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Analysis of in vivo skin penetration enhancement by oleic acid based on a two-layer diffusion model with polar and nonpolar routes in the stratum corneum

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Abstract

The enhancement effect of oleic acid on the in vivo percutaneous absorption of mannitol, 6-mercaptopurine, and butylparaben was evaluated in rats. A deconvolution method was applied to estimate skin permeation from urinary excretion profiles of drugs. Oleic acid markedly increased skin permeation of mannitol and 6-mercaptopurine, while it slightly increased the permeation of butylparaben at the low dose but decreased it at the high dose. To elucidate the mechanism of enhancement of skin permeation by oleic acid, the estimated in vivo penetration profiles were analyzed based on a two-layer skin diffusion model with polar and nonpolar routes in the stratum corneum. With respect to the polar route, oleic acid did not change the diffusion parameter but increased the partition parameter, suggesting that it increased hydration of the stratum corneum and enlarged the aqueous pore pathway. On the other hand, oleic acid increased both the diffusion and partition parameters of 6-mercaptopurine in the nonpolar route. The mechanism of action of oleic acid was compared to those determined in a previous in vitro study using guinea pig skin. It was revealed that the basic mechanism of oleic acid for enhancing drug permeation was comparable between the present and previous studies, and that the difference in the apparent enhancement effect of oleic acid was due to the in vitro-in vivo difference in the degree of hydration of the stratum corneum and the diffusion length of the dermis layer.

Keywords: In vivo percutaneous absorption; Penetration enhancer; Oleic acid; Diffusion model; Urinary excretion; Deconvolution

1. Introduction

Oleic acid is a well-known percutaneous penetration enhancer and its mechanism of action has been studied from various aspects such as infrared spectroscopy (Golden et al., 1987; Francoeur et al., 1990; Ongpipattanakul et al., 1991) and permeation study (Cooper, 1984; Barry and Bennett, 1987). In our previous study, we examined the effect of oleic acid on in vitro skin permeation of three drugs with different lipophilicities (Koyama et al., 1994). Analysis of

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the penetration profiles based on a two-layer skin diffusion model with polar and nonpolar routes in the stratum corneum (Yamashita et al., 1993a) revealed that oleic acid mainly affects the nonpolar route of the stratum corneum and increases both diffusivity and partitioning of drugs.

In addition to these in vitro examinations, generally, the action of an enhancer should be elucidated in the in vivo system especially from a practical viewpoint. In a comparative study between the in vitro and in vivo cases, we demonstrated the fundamental mechanism of the enhancement effect of 1-geranylazacycloheptan-2-one (GACH) in the in vivo system which was apparently different from that in the in vitro system (Yamashita et al., 1994). Although it has been shown in some reports that oleic acid accelerates in vivo drug absorption (Yamada et al., 1987; Green et al., 1988), there are few systematic studies on the enhancement effect of oleic acid in the in vivo system.

In this investigation, we selected three drugs with different lipophilicities and performed in vivo percutaneous absorption experiments in the presence of oleic acid. Further, we analyzed their penetration profiles based on a two-layer skin model with parallel routes in the stratum corneum and then compared the mechanism of action of oleic acid with our previous in vitro results.

2. Materials and methods

2.1. Materials

Oleic acid (Nissan Extra Oleic 99) was kindly supplied by Nippon Oil and Fats Co., Ltd, Japan. Mannitol and 6-mercaptopurine were obtained from Nacalai Tesque Inc., Japan, and butylparaben from Tokyo Chemical Ind., Japan. [^{14}C]Mannitol and [^{14}C]butylparaben were purchased from Daiichi Pure Chemicals, Japan. 6-[^{14}C]Mercaptopurine was obtained from Commissariat A L'Energie Atomique, France.

2.2. In vivo skin absorption experiment

The in vivo absorption experiment was performed as previously reported (Yamashita et al., 1994). Under anesthesia induced by intraperitoneal injection of 1 g/kg urethane, the abdominal hair of a male Wistar strain rat weighing about 200 g was removed using electric hair clippers and a glass half-chamber (effective area 3.14 cm^2) was attached to the abdominal surface using a cyanoacrylate adhesive (Aron Alpha A, Sankyo Co., Japan). The skin surface was pretreated with 0.2 ml ethanolic solution dissolving 0, 15.7, 62.7 μmol of oleic acid. 6 h later, ethanol remaining in the donor cell was removed by evaporation with a hair dryer. Then, the urinary bladder was cannulated with vinyl tubing (i.d. 0.50 mm, o.d. 0.90 mm, Dural Plastics and Engineering, Australia) and a 1 ml aliquot of drug solution was placed in the glass chamber. Urine was collected every 15 min for 4 h by injecting 0.2 ml of 0.9% NaCl solution through the tubing twice before each sampling time. At the end of the experiment, the drug remaining in the donor cell was recovered and the skin was excised. After solubilization of the urine and skin samples with Soluene-350 (Packard Instrument, IL), the radioactivity was evaluated using a liquid scintillation counter (LSC-5000, Beckman, Japan).

In vivo absorption through tape-stripped skin was also evaluated. After the hair was removed with hair clippers, the abdominal skin was stripped 15 times with adhesive tape (Scotch® tape, Sumitomo 3M, Japan). After a glass chamber was attached to the surface, the skin was pretreated with 0.9% NaCl solution for 6 h. 6 h later, the solution was blotted using tissue paper. The drug was applied in the form of 0.9% NaCl solution to reduce osmotic convective flow. The remainder of the experimental procedures were the same as that for intact skin.

2.3. Intravenous injection experiment

The intravenous injection experiment was performed to elucidate the disposition behavior of the tested drugs and to estimate their absorption profiles by a deconvolution method (Kiwada et al., 1977). Under urethane anesthesia, 0.2 ml of 0.9% NaCl solution of drugs was injected into the femoral vein. Urine was collected by the same method as described above and the radioactivity was measured.

2.4. Data analysis

The penetration profiles obtained in the in vivo study were analyzed based on a model in which a receptor phase under perfect sink conditions was connected with a viable layer (Yamashita et al., 1994). Based on this model, the Laplace transform for the amount of drug penetrated through the intact skin is expressed as follows (Yamashita et al., 1993a):

$$\overline{Q} = Z_d X_0 (Z_{np} \sinh d_p + Z_p \sinh d_{np})/s/g(s) \quad (1)$$

where s is the Laplace operator with respect to time and X_0 denotes the initially applied dose. Also,

$$d_p = L_s (s/D_p)^{1/2} \quad (2)$$

$$d_{np} = L_s (s/D_{np})^{1/2} \quad (3)$$

$$d_d = L_d (s/D_d)^{1/2} \quad (4)$$

$$Z_p = K_p V_p / d_p \quad (5)$$

$$Z_{np} = K_{np} V_{np} / d_{np} \quad (6)$$

$$Z_d = K_d V_d / d_d \quad (7)$$

$$\begin{aligned} g(s) = V_v (& Z_p \cosh d_p \sinh d_{np} \sinh d_d \\ & + Z_{np} \sinh d_p \cosh d_{np} \sinh d_d \\ & + Z_d \sinh d_p \sinh d_{np} \cosh d_d) \\ & + Z_p \{ Z_p \sinh d_p \sinh d_{np} \sinh d_d \\ & + Z_{np} \sinh d_d (\cosh d_p \cosh d_{np} - 1) \\ & + Z_d \cosh d_p \sinh d_{np} \cosh d_d \} \\ & + Z_{np} \{ Z_{np} \sinh d_p \sinh d_{np} \sinh d_d \\ & + Z_p \sinh d_d (\cosh d_p \cosh d_{np} - 1) \\ & + Z_d \sinh d_p \cosh d_{np} \cosh d_d \} \end{aligned} \quad (8)$$

where V_v is the volume of vehicle; D_i, K_i, and V_i (i = p, np, or d) are the diffusion coefficient in the i-th domain, the partition coefficient between the i-th domain and vehicle, and the effective volume of the i-th domain for diffusion; subscripts s, p, np, and d represent the stratum corneum, polar route, nonpolar route and second layer, respectively. V_i is obtained from the area (A), area fraction of the polar route (f), and diffusion length (L_i) as:

$$V_p = AfL_s \quad (9)$$

$$V_{np} = A(1-f)L_s \quad (10)$$

$$V_d = AL_d \quad (11)$$

Curve fitting to in vivo data was performed for a urinary excretion profile in order to minimize the error that occurred during numerical calculation (Yamashita et al., 1993b). Expressing the excretion profile by a bi-exponential function ($dX_u/dt = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t}$), the Laplace-transformed equation for urinary excretion of topically applied drug is as follows (Yamashita et al., 1993b, 1994):

$$\overline{X}_u = \overline{Q} \cdot \{ A/(s+\alpha) + B/(s+\beta) \} \quad (12)$$

This equation was fitted to urinary excretion data after topical application. Curve fitting to data was performed using the nonlinear regression program with a fast inverse Laplace transform algorithm MULTI(FILT) (Yano et al., 1989) on the M-382 mainframe computer of the Kyoto University Data Processing Center.

Due to the difficulty in determining the real diffusion length, we defined two parameters for drug diffusion and partitioning involving the diffusion length as follows (Okamoto et al., 1988; Yamashita et al., 1993a, 1994):

$$D_i' = D_i / L_i^2 \quad (13)$$

$$K_i' = K_i V_i \ (i = \text{p,np, or d}) \quad (14)$$

The proposed six hybrid parameters for each drug were determined according to the procedures reported previously (Yamashita et al., 1993a,1994; Koyama, et al., 1994). In this analysis, three assumptions were introduced: (1) The polar route is filled with water and the partition coefficient between the polar route and the aqueous vehicle is unity; (2) diffusion in the polar route obeys the Einstein-Stokes equation; and (3) oleic acid does not affect the permeability of the viable layer.

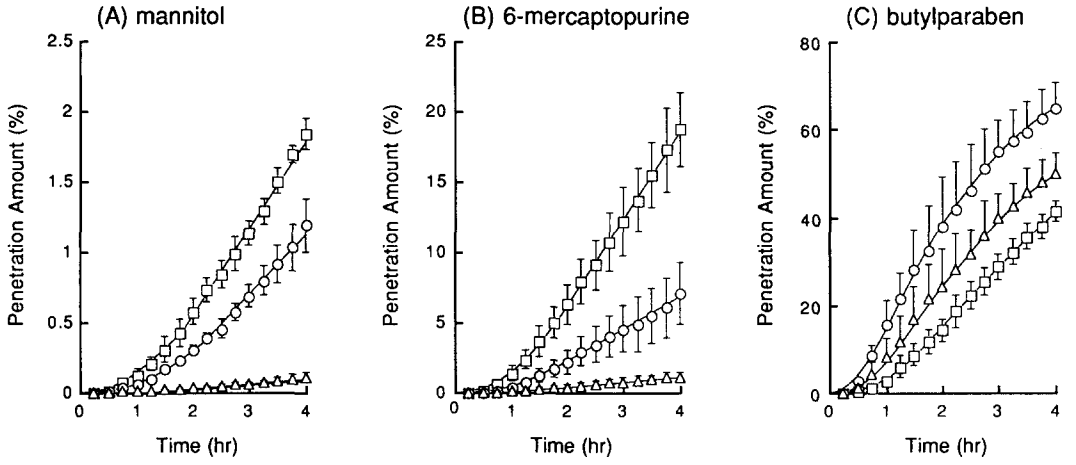

Fig. 1. In vivo absorption profiles of mannitol (A), 6-mercaptopurine (B), and butylparaben (C) through rat skin pretreated with an ethanolic solution of 0 (△), 15.7 (○), and 62.7 (□) μmol of oleic acid. The profiles were calculated using a deconvolution method. Each point represents the mean ± S.D. value of at least three experiments.

3. Results

Urinary excretion profiles of drugs after intravenous injection were approximated with a two-exponential function as follows by least-squares regression analysis:

$$dX_u/dt = 146 \cdot \exp(-2.24 \cdot t) + 17.8 \cdot \exp(-0.689 \cdot t) \text{ (for mannitol)}$$

$$dX_u/dt = 108 \cdot \exp(-11.0 \cdot t) + 30.4 \cdot \exp(-0.692 \cdot t) \text{ (for 6-mercaptopurine)}$$

$$dX_u/dt = 250 \cdot \exp(-6.75 \cdot t) + 69.7 \cdot \exp(-1.05 \cdot t) \text{ (for butylparaben)}$$

where dX_u/dt expresses the urinary excretion rate (% of dose/h).

Table 1
Amount of drugs recovered at the end of in vivo percutaneous absorption experiment

Drug	Amount of oleic acid (μmol)	Amount recovery (% applied) [a]				
		Donor	Skin	Urine	Absorbed [b]	Total [c]
Mannitol	0	86.32 ± 8.79	0.35 ± 0.12	0.08 ± 0.02	0.11 ± 0.03	86.79 ± 8.72
	15.7	90.88 ± 5.39	0.55 ± 0.12	1.04 ± 0.34	1.19 ± 0.19	92.62 ± 5.70
	62.7	79.44 ± 0.03	1.01 ± 0.02	1.34 ± 0.08	1.84 ± 0.11	82.29 ± 0.15
	stripping	39.29 ± 2.11	1.09 ± 0.60	42.06 ± 3.36	54.67 ± 4.18	95.05 ± 1.52
6-Mercaptopurine	0	98.06 ± 5.82	2.17 ± 0.67	0.43 ± 0.14	1.12 ± 0.36	101.36 ± 5.84
	15.7	86.65 ± 3.15	3.57 ± 0.17	2.52 ± 0.86	7.02 ± 2.20	97.24 ± 4.51
	62.7	63.65 ± 14.98	4.55 ± 1.19	6.89 ± 1.11	18.76 ± 2.62	86.96 ± 12.12
	stripping	32.68 ± 4.48	4.91 ± 0.19	32.62 ± 3.29	78.88 ± 7.01	116.47 ± 10.89
Butylparaben	0	18.18 ± 5.88	12.31 ± 7.47	45.17 ± 5.36	50.02 ± 4.66	80.50 ± 3.07
	15.7	10.25 ± 5.74	7.50 ± 3.43	59.78 ± 7.00	64.68 ± 6.11	82.43 ± 7.65
	62.7	18.88 ± 2.99	29.06 ± 8.16	35.63 ± 2.64	41.26 ± 2.37	89.20 ± 3.71
	stripping	2.01 ± 0.38	0.34 ± 0.14	80.31 ± 3.29	80.37 ± 2.36	82.72 ± 1.93

[a] Data are expressed as mean ± S.D. of at least three experiments.
[b] Values were calculated using a deconvolution method (Kiwada et al., 1977).
[c] Values represent the sum of drug amounts in the donor, in the skin, and absorbed.

Fig. 1 shows in vivo absorption profiles of tested drugs obtained by a deconvolution method. Oleic acid markedly enhanced the absorption of mannitol and 6-mercaptopurine. The absorption of butylparaben was slightly increased by the low dose of oleic acid but decreased by the high dose. Table 1 summarizes the drug amount recovered in the donor, skin, and urine, and that absorbed in the systemic circulation at the end of the 4 h absorption experiment. For all drugs, oleic acid increased the amount in the skin at the end of the experiment.

The penetration profiles were analyzed based on a two-layer skin model with polar and nonpolar routes in the stratum corneum. Table 2 summarizes the penetration parameters of the drugs tested for the polar route, nonpolar route, and a lower viable layer. The parameters for the polar route were estimated assuming that mannitol diffuses only through the polar route in the stratum corneum because of its high hydrophilicity. Oleic acid considerably increased the partition parameter for the polar route, while it had little efect on the diffusion parameter.

The effect of oleic acid on the parameters for the nonpolar route was evaluated using 6-mercaptopurine and butylparaben. Oleic acid increased both diffusion and partition parameters of 6-mercaptopurine for the nonpolar route. For butylparaben, the effect of oleic acid on its diffusion and partition parameters was not clear.

4. Discussion

To understand the enhancement effect of penetration enhancers quantitatively and systematically, we have analyzed the skin permeation of drugs with the enhancers based on a two-layer model with polar and nonpolar routes in the stratum corneum (Yamashita et al., 1993a, 1994; Koyama, et al., 1994). This approach enables us to discuss the mechanism of action of enhancers from a fundamental viewpoint, i.e., in terms of drug diffusivity and partition coefficient in each domain. In this study, the mechanism of action of oleic acid in the in vivo system was analyzed using the diffusion model.

Oleic acid increased the partition parameter for the polar route in vivo (Table 2), whereas it did not affect the parameter in the previous study using excised guinea pig skin (Koyama et al., 1994). The diversity between both results might be due to the in vitro-in vivo difference rather than to the species differences. In our other report (Yamashita et al., 1994), the in vitro-in vivo difference in the effect of GACH was investigated in rats. GACH also increased the partition parameter for the polar route in vivo much more than in vitro in rats, while the in vitro result in rats was similar to that in guinea pigs. The mechanisms for enhancing drug penetration through the polar route might be similar between the two enhancers: That is, oleic acid may induce

Table 2
Penetration parameters of mannitol, 6-mercaptopurine, and butylparaben in each route [a]

Drug	Amount of oleic acid (μmol)	Polar route		Nonpolar route	
		D'_p (h^{-1})	K'_p ($\times 10^5$) (cm^3)	D'_{np} (h^{-1})	K'_{np} (cm^3)
Mannitol	0	60.9 ± 1.1	0.586 ± 0.011	–	–
	15.7	67.3 ± 1.1	6.67 ± 0.21	–	–
	62.7	65.4 ± 1.4	9.65 ± 0.26	–	–
	stripping	D'_d = 0.691 ± 0.075 (h^{-1})		K'_d = 0.359 ± 0.049 (cm^3)	
6-Mercaptopurine	0	64.7 ± 1.2	0.586 ± 0.011	4.19 ± 0.68	0.00112 ± 0.00019
	15.7	71.5 ± 1.2	6.67 ± 0.21	11.7 ± 1.1	0.00226 ± 0.00020
	62.7	69.5 ± 1.5	9.65 ± 0.26	14.7 ± 0.2	0.00745 ± 0.00011
	stripping	D'_d = 0.352 ± 0.008 (h^{-1})		K'_d = 1.37 ± 0.05 (cm^3)	
Butylparaben	0	59.6 ± 1.1	0.586 ± 0.011	0.544 ± 0.022	4.04 ± 0.30
	15.7	65.9 ± 1.1	6.67 ± 0.21	1.08 ± 0.02	2.27 ± 0.08
	62.7	64.0 ± 1.4	9.65 ± 0.26	0.243 ± 0.002	4.03 ± 0.27
	stripping	D'_d = 1.13 ± 0.17 (h^{-1})		K'_d = 2.04 ± 0.58 (cm^3)	

[a] Parameters represent means ± computer-calculated S.D.

hydration of the stratum corneum by perturbing its lipid structure in vivo, differently from the in vitro system where the skin is fully hydrated (Lambert et al., 1989).

This speculation is supported by other investigators' observations that oleic acid increased transepidermal water loss (TEWL), which provides an index of the hydration of the stratum corneum (Green et al., 1988). In addition, a Fourier transform infrared spectroscopy (FT-IR) study suggested that oleic acid forms defects at the fluid-solid interface in the stratum corneum lipids, with which water may be associated (Francoeur et al., 1990; Ongpipattanakul et al., 1991). The formation of the interfacial defects may be related to enlargement of the polar route by oleic acid.

Penetration of 6-mercaptopurine with oleic acid was much greater in extent than that of mannitol, a marker of polar route permeation (Fig. 1). This means that for most drugs, except for very hydrophilic drugs, the major mechanism of enhancement by oleic acid is an increase in permeation not through the polar route but through the nonpolar route. Oleic acid increased both diffusivity and partitioning in the nonpolar route for 6-mercaptopurine (Table 2). If the enhancer disrupts the intermolecular forces between the hydrocarbon chains, the energy required for partitioning of the drug into lipids may be lower than that without enhancer, resulting in an increase in drug partitioning. Both diffusivity and partitioning of chemicals are known to be increased by temperature-induced fluidization of lipid layers (Dix et al., 1978). Further, the influence of oleic acid on nonpolar route permeation in the in vivo system was consistent with that found in the previous in vitro permeation study (Koyama et al., 1994). This indicates that the mechanism of action of oleic acid is fundamentally similar between the in vitro and in vivo cases.

In the case of butylparaben, the effect of oleic acid on its penetration parameters was unclear. The diffusion process through the viable layer is considered to play an important role in the total penetration of butylparaben, since its rate of penetration through the stratum corneum was very rapid (Fig. 1 and Table 1). In this case, the reliability of the parameters in the nonpolar route as estimated from the urinary excretion profile should be low. Thus, the irregular change in parameters of butylparaben by oleic acid might be due to experimental error rather than to a special mechanism.

The in vitro-in vivo difference in skin permeation of drugs and its enhancement basically occurs due to the difference in diffusion length of the dermis between both experimental systems, as well as the degree of hydration of the stratum corneum (Yamashita et al., 1994). Resistance of the viable layer to drug diffusion is less in vivo, since the drug is transported away at the upper dermis by blood microcirculation. Therefore, the effect of the enhancer is greater in vivo when it affects the stratum corneum. The enhancement effect of oleic acid on the permeation of 6-mercaptopurine was stronger than that observed in the previous in vitro study (Koyama et al., 1994). Further, oleic acid slightly affected the permeation of butylparaben, of which the rate-limiting step is in viable epidermis and dermis layer in vitro (Yamashita et al., 1994). The present results might be related to the difference in diffusion length of the dermis between the in vitro and in vivo cases.

In this investigation, the normal rat was used as a model animal under standardized experimental conditions, while the skin of hairless mouse is preferred in some cases (Stoughton, 1975; Durrheim et al., 1980). Although the permeabilities of most drugs through rat skin are reported to be greater than those through human skin (Wester and Maibach, 1989), the basic mechanism of drug permeation is expected to be similar between the two species. Since the present model analysis enables us to evaluate skin permeation of drugs and its enhancement during each penetration process in terms of physicochemical and physiological parameters, it should be possible to discuss species differences in percutaneous absorption from the present data, as well as the in vitro-in vivo difference (Yamashita et al., 1994).

In conclusion, the basic mechanism of enhancement by oleic acid for the permeation of drugs is to increase their diffusivity and partition-

ing in the nonpolar route, irrespective of whether the in vitro and in vivo situation is considered. The in vitro-in vivo differences in the degree of hydration of the stratum corneum and the diffusion length of dermis should affect the apparent enhancement effect of oleic acid.

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