Effect of Thermodynamic Activity on Skin Permeation and Skin Concentration of Triamcinolone Acetonide

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Effects of thermodynamic activity and the state (solution/suspension) of triamcinolone acetonide (TA) on skin permeation and concentration were physicochemically and kinetically analyzed. Permeation of TA through a silicone membrane, hairless rat skin (full-thickness skin or stripped skin) or a three-dimensional cultured human skin model (LSE-high) was determined and a permeability coefficient (*P*), partition coefficient (*K*) , diffusion coefficient (*D*) and steady-state flux (*J*) were calculated. The resulting *J* values proportionally increased with an increase in the TA activity in the drug solution and similar *P*, *K* and *D* values were obtained independent of the TA state (solution/suspension) in all membranes except for full-thickness hairless rat skin. On the other hand, the TA permeation through full-thickness hairless rat skin with the 1000 μg/ml suspension was higher than that expected judging by the thermodynamic acidity of TA. Higher *D* and *P* values were also obtained in the skin permeation of TA from the 1000 μg/ml suspension. Morphological observation of the skin surface by scanning electron microscope (SEM) showed the presence of TA solids in the hair follicles after application of the TA suspension. These results suggest that dissolved TA may be permeated predominantly through the stratum corneum, but that solid TA may be passed through the hair follicles to enter the dermis. The present physicochemical and kinetic analysis provides useful information to develop topical steroid formulations.

Key words thermodynamic activity; steroid; topical formulation; skin permeation; skin concentration; diffusion coefficient

Studies on topical formulations containing steroidal drugs were started in 1952 by Goldman *et al.*,[1] who firstly used topical formulations containing a steroid, cortisone acetate, to treat skin diseases. Dumas and Scholtz reported typical concentration dependence of steroidal drugs on the pharmacological effect against psoriasis.[2] The effect of topical steroids is closely related to the steroidal concentration at the skin disease site. The skin concentration is usually determined by partition and diffusivity of the steroid into and through the skin. The calculated concentration is sometimes more useful than the directly observed skin concentration.[3] The partition and diffusion properties are affected by the thermodynamic activity and state (solution/suspension) of steroids, as well as the barrier function of the skin. Generally, the thermodynamic activity of drugs shows their escaping tendencies from formulations.[4] Skin permeation is a transport phenomenon from a high activity-site to a low activity-site, indicating that skin permeation occurs due to an activity difference.[5] Thermodynamic activity of drugs must be considered when designing topical[6—8] and cosmetic formulations.[9] Most studies on steroidal drugs focused on their pharmacological effects,[1,2,10,11] but not on steroid disposition in the skin.[11,12] There are very few studies, however, on the effect of thermodynamic activity and state (solution/suspension) of steroids in topical formulations on skin permeation and skin disposition.

A medium strength steroid, triamcinolone acetonide (TA), was selected as a model drug in the present study. The effects of thermodynamic activity and TA state were evaluated for the TA concentration in the stratum corneum and viable epidermis and dermis. One-layered and two layered models were used to analyze the membrane permeation and concentration of TA by Fick's law of diffusion. Distilled water was used as a formulation vehicle to simplify evaluate the effect of thermodynamic activity and TA state.

Experimental

Materials and Experimental Animals A powder of triamcinolone acetonide (TA) (MW; 434.5, mp; 290 °C, Log P; 2.53, solubility in water at 32 °C; 22.05±0.05 μg/ml, and solubility parameter; 9.45 (cal/cm^3)$^{1/2}$) was purchased from Wako Pure Chemical Industries, Ltd. (Chuo, Osaka, Japan). A disposable syringe filter unit (DISMIC®-13HP 0.20 μm) was purchased from Toyo Roshi Kaisha, Ltd. (Bunkyo, Tokyo, Japan). Other chemicals and solvents were of reagent grade or HPLC grade and used without further purification. A silicone membrane (LTC-S1-75, thickness of 75 μm) was a kind gift from Lintec Co., Ltd. (Itabashi, Tokyo, Japan). A three-dimensional cultured human skin model (LSE-high: TMLSE-003) was purchased from Toyobo Co., Ltd. (Kita, Osaka, Japan). Male hairless rats (WBM/ILA-Ht, 8—9 weeks-old, body weight: 230—260 g) were obtained from the Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animals (Fukaya, Saitama, Japan). The animal feeding and experiments were conducted according to the ethical committee at Josai University.

Preparation of Triamcinolone Acetonide Aqueous Solution and Suspension TA aqueous solution (5, 10 μg/ml, pH; 6.3), saturated solution (22.05 μg/ml, written as 22 μg/ml hereafter, pH; 6.4) and suspended solution (30, 1000 μg/ml, pH; 6.5) were prepared with distilled water.

***In Vitro* Skin Permeation Experiments** Male hairless rats were fixed on their backs after anesthesia with an intraperitoneal (i.p.) injection of sodium pentobarbital, and hair on the abdomen was shaved using an electric shaver. The skin was excised and excess fat was trimmed off. Stripped skin from the hairless rat was prepared by 20 consecutive tape-strippings of the stratum corneum and stripped LSE-high was obtained by forceps according to our previous study.[13] The excised abdominal skin from the hairless rat (full-thickness skin and stripped skin), LSE-high (full-thickness skin and stripped skin) and silicone membrane were set in a side-by-side diffusion cell with an effective permeation area of 0.95 cm^2. The test solution (TA solution or suspension, 3.0 ml each) was applied to the epidermal side, and pH 7.4 phosphate buffered saline (PBS) (3.0 ml) was applied to dermal side. The diffusion cells were kept at 32 °C with a water jacket connected to a water bath. The donor and receiver cells were agitated using a magnetic stirrer and stirrer bar. At predetermined times, an aliquot (600 μl) was withdrawn from the dermal side and the same volume of fresh PBS was added to keep the volume constant. In addition, the amount of TA retained in the skin was also determined after the permeation experiment as follows. The skin sample was removed, and the skin surface was cleaned off a few times with ethanol (99.5%) and once with PBS. The obtained skin sample was kept at −20 °C until analysis. The frozen skin was finely cut and homogenized with 5 ml of PBS on ice. This skin homogenate was used to determine the TA

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concentration.

Determination of Triamcinolone Acetonide Each 600-μl sample from the *in vitro* skin permeation experiment or skin homogenate was mixed with the same volume of internal standard for HPLC determination (1.0 μg/ml of methyl 4-hydroxybenxoicate in chloroform) or LC/MS determination (1.0 μg/ml of prednisolone in chloroform). Then, the chloroform layer (500 μl) was used after centrifugation (18800×***g***, 5 min, 4 °C). The chloroform solution was evaporated by nitrogen gas purge, and reconstituted with 50 μl of water : acetonitrile (65 : 35). An aliquot (20 μl) was injected into the HPLC or LC/MS. The HPLC system consisted of a pump (LC-20AS; Shimadzu, Chukyo, Kyoto, Japan), a column (CAPCELL PAK C18 UG120 5 μm, 4.6×250 mm; Shiseido, Chuo, Tokyo, Japan), an auto-injector (SIL-20A; Shimadzu), a UV detector (SPD-20A; Shimadzu), and an analysis system (LC solution; Shimadzu). The mobile phase used distilled water : acetonitrile (65 : 35) and the flow rate was 1.0 ml/min. Detection was performed at UV 240 nm. The LC/MS system consisted of an atmospheric pressure chemical ionization/ion trap mass spectrometer (LCQ DECA XP plus, Thermo Fisher Scientific K.K., Yokohama, Kanagawa, Japan), a pump (paradigm MS4; AMR, Meguro, Tokyo, Japan), an auto-injector (CTC HTS-PAL; AMR), and a column (Hypersil GOLD 3 μm, 2.1×50 mm; Thermo Fisher Scientific K.K.). The mobile phase used an A : B solution (65 : 35) (A; acetonitrile : distilled water : formic acid=90 : 10 : 0.1, B; acetonitrile : distilled water : formic acid=2 : 98 : 0.1) and the flow rate was 0.2 ml/min. Detection was performed in the positive ion mode.

Scanning Electron Microscope Observation of the Hairless Rat Full-Thickness Skin Surface after Application of the Triamcinolone Acetonide Suspension The TA suspension (1000 μg/ml) was applied to full-thickness skin on hairless rats, and the suspension was immediately removed from the skin surface. Then, the skin was excised and conducted with a metal coating. The skin surface was observed in a scanning electron microscope (SEM) (S-3000N, Hitachi, Chiyoda, Tokyo, Japan).

Theoretical

Drug Thermodynamic Activity Steady-state flux of a drug through skin , J, is expressed using the thermodynamic activity of the drug in a formulation, A_v, as follows:

$$J = \frac{K \cdot C_0 \cdot D}{L} = \frac{\gamma_v \cdot C_0 \cdot D}{\gamma_s \cdot L} = \frac{A_v \cdot D}{\gamma_s \cdot L} \quad (1)$$

where K, C_0, D, γ_v and γ_s are the partition coefficient, initial concentration in the formulation, diffusion coefficient, activity coefficient in the formulation and that in the skin barrier of the drug, and L is thickness of the skin barrier, respectively. Equation 1 is Fick's 1st law of diffusion. J is proportional to the chemical potential, μ, so Eq. 1 can be replaced by the following Nernst–Planck equation.

$$J = -KC_0 u \frac{d\mu}{dx} \quad (2)$$

where u is drug mobility, and this is a proportional constant between the permeability coefficient, $P(=KD/L)$ and the driving force to move the drug through the membrane, f (*i.e.*, $P=uf$).[6] Thus, the Nernst–Planck equation is equivalent to Fick's law. The chemical potential, μ, of a unionized drug can be expressed under a constant temperature and constant pressure as follows:

$$\mu = \mu^0 + RT \ln X \quad (3)$$

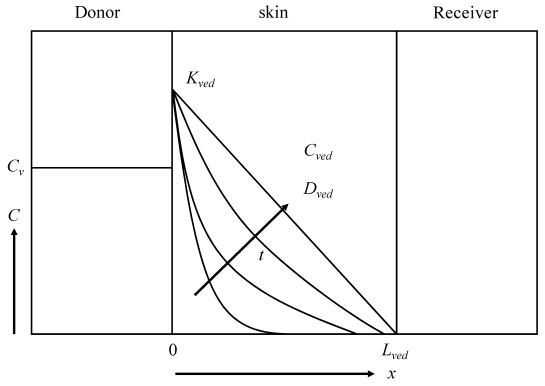
where μ^0 is the standard chemical potential, R is the gas constant, T is the absolute temperature, and X is the drug molar fraction. When the drug is solid (powder, crystal) and no solvent is found around the drug, X reaches unity (=1.0). Chemical potential μ_{solid} must be equal to μ^0 in this situation. The chemical potential of a drug in a regular solution, μ_{liquid} becomes,

$$\mu_{liquid} = \mu^0 + RT \ln A_v' \quad (4)$$

The parameter A_v' is also an activity like A_v, although the unit of A_v' is a molar fraction. When a suspended solution is used in formulation, A_v' reaches unity and then $\mu_{liquid}=\mu_{solid}=\mu^0$. On the other hand, when a regular solution is used, $A_v'<1$ and μ_{liquid} is less than μ_{solid}. Under a constant temperature, μ^0 can be determined for the fixed drug. Thus, J is a function of only A_v'. When using suspended solutions, A_v' is unity-independent of vehicles and the skin permeation rate is the same independent of the vehicles as explained by T. Higuchi.[4]

Analysis of Membrane and Skin Permeation by the Difference Method According to Fick's 2nd Law Permeation profiles of TA through a silicone membrane, rat skin and LSE-high were analyzed by Fick's 2nd law of diffusion. The analytical method was shown in our previous study.[14,15] A one-layered model was used for the silicone membrane and stripped skin (hairless rat, porcine and LSE-high), and a two-layered model was for the full-thickness skin. Figures 1a and b illustrate typical concentration-distance profiles through one-layered and two-layered membrane models, respectively.

a) One-layered Diffusion Model for Stripped Skin



b) Two-layered Diffusion Model for Intact Skin

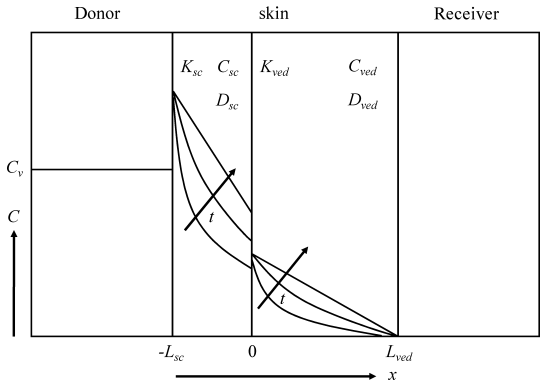


Fig. 1. Typical Concentration-Distance Profiles in One-Layered (a) and Two-Layered (b) Membrane Diffusion Models during Membrane Permeation of Triamcinolone Acetonide (Infinite Dose System)

Abbreviations C, C_v, C_s, C_{ved}: TA concentration, x: position of the membrane, t: time after starting the permeation experiment, L_{ved}, L_{sc}: thickness, D_{ved}, D_{sc}: diffusion coefficient, K_{ved}, K_{sc}: partition coefficient.

Results and Discussion

Silicone Membrane Permeation of Triamcinolone Acetonide from Solution and Suspension Effects of thermodynamic activity and state of TA on the TA permeation through a silicone membrane were evaluated. Three different TA solutions (TA conc.; 5, 10, 22 μg/ml) or two TA suspensions (30, 1000 μg/ml) were applied to the silicone membrane. The time course of the membrane permeation was followed and the permeation parameters of TA, K, D, P and J, were calculated by the TA permeation profiles.

Figure 2a shows the time course of the cumulative amount of TA that was permeated through the silicone membrane, and Table 1 summarizes the calculated permeation parameters. As shown in Fig. 2a, the cumulative amounts of TA permeated over 24 h were 0.11 ± 0.03 μg/cm^2 after application of a 5 μg/ml TA solution, 0.17 ± 0.06 μg/cm^2 for a 10 μg/ml

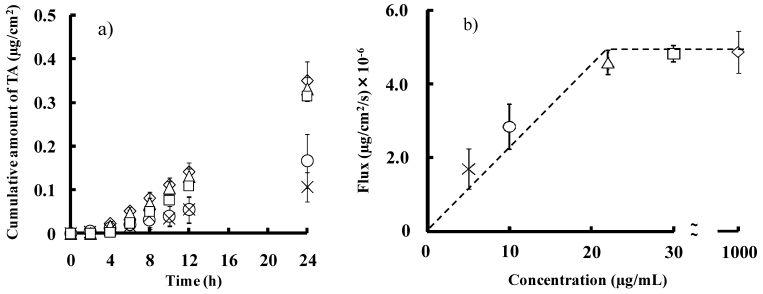


Fig. 2. Triamcinolone Acetonide Permeation through a Silicone Membrane

(a) Cumulative amount of triamcinolone acetonide permeated through the silicone membrane. (b) Correlation of applied concentration and flux of triamcinolone acetonide. Symbols: 5 (×), 10 (○) and 22 (△) triamcinolone acetonide solutions and 30 (□) and 1000 μg/ml (◇) triamcinolone acetonide suspensions. Each value shows the mean±S.E. (n=4—8).

Table 1. Calculated Parameters Using Permeation Data through the Silicone Membrane

C_v (μg/ml)	5 μg/ml (solution)	10 μg/ml (solution)	22 μg/ml (saturation)	30 μg/ml (suspension)	1000 μg/ml (suspension)
A_v'	0.23	0.45	1.00	1.00	1.00
K	4.96±3.20	6.89±1.09	2.55±0.36	3.95±0.20	2.47±0.33
D (cm²/s)×10⁻¹⁰	10.8±4.1	4.40±1.99	6.44±0.91	4.16±0.04	7.62±1.41
P (cm/s)×10⁻⁷	3.39±1.08	2.84±0.61	2.08±0.15	2.19±0.10	2.19±0.26
J (μg/cm²/s)×10⁻⁶	1.69±0.54	2.84±0.61	4.58±0.33	4.82±0.26	4.82±0.57

Table 2. Calculated Parameters by Permeation Data through Full-Thickness or Stripped Hairless Rat Skin

C_v (μg/ml)	5 μg/ml (solution)	10 μg/ml (solution)	22 μg/ml (saturation)	30 μg/ml (suspension)	1000 μg/ml (suspension)
A_v'	0.23	0.45	1.00	1.00	1.00
K_{sc}	10.7±3.8	10.3±1.3	13.3±4.9	11.5±6.0	15.1±3.7
K_{ved}	5.82±1.49	11.4±1.7	9.11±2.23	6.66±2.71	8.00±1.07
D_{sc} (cm²/s)×10⁻¹¹	2.85±0.09	2.65±0.07	2.18±0.20	3.23±0.62	3.99±0.55
D_{ved} (cm²/s)×10⁻⁸	9.44±0.97	7.02±0.75	8.94±1.57	12.9±0.4	10.1±0.3
P_{sc} (cm/s)×10⁻⁷	2.02±0.69	1.82±0.29	1.75±0.43	1.82±0.54	3.42±0.18
P_{ved} (cm/s)×10⁻⁶	8.65±1.47	12.4±0.91	10.3±1.9	9.58±2.28	11.2±1.0
J_{sc} (μg/cm²/s)×10⁻⁶	1.01±0.35	1.82±0.23	3.86±0.94	4.00±1.20	7.55±0.39
J_{ved} (μg/cm²/s)×10⁻⁵	4.33±0.74	12.4±0.91	22.6±4.1	21.1±5.0	24.7±2.3

solution, 0.33±0.03 μg/cm² for 22 μg/ml saturated solution, 0.31±0.01 μg/cm² for a 30 μg/ml suspension, and 0.35±0.04 μg/cm² for a 1000 μg/ml suspension. Figure 2b shows the relation between the J value (flux) and TA application concentration. These permeated amounts and J values were proportionally increased by the TA concentration in the solution applied to the silicone membrane, but became constant over the saturation level (22 μg/ml) of TA in water. Similar K, D and P values were obtained independent of thermodynamic activity of TA in the solutions (Table 1). These profiles could be simply analyzed by the thermodynamic activity of TA in the aqueous solution.

Hairless Rat Skin Permeation of Triamcinolone Acetonide from Solution and Suspension Next, hairless rat skins (full-thickness skin and stripped skin) were used to evaluate the effect of thermodynamic activity and state of TA on skin permeation. The same sets of TA solutions were used as in the permeation experiments with the silicone membrane. The thickness of the stratum corneum and viable epidermis and dermis, L_{sc} and L_{ved}, were fixed to 15 and 585 μm, respectively, as in our previous study.[13] Parameters, K_{ved}, D_{ved}, P_{ved} and J_{ved} were obtained by the permeation data through stripped skin, and K_{sc}, D_{sc}, P_{sc} and J_{sc} were those through full-thickness skin. Figures 3a and b show the time course of the cumulative amount of TA that was permeated through full-thickness skin and stripped skin, respectively, and Table 2 summarizes permeation parameters which were obtained using a difference equation of Fick's law of diffusion. Three kinds of TA solutions (TA conc.; 5, 10, 22 μg/ml) and two TA suspensions (30, 1000 μg/ml) were applied. The cumulative amounts of TA through stripped skin over 8 h were 1.04±0.20 μg/cm² for a 5 μg/ml TA solution, 2.50±0.22 μg/cm² for a 10 μg/ml solution, 4.74±1.11 μg/cm² for a 22 μg/ml saturated solution, 4.68±0.86 μg/cm² for a 30 μg/ml suspension, and 5.61±0.86 μg/cm² for a 1000 μg/ml suspension (Figs. 3b, d). These amounts of TA permeated were proportionally increased with an increase in the thermodynamic activity of TA (Fig. 3b). Similarly, J_{ved} was proportional to the activity of TA in the applied solution on the stripped skin (Fig. 3d). Each K_{ved}, D_{ved} and P_{ved} showed the similar values (Table 2).

On the other hand, the cumulative amounts of TA permeated through full-thickness hairless rat skin 24 h after starting the *in vitro* permeation experiment were 0.07±0.03 μg/cm² for 5 μg/ml, 0.09±0.01 μg/cm² for 10 μg/ml, 0.18±0.05 μg/cm² for 22 μg/ml, and 0.23±0.06 μg/cm² for 30 μg/ml. These

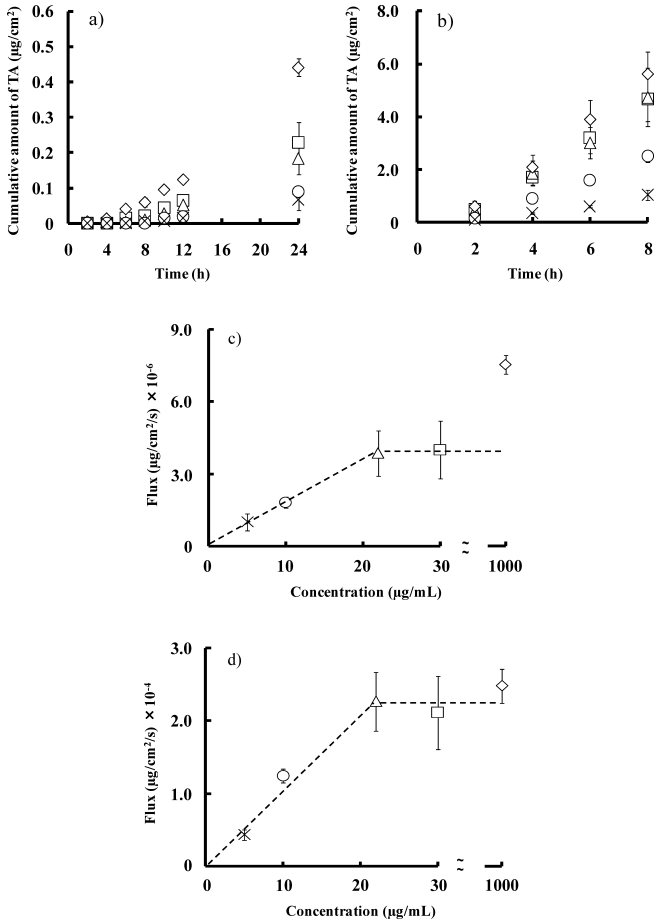


Fig. 3. Triamcinolone Acetonide Permeation through Full-Thickness (a, c) and Stripped Hairless Rat Skin (b, d)

(a, b) Cumulative amount of triamcinolone acetonide permeated through full-thickness (a) and stripped (b) hairless rat skin. (c, d) Correlation of applied concentration and flux of triamcinolone acetonide after application on full-thickness (c) and stripped (d) hairless rat skin. Symbols: the same in Fig. 2. Each value shows the mean±S.E. (n=4—12).

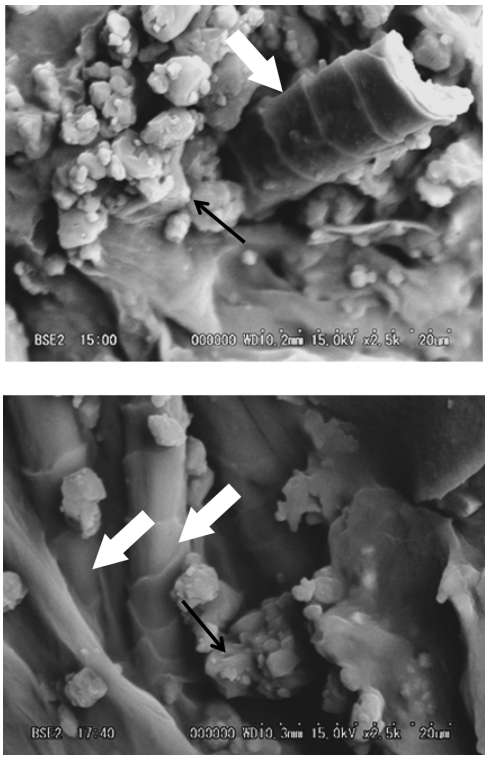


Fig. 4. Two Typical Images from SEM Observation of Full-Thickness Skin Surface in Hairless Rats after Application of Triamcinolone Acetonide Suspension

Large white arrows show hair shafts, and small black arrows show TA crystals. TA solid was 5—10 μm in diameter, as shown in Fig. 4.

values were proportional to the thermodynamic activity of TA in the solution. However, the cumulative amount of TA 24 h after application of 1000 μg/ml TA (suspension) was 0.44±0.02 μg/cm^2, which was about twice that compared to 22 μg/ml or 30 μg/ml TA, although these three groups showed the same TA activity (Fig. 3b). P_{sc} (3.42×10^{-7} cm/s), D_{sc} (3.99×10^{-11} cm^2/s) and J_{sc} (7.55×10^{-6} μg/cm^2/s) for 1000 μg/ml TA suspension were also about twice that of those for a TA saturated solution (22 μg/ml) ($P_{sc}=1.75\times10^{-7}$ cm/s, $D_{sc}=2.18\times10^{-11}$ cm^2/s, and $J_{sc}=3.86\times10^{-6}$ μg/cm^2/s) (Table 2, Fig. 3c). TA solid itself may damage the stratum cornium. To confirm the effect of TA solid, skin surface was observed by scanning electron microscope (SEM). As result, no skin abrasion was observed on the TA applied site.

These results suggest that the state of TA, as well as its thermodynamic activity in the vehicle, affect the full-thickness skin permeation properties (P_{sc}, D_{sc}, J_{sc}) of TA. Theoretical analysis using diffusion theory suggests that unexpectedly higher values were due to a higher effective diffusion coefficient in the stratum corneum, D_{sc}. Since TA diffusion through the stratum corneum must be unchanged independent of the concentration or state (solution or suspension) of TA, the drug solid or crystal in the suspended solution may penetrate into the hair follicles and dissolve, increasing the D_{sc} value. Barrier function in the stratum cornium is markedly higher than that in the hair follicle as well as viable epidermis and dermis. Thus, contribution of hair follicles must be high to the permeation of full-thickness skin and not so high to that of stratum corneum-stripped skin.

Scanning Electron Microscope Observation of the Skin Surface in Hairless Rats after Application of Triamcinolone Acetonide Suspension Unexpectedly higher skin permeation of TA from the 1000 μg/ml TA suspension may have been due to direct migration of solid TA into the hair follicles, as suggested by theoretical analysis of TA skin permeation profiles. In the literature, a major contribution of hair follicles to the skin permeation of steroids has already been reported.[16—18] Next, the hairless rat skin surface was observed in a SEM after application of a TA suspended solution at a concentration of 1000 μg/ml. As shown in the two pictures in Fig. 4, many solid TA particles were concentrated around the hair follicles. Thus, hair follicles must be considered as a drug permeation pathway, in addition to the stratum corneum.

LSE-High Permeation of Triamcinolone Acetonide To evaluate the skin permeation of TA, especially solid TA, LSE-high, a three-dimensional cultured human skin model was used. A TA solution (10 μg/ml) or suspension (1000 μg/ml) was applied. The thickness of the stratum corneum, L_{sc}, and viable epidermis and dermis, L_{ved}, of LSE-high were 15 and 285 μm, respectively, according to our previous study.[13,15] Figures 5a and b show the time course of the cumulative amount of TA permeated through full-thickness and

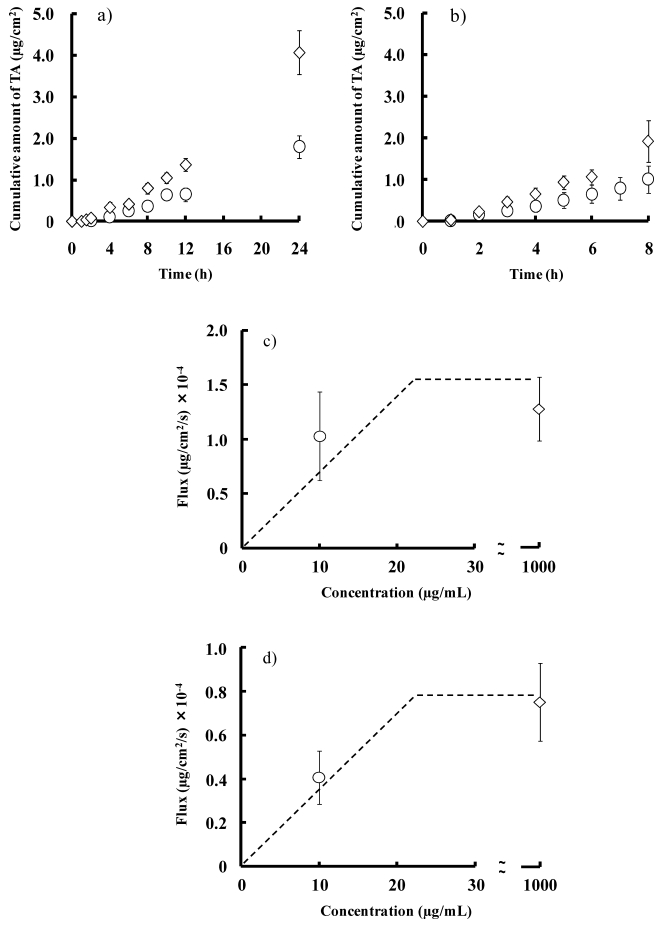


Fig. 5. Triamcinolone Acetonide Permeation through LSE-High (a, c) and Stripped LSE-High (b, d)

(a, b) Cumulative amount of triamcinolone acetonide permeated through LSE-high (a) and stripped (b) LSE-high. (c, d) Correlation of applied concentration and flux of triamcinolone acetonide after application on the LSE-high (c) and stripped LSE-high (d). Symbols: the same as in Fig. 2. Only 10 μg/ml triamcinolone acetonide solution and 1000 μg/ml triamcinolone acetonide suspended solution were used. Each value shows the mean±S.E. (n=3—9).

stripped LSE-high, respectively, after application of 10 μg/ml TA solution and 1000 μg/ml TA suspension. Table 3 summarizes the obtained permeation parameters. The cumulative amounts of TA permeated through full-thickness LSE-high over 24 h were 1.80±0.27 μg/cm^2 after application of 10 μg/ml TA solution and 4.07±0.53 μg/cm^2 after application of 1000 μg/ml TA suspension, and those through stripped LSE-high over 8 h were 1.01±0.32 μg/cm^2 after application of 10 μg/ml TA solution and 1.92±0.50 μg/cm^2 for the 1000 μg/ml suspension. Both the J_{ved} and J_{sc} were proportional to the thermodynamic activity of TA independent of the state of TA in the solutions (Figs. 5c, d). Permeation parameters (K_{ved}, K_{sc}, D_{ved}, D_{sc}, P_{ved} and P_{sc}) for LSE-high were similar between the two application concentrations (Table 3). These profiles were similar to those through the silicone membrane, but not hairless rat full-thickness skin. This may be explained by the presence or absence of hair follicles.

Skin Concentration of Triamcinolone Acetonide Table 4 summarizes skin levels of TA 24 h after application on hairless rat full-thickness skin and 8 h after application on stripped skin. The skin samples were withdrawn after the skin permeation experiments. The skin concentration of TA was increased with an increase in TA activity in the donor solution under saturation, but extremely high skin levels of TA were observed for the application groups of TA suspension, especially on the full-thickness skin. These results suggest that state of TA, as well as its thermodynamic activity in the vehicle, affect the skin concentration of TA.

Table 3. Calculated Parameters by Permeation Data through Full-Thickness or Stripped LSE-High

C_v (μg/ml)	10 μg/ml (solution)	22 μg/ml (suspension)
A_v'	0.45	1.00
K_{sc}	45.2±10.0	53.7±7.0
K_{ved}	4.57±0.81	4.00±1.34
D_{sc} (cm^2/s)×10^{-10}	2.87±0.66	1.59±0.30
D_{ved} (cm^2/s)×10^{-8}	2.26±0.32	2.84±0.46
P_{sc} (cm/s)×10^{-6}	10.3±4.1	5.77±1.33
P_{ved} (cm/s)×10^{-6}	4.07±1.21	3.38±0.80
J_{sc} (μg/cm^2/s)×10^{-4}	1.03±0.41	1.27±0.29
J_{ved} (μg/cm^2/s)×10^{-5}	4.07±1.21	7.46±1.76

Table 4. Skin Concentration of Triamcinoone Acetonide 24 h after Application on the Hairless Rat Full-Thickness Skin and 8 h after Application on the Hairless Rat Stripped Skin

C_v (μg/ml)	Full-thickness skin (μg/g)	Stripped skin (μg/g)
5	0.67±0.11	5.63±0.64
10	0.77±0.05	12.4±1.6
22 (saturation)	1.54±0.10	21.3±1.2
30	3.56±1.14	29.9±7.2
1000	23.5±4.6	36.5±5.6

Conclusion

Thermodynamic activity of TA in the applied solution greatly affected the TA permeation through a silicone membrane or LSE-high. No effect was found on the membrane permeation due to the state (solution or suspension) of TA. These phenomena were proved by T. Higuchi more than four decades ago. On the other hand, TA permeation through full-thickness hairless rat skin after TA suspension was much higher than that estimated by the thermodynamic activity of TA in the vehicle, although the skin permeation of TA from the regular solution was proportional to its activity. The state of TA especially increased the D_{sc} value, which was established with theoretical analysis of the skin permeation profile using Fick's law of diffusion. Morphological evaluation of the skin surface suggested that solid TA probably dissolved and penetrated into the hair follicles. Thus, the thermodynamic activity and state of a steroid must be taken into account when developing suitable topical formulations and dosage regimens for steroids, especially when determining concentration of the steroid in the formulations.

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