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In vitro percutaneous absorption of prednisolone derivatives based on solubility parameter

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Abstract

A series of ester derivatives of prednisolone (I–VII) with various lipophilicities was synthesized to investigate their in vitro percutaneous absorption and their distribution and accumulation in the skin. Experimental findings were supported with theoretical calculations, using the solubility parameter as an indicator of the lipophilicity of the derivatives. The solubility parameters of the derivatives were well correlated with their partition coefficients in an octanol–water system and increase in lipophilicity was well correlated with the theoretical and experimental values of drug distribution into the skin. Drug distribution into the skin was increased, but the drug diffusion rate was decreased with increasing derivative lipophilicity. These findings indicated that accumulation of the derivatives in the skin increased with increasing derivative lipophilicity. This suggests that retention of prednisolone derivatives in the skin is a function of their solubility parameters and that derivatives are partitioned in the skin relative to their lipophilicities. © 1997 Elsevier Science B.V.

Keywords: Prednisolone derivatives; Solubility parameter; In vitro percutaneous absorption; Lipophilicity; Distribution; Accumulation

1. Introduction

Topical steroids are very useful for the treatment of dermatological disorders, e.g. atopic dermatitis. Recently, however, consecutive application of steroids has elicited concern regarding not only the possibility of local adverse reactions but also that of systemic side effects. In an attempt to deal with these concerns, the antidrug steroids have been developed. These are molecularly modified steroids with few adverse reactions which are converted to weak steroids following their metabolism in skin (Brattsand and Sarnstrand, 1989; Flynn and Yalkowsky, 1972). However, it is thought that these drugs have failed to reduce adverse reactions to a satisfactory extent.

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We have examined several steroid prodrugs which are retained in the skin for a long period of time, are expected to exert long-term effects and to a small extent may be absorbed into the body. Increasing the lipophilicity of a drug is frequently required to increase its accumulation in the skin. Hence, our investigation examines derivatives which will increase the steroid lipophilicity.

Since the stratum corneum is thought to be a lipophilic layer, it is composed mainly of keratin protein and lipids, improvement of drug lipophilicity is expected to enhance the process of drug dissolution into lipophilic material of the stratum corneum, resulting in increased drug distribution into the skin. Numerous reports and reviews are available on the relationship between lipophilicity and percutaneous absorption of drugs. It has been reported that a parabolic correlation of convex type exists between lipophilicity and the permeability coefficient of drugs, and that there exist optimal values for the partition coefficients of drugs. Numerous studies have been published concerning the prediction of percutaneous absorption of drugs on the basis of their physicochemical properties, e.g. partition coefficient and molecular weight (Schuplein et al., 1969; Tayer et al., 1991; Rougier et al., 1987; Lin et al., 1995). The majority of these studies were concerned with the improvement of permeability. To achieve this, the rate of diffusion in the skin, especially in the stratum corneum, which possesses high resistance against percutaneous absorption, must be improved. However, in dermatological topical application of drugs, e.g. steroids or antifungal agents, it is thought that enhancement of drug distribution and accumulation in the stratum corneum is more important for drug efficacy and safety than the enhancement of percutaneous drug absorption. In this investigation, to examine the effect of steroid lipophilicity on distribution into the skin, we compared the theoretical and experimental values of drug distribution using the method proposed by Waranis et al. (1987), with the solubility parameter as an indicator of the lipophilic parameter. We synthesized several prednisolone derivatives with different degrees of lipophilicity and used them for an in vitro percutaneous absorption study.

2. Materials and methods

2.1. Materials

Prednisolone was purchased from Sigma (St. Louis, MO). Heptane sulfonic acid (special grade), acetic acid, propionic acid, butyric acid, valeric acid, hexanoic acid, enantoic acid and octanoic acid were purchased from Wako Pure Chemical Industries, (Osaka, Japan). Diethyl sebacate (DES) was purchased from Nikko (Tokyo, Japan). Other reagents were of analytical grade. Hairless mice were purchased from Kyudo (Tosu, Japan).

2.2. Synthesis of prednisolone derivatives and measurement of their partition coefficients

The derivatives of prednisolone were synthesized in methylene chloride by condensation of prednisolone with aliphatic carboxylic acids using dicyclohexylcarbodiimido, with 4-dimethyl aminopyridine as a catalyzer. The partition coefficients (log P) of these derivatives in an octanol–water system were calculated from the retention time, which was determined by HPLC using the method proposed by Miyake and Terada (1982). The conditions of HPLC and the measurement of retention time is described in Section 2.6. We used dibenzyl (log $P_{\text{octanol/water}} = 4.81$) and benzene (log $P_{\text{octanol/water}} = 2.99$) as reference substances for the estimation of log P (Tomita et al., 1984).

2.3. Solubility parameters of hairless mouse skin

The solubility parameter of hairless mouse skin was determined using the method proposed by Liron and Choen (1984). The in vitro percutaneous absorption experiment was conducted at 25 ± 2°C as follows: the abdominal skin of a female hairless mouse (8 weeks old) was excised and fixed between the donor and the receptor chambers of a diffusion cell similar to that used by Loftsson and Bodor (1981). The volume of the receptor chamber was 5 cm^3 and the effective diffusion area was 0.785 cm^2. The receptor phase facing the dermis side of the excised skin was filled with saline and 0.5 ml of alkanoic acids (2–8

carbons) was applied to the stratum corneum side. This 0.5 ml portion of receptor solution was periodically removed for analysis and replaced with the same volume of fresh receptor solution.

Alkanoic acids in the receptor solution were assayed using the procedure described in Section 2.6. However, the following mobile phases were used: 10 mM $HClO_4$ for the assay of acetic acid and propionic acid; a mixture (1:3) of acetonitrile and 10 mM $HClO_4$ for the assay of butyric acid and valeric acid; and a mixture (1:1) of acetonitrile and 10 mM $HClO_4$ for the assay of hexanoic acid, enantoic acid and octanoic acid. The UV detection wavelength was 210 nm and the flow rate was 0.8 ml min^{-1}.

2.4. Skin permeability experiment with derivatives of prednisolone

A mixture (1:1) of DES and ethanol (DES/EtOH) was used as the solvent to prepare test solutions, i.e. 1% solutions of the prednisolone derivatives. These test solutions were used to conduct a skin permeability test similar to that described for solubility parameter measurement. However, the receptor phase was filled with a mixture of ethanol and water (8:2) and the test solution was applied to the stratum corneum side. Drugs in the collected receptor solution were assayed by HPLC.

2.5. Assay of drugs in the skin

After 48 h of the in vitro percutaneous absorption study, the skin was removed from the cells, wiped with dry gauze and then twice with gauze soaked in ethanol. The skin was washed with 1 ml of 50% (v/v) aqueous methanol solution and wiped with a dry gauze. The skin was then cut into pieces with scissors and homogenized after the addition of 2 ml methanol. The homogenized solution was centrifuged at 3000 rpm for 10 min. The agents in the supernatant were determined by the analytical method described in Section 2.6.

2.6. Method of analysis

The derivatives were assayed by HPLC. The HPLC system was comprised of an LC-6A system (Shimadzu, Kyoto, Japan) including SCL-6A system controller, LC-6A pump, SIL-6A autoinjector, SPD-6AV detector, CTO-6A column oven and C-R6A integrator. An ODS-120T® column (4.6 × 150 mm; Tosoh, Tokyo, Japan) was used for analysis and a temperature of 40°C was maintained. The UV detection wavelength was 254 nm. The mobile phase was a mixture of acetonitrile and water (35:65). A flow rate of 1.0 ml min^{-1} was maintained. Propylparaben was used as an internal standard.

2.7. Analysis of data

According to the regular solution theory proposed by Davis (1970), the mole fraction solubility ($X_{v,i}$) of compound i in the solvent v in relation to the thermodynamic activity compound (a_i) and the activity coefficient r is expressed by the following formulas:

$$a_i = rX_{v,i} \tag{1}$$

$$\ln r = \{V_i\phi_v^2(\delta_v - \delta_i)^2\}/RT \tag{2}$$

Eq. (1) can be converted to the following:

$$\ln X_{v,i} = \ln a_i - \{V_i\phi_v^2(\delta_v - \delta_i)^2\}/RT \tag{3}$$

where V_i and δ_i denote the molar volume and the solubility parameter, respectively, for the compound i, while ϕ_v and δ_v denote the partial molar volume and the solubility parameter of the solvent v, repectively.

The partition coefficient P between octanol and water is expressed as follows:

$$P = (X_{v,i})_{\text{octanol}}/(X_{v,i})_{\text{water}} \tag{4}$$

Therefore, $(\phi_v)_{\text{octanol}}$ is almost equal to $(\phi_v)_{\text{water}}$, and Eq. (3) can be expressed as follows:

$$\log P = \frac{V_i\phi_v^2}{2.303\,RT}[(\delta_w - \delta_i)^2 - (\delta_o - \delta_i)^2] \tag{5}$$

where δ_o and δ_w denote the solubility parameters of octanol and water, respectively.

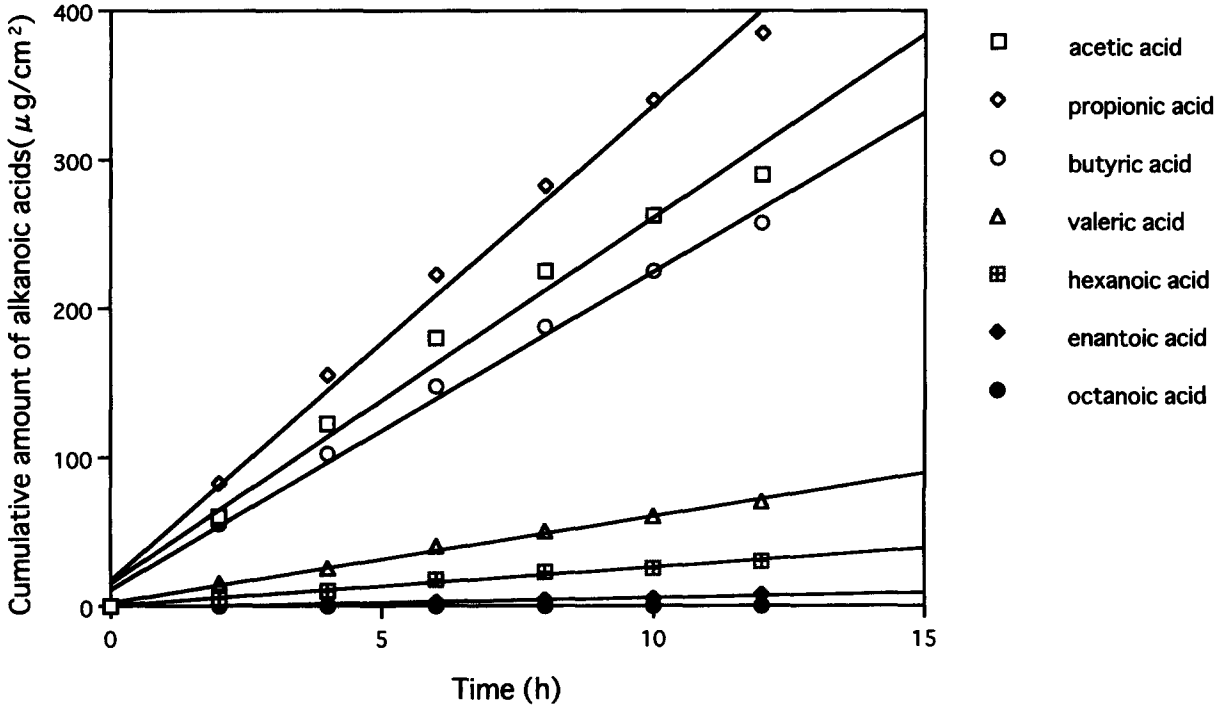


Fig. 1. Percutaneous absorption profiles of alkanoic acids through excised hairless mouse skin.

Similarly, the distribution of the compound i from the vehicle into the skin in the process of percutaneous absorption can be expressed as the ratio between the solubility of the drug i in the vehicle and the solubility of the drug in the skin. Therefore, the parameter K for distribution between the skin and the vehicle under this condition can be expressed as follows:

$$\log K = \frac{V_i \phi_v^2}{2.303\, RT} [(\delta_v - \delta_i)^2 - (\delta_s - \delta_i)^2] \qquad (6)$$

where δ_v and δ_s denote the solubility parameters of the vehicle and skin, respectively. The distribution of drug i into the skin is considered to be dependent on the solubility parameter of the vehicle alone.

3. Results and discussion

3.1. Measurement of the solubility parameter of hairless mouse skin

The cumulative amounts of permeation-time curves for alkanoic acids of 2–8 carbons are shown in Fig. 1. The highest flux was obtained with propionic acid (carbon $n = 3$). The flux decreased with an increasing number of carbons in the alkanoic acid. The relationship between the permeability coefficient (K_p) obtained from these curves and the solubility parameters of the alkanoic acids is shown in Fig. 2. The K_p values formed a parabolic curve of convex type when plotted against the solubility parameter. A solu-

bility parameter of 10.2 yielded the maximum K_p, which suggested that the solubility parameter of hairless mouse skin would be close to this value. This value was slightly higher than 9.7–10, the solubility parameter of pig skin as reported by Liron and Choen (1984). However, this value appears to be correct given the fact that sebaceous glands are poorly developed in hairless mice. Therefore, we used this value (10.2) as the solubility parameter of hairless mouse skin in subsequent steps of the experiment.

3.2. Distribution profile of prednisolone derivatives for in vitro percutaneous absorption

The structural formulas and physicochemical properties of prednisolone prodrugs, which were synthesized systematically by esterifying the OH group at the C-21 position of prednisolone with aliphatic carboxylic acids, are shown in Table 1. All the compounds obtained were white crystalline powders, whose melting points decreased with elongation of their side chains. However, the derivatives with molecular weights above 500 had almost the same melting point. Both the experimental and theoretical values, which were obtained from Eq. (5), of log P in the octanol–water system increased in proportion to the number of carbons in the side chain. This was confirmation that the lipophilicity of these derivatives was enhanced by an increasing carbon number in the side chains. On the other hand, the solubility

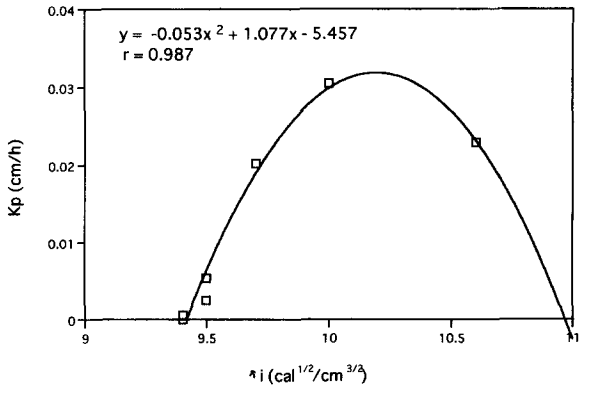


Fig. 2. Correlation of the permeability coefficient (K_p) and the solubility parameter (δ_i) of straight-chain alkanoic acids through excised hairless mouse skin at 25°C.

parameter was decreased with an increase in lipophilicity and a significant negative correlation existed between these two parameters. Therefore, we used the solubility parameter as an indicator of the lipophilicity of the derivatives.

An in vitro percutaneous absorption experiment was subsequently performed with the excised abdominal skin of the hairless mouse to examine the effects of lipophilicity of these derivatives on the distribution and diffusion parameters of the skin. In this experiment, we considered the stratum corneum to constitute the main barrier to drug distribution and retention in the skin. Therefore, suppression of drug metabolism in the skin was required in order that the lipophilic properties of the derivatives themselves might be reflected in the process of percutaneous absorption. Thus, 80% aqueous ethanol was added to the receptor solution and was simultaneously used to maintain the sink condition.

The derivatives of prednisolone were dissolved in DES/EtOH. The permeation profile curve obtained from the results of percutaneous absorption experiments is shown in Fig. 3.

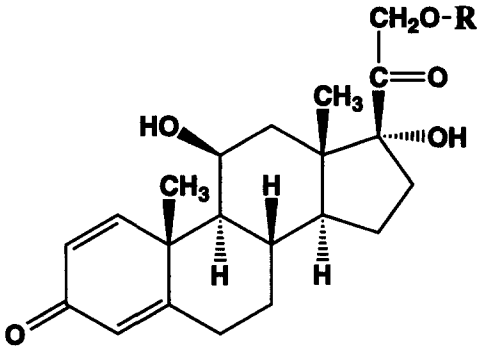
In the one-layer model, the total amount of drugs Q which penetrates through the excised hairless mouse skin in time t from the donor phase at constant concentration C to the receptor phase is given as follows (Komatzu et al., 1986):

$$Q = AKLC\left(\frac{Dt}{L^2} - \frac{1}{6} - \frac{2}{\pi^2}\cdot\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\exp\left(-Dn^2\pi^2t/L^2\right)\right) \quad (7)$$

where A is area of application, K is the partition coefficient, L is thickness of skin and D is the diffusion coefficient. Based on Eq. (7), D and K were determined by the method of nonlinear least squares (Yamaoka et al., 1981).

The permeation parameters obtained from the in vitro percutaneous absorption study are shown in Table 2. The experimental distribution values obtained and the results of plotting the theoretical values obtained from Eq. (6) in terms of the drug solubility parameter are shown in Fig. 4, where δ_v is 11.2. δ_v was obtained by using solubility parameter values of 9.6 for DES, calculated by the method of Fedors (1974), and 12.7 for EtOH

Table 1
Synthesized derivatives of prednisolone 21-esters



Number	Prednisolone 21-ester	R	m.p. (°C)	Mol. wt.	log P [a]	log P [b]	δ_i [c] ($cal^{1/2}$ $cm^{-3/2}$)	V_i [d] (cm^3 mol^{-1})
I	Heptanoate	$-CO(CH_2)_5CH_3$	186–189	472.62	4.08	33.80	12.02	370.2
II	Octanoate	$-CO(CH_2)_6CH_3$	159–161	486.65	4.39	36.23	11.89	386.3
III	Nonanoate	$-CO(CH_2)_7CH_3$	131–134	500.68	4.66	38.57	11.78	402.4
IV	Decanoate	$-CO(CH_2)_8CH_3$	145–147	514.70	5.15	40.99	11.67	418.5
V	Undecanoate	$-CO(CH_2)_9CH_3$	129–132	528.73	5.54	43.39	11.57	434.6
VI	Tridecanoate	$-CO(CH_2)_{11}CH_3$	140–143	556.78	6.02	48.19	11.39	466.8
VII	Pentadecanoate	$-CO(CH_2)_{13}CH_3$	139–141	584.84	6.79	53.02	11.23	499.0

[a] Represents partition coeffcients in octanol–water determined with HPLC method.
[b] Represents partition coeffcients in octanol–water calculated by Eq. (5). $\phi_v = 0.992$, $\delta_w = 23.4$ and $\delta_o = 10.3$ (Barton, 1975) were used for calculation.
[c] Represents solubility parameters calculated by the method of Fedors (1974).
[d] Represents molar volumes calculated by the method of Fedors (1974).

(Barton, 1975). The values for DES/EtOH were then calculated by the method of Chertkoff and Martin (1960) where ϕ_v was almost 1.0. As shown in Table 2 and Fig. 4, distributions of these derivatives into the skin were estimated to be enhanced with an increase in their lipophilicity, while the diffusion coefficients were estimated to decrease with such increases (Fig. 5). In Fig. 4, the difference between experimental and calculated data appears to be caused by being out of the regular solution, and influence of the receptor solution. It was thought that an increase of the diffusion coefficient is due to affinity for the stratum corneum. However, no large changes were noted in K_p over change in lipophilicity. These findings suggested that drugs with high distribution and low diffusion into the skin will show high accumulation in the skin. Therefore, drug concentrations in the skin appear to increase with increases in drug lipophilicity. In fact, the experimental values of drug distribution between vehicle and skin were well correlated with theoretical values. When drug concentrations in the skin were directly measured following the termination of the experiment, highly lipophilic derivatives tended to have higher concentrations in the skin (Table 3). These findings suggest that the stratum corneum plays a major role as a drug reservoir for highly lipophilic drugs and that highly lipophilic derivatives accumulate in the skin more than prednisolone itself. The derivatives lipophilicity, which improves percutaneous absorption kinetics, was confirmed to contribute to drug accumulation in the skin.

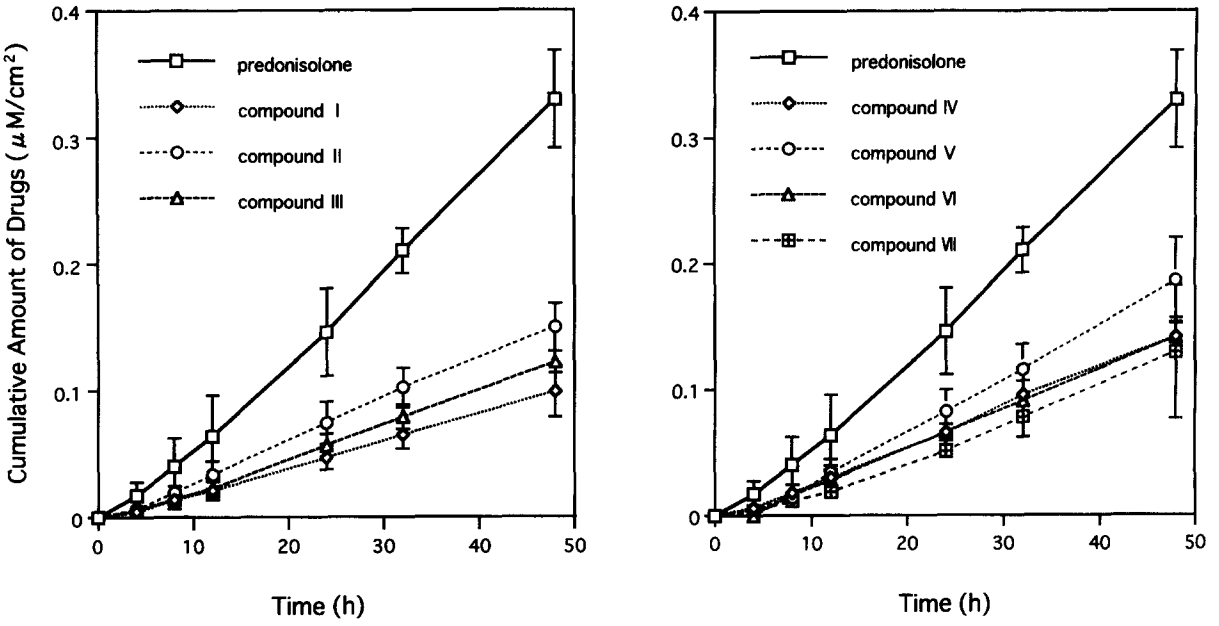


Fig. 3. Percutaneous absorption profiles of prednisolone derivatives in DES/EtOH (1:1) solution through the excised hairless mouse skin. Each symbol and bar represent the mean ± SD of three determinations.

4. Conclusions

The increased lipophilicity of derivatives of prednisolone contributed to their distribution into the stratum corneum of the skin. Although the permeation constants of the prednisolone derivatives were lower than that of prednisolone itself, drug retention in the skin increased and diffusion rate decreased with increase in the lipophilicity of the derivatives. These findings suggested that derivatives of steroids with increased lipophilicity are retained in the skin for a long time, resulting in lower systemic absorption, which may reduce adverse reactions. Furthermore, with regard to the effects of the derivatives on drug accumulation in the skin, both theoretical and experimental values of the drug solubility parameter were in agreement. We therefore consider it possible to predict the degree of accumulation of a series of derivatives in the skin, using the solubility parameter as an indicator of the lipophilic parameter.

Table 2
Permeation absorption parameters of prednizolone derivatives through hairless mouse skin

Drug	D (cm^2 h^{-1})	K	K_p(cm h^{-1})
Compound I	1.37×10^{-4}	0.023	0.77×10^{-4}
Compound II	1.96×10^{-4}	0.023	1.10×10^{-4}
Compound III	0.89×10^{-4}	0.042	0.92×10^{-4}
Compound IV	1.14×10^{-4}	0.039	1.11×10^{-4}
Compound V	0.59×10^{-4}	0.102	1.49×10^{-4}
Compound VI	0.79×10^{-4}	0.084	1.06×10^{-4}
Compound VII	0.42×10^{-4}	0.093	0.98×10^{-4}
Prednisolone	1.59×10^{-4}	0.074	3.14×10^{-4}

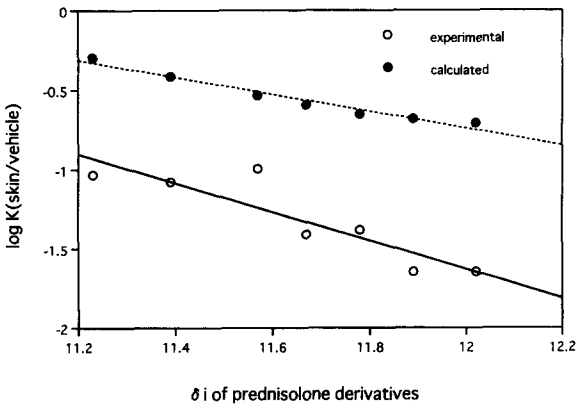


Fig. 4. Comparison between experimental and calculated data in log K (skin/vehicle). Experimental data were obtained from in vitro permeation study through the excised hairless mouse skin and calculated data were obtained from Eq. (6).

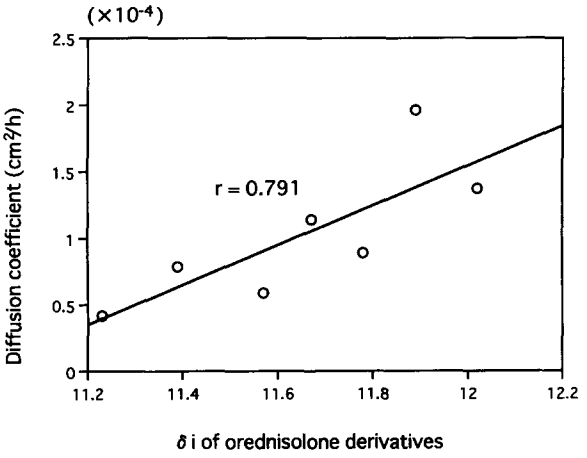


Fig. 5. Correlation of solubility parameters (δ_i) and diffusion coefficients of prednisolone derivatives through excised hairless mouse skin.

Our results suggest that theoretical calculations based on the solubility parameter are useful for molecular design of derivatives for local use, which takes percutaneous absorption kinetics into account.

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Table 3
Amount of prednisolone derivatives in the excised hairless mouse skin

Drug	Amount of drugs in the skin (μM cm^{-2})
Compound I	0.000364 ± 0.000050
Compound II	0.000212 ± 0.000055
Compound III	0.000733 ± 0.000163
Compound IV	0.000594 ± 0.000071
Compound V	0.002388 ± 0.000609
Compound Vl	0.002777 ± 0.001222
Compound VII	0.014047 ± 0.003091

These values represent the mean ± SD for three examinations which were determined after a 48 h permeation study. DES/EtOH mixture (1:1) solution was used as a vehicle.

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