan), forming a disk-shaped space inside the ring and two membranes, and placed in a two-chamber glass diffusion cell. After filling the space inside the ring and membranes with the same pH 10 buffer used in the experiments discussed above, ketotifen suspension in the same buffer and the buffer without ketotifen were simultaneously added to the donor chamber and receptor chamber, respectively. Both chambers were hermetically closed to prohibit solvent flow across the porous membrane. According to the procedure for determining the permeability coefficient in the skin, the permeability coefficient of ketotifen across the aqueous layer inside the ring and membranes $P_{(KT)aq}$ was obtained. The diffusion coefficient of KT in aqueous solution $D_{(KT)aq}$ was calculated by multiplying $P_{(KT)aq}$ with the thickness of the aqueous space (0.5 cm).

RESULTS

Permeation Through Full-Thickness and Tape-Stripped Skin

Figure 2 shows the cumulative permeation of KT through a unit area of the skin from a suspension in silicon medical fluid. The donor concentration was kept at saturation by excess solid particles of KT, and the receptor concentration was sufficiently low (less than 1/100 of the solubility) at the final sampling time so that the concentration gradient between the donor and receptor phases was maintained approximately constant. The linear relationship between the amount of permeated drug and time was obtained from 1.6–6 hr in both intact and tape-stripped skin, and the permeation flux was calculated using the data in this time range. The result was 0.01 μmol/hr/cm^2 and 0.22 μmol/hr/cm^2 for full-thickness and tape-stripped skin, respectively.

Permeation Through Intact and Delipidized Skin

Figures 3a–3c, respectively, represent the permeation profile of ketotifen from pH 6, pH 9, and pH 10 donor through intact (closed square) and delipidized (open diamond) skin. In every profile, linearity was good ($r^2 > .99$) in 5–40 hr, and the change of the concentration gradient between donor and receptor during that period was minimal. Therefore, the steady-state permeation flux was calculated using the data in this time range.

The permeability coefficient was calculated with the permeation flux thus obtained and the ketotifen concen-

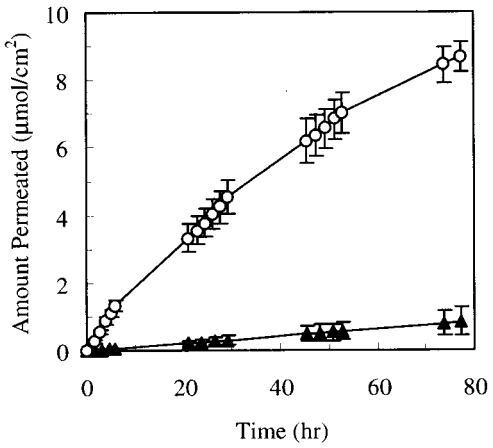
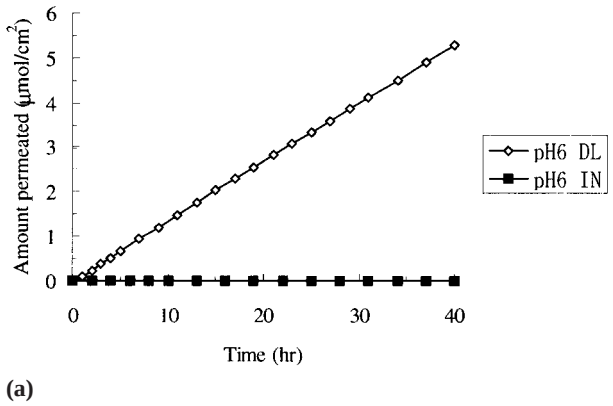
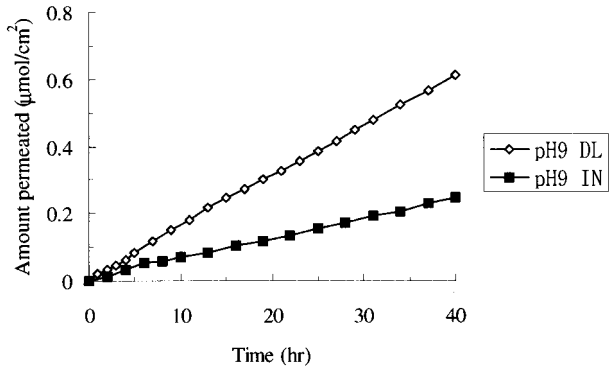


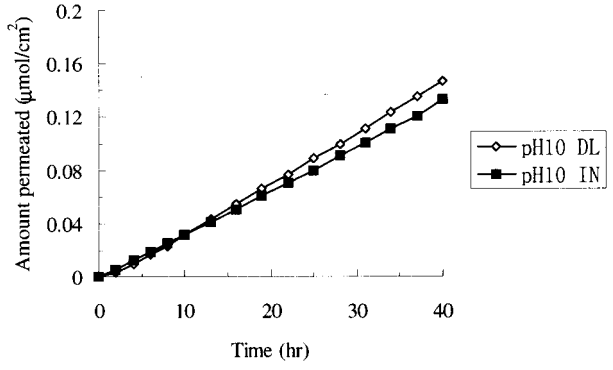
Figure 2. Comparison of the permeability of KT through intact (closed triangle) and tape-stripped (open circle) skin. Each data point is the mean ± SD of three permeation experiments.



(a)



(b)



(c)

Figure 3. The permeation profiles through intact (closed square) or delipidized (open diamond) skin: (a) KTH+, with a donor pH of 5.4 for intact skin and 6.3 for delipidized skin versus a pK_a of 8.5; (b) the total of KTH+ and KT, with a donor pH of 9.1 for intact skin and 8.6 for delipidized skin; (c) KT, with a donor pH of 10.5 for intact skin and 10.3 for delipidized skin.

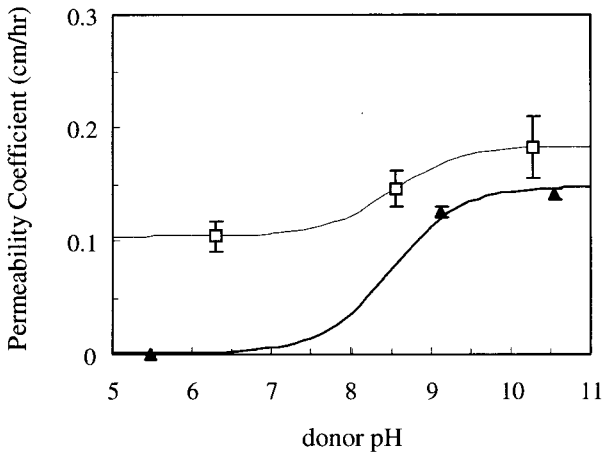


Figure 4. pH profiles of the permeability coefficient of ketotifen in aqueous solution across intact (closed triangle) or delipidized (open square) hairless mouse skin. Solid curves represent regression curves based on Eq. 2.

tration in the donor phase at the end of the experiment. The result is plotted against donor pH in Fig. 4. The intrinsic permeability coefficients $P_{(KTH+)intact}$, $P_{(KT)intact}$, $P_{(KTH+)delip}$, and $P_{(KT)delip}$, obtained by regression analysis with these data and Eq. 2, are listed in Table 1. Figure 4 exhibits good agreement between the data and regression curve in both intact and delipidized skin, implying the applicability of Eq. 2.

Partition Between Either Intact or Delipidized Stratum Corneum and Aqueous Solution

Intact and delipidized SC/water partition coefficient is plotted against pH of aqueous phase in Fig. 5. Besides the large variation in the data, sigmoid pH profiles were displayed in both intact and delipidized SC. The intrinsic partition coefficients $K_{(KTH+)intact}$, $K_{(KT)intact}$, $K_{(KTH+)delip}$, and

Table 1

Intrinsic Permeability Coefficients (cm/hr) of KTH+ and KT Across Intact and Delipidized Skin

Intact Skin		Delipidized Skin	
$P_{(KTH+)intact}$	$P_{(KT)intact}$	$P_{(KTH+)delip}$	$P_{(KT)delip}$
1.1×10^{-3}	1.5×10^{-1}	1.0×10^{-1}	1.8×10^{-1}

(KTH+) or (KT) indicate the permeation species. The inferior terms intact or delip mean permeation across intact or delipidized skin, respectively.

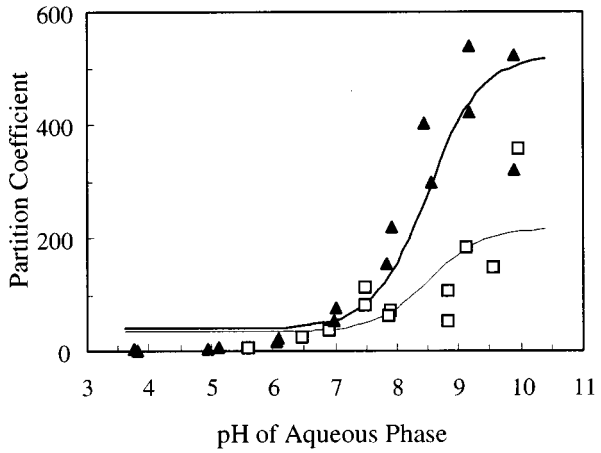


Figure 5. The partition coefficient of ketotifen between intact (closed triangles) or delipidized (open squares) stratum corneum and aqueous solution. Solid curves represent regression curves based on Eq. 2.

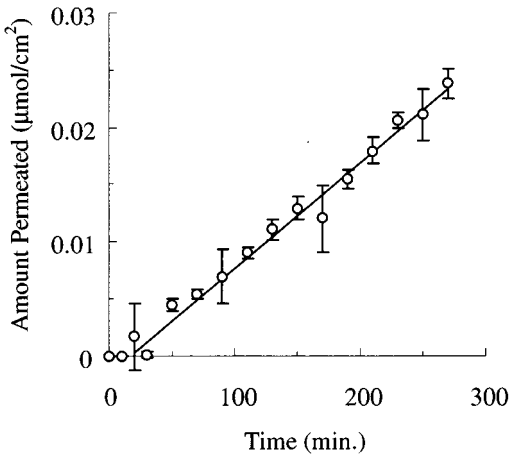


Figure 6. Permeation profile of ketotifen through stationary aqueous layer (pH 10, 0.5 cm thickness). Open circles and error bars represent mean values and standard deviations, respectively ($n = 3$). The solid line represents a regression line calculated using the data from 110 to 270 min.

$K_{(KT)delip}$, obtained by regression analysis with data in Fig. 5 and Eq. 2 are listed in Table 2.

Diffusion Coefficient of Ketotifen in Aqueous Solution

Figure 6 shows the permeation profile of KT through an aqueous layer between two porous membranes. From 110 min after the beginning to the end of this experiment, the linear relationship between time and cumulative amount of permeated drug was attained under an approximately constant concentration gradient. The concentration of ketotifen in the filtrate of donor solution at the end of the experiment (270 min) was 4.2 ± 10^{-2} μmol/ml, whereas that in the receptor was $8.8 \pm 10^{-}4$ μmol/ml. The steady-state permeation flux obtained from the data was 0.55 ± 10^{-3} μmol/hr/cm^2. Using these data, the diffusion coefficient of KT in aqueous solution was calculated as 6.5 ± 10^{-2} cm^2/hr.

Table 2

Partition Coefficients of KTH+ and KT

Intact Skin		Delipidized Skin	
$K_{(KTH+)intact}$	$K_{(KT)intact}$	$K_{(KTH+)delip}$	$K_{(KT)delip}$
40	523	34	217

$K_{(KTH+)intact}$ and $K_{(KT)intact}$ are the partition coefficients, respectively, of KTH+ and KT between intact SC and aqueous solution.

$K_{(KTH+)delip}$ and $K_{(KT)delip}$ are the partition coefficients, respectively, of KTH+ and KT between delipidized stratum corneum and aqueous solution.

DISCUSSION

Rate-Limiting Barrier in the Permeation Through Full-Thickness Skin

As shown in Fig. 2, the permeation flux from KT suspension in silicon medical fluid was around 20 times larger for tape-stripped skin than for full-thickness skin. This suggests that more than 95% of the total resistance for KT resides in the SC layer. Since tape-stripped skin consists of highly hydrated viable tissues, it is presumed that the permeability of KTH+, more hydrophilic species than KT, through tape-stripped skin is equivalent to or larger than that of KT. In addition, it is presumed that the permeability coefficient of KTH+ through SC is equivalent to or less than that of KT. Hence, the permeability measurement through the full-thickness skin should approximately give the permeability coefficient through SC for not only KT, but also for KTH+.

Strictly speaking, the permeability of a boundary layer should be assessed by in vitro permeation experiments. Assuming a boundary layer thickness of 300 μm for the diffusion cell utilized (22), a permeability coefficient for aqueous boundary layer–controlled transport is calculated to be 2.2 cm/hr using the diffusion coefficient of 6.5 ± 10^{-2} cm^2/hr for KT. The value is 10 times larger

than the permeability coefficient shown in Table 1. Thus, it is confirmed that the permeation process in our experiment was not boundary-layer controlled.

Permeation and Uptake in the Lipid and Proteinaceous Phase

In the case of KTH+, the permeability coefficient through delipidized skin $P_{(KTH+)delip}$ was 100 times larger than that through intact skin $P_{(KTH+)intact}$ (Table 1). This result is consistent with the existing knowledge that the lipid phase in SC functions as a barrier against the hydrophilic compound.

In contrast, the permeability coefficient of KT through delipidized skin $P_{(KT)delip}$ was comparable to that through intact skin $P_{(KT)intact}$ (Table 1), which suggests that the permeability of KT through the lipid phase is as high as that through the aqueous phase of delipidized SC. This finding appears contradictory to the existing knowledge (23) that the dense packing of lipid molecules inhibits the diffusion of penetrant and keeps the diffusivity in the lipid phase lower than that in the aqueous phase. The partition coefficient of KT in intact SC $K_{(KT)intact}$ is 300 times larger than that in delipidized SC $K_{(KT)delip}$ (Table 2). Uptake of KT in the lipid phase is then suggested to be a few hundred times larger than that in the aqueous phase. It is then presumed that such high uptake overcomes the low diffusivity in the lipid phase.

To evaluate the barrier function of the proteinaceous phase, the permeability coefficient of KT through delipidized SC was compared with that through a homogeneous aqueous layer, which has the same thickness as SC, approximately 10 μm (18,21). As the diffusion coefficient of KT in a homogeneous aqueous phase was found to be 6.4 ± 10^{-2} cm^2/hr, the permeability coefficient of KT through the aqueous layer was estimated as 64 cm/hr, which is much greater (34 times) than that through delipidized SC. If the permeability of KT through the proteinaceous phase is equivalent to that through the aqueous phase, KT can be assumed to pass through both the proteinaceous phase and the aqueous phase consecutively as if permeating in a homogeneous phase, and then the permeability coefficient of KT through delipidized SC should be equivalent to that through a homogeneous aqueous layer. Therefore, lower permeability through delipidized SC compared to that through the aqueous layer suggests that the permeability of KT in the proteinaceous phase is much lower than that in the aqueous phase. Since the permeability of KT in the aqueous phase is similar to that in the lipid phase, as discussed above, it can be presumed that the permeability of KT in the proteinaceous phase is much lower than that in the lipid phase. It is then concluded that the main permeation pathway of KT through intact SC resides in the lipid phase, and that the proteinaceous phase functions as a barrier that inhibits the permeation of KT.

On the other hand, the partition data 523 as $K_{(KT)intact}$ and 217 as $K_{(KT)delip}$ suggest that KT is highly taken up in both lipid and proteinaceous phases from aqueous solution. As discussed before, it is expected that a high uptake of KT in the lipid phase results in a positive effect for skin permeability. However, high uptake of KT in the proteinaceous phase seems meaningless because the permeability of KT through the proteinaceous phase is very low. Thus, the proteinaceous phase does behave as a depot in which KT is trapped before it permeates across the skin.

In the past few decades, many studies have been conducted concerning whether the drug predominantly permeates through the lipid phase or the proteinaceous phase. Michael, Chandrasekaran, and Shaw (7) conducted a mathematical analysis using an ordered-brick model and concluded that the diffusion coefficient of a drug is generally 500 times greater in the proteinaceous phase than in the lipid phase regardless of the type of drug. In contrast, Elias and Friend (24) pointed out the possibility that Michael et al. might have underestimated the diffusion coefficient of drugs in the lipid phase by an incorrect estimation of the volume fraction of the lipid phase in the SC. In addition, Barry (25) commented in his review that the case of lipid-phase-mediated drug permeation had been reported more frequently than the case of proteinaceous-phase-mediated drug permeation. Our study evidently revealed that ketotifen is also a typical drug that permeates predominantly through the lipid pathway.

The delipidized SC/water partition coefficient of KT $K_{(KT)delip}$ was written as

$$217\ (\text{mmol/cm}^3\ \text{SC})/(\text{mmol/cm}^3\ \text{aqueous phase}) = 360\ (\text{mmol/g SC})/(\text{mmol/g aqueous phase})$$

using the density of SC as 0.6 g/cm^3 (our data). On the other hand, Raykar et al. (15) found a linear relationship between K_{delip} and its log $P_{octanol/water}$ for a series of hydrocortisone with log $P_{octanol/water}$ ranging from 0.5 to 5. 5. In addition, Surber et al. (26) reported the K_{delip} of testosterone and progesterone, which have log $P_{octanol/water}$ close to that of KT, as log $P_{octanol/water}$ of testosterone, progesterone, and KT are 3.3, 3.9, and 3.7, respectively. The $K_{(testosterone)delip}$ and $K_{(progesterone)delip}$ reported are 74 and 214 (mmol/g of SC)/(mmol/g of aqueous phase), respectively. Therefore, according to the relationship, $K_{(KT)delip}$ is predicted to be 167 (mmol/g of SC)/(mmol/g of aqueous phase). The

observed $K_{(KT)delip}$ of 360 is more than two times larger than the predicted value. This suggests that KT has an extraordinarily strong affinity to the proteinaceous phase considering its log $P_{octanol/water}$, probably due to an interaction between the KT molecule and keratin protein.

Designing a Transdermal Formulation of Ketotifen

Based on the above discussion about the permeation pathway and the barrier function of SC, a transdermal formulation of ketotifen was designed as follows. Ketotifen can be formulated as KTH+ and/or KT. In the case of an enhancer-free formulation, KT should be used since KT has greater skin permeability than KTH+ when applied to intact skin. On the other hand, if an enhancer is formulated, the type of enhancer would govern the appropriate drug species. KTH+ should be used in the case of an enhancer that increases the area of aqueous pores in SC by extracting lipid from SC, as in delipidization. In contrast, KT should be chosen when an enhancer increases the permeability in the lipid phase by increasing the solubility of drug in the lipid phase or by increasing the fluidity of the lipid molecules.

REFERENCES

1. J. A. A. Hunter and B. Finlay, Scanning electron microscopy of connective tissues in health and disease, Int. Rev. Connective Tissue Res., 6, 217–255 (1973).
2. I. C. Mackenzie, An examination of cellular organization within the stratum corneum by a silver staining method, J. Invest. Dermatol., 61, 245–250 (1973).
3. K. J. McGinley, R. R. Marples, and G. Plemig, A method for visualizing and quantitating portion of the stratum corneum. J, Invest. Dermatol., 53, 107–111 (1969).
4. I. C. Mackenzie, Ordered structure of the stratum corneum of mammalian skin, Nature, 222, 881–882 (1969).
5. E. Christophers, Cellular architecture of the stratum corneum of mammalian skin, J. Invest. Dermatol., 56, 165–169 (1971).
6. R. J. Scheuplein, Properties of the skin, Adv. Biol. Skin, 12, 125–152 (1972).
7. A. S. Michael, S. K. Chandrasekaran, and J. E. Shaw, Drug permeation through human skin: theory and in vitro experimental measurement, AIChE J., 21(5), 985–996 (1975).
8. S. K. Chandrasekeran and J. E. Shaw, Factors influencing the percutaneous absorption of drugs, Curr. Probl. Dermatol., 7, 142–155 (1978).
9. P. M. Elias, Epidermal lipids, barrier function, and desquamation, J. Invest. Dermatol., 80, 44s–49s (1983).
10. K. Tojo, Random brick model for drug transport across stratum corneum, J. Pharm. Sci., 76(12), 889–891 (1987).
11. R. O. Potts and R. H. Guy, *Mechanism of Transdermal Drug Delivery*, Marcel Dekker, New York, 1997, pp. 1–40.
12. M. A. Lampe, M. L. Williams, and P. M. Elias, Human epidermal lipids: characterization and modulations during differentiation, J. Lipid Res., 24, 131–140 (1983).
13. P. C. Kuo, J. C. Liu, S. F. Chang, and Y. W. Chien, In vitro transdermal permeation of oxycodone: (I) effect of pH, delipidization and skin stripping, Drug Dev. Ind. Pharm., 15(8), 1199–1215 (1989).
14. K. Harada, T. Murakami, N. Yata, and S. Yamamoto, Role of intercellular lipids in stratum corneum in the percutaneous permeation of drugs, J. Invest Dermatol., 99(3), 278–282 (1992).
15. P. V. Raykar, M. C. Fung, and B. D. Anderson, The role of protein and lipid domains in the uptake of solubility by human stratum corneum, Pharm. Res., 5(3), 140–150 (1988).
16. E. Aspe, R. H. Guy, W. A. Lee, J. A. Kennedy, G. C. Visor, and R. D. Ennis, Optimization of in vitro flux through hairless mouse skin of cidofovir, a potent nucleotide analog, J. Pharm. Sci., 84(6), 750–754 (1995).
17. N. Higo, R. S. Hinz, D. T. W. Lau, L. Z. Benet, and R. H. Guy, Cutaneous metabolism of nitroglycerin in vitro. II. Effects of skin condition and penetration enhancement, Pharm. Res., 9(3), 303–306 (1992).
18. H. S. Shah, K. Tojo, and Y. W. Chien, Transdermal controlled delivery of verapamil: characterization of in vitro skin permeation, Int. J. Pharm., 86, 167–173 (1992).
19. P. W. Werts and D. Downing, Covalent attachment of ω-hydroxyacid derivatives to epidermal macromolecules: a preliminary characterization, Biochem. Biophys. Res. Commun., 137(3), 992–997 (1986).
20. K. Knutson, S. L. Krill, W. J. Lambert, and W. I. Higuchi, in *Controlled Release Technology: Pharmaceutical Applications* (P. I. Lee and W. R. Good, Eds.). ACS Symposium Series 348, Washington, D.C., 1987, pp. 241–266.
21. J. C. Tsai, N. Weiner, G. L. Flynn, and J. J. Ferry, Drug and vehicle deposition from topical applications of minoxidil with skin strata of the hairless mouse, Skin Pharmacol., 7(5), 262–269 (1994).
22. G. L. Flynn, H. Durrheim, and W. I. Higuchi, Permeation of hairless mouse skin II: membrane sectioning techniques and influence on alkanol permeabilites, J. Pharm. Sci., 70(1), 52–56 (1981).
23. E. R. Cooper, Increased skin permeability for lipophilic molecules, J. Pharm. Sci., 73(8), 1153–1156 (1984).
24. P. M. Elias and P. S. Friend, The permeability barrier in mammalian epidermis, J. Cell Biol., 65, 180–191 (1975).
25. B. W. Barry, in *Dermatological Formulations: Percutaneous Absorption* (B. W. Barry, Ed.), Marcel Dekker, New York, 1983, pp. 107–116.
26. C. Surber, K. P. Wilhelm, M. Hori, H. I. Maibach, and R. H. Guy, Optimization of topical therapy: partitioning of drugs into stratum corneum, Pharm. Res., 7(12), 1320–1324 (1990).

Copyright © 2002 EBSCO Publishing

Copyright of Drug Development & Industrial Pharmacy is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.