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# Effect of temperature on percutaneous absorption of terodiline, and relationship between penetration and fluidity of the stratum corneum lipids

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**Abstract**

The effect of temperature on the skin permeation of terodiline (TD) hydrochloride and the free base form was examined. The in vitro penetration experiment at 25–50°C was carried out using the full-thickness skin (FS) and the stratum corneum (SC) sheet of Wistar rat. The fluidity of the stratum corneum lipids was measured by ESR. The relationship between the flux and the phase state of the SC lipids was evaluated based on the data obtained. The effect of heating on the in vivo percutaneous absorption was also estimated. Increasing temperatures resulted in increased penetration of both hydrochloride and free base forms. A significant difference in the penetration rates through the FS was not observed between the hydrochloride and free base forms, whereas the fluxes of the free base form through the SC sheet were slightly higher than those of the hydrochloride form at each temperature. The Arrhenius plots of permeability coefficient ($K_p$) yielded straight lines for both FS and SC sheet. The activation energies (15.5 and 20.0 kJ/mol), calculated from the slope of curves, for the SC sheet were smaller than those (45.7 and 39.3 kJ/mol) for the FS. The spin label mobility of the SC lipids, measured by ESR, was increased with rising temperatures. The plots of apparent rotational correlation time ($\tau_c$) versus temperature represented the temperature dependence, with abrupt breaks at $41.3 \pm 0.7$°C and $68.1 \pm 1.4$°C ($n = 4$), suggesting the phase transition of the lipids. When $1/\tau_c$ was plotted against the $K_p$ for the free base form at four temperatures, straight lines were obtained for both skins ($r^2 = 0.842$ and 0.938). This indicates that the penetration of TD free base through the skin was dependent on the temperatures of the SC lipids and the drug penetrated via the lipoidal pathway within the SC. A notable result was not obtained from heating the transdermal system using a heat patch, because of the lesser efficiency of the patch. 

*Keywords:* Terodiline; Percutaneous penetration; Temperature effect; Lipid fluidity; Fluidity/penetration; Arrhenius plot

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## 1. Introduction

The stratum corneum (SC) barrier function has been attributed to the interstitial lipids for lipophilic solutes, or to a more polar pathway for polar solutes (Scheuplein, 1965; Scheuplein and Blank, 1971; Behl et al., 1980; Knutson et al., 1985). The composition and physical structures of lipoidal and polar pathways affect the barrier functions of the pathways. A principal physicochemical factor which controls the passive diffusion of a solute from a vehicle into the skin arises from the skin temperature. It is an established fact that skin temperature rise increases penetration of solute (Fritsch and Stoughton, 1963; Barry, 1983). Additionally, temperature enhanced permeabilities of the solutes were associated with the gel to liquid-crystalline transition of lipid hydrocarbon chains (Knutson et al., 1987) and increasing temperatures of the SC resulted in increased fluidity (rotational disorder) of the intercellular lipids (Golden et al., 1986; Ogiso et al., 1996). We have also reported that the activity coefficients of indomethacin and ibuprofen at 37°C decreased to one-third of those at 27°C, with the increased fluxes and permeability coefficients (Ito et al., 1988).

In spite of the well documented enhanced delivery with increasing temperature, the relationship between transdermal flux and SC lipid disorder is still a matter for discussion. To estimate the enhancement effect with a rise of temperature, terodiline (TD), which could rapidly penetrate through rat skin without enhancers (Ogiso et al., 1993), was selected as a model drug.

The present study was undertaken in order to further clarify the mechanism involved in the enhancement of percutaneous absorption resulting from increasing temperatures. The in vitro penetration experiment at 25–50°C was carried out using TD and rat skin, without any enhancers. The fluidity of the SC lipids was measured using a spin labeling technique and a deeper probe, 16-doxylstearic acid, in lipid bilayer. The relationship between the phase state of the SC lipids and skin penetration of the drug was evaluated based on the data obtained. The in vivo percutaneous absorption under a heated condition was also investigated to estimate the efficiency of heat delivery.

## 2. Materials and methods

### 2.1. Materials

TD hydrochloride was a generous gift of Pharmacia AB (Stockholm, Sweden). Propiverine hydrochloride, an internal standard for HPLC, was obtained from Taiho Pharmaceutical Co. (Tokyo, Japan). Hiviswako® 104 (carboxyvinyl polymer), a gel base, and diisopropanolamine were purchased from Wako Pure Chemical Industries (Osaka, Japan) and Nacalai Tesque (Kyoto, Japan), respectively. 16-Doxylstearic acid, a spin label, was obtained from Aldrich (Milwaukee, WI). Sennenkyu Taiyo®, a heat patch (40–50°C, for 3 h), was purchased from Sennenkyu (Shiga, Japan). All other chemicals and solvents used were of reagent grade or HPLC quality. Free TD was prepared from the hydrochloride salt by the same method as described previously (Ogiso et al., 1993). Male Wistar rats, weighing 230–260 g (Japan SLC, Shizuoka, Japan), were used throughout this experiment. The animals had free access to MF diet (Oriental Yeast, Tokyo, Japan) for 3–4 days prior to and during the experiment.

### 2.2. Skin preparations

On the day before the experiment, the hair of the abdominal area of rat was removed with an electric clipper and an electric razor. On the next day, pieces ($3 \times 3$-cm area) of full-thickness abdominal skin were excised from the rats. The adherent fat and other visceral debris were removed from the undersurface (full-thickness skin, FS). The SC was prepared by heating at 60°C for 2 min (Kligman and Christophers, 1963), followed by teasing the dermis with a spatula (SC sheet).

### 2.3. Preparation of gel formulations and their transdermal systems

Hiviswako® 104 was swollen with water and the pH value was neutralized with diisopropanolamine (gel base). TD free base or the hydrochloride salt dissolved in a mixture of propylene glycol and ethanol was mixed with the gel base. Gentamicin sulfate solution (10 mg/ml,

Sigma, St Louis, MO) was added to the mixture (Table 1). The transdermal systems, containing 0.7 g (TD 14 mg) of formulation and having an absorption area of 6.0 $cm^2$, were prepared using a corresponding gel formulation described in Table 1 as reported previously (Ogiso et al., 1991).

### 2.4. In vitro percutaneous penetration experiment

The dermal sides of the FS and SC sheet were soaked in a buffer solution (0.85% NaCl-10 mM phosphate buffer, pH 7.4) for 12 h at 5°C to equilibrate the skin. Then, a 0.25-g gel formulation was uniformly spread over the SC surface of the skin, which was mounted in a Franz diffusion cell (reservoir volume, 10.5 ml; 1.6 cm i.d. *O*-ring flange), and occluded with a sheet of aluminum foil. Gentamicin sulfate solution (10 mg/ml) was added to the receptor fluid (0.85% NaCl-10 mM phosphate buffer, pH 7.4) at a ratio of 1:100 (v/v). The receptor compartment was thermostated at 25, 35, 40 and 50°C and stirred. Aliquots (100 $\mu$l) of receptor fluid were withdrawn periodically up to 10 h.

### 2.5. In vivo percutaneous absorption experiment

On the day before the experiment, the jugular vein of the rats was cannulated with silicone tubing and the hair of the abdominal area was carefully removed with an electric clipper and an electric razor. On the next day, the transdermal system (0.7 g/6 $cm^2$) was applied to the abdomen of the rats. The system was fixed with an adhesive and immediately occluded with an adhesive tape. In some experiments, a heat patch (3.14 $cm^2$) was put on the system for 10 h. Blood samples (200 $\mu$l) were collected periodically for 10 h after dosing. The plasma was separated immediately by centrifugation and stored frozen until the time of assay.

Table 1
Composition of terodiline gel formulations

| Composition (g) | Terodiline | |
|---|---|---|
| | Free base | Hydrochloride |
| Hiviswako® 104 | 1.0 | 2.0 |
| Propylene glycol | 20.0 | 20.0 |
| Ethanol | 30.0 | 30.0 |
| Diisopropanolamine | 1.1 | 2.0[a] |
| Terodiline | 2.0 | — |
| Terodiline-HCl | — | 2.3 |
| Gentamicin sol. | 1.0 | 1.0 |
| Dist. water ad. | 100.0 | 100.0 |

[a] Containing the amount equivalent to neutralizing hydrochloride.

### 2.6. Determination of TD

TD in sample was determined by HPLC methods as follows. A 100-$\mu$l portion of receptor fluid was mixed with 200 $\mu$l of the internal standard solution (5 $\mu$g/ml in acetonitrile). Following centrifugation, the supernatant was filtered through a membrane filter (0.45 $\mu$m, Chromatodisc, GL Sciences, Tokyo, Japan) and then 10–50 $\mu$l of the filtrate was injected onto a reversed-phase Inertsil ODS column (4.6 × 150 mm, 5 $\mu$m, GL Sciences) using Shimadzu liquid chromatography (model LC-6A, Kyoto, Japan) equipped with an ultraviolet (UV) detector (model SPD-6AV). The mobile phase was acetonitrile:10 mM phosphate buffer, pH 3.5, (38:62, v/v) containing 0.6% nonylamine. The flow rate was 1.2 ml/min and the detection was 210 nm. For the determination of drug in plasma, 100 $\mu$l of plasma was mixed with 50 $\mu$l of the internal standard solution (10 $\mu$g/ml in distilled water) and 0.4 ml of 0.5 N NaOH. The mixture was extracted with hexane:ether (4:1, v/v, 5 ml). Following centrifugation, the supernatant was evaporated under reduced pressure and the residue was dissolved in 100 $\mu$l of methanol. The solution, after filtration, was injected onto a reversed-phase column, as mentioned above. Linearities of the standard curves were found in the range from 0.2 to 100 $\mu$g/ml ($r^2 > 0.999$). Intra- and interday variabilities were $< 10\%$.

### 2.7. ESR Measurement

The SC from rat abdominal skin was prepared by trypsin treatment (Kligman and Christophers, 1963) and dried under vacuum. The dried SC (280 mg) was homogenized for 1.5 min in 8 ml of 0.85% NaCl-40 mM Tris–HCl buffer, pH 7.4, by

use of a microhomogenizer (Physcotron NS-10, Nichion, Tokyo). The spin label (0.28 mg, 16-doxylstearic acid) dissolved in 5% albumin (0.2 ml) was added to the homogenate (0.5 ml), and the suspension was kept on ice for 1.5 h. After three washes with the buffer and centrifugation, the pellet, suspended in the buffer, was packed in a hematocrit capillary tube (1.1 mm i.d.; non-heparinized). The ESR spectra of the packed samples were recorded with a JES-RE2X ESR spectrometer equipped with a variable-temperature accessory. The rotational correlation time, $\tau_c$, was calculated by the following formula (Singer, 1977): $\tau_c = 6.5 \times 10^{-10} W_0 (h_0/h_{-1})^{1/2} - 1$, where $W_0$, and $h_0$ are the width and height, respectively, of the medium-field line of the spectrum and $h_{-1}$ is the height of the high-field line.

### 2.8. Data analysis

The in vitro penetration parameters were calculated from the penetration data by using the following equations:

$$D = \frac{\delta^2}{6\tau}$$

$$J_s = \frac{D \cdot K_m \cdot C_s}{\delta} = K_p \cdot C_s$$

where $J_s$ is the penetration rate, $K_m$ denotes the skin/vehicle partition coefficient of drug, $D$ is the diffusion constant within skin, $\tau$ represents the lag time, and $\delta$ the thickness (0.002 cm) of the stratum corneum, $K_p$ denotes the permeability coefficient through the skin, and $C_s$ is the drug concentration in the gel formulation.

The activation energy ($\Delta E$) was calculated from the penetration rates at 25–50°C according to the Arrhenius equation:

$$\Delta E = \frac{2.303 R T_1 T_2}{T_1 - T_2} \log \frac{J_{s1}}{J_{s2}}$$

where $T_1$ and $T_2$ are the absolute temperatures, $J_{s1}$ and $J_{s2}$ represent the penetration rates at lower and higher temperatures, respectively. $R$ is the gas constant.

The means of all data are presented with their standard deviation (S.D.). Statistical analysis was performed using an unpaired Student's $t$-test, and the significance level adopted was $p < 0.05$.

## 3. Results

### 3.1. In vitro percutaneous penetration

In this study, no enhancers were used to estimate the effect of temperature on the penetration. The penetration profiles of TD hydrochloride and the free base through rat skins (FS and SC sheet) at 25, 35, 40 and 50°C are shown in Fig. 1. A significant difference in the penetration rates through the FS was not observed between the hydrochloride and free base forms of TD at each temperature. On the other hand, there was considerable difference in the penetration through the SC sheet between both forms of TD; the free base penetrated more easily through the SC sheet than the hydrochloride form, reflecting the difference in the penetration rates of each form through the viable skin. Increasing temperatures resulted in increased penetration of both forms. Compared to the fluxes at 25°C, the flux through the FS at 50°C was 4.2-fold greater for the free base and 3.4-fold greater for the hydrochloride form. However, the increased extent of the penetration of the free base form through the SC sheet with increasing temperatures was relatively small compared with that via the FS.

The penetration parameters are listed in Table 2. Reflecting the penetration differences as shown in Fig. 1, the fluxes of the free base form through the SC sheet were slightly higher then those of the hydrochloride form at each temperature. This suggests that the penetration through the lipophilic pathway, such as the intercellular lipids, of the SC sheet was slightly more rapid than that through the hydrophilic routes. The small $D$ values in the SC sheet penetration also indicate the facile diffusion of both forms through the SC. The temperature dependence of the $J_s$ and $K_p$ values was observed in these experiments, whereas the dependence was not observed with the $D$ values.

We constructed Arrhenius-type plots which illustrate the effect of temperature on the transport

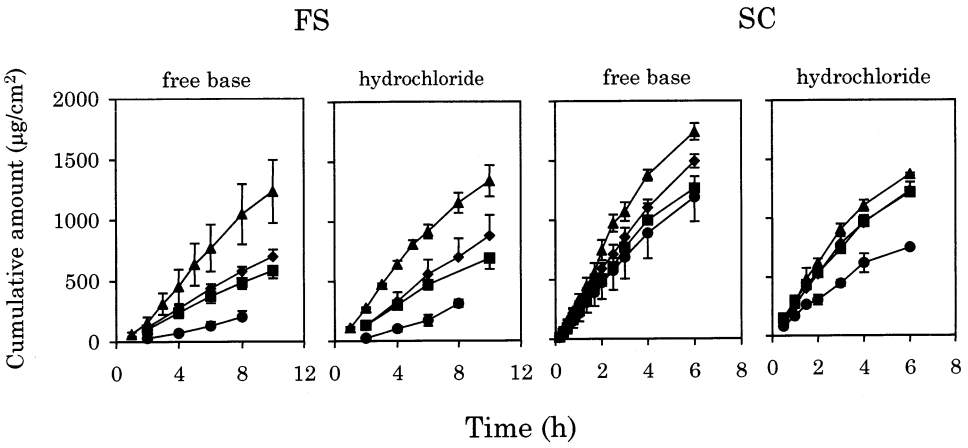


Fig. 1. Penetration profiles of terodiline through rat skin after application of terodiline gel formulations at various temperatures. Each point represents the mean ± S.D. ($n = 3–6$). FS, full-thickness skin; SC, stratum corneum sheet. Penetration at (●) 25°C; (■) 35°C; (◆) 40°C; (▲) 50°C.

of TD across the SC and FS. The Arrhenius plots of $K_p$ yielded straight lines ($r^2 > 0.933$) for both SC sheet and FS (Fig. 2). This indicates that Fick's law of diffusion is applicable to TD penetration through the skins, and the penetration is attributed to the passive diffusion. The activation energies (15.5 and 20.0 kJ/mol) calculated from the slope of curves for the SC were much smaller than those for the FS, although the $\Delta E$ (45.7 and 39.3 kJ/mol) for the FS was also relatively small, probably due to the partial diffusional resistance of the dermis.

### 3.2. Thermal transition pattern of SC lipids

The typical ESR spectra were obtained with Wistar rat SC, labeled with 16-doxylstearic acid, at various temperatures. Since the paramagnetic group of this probe is located in the inner hydrocarbon core of the lipid bilayer (Schreier-Muccillo et al., 1976), the lipids of intercellular domains were judged to be definitely labeled by this probe. The spin label mobility was increased with rising temperatures, consequently resulting in decreasing the $\tau_c$ values. Fig. 3 denotes the representative plots of apparent $\tau_c$ versus temperature for 16-doxylstearic acid spectra of the SC lipids. The curves represent the temperature dependence of $\tau_c$ values, indicating the increased fluidity (rotational disorder) of the lipids with increasing temperatures. The plots of $\tau_c$ values indicated an abrupt inflection at $41.3 \pm 0.7$°C and $68.1 \pm 1.4$°C ($n = 4$). Since values of $\tau_c$ at given temperature serve as an index of membrane lipid viscosity (Morse et al., 1975), the break temperatures represent the phase transition of the lipids from gel to liquid-crystalline phase (White et al., 1988). Our data also roughly agreed with the results obtained by DSC measurements, in which thermal profiles show three peaks near 35–40°C, 65–70°C and 80–85°C for the SC (Knutson et al., 1985; Golden et al., 1987; Potts, 1989; Schückler and Lee, 1992).

### 3.3. Relationship between fluidity of SC lipids and permeability coefficient

When the reciprocals of $\tau_c$ were plotted against the $K_p$ of the free base form at four temperatures, straight lines for both skins were obtained ($r^2 = 0.842$ and 0.938) (Fig. 4), indicating that the penetration of TD free base through the skin depended on the SC lipids and that the drug penetrated via the lipoidal pathway within the SC. The $r^2$ value (0.938) for the SC sheet was slightly larger than that (0.842) for the FS. This also suggests that the penetration of the lipophilic drug was slightly more facile through the SC sheet than via the FS containing the dermis, since the steady-

Table 2
In vitro skin penetration parameters and activation energies for terodiline

| Skin | Drug form | Temperature (°C) | $\tau$ (h) | $J_s$ ($\mu$g/h·cm$^2$) | $D$ ($\times 10^{-4}$cm$^2$/h) | $K_p$ ($\times 10^{-4}$cm/h) | $\Delta E$ (kJ/mol) |
|---|---|---|---|---|---|---|---|
| FS | Free base | 25 | 2.14 ± 0.90 | 35.1 ± 3.0 | 4.3 ± 1.5 | 17.5 ± 1.5 | |
| | | 35 | 0.47 ± 0.36 | 66.9 ± 7.3 | 55.1 ± 7.4 | 33.4 ± 3.6 | 45.65 |
| | | 40 | 0.41 ± 0.25 | 76.3 ± 3.7 | 26.4 ± 15.4 | 38.1 ± 1.9 | |
| | | 50 | 0.83 ± 0.19 | 146.1 ± 33.3 | 10.5 ± 2.2 | 73.1 ± 16.7 | |
| | Hydrochloride | 25 | 1.77 ± 0.27 | 47.1 ± 19.5 | 4.8 ± 0.8 | 23.5 ± 1.0 | |
| | | 35 | 0.29 ± 0.20 | 80.5 ± 3.1 | 38.9 ± 27.1 | 40.2 ± 1.5 | 39.33 |
| | | 40 | 0.56 ± 0.20 | 96.2 ± 22.3 | 16.6 ± 6.8 | 48.1 ± 11.1 | |
| | | 50 | 0.18 ± 0.04 | 161.2 ± 6.6 | 47.6 ± 8.3 | 80.5 ± 3.3 | |
| SC | Free base | 25 | 0.13 ± 0.07* | 243.8 ± 60.5* | 0.08 ± 0.07* | 122.1 ± 30.2* | |
| | | 35 | 0.13 ± 0.05* | 264.2 ± 28.3* | 0.06 ± 0.03* | 132.0 ± 14.2* | 15.48 |
| | | 40 | 0.09 ± 0.05* | 307.4 ± 27.9* | 0.10 ± 0.06* | 153.3 ± 13.9* | |
| | | 50 | 0.14 ± 0.02* | 395.0 ± 36.1* | 0.05 ± 0.01* | 198.1 ± 18.1* | |
| | Hydrochloride | 25 | 0.14 ± 0.01* | 183.1 ± 28.7* | 0.05 ± 0.00* | 91.5 ± 14.4* | |
| | | 35 | —[a] | 242.0 ± 11.8* | —[a] | 121.1 ± 5.9* | 19.96 |
| | | 40 | 0.09 ± 0.06* | 280.2 ± 22.1* | 0.13 ± 0.09* | 140.0 ± 11.0* | |
| | | 50 | 0.18 ± 0.13* | 341.0 ± 17.0* | 0.07 ± 0.08* | 171.3 ± 8.5* | |

Each value represents the mean ± S.D. ($n$ = 3-6). $\Delta E$, activation energy at 25–50°C; FS, full-thickness skin; SC, stratum corneum sheet.
[a] Not calculated for the negatives.
* $p < 0.05$ compared with the correspondent form.

state rate of permeation is proportional to the solubility of drug in the SC or in the viable skin (Tojo et al., 1987). This result demonstrated that the transdermal flux of TD is related to packing in the intercellular lipid domains of the SC.

### *3.4. Effect of temperature on in vivo percutaneous absorption*

In order to estimate the effect of heating on the in vivo percutaneous absorption, a heat patch (Sennenkyu Taiyo®) was combined with the transdermal system containing TD free base. The plasma concentration profiles of TD after applying the heat patch are shown in Fig. 5. The plasma concentrations at the initial time were slightly higher than those after the transdermal system without a heat patch, however after that the plasma levels were not significantly different between both systems. The reason why the effect of the heat patch was less than expected was because the rise in skin temperature was small, since the patch was applied above the transdermal system.

## 4. Discussion

The success of transdermal systems depends on the ability of the drug to permeate skin in sufficient quantities to achieve its desired therapeutic effect. However, many drugs do not intrinsically possess any great ability to cross the skin, and ways must be found to modify the diffusional barrier. A possible method of accelerating the transport of drugs across the skin is to apply heat to the skin. The SC resists heat damage, tolerating temperatures as high as 60°C for several hours without serious alteration to its barrier properties (Blank and Scheuplein, 1964). Thus, it is of interesting to estimate the enhancement effect of heat on the skin penetration of drug.

There was a temperature dependent enhancement of flux and permeability coefficient of TD (hydrochloride and free base forms) through the FS and SC. However, no significant difference between the penetration parameters of TD hydrochloride and free base forms through the FS was observed (Fig. 1). A possible explanation for the similarity of penetration is that at pH 7.0 (pH

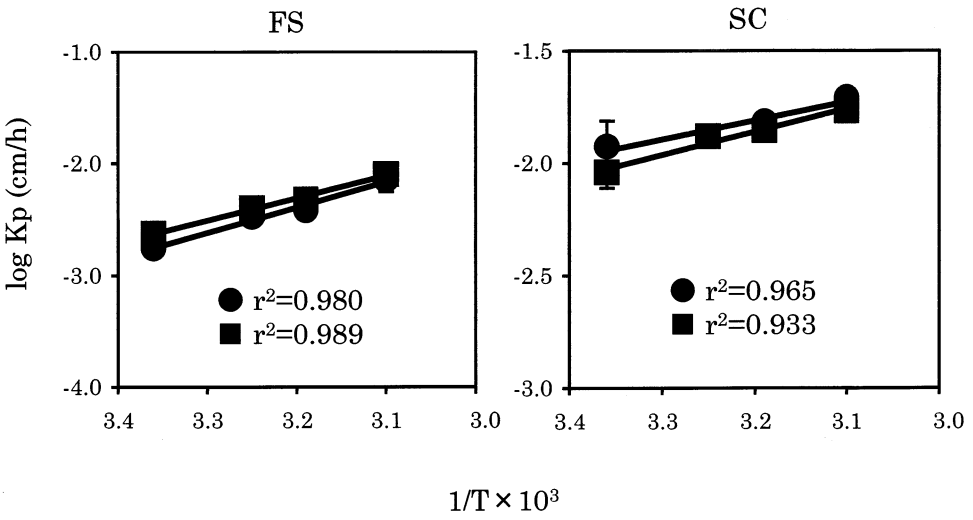


Fig. 2. Arrhenius plots of permeability coefficient ($K_p$) of terodiline. Each point represents the mean ± S.D. ($n = 3$–6). FS, full-thickness skin; SC, stratum corneum sheet. (●) Free base form; (■) hydrochloride form.

of gel formulation) TD (p$K_a$, 9.85; Hallén et al., 1988) dissociates to the ionized form to a great extent (ionized form:unionized form = 708:1), and so the concentrations of the unionized or ionized form in the gel formulation would not be different between both formulations. TD existing as unionized molecules would mainly penetrate through the intercellular lipid of the SC. Assuming that both the ionized and non-ionized species contribute to the steady state flux of TD, the total observed flux ($J_{s,obs}$) is dependent on the flux of ionized fraction ($J_{s,i}\alpha$) and the flux of the unionized fraction ($J_{s,u}$, $1-\alpha$) and can be expressed by the following equation:

$$J_{s,obs} = \alpha J_{s,i} + (1-\alpha)J_{s,u}$$

Thus, a plot of $J_{s,obs}/\alpha$ against $(1-\alpha)/\alpha$ makes it possible to estimate the flux of the ionized and unionized species. Judging from the high concentration of the ionized form in the gel formulation and the large flux of the hydrochloride form, the ionized form of TD can easily penetrate through the SC. It is generally accepted that ionized materials penetrate the skin poorly. Therefore, there is a possibility that the ionized species could rapidly penetrate through the follicles and the aqueous layers of intercellular spaces of the SC. This may be mainly due to the molecular structure of TD. The secondary amino group of TD is masked by four methyl residues, as shown in our previous paper (Ogiso et al., 1995), and thus TD would probably prevent the charge interaction with epidermal components. Consequently, this makes it possible to facilitate the transport through the skin, especially through the intercellular pathway. It is shown that counter ions such as fixed, negative-charge-bearing end groups (e.g. carboxylic and phosphate groups) contained in intercellular lipids (Elias et al., 1977) would potentiate cationic drug binding by electrostatic interaction. Our data

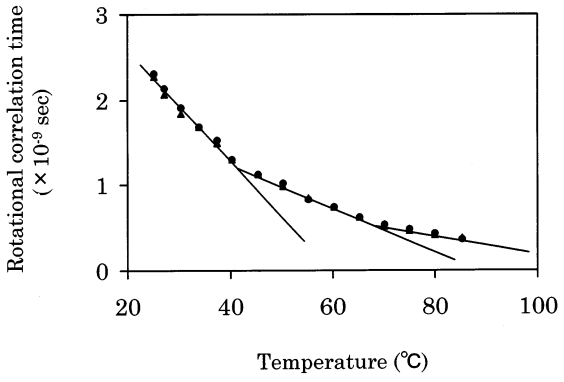


Fig. 3. Temperature dependence of motion parameters of lipid spin label in stratum corneum. Point (●, No. 1; ▲, No. 2) and line are the observed value and regression line, respectively.

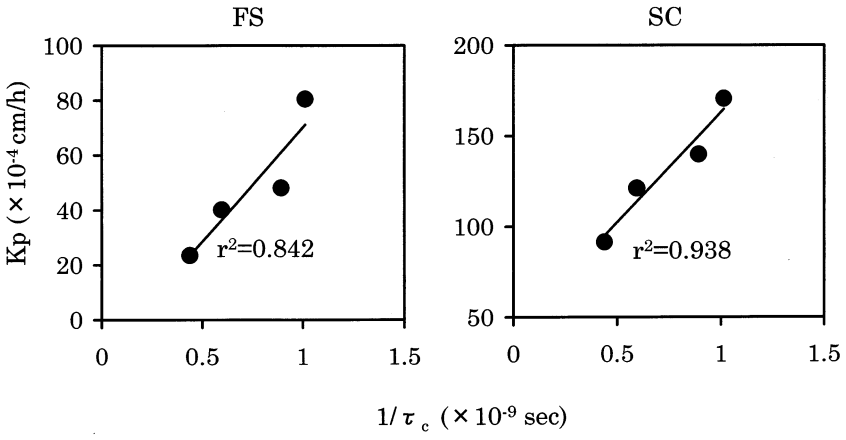


Fig. 4. The relationship between permeability coefficient ($K_p$) for terodiline free base and rotational correlation time ($\tau_c$) of stratum corneum lipids. FS, full-thickness skin; SC, stratum corneum sheet.

proved that TD hydrochloride, having a hydrophilic property, could also penetrate through the skin like the free base form.

The facile penetration of the hydrochloride form was also demonstrated by the low activation energies (20.0 kJ/mol) for penetration through the SC. TD free base form showed a lower activation energy (15.5 kJ/mol) compared with the $\Delta E$ (86.2–186.2 kJ/mol) of other drugs (Ito et al., 1988; Cornwell and Barry, 1993; Ogiso et al., 1996), indicating that the free base form rapidly penetrates via the SC.

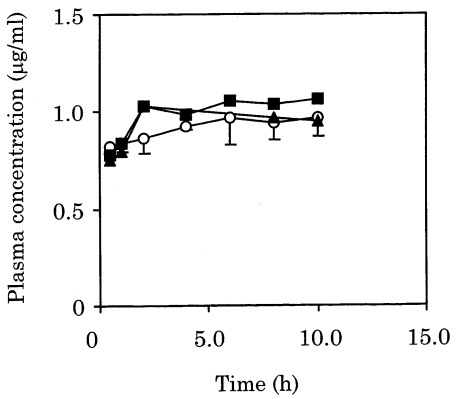


Fig. 5. Plasma concentration-time curves for terodiline after application of percutaneous absorption systems to rats. (○) Transdermal system without heat patch; (■) and (▲) transdermal system with heat patch, No. 1 and No. 2, respectively. Transdermal system (6.0 $cm^2$) contained 0.7 g of the free base formulation.

Knutson and coworkers showed an approximately doubling of flux with each 6–8°C increase in temperature from 10 to 60°C (Knutson et al., 1985). Our data on the flux through the FS approximately resemble their results.

The spin label technique showed that the fluidity of the SC lipids was increased with rising temperatures, although the extent of increase was a little above 70°C. The plots of $\tau_c$ versus temperature for the SC indicated breaks at 41 and 68°C. The SC lipids of rat probably exist as the gel, crystalline state below 41°C and the fluid, liquid-crystalline state at temperatures above 68°C. The lipids between 41 and 68°C are thought to coexist as gel- and liquid-crystalline states. This is suggested by the data obtained in X-ray diffraction and FTIR experiments that crystalline and liquid alkyl chains coexist in untreated SC of murine (Knutson et al., 1987; White et al., 1988). Our data on the inflection approximately agreed with the data obtained by Knutson et al. (1985), who found lipid transitions near 40 and 70°C for desiccated samples of hairless mouse SC. These breaks in the plots closely resemble the results (39–40°C and 63–64°C) obtained with hairless rat SC reported previously (Ogiso et al., 1996, 1997). Thus, the rapid penetration of TD at 50°C was ascribed to permeation through the intercellular lipids being partially melted and less viscous, and the hydrophilic routes partly opened.

The increased fluidity of the SC lipids should affect the diffusion of drug in the skin. However, the break of the Arrhenius plots of $K_p$ was not observed in this experiment, being opposite to the results from the plots of $\tau_c$ versus temperature. This suggests that the penetration of TD through the skin would mainly depend on the temperature of the SC and that the effect of increased fluidity of the SC lipids on the penetration would be relatively small between 25 and 50°C, although it must be considered that the SC temperature was slightly lower than that used in the experiment.

In order to find a simple and possible method for the enhancement of percutaneous absorption, the effect of heating on the in vivo percutaneous absorption was estimated using a heat patch. However, a notable result was not obtained in this study. The skin temperature below the transdermal system was 36°C when the heat patch was applied, being slightly higher than skin temperature (32°C) without the patch, and the temperature of 36°C was only maintained for 3 h. Therefore, if a much more efficient heating method (e.g. at 43–45°C for several hours) was used for the transdermal system, the percutaneous absorption would improve dramatically.

In conclusion, increasing temperatures resulted in increased penetration of both TD hydrochloride and the free base forms through the FS and SC sheet. The fluxes of the free base form via the SC sheet were slightly higher than those of the hydrochloride form at each temperature. The Arrhenius plots of $K_p$ gave straight lines for both FS and SC sheet, and the activation energies for permeation calculated were low values. The spin label mobility measured by ESR was increased with rising temperatures. The plots of $\tau_c$ versus temperature indicated two breaks at about 41 and 68°C. Additionally, the plots of $1/\tau_c$ versus $K_p$ also yielded a straight line, indicating that the penetration of TD was largely affected by the disorder of the intercellular lipids.

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