Binding of Prednisolone and Its Ester Prodrugs in the Skin

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Purpose. Skin binding of prednisolone and its esters was investigated in the hairless mouse skin *in vitro*.

Methods. The distribution of the amount of drugs bound in the skin was determined by a skin slicing technique. The model drugs used were prednisolone (PN, M.W. 360) and its esters, senesyonate (PN-C_5, M.W. 442), geranate (PN-C_{10}, M.W. 510), farnesylate (PN-C_{15}, M.W. 578), and geranylgeranate (PN-C_{20}, M.W. 646).

Results. The distribution of bound drug was nonhomogeneous in the skin; the concentration of PN-C_{10} and PN-C_{15} in the skin increased gradually with the distance from the skin surface. The parent drug, PN, however, was hardly bound in the viable skin.

Conclusions. These findings suggest that the prodrugs of prednisolone may prolong the dermal retention of the parent drug and minimize to delivery into the systemic circulation of the prodrug and metabolite.

KEY WORDS: skin binding; percutaneous absorption; prednisolone; prednisolone esters.

INTRODUCTION

It is well established that skin is a physical and biochemical barrier membrane against the entry of foreign substances including drugs (1,2). The barrier capacity of the skin may not be strongly influenced by skin binding (3,4). The bound drug molecules reside in the skin for a significantly longer period of time compared with the dissolved free molecules, have a long time lag before reaching a steady state, and show a reservoir function when the transdermal therapeutic system is removed (5,6). The bound drug may also induce the dermal toxicity due to a high concentration localized in the skin. It is therefore important to elucidate the binding of drug in the skin to improve the bioavailability for systemic and topical application.

In this report, we have investigated skin binding of drugs; the effect of the side chain length of prednisolone esters on skin binding was quantitatively studied in the hairless mouse skin *in vitro*. The effects of the alkyl chain length of the esters on binding capacity and penetration of the parent drug, prednisolone, have been studied by a skin slicing technique (7).

MATERIALS AND METHODS

Materials

Prednisolone (PN) and its esters, senesyonate (PN-C_5, m.p. 124–126°C, purity 99.9%), geranate (PN-C_{10}, m.p. 148–150°C, purity 99.9%), farnesylate (PN-C_{15}, m.p. 155–156°C, purity 99.9%), and geranylgeranate (PN-C_{20}, m.p. 139–140°C, purity 97.0%) synthesized by Kuraray Co. (Kurashiki, Japan) (8) were received from Kuraray Co. and Taihou Pharmaceuticals Co. (Tokushima, Japan).

Figure 1 shows the chemical structures of these compounds. Polyethylene glycol 400 (PEG400), HIVISWAKO® 104 (carboxyl vinylpolymer), potassium dihydrogen phosphate, diisopropylfluorophosphate (DFP), methanol (HPLC grade), and distilled water (HPLC grade) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Female hairless mice (Hr^-/Kud strain, 8 to 10 weeks old) were obtained from Kyudo (Tosu, Japan).

In Vitro Skin Penetration and Metabolism Experiment

Either intact or stripped skin excised from the abdomen of the hairless mouse was mounted between the two half cells of *in vitro* side-by-side permeation systems (the effective volume is 5.0 mL and the effective membrane area is 0.64 cm^2) (6). The stratum corneum was completely removed by stripping 20 times with cellophane tape (Nichiban LP-24, Nichiban, Tokyo, Japan). The temperature in the *in vitro* system was maintained at 37°C, because this research discussed mainly metabolism in skin. A 40% PEG400 solution without drugs (5 mL) was charged in the receptor cell to maintain a sink condition and to achieve measurable concentrations of both prodrugs and the parent drug. A drug suspension in 40% PEG400 solution (5 mL) was then loaded in the donor cell. At predetermined time intervals, 100 μL samples were withdrawn from the receptor cell and assayed for the concentration of prodrugs and the parent drug by HPLC. We found previously that the skin metabolism of a prednisolone ester was inhibited in the presence of PEG400 (9). In the present study, however, the effect of PEG400 concentration on skin metabolism is not investigated because the prodrug concentrations appeared in the receptor solution are lower than the detectable level at PEG400 concentrations less than 20%. Metabolism due to enzymes leached out from the skin was also previously found to be negligible (11).

Reservoir Effects in the Skin

The intact or stripped skin of hairless mouse was clamped in the modified Franz cell (the effective volume is 11.5 mL and the effective membrane area is 1.77 cm^2) (10). In this experiment, a drug containing hydrogel was used as the donor

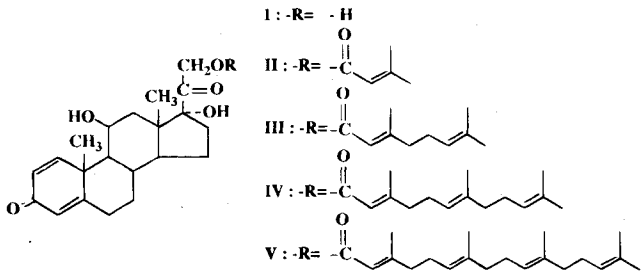

Fig. 1. Chemical structure of prednisolone (PN) and its prodrugs used. I: PN (M.W. 360), II: PN-C_5 (M.W. 442), III: PN-C_{10} (M.W. 510), IV: PN-C_{15} (M.W. 578), V: PN-C_{20} (M.W. 646).

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formulation; PN-C_5 (1.07 w/w %) or PN-C_{10} (1.23 w/w %) were well mixed into the blank hydrogel (HIVISWAKO® 104, Taihou Pharmaceuticals Co.) just prior to the experiment. A 40% PEG400 solution without drug (11.5 mL) was charged into the receptor compartment, then the hydrogel (0.2 mL) was loaded onto the skin. Since the penetration completely reached steady-state in the skin of hairless mouse within 24 hrs and 12 hrs for intact and stripped skin, respectively (10), both the hydrogel on the skin and the receptor solution were completely removed after 24 hrs and 12 hrs, respectively. A 40% PEG400 solution without drug was immediately added into the receptor compartment and thereafter the amount of drug released from the skin was measured to evaluate the reservoir capacity of the skin. PEG400 may have some effects on the reservoir (binding) capacity of skin. However, little is known at this stage of research with respect to the effect of PEG400 concentration on skin binding.

Distribution of Binding Capacity in the Skin

The distribution of binding capacity in the skin was investigated using a skin slicing technique (7). After the abdominal intact skin was frozen in TISSUE-TEK® O.C.T. (Optimum Cutting Temperature) compound (polyvinyl alcohol and polyethylene glycol, Milles Sankyo Co., Tokyo, Japan) at −20°C, the skin was consecutively sliced from the surface by 20 μm thickness with microtome Cryostat (Milles Sankyo Co.). Two consecutive slices were placed into 2 mL of 40% PEG400 solution with a known amount of PN esters, then incubated at 37°C for 48 hrs. In order to inhibit skin metabolism, 1 mM DFP was added to the drug solution. The sample (600 μL) was withdrawn from the drug solution. In order to remove protein and O.C.T. compound used for skin preparation, 200 μL of ethanol was added to the sample (600 μL) and the mixture was centrifuged at 12,000 rpm (MRX-152, TOMY, Tokyo, Japan) for 10 min. The supernatant, the mixture of drug solution and ethanol, was obtained by filtration (0.45 μm chromatodisk 13A, Kurabou Ltd., Osaka, Japan). The drug concentration in the supernatant was assayed by HPLC. On the other hand, the sliced skins under the equilibrium condition were washed twice with a 500 μL fresh 40% PEG400 solution to remove drugs on the skin surface. The sliced skins were then placed in 1 mL ethanol and incubated at 37°C for 24 hrs for total extraction. The ethanol solution was analyzed for the drug concentration by HPLC. When the drug in the sliced skins was extracted three times by fresh ethanol, a negligible amount appeared in the second and third extractions.

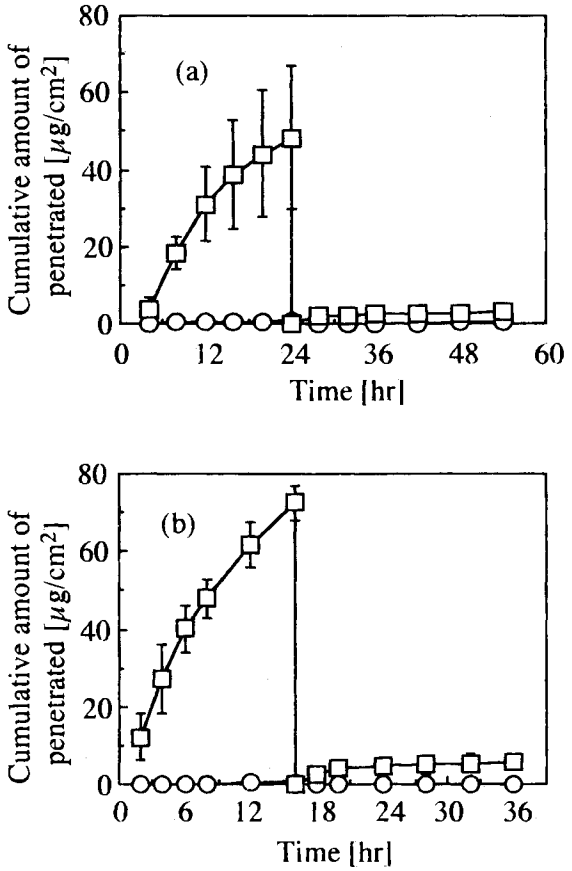

Fig. 2. Penetration and release profiles of PN-C_5 (□) and its metabolite (PN, ○) across intact skin (a) and stripped skin (b).

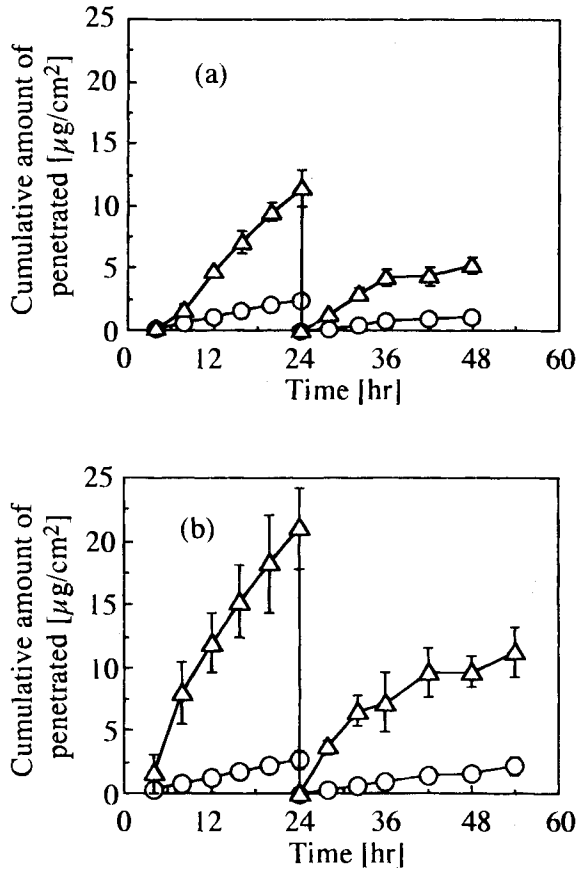

Fig. 3. Penetration and release profiles of PN-C_{10} (△) and its metabolite (PN, ○) across intact skin (a) and stripped skin (b).

Assay Methods

The assay procedure for PN and PN-C_{15} was previously reported (6). PN-C_5, PN-C_{10} and PN-C_{20} were assayed by a modified HPLC method; the mobile phase was the mixture of methanol and 20 mM KH_2PO_4 buffer solution. The methanol

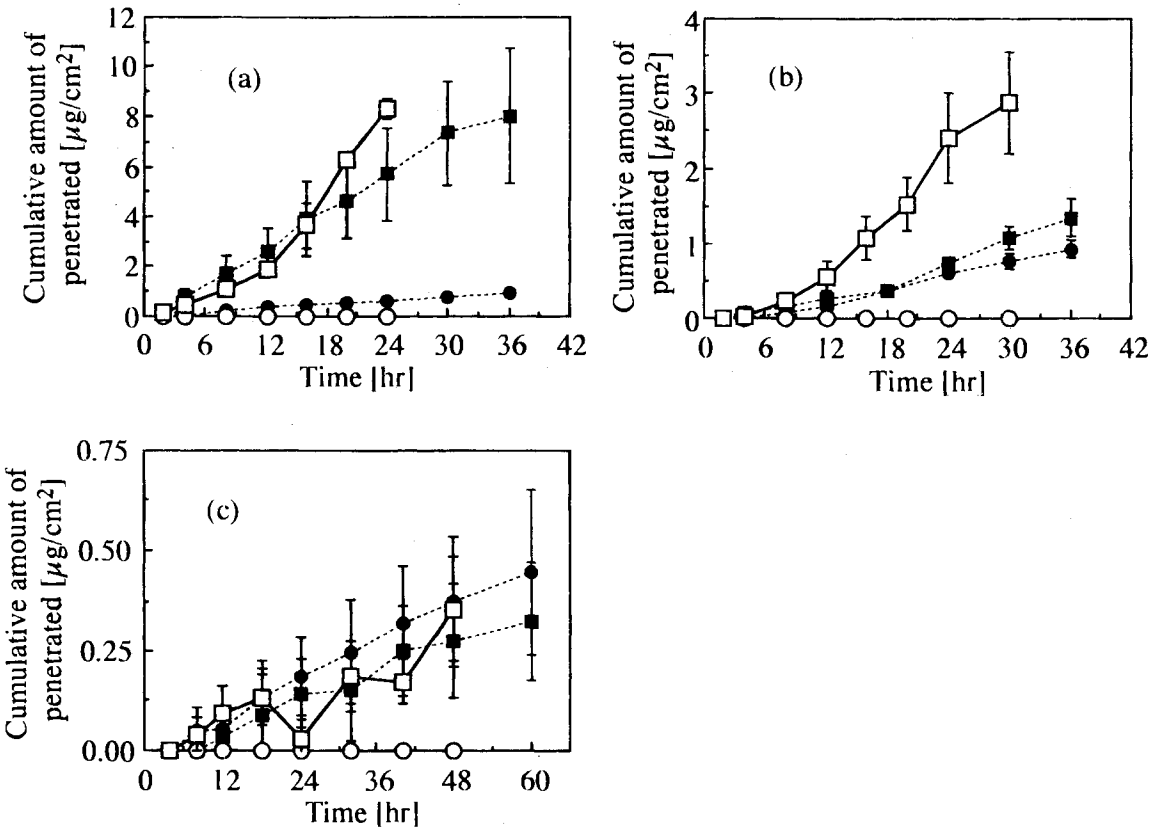

Fig. 4. Effect of diisopropylfluorophosphate (DFP) on time courses of PN-C_5 (a), PN-C_{10} (b), and PN-C_{15} (c) across stripped skin. □: PN esters with DFP, ○: metabolite with DFP, ■: PN ester without DFP and ●: metabolite without DFP.

fraction varied for each PN ester to separate the PN esters and their metabolite PNs completely.

RESULTS AND DISCUSSION

In Vitro Skin Penetration and Metabolism Experiment

The steady-state penetration rate of the parent drug (PN) and its esters is summarized in Table I. PN-C_5, PN-C_{10} and PN-C_{15} were extensively metabolized to PN in hairless mouse skin. However, PN-C_{20} was little penetrated across the intact skin and stripped skin. The steady-state penetration rate in the stripped skin shows that the fraction of the metabolite to PN-C_5, PN-C_{10}, and PN-C_{15} is, respectively, 12%, 51%, and 65% of the total flux of PN ester and its metabolite. These findings may indicate that the increase in the alkyl chain length decreases partitioning to the viable skin due to the lipophilicity of PN esters ($K_{o/w}$ is in the range of 9050.5 ± 4561.6, PN-C_5, to 28075.2 ± 8752.8, PN-C_{15} (11)) and decreases in the diffusion coefficient for the prodrugs with the increased molecular weight.

Table I. Steady-State Penetration Rate of Prednisolone (PN) and Its Ester Prodrugs Across Hairless Mouse Skin

		dQ/dt [μg/cm²/hr]	
		Prednisolone or metabolite (PN)	PN ester
Intact skin	Prednisolone	0.112±0.034	—
	PN-C_5 ester	0.002±0.001	0.024±0.005
	PN-C_{10} ester	0.015±0.004	0.025±0.007
	PN-C_{15} ester	0.009±0.003	0.007±0.004
	PN-C_{20} ester	N.D.	N.D.
Stripped skin	Prednisolone	8.458±3.384	—
	PN-C_5 ester	0.027±0.003	0.251±0.062
	PN-C_{10} ester	0.030±0.003	0.041±0.006
	PN-C_{15} ester	0.008±0.004	0.007±0.003
	PN-C_{20} ester	N.D.	N.D.

Note: Each values are the average ±S.D. (n=3). N.D.: Not Detected.

The steady-state penetration rates of PN-C_5 across intact skin and stripped skin were, respectively, 3.4 and 35.9 times higher than those of PN-C_{15}. This finding may suggest that the viable skin provides a significant barrier capacity for highly lipophilic compounds such as PN-C_{15}.

Reservoir Effect in the Skin

Figure 2 shows the penetration and release profiles of PN-C_5. In the release experiment (≥ 24 hrs for the intact skin, ≥ 16 hrs for the stripped skin), the release amount of PN-C_5 reached a plateau within 4 hrs. The metabolite PN hardly appeared in the receptor solution during the entire period of the experiment. Since the time lag to reach steady-state penetration for drug molecules with the molecular weight of 200–300 is about 4 hrs unless skin binding occurs (10), the present finding may suggest that the skin binding of PN-C_5 is negligible.

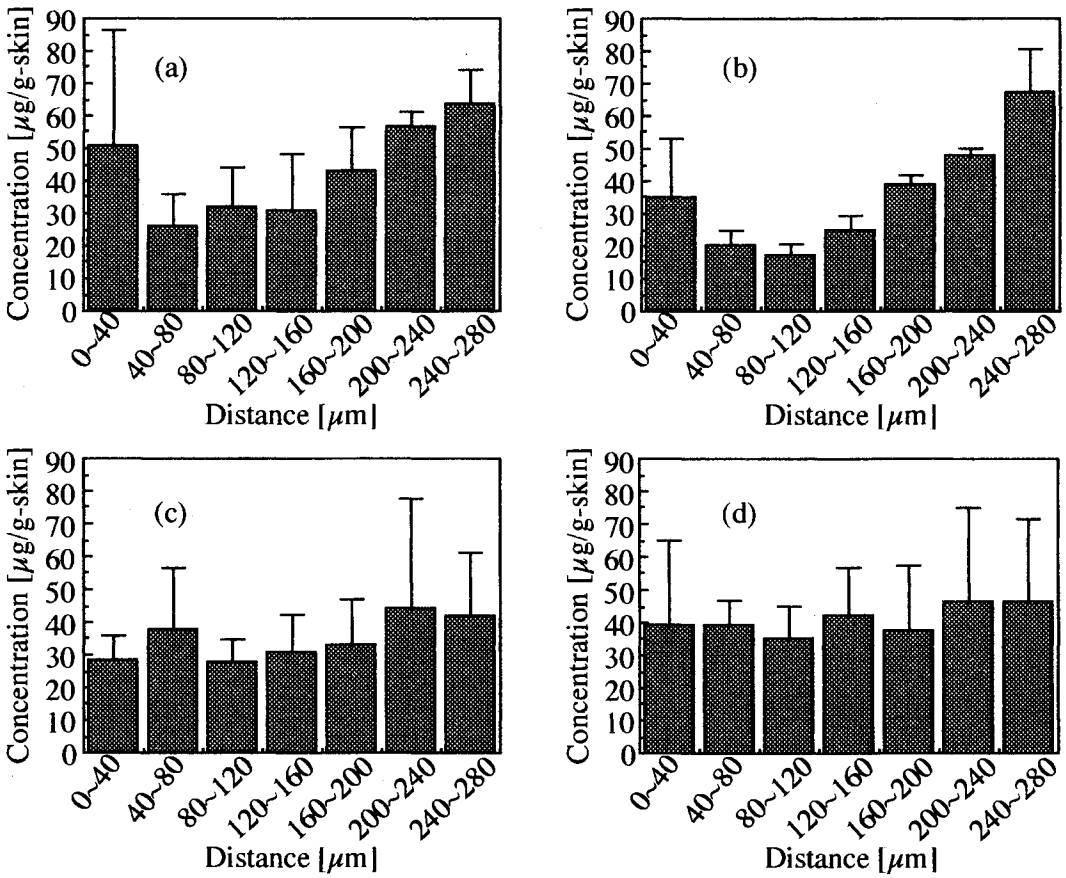

Fig. 5. Distribution of the equilibrium concentration of prednisolone and its prodrugs in the skin. (a): PN-C_{15}, (b): PN-C_{10}, (c): PN-C_5 and (d): PN.

Figure 3 shows the penetration and release profiles of PN-C_{10}. In the release experiment ($\geq$ 24 hrs for the intact and the stripped skin), the steady-state penetration rate of PN-C_{10} decreased beyond 36 hrs. Interestingly, however, the metabolite was continuously released for many hours, indicating that a part of PN-C_{10} bound in the skin was continuously metabolized to PN, and the intact prodrug was also released. The present finding suggests that PN-C_{10} was bound not only in the stratum corneum but in the viable skin as well.

We found previously that water content in the skin was decreased in the presence of PEG400 (9). Therefore, PEG400 may have some effects on the reservoir (binding) capacity of skin. However, little knowledge was obtained with respect to the effect of PEG400 concentration on skin binding, because PN esters could hardly dissolve in low concentrations of PEG400 solution.

Inhibition of Bioconversion in the Skin

Guzek et al. reported that the hydrolysis of salicylate esters in the skin was inhibited by DFP and phenylmethylsulfonylfluoride (12). Ghosh and Mitra also reported that 70–80% of hydrolyzing activity for 5-iodo-2′-deoxyuridine ester resulted from cholinesterase (13). We therefore employed DFP as a cholinesterase inhibitor for PN esters. Figure 4 shows penetration profiles of PN esters and their metabolite PN across stripped skin. The steady-state appearance rate of the metabolite of PN-C_5, PN-C_{10}, and PN-C_{15} were, respectively, 0.026 $\pm$ 0.005, 0.030 $\pm$ 0.003, and 0.008 $\pm$ 0.000 $\mu g/cm^2/hr$. However, the hydrolysis of PN ester was completely inhibited by adding 1 mM DFP in both donor and receptor solutions. The amount of PN ester penetrated was approximately equal to the total amount of the PN ester and its metabolite together with no inhibition. The effect of DFP on the penetration of PN ester was insignificant except for inhibiting the hydrolysis. Consequently, the complete inhibition of metabolism by DFP may suggest that the enzymes responsible for the skin bioconversion of PN esters is cholinesterase.

Table II. The Intrinsic Partition Coefficient (Kd) Between the Sliced Skin and the Medium of Prednisolone (PN)

Distance from skin surface [μm]	Kd [mL/g-skin]
0~40	0.91
40~80	0.67
80~120	0.69
120~160	0.80
160~200	0.69
200~240	0.67
240~280	0.78

Table III. The Values of the Model Parameters (Kd, Pt and b) for PN-C_{15} Ester in Langmuir Type Binding Equation

Distance from skin surface [μm]	Kd [mL/g-skin]	Pt [μg/g-skin]	b [g-skin/μg]
0~40	201.71	—	—
40~80	128.87	42.2	0.06
80~120	76.81	67.8	0.03
120~160	133.21	25.5	0.3
160~200	97.35	59.6	0.09
200~240	116.32	81.2	0.02
240~280	227.34	50.9	0.02

Spatial Distribution of Skin Binding

Figure 5 shows the relationship between the amount of drug extracted from sliced skin layers and the distance from the skin surface. Since the outermost layer (0–40 μm samples) contains the stratum corneum, we neglected these layers to analyze the concentration distribution of drug in the viable skin.

The amount of PN-C_{15} (Fig. 5 (a)) extracted from the skin layers increased gradually toward the deep layers of skin, indicating that the binding capacity of skin increases with the distance from the skin surface. Esumi *et al.* found previously that the concentration of PN-C_{15} in rat skin was 1.5 times higher beneath the site of application than that of PN, when both drugs were applied transdermally *in vivo* (14). However, the blood concentration of PN-C_{15} was about 17% of that of PN (14). The cumulative amount of PN-C_{15} excreted in urine and feces was about 7% of PN (14). The present study may suggest that PN-C_{15} would bind in the skin *in vivo* and minimize the systemic circulation.

The distribution profile of the concentration extracted from sliced skins for PN-C_{10} (Fig. 5 (b)) was similar to that for PN-C_{15} (Fig. 5 (a)), indicating a similar binding capacity for PN-C_{10} and PN-C_{15} in hairless mouse skin. On the other hand, the amount of PN-C_5 (Fig. 5 (c)) extracted was constant throughout the sliced skin layers, indicating that the binding capacity for PN-C_5 is homogeneous in the viable epidermis. PN was also uniformly distributed in the viable skin (Fig. 5 (d)). This uniform distribution of PN was found to be independent of the drug concentration, suggesting that the binding capacity for PN was constant throughout the sliced skin layers.

If we assume that PN and PN-C_{15} follows Langmuir-type binding (15), the total equilibrium concentration in the sliced skin, C_t [μg/mL] can be represented by:

$$C_t = K_d C_d + \frac{P_t b K_d C_d}{1 + b K_d C_d} \quad (1)$$

Table IV. The Values of the Redetermined Model Parameters in Langmuir Type Binding Equation

Distance [μm]	Kd [mL/g-skin]	Pt [μg/g-skin]	b [g-skin/μg]
40~80	110.57	38.6	1.13
80~120	110.57	18.6	0.78
120~160	110.57	56.3	0.04
160~200	110.57	69.9	0.04
200~240	110.57	81.7	0.03

where C_d [μg/mL] is the medium concentration, K_d [mL/g-skin] is the intrinsic partition coefficient between the sliced skin and the medium, b [g-skin/μg] is the association constant, and P_t [μg/g-skin] is the total concentration responsible for binding.

The amount of PN extracted from the sliced skin was found to be proportional to the medium concentration. The intrinsic partition coefficients, K_d, was constant throughout the sliced skin layers except the surface layer of 0–40 μm including the stratum corneum as summarized in Table II. This finding may indicate that PN hardly binds in the skin. The values of parameters in equation (1) for PN-C_{15} were summarized in Table III. K_d in the 0–40 and 240–280 μm layers was approximately twice that for the other layers. This is probably due to the effect of stratum corneum in the surface layer and the subcutaneous fat or O.C.T. compound used for skin preparation in the deeper layer, respectively. K_d in the middle layers was almost constant, indicating the solubility of PN-C_{15} in the viable skin was constant. The model parameters in equation (1), P_t and b, for PN-C_{15} were summarized in Table IV. P_t; the total amount of binding in the sliced skin increased gradually with increasing distance from the surface of the skin. The amount of binding in the 200–240 μm layer was approximately 4.4 times larger than in the 80–120 μm layer. Therefore, the binding capacity of PN-C_{15} is not uniformly distributed in the viable skin.

CONCLUSIONS

The effects of the alkyl chain length of PN esters on binding capacity and penetration of the parent drug have been investigated in hairless mouse skin by a skin slicing technique and penetration/metabolism experiment. The binding capacity in skin of PN-C_{10} and PN-C_{15} increased gradually with an increase in the distance from the skin surface. However, PN and PN-C_5 were uniformly distributed throughout the viable skin. The steady-state penetration rate of PN esters decreased with increasing alkyl chain length, and PN-C_{20} was little penetrated into skin. The fraction of metabolite to esters penetrated across skin increased with increasing alkyl chain length.

The present experiment indicated that binding capacity and the rate of penetration of PN esters in the skin could be controlled by the length of alkyl chain of prodrugs. PN-C_{10} and PN-C_{15} bind strongly in the dermis for a long time, suggesting that the ester prodrugs of prednisolone with alkyl chains may prolong the dermal retention of the parent drug and minimize to delivery into the systemic circulation of the prodrug and metabolite.

REFERENCES

1. A. Pannatier, P. Jenner, B. Testa, and J. C. Etter. *Drug Metabolism Reviews* **8**(2):319–343 (1978).
2. P. Liu, W. I. Higuchi, W. Song, T. Kurihara-Bergstrom, and W. R. Good. *Pharm. Res.* **8**:865–872 (1991).
3. E. Menczel, D. Bucks, H. Maibach, and R. Wester. *Arch. Dermatol. Res.* **275**:403–406 (1983).
4. J. R. J. Baker, R. A. Christin, P. Simpson, and A. W. White. *Br. J. Dermatol.* **96**:171–178 (1977).
5. B. Mollgaard, and A. Haelgarrd. *Int. J. Pharm.* **15**:185–197 (1983).
6. T. Hikima, K. Yamada, A. Yamashita, and K. Tojo. *Drug Deliv. Syst.* **7**:453–457 (1992).

7. T. Hikima, K. Yamada and K. Tojo. *Proc. 22nd Intern. Symp. Control. Rel. Bioact. Mater.*, #3144, 1995, p. 720–721.
8. Y. Esumi, Y. Jin, K. Hori, K. Ichige, M. Komuro, H. Kakuo, T. Shindo, A. Kageyu, and K. Sudo. *Jpn. Pharmacol Ther.* **20**:4411–4420 (1992).
9. T. Hikima and K. Tojo. *Drug Deliv. Syst.* **8**:291–295 (1993).
10. T. Hikima and K. Tojo. *Yakuzaigaku.* **55**:122–126 (1995).
11. T. Hikima. Ph. D. thesis, Kyushu Institute of Technology (1996).
12. D. B. Guzek, A. H. Kennedy, S. C. McNeill, E. Wakshull, and R. O. Potts. *Pharm. Res.* **6**:33–39 (1989).
13. M. K. Ghosh and A. K. Mitra. *Pharm. Res.* **7**:251–255 (1990).
14. Y. Esumi, Y. Jin, K. Hori, K. Ichige, M. Komuro, H. Kakuo, T. Shindo, A. Kageyu, and K. Sudo. *Jpn. Pharmacol. Ther.* **20**:151 (4421)–168 (4438) (1992).
15. W. R. Vieth. Oxford University Press, New York, 1988.