Enhancing Effect of *N*-Dodecyl-2-pyrrolidone on the Percutaneous Absorption of 5-Fluorouracil Derivatives

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The enhancing effects of *N*-dodecyl-2-pyrrolidone (NDP) on the percutaneous absorption of doxifluridine (DOX), 5-fluorouracil (5-FU), tegafur (TEG) and carmofur (CAR) were examined using an *in vitro* penetration technique and rat skin. Phosphate buffered isotonic saline (PBS), propylene glycol (PG) and PG containing 0.4 M NDP (PGNDP) were applied as the donor solution. The correlation between the *n*-octanol/water partition coefficients and the permeability coefficients of DOX, 5-FU and TEG was investigated using both logarithmic plots. It was determined that the permeability coefficients are significantly correlated with their *n*-octanol/water partition coefficients on PBS. This result suggested that the non-polar stratum corneum lipid lamella in the skin might act as a rate limiting step on the skin penetration of DOX, 5-FU and TEG. The permeability coefficient of DOX, 5-FU and TEG was increased on PGNDP. The enhancing effect of NDP on the permeability coefficient was more effective at higher hydrophilic drugs, the values of the permeability coefficient had almost the same values on PGNDP and the dependency of the permeability coefficient on the *n*-octanol/water partition coefficient disappeared in the presence of NDP. These results indicated that the enhancing effect of NDP on the percutaneous absorption of DOX, 5-FU and TEG might be closely related to the perturbation of stratum corneum lipid lamella. Since it has been well recognized that CAR is decomposed into 5-FU in neutral and alkaline solution, the decomposition rate of CAR was measured using PBS solution and was found to be very rapid ($K_d = 3.17\ h^{-1}$, $t_{1/2} = 13.1$ min). The total concentrations of CAR plus 5-FU in the acceptor compartment were used to determine the permeability coefficient of CAR. The obtained value of the permeability coefficient of CAR on PG was almost the same as that of TEG on PG (CAR: 1.11×10^{-3} cm/h, TEG: 1.24×10^{-3} cm/h), while that of CAR on PGNDP was smaller than that of TEG on PGNDP (CAR: 6.06×10^{-3} cm/h, TEG: 1.24×10^{-2} cm/h). To determine the lipophilic property of CAR, the lipophilic index ($\log k'$) was measured by HPLC. The value of the lipophilic index of CAR was 92 times higher than that of TEG. These results indicated that CAR is a higher lipophilic compound, and the smaller value of the permeability coefficient of CAR compared with that of TEG on PGNDP might be caused by the strong binding of CAR to the rat skin (dermis). The dermis might act as a rate limiting step on the skin penetration of CAR, and the percutaneous absorption of CAR might be controlled by both the stratum corneum and the dermis.

Key words percutaneous absorption; doxifluridine; 5-fluorouracil; carmofur; tegafur; rat skin

Doxifluridine (DOX), tegafur (TEG) and carmofur (CAR) which are masked forms of 5-fluorouracil (5-FU) have been used for treatment of carcinoma.[1] The side effects of these drugs, diarrhea, nausea and vomiting[2] might be caused by their high initial plasma concentration after oral administration. The development of a transdermal delivery system for these anticancer drugs might be useful in avoiding such side effects and for long-term maintenance chemotherapy of the drugs. It has been demonstrated that the pyrrolidone derivatives are effective penetration enhancers of the percutaneous absorption of several drugs.[3] Phillips and Michniak demonstrated that *N*-dodecyl-2-pyrrolidone (NDP) is a promising enhancer for the skin penetration of hydrophilic drugs including 5-FU, and that its enhancing effect is greater than that of Azone[4]; therefore, NDP was used as the penetration enhancer in this study. It was learned in a previous study that the permeability coefficients of parabens were correlated with their *n*-octanol/water partition coefficients, and that this dependency almost disappeared in the presence of penetration enhancers including NDP; the perturbation of stratum corneum lipid lamella might be related with the enhancement of the absorption of the hydrophilic parabens.[5] This study was designed to identify the correlation between the *n*-octanol/water partition coefficients and the permeability coefficients of 5-FU derivatives, and the effect of NDP on this correlation.

It is well recognized that CAR is decomposed into 5-FU and hexylamine or 1,3-dihexylurea in neutral and alkaline solution (>pH 5).[6] Therefore, CAR was believed to decompose into 5-FU in pH 7.4 phosphate buffered isotonic saline (PBS) solution which was used as acceptor solution in this study. Understanding of the degradation (metabolism) of drugs on the percutaneous absorption is important in explaining the skin penetration behavior of a parent drug (prodrug) and productive drug (metabolite). Usefulness of the diffusion/bioconversion model (two-layer skin diffusion model with polar and nonpolar routes in the stratum corneum) has been demonstrated for comprehensive discussions on the percutaneous penetration of drugs with regard to the diffusion, partition and metabolism of drugs in the skin.[7] In the present study, it was assumed that the influx of CAR into the acceptor compartment through the rat skin is dependent on the zero-order absorption rate, and that the decomposition from CAR to 5-FU in the acceptor compartment follows the typical first-order kinetics. The zero-order absorption rate constant (permeability coefficient) of CAR could be determined using the total concentration of CAR plus the productive 5-FU in the acceptor compartment. The relationship between the lipophilic property and the permeability coefficient of CAR and the effect of NDP on this relationship were investigated to explain the skin penetration behavior of CAR.

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Experimental

Chemicals 5-FU (Wako Pure Chemical Industries, Osaka, Japan) and TEG (Sigma, St. Louis, MO) were purchased commercially. DOX and CAR were generously supplied by Nippon Roche K.K. (Kamakura, Japan) and Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan), respectively. These drugs were used without further purification. Propylene glycol (PG) was also purchased from Wako Pure Chemical Industries. NDP was purchased commercially from Aldrich Chemical Company (Milwaukee, WI). All other chemicals were of reagent grade and were obtained commercially from Wako Pure Chemical Industries.

***n*-Octanol/Water Partition Coefficient** *n*-Octanol/water partition coefficients of DOX, 5-FU, and TEG were determined using an *n*-octanol (5 ml)–PBS (pH 7.4, 5 ml) system after stirring for 5 h at 32 °C. The drug concentrations in the aqueous phase after stirring were measured by high-performance liquid chromatographic (HPLC) assay (described below). The values of the *n*-octanol/water partition coefficients were calculated by the usual equation.[8] Since it is known that CAR is decomposed into 5-FU in neutral and alkaline solution (>pH 5), the *n*-octanol/water partition coefficient of CAR was not measured.

Determination of the Lipophilic Index Since the value of the *n*-octanol/water partition coefficient of CAR could not be measured using PBS solution, the lipophilic index ($\log k'$) of DOX, TEG and CAR was determined by HPLC method.[9] The values of the lipophilic index were calculated by the following equation: $\log k' = \log((t_r - t_0)/t_0)$, where t_r and t_0 are the retention time of the sample and the solvent peak, respectively. The column was Supelcosil LC-18-DB (4.6 mm i.d. × 250 mm, 5 μm particle size, SUPELCO, PA, U.S.A.). A mixture of 0.05 M potassium phosphate–methanol (75 : 25, v/v) was used as the mobile phase. The flow rate was 1.0 ml/min.

Drug Solubility The solubility of DOX, 5-FU, TEG and CAR in PBS, PG and PG containing 0.4 M NDP (PGNDP) were determined by the usual method at 32 °C. The model drugs were dissolved using PBS, PG and PGNDP in a screw cap test tube until visual saturation was observed after 24 h. The solutions containing the model drugs were stirred throughout the 24 h period and drug was added as needed so that saturation was achieved. After 24 h, the solutions were transferred to centrifuge tubes, and these tubes were centrifuged at 6700 × ***g*** for 2 min. The supernatant (250 μl) was withdrawn and diluted using methanol (25 ml). Drug concentrations in the diluted solution were measured by HPLC assay (described below). Since CAR is known to decompose into 5-FU in neutral and alkaline solution (>pH 5), the solubility of CAR in PBS was not measured.

***In Vitro* Skin Penetration Experiments** Franz cells (with a surface area of 1.651 cm^2 and an acceptor volume of 10 ml) were used in this study. The temperature was maintained at 32 °C using a water bath (Thermo Minder SM-05, Taitec, Tokyo). The hair from male Wistar rats (7 weeks old, Sankyo Lab Service Corporation Inc., Shizuoka, Japan) was removed with animal clippers and the animals were sacrificed with ether. Excised lumbar skins with adhering fat removed were placed over the acceptor compartments; these compartments were filled with PBS (pH 7.4, 10 ml) to ensure good hydration and the acceptor solutions were continuously stirred. The solutions (PBS, PG and PGNDP without the model drugs) were placed on each skin (donor compartment) prior to the drug penetration experiment. Twenty hours later, the acceptor solution was discarded and the solution on the skin was blotted lightly with paper. Fresh PBS solutions were placed in the acceptor compartments and a saturated suspension of the model drugs in PBS, PG and PGNDP (0.5 ml) was placed on each skin. The donor and acceptor compartments were occluded with parafilm. Samples (0.4 ml) were withdrawn from the acceptor compartments over 8 h and replaced with the same volume of fresh buffer. The concentration ($C_{t-(t-1)}$) of the model drugs penetrating into the acceptor compartment during each sampling period was calculated by the following equation: $C_{t-(t-1)} = C_t - (C_{(t-1)} - C_{(t-1)} \times V_{sample}/V_{Cell})$; C_t, V_{sample} and V_{Cell} are the drug concentration at each sampling time, the sampling volume and the cell volume, respectively. In the CAR study, 4 M of phosphoric acid (10 μl) was added to the sample immediately to prevent the degradation of CAR. No skin penetration study of CAR on PBS was performed.

Decomposition of CAR The decomposition of CAR in PBS, rat skin homogenate (2% and 10% in PBS), PG and PGNDP was observed at 32 °C. The rat skin homogenate was prepared by a polytron homogenizer (PT 10-35, Kinematica, Switzerland) at 15000 rpm for 3 min in PBS solution. Ten ml of PBS, the rat skin homogenate, PG and PGNDP solutions were placed in a screw cap test tube and these solutions were continuously stirred. One hundred μl of CAR solution (38.9 mM in methanol) was added to these solutions. Samples (0.2 ml) were collected at 10, 20, 30, 40, 50, 60, 80, 100, 120 and 140 min after the dosing of CAR. The rat homogenate samples were centrifuged at 6700 × ***g*** for 2 min. The CAR and 5-FU concentrations were measured by HPLC assay (described below).

Assay Methods for Drug Concentration The concentrations of DOX, 5-FU, TEG and CAR were determined by a modification of the HPLC assay of Itoh *et al.*[10] with 5-chlorouracil (5-CLU, Nacalai Tesque Inc., Kyoto, Japan) as the internal standard. The samples obtained from the acceptor compartment were centrifuged at 6700 × ***g*** for 2 min. The clear supernatant (0.3 ml) and the internal standard solution (5-CLU, 1 mg/ml, 0.025 ml) were pipetted into a tube and mixed well. Samples for the measurement of the *n*-octanol/water partition coefficient and the solubility of these drugs were prepared by the same procedure. The obtained mixture solutions were injected onto the HPLC system. A reversed-phase HPLC column (Supelcosil LC-18-DB, SUPELCO, PA, U.S.A.) was used. The solvent delivery system (L-5000 LC controller and 655A-11 pump, Hitachi, Tokyo, Japan) was equipped with a variable-wavelength ultraviolet absorbance detector (L-4000 UV detector, Hitachi) at 265 nm with an auto-sampler (AS-8010, Tosoh, Tokyo, Japan) and with a chromatointegrator (D-2500, Hitachi). A guard column (Supelcosil LC-18-DB, SUPELCO) was placed between the auto-sampler and the analytical column. Potassium phosphate solution, 0.05 M was used as the mobile phase (A) for 5-FU. A mixture of 0.05 M potassium phosphate–methanol (90 : 10, v/v) was used as the mobile phase (B) for DOX and TEG and a mixture of 0.05 M potassium phosphate–methanol (30 : 70, v/v) was used as the mobile phase (C) for CAR. The mobile phases were filtered by passing them through a 0.4 μm pore size membrane filter (Fuji Photo Film Co., Ltd., Tokyo). The flow rate was 1.0 ml/min. In the CAR study, CAR and 5-FU concentrations were measured simultaneously by a gradient method using the mobile phases (A) and (C). To test the linearity of the calibration graph, various amounts of DOX, 5-FU, TEG and CAR ranging from 0.25 to 100 μg were prepared. Linear relationships were obtained from both logarithmic plots, and the regression lines for the DOX, 5-FU, TEG and CAR concentrations were $\ln y = 0.990 \ln x - 2.777$ ($\gamma = 0.9997$), $\ln y = 0.935 \ln x - 3.206$ ($\gamma = 0.9966$), $\ln y = 0.984 \ln x - 2.963$ ($\gamma = 0.9978$), $\ln y = 1.023 \ln x + 1.252$ ($\gamma = 0.9901$), respectively, where y is the peak-area ratio and x is the amount of DOX, 5-FU, TEG and CAR. The coefficient of variation was 5% or less in these concentration ranges for calibration graphs.

Estimation of Permeability Coefficient for DOX, 5-FU and TEG The permeability coefficients of DOX, 5-FU and TEG were calculated by the usual method.[11] The cumulative concentration of these model drugs penetrating into the acceptor compartment (mM) were plotted against time. The steady state flux (Flux, mM/h) was calculated from the obtained slope values of the linear portion of these plots. The permeability coefficients (K_p, cm/h) were calculated by Eq. 1:

$$K_p = \frac{\text{Flux} \times V}{A \times C_d} \tag{1}$$

where V is the volume of the acceptor compartment (ml), A is the diffusion area (cm^2), and C_d is the drug concentration in the donor compartment (mM, saturated concentration of the model drugs). The value of the permeability coefficient was determined from individual data, and then the mean value and the value of the standard deviation were calculated.

Estimation of the Decomposition Rate Constant from CAR to 5-FU A typical first-order kinetic model was used to estimate the decomposition rate constant from CAR to 5-FU. The following Eqs. 2 and 3 represent the time course of CAR and 5-FU concentration in PBS after the dosing of CAR, respectively:

$$C_{CAR} = C_0 e^{-K_d t} \tag{2}$$

$$C_{5\text{-FU}} = C_0(1 - e^{-K_d t}) \tag{3}$$

where C_{CAR}, $C_{5\text{-FU}}$ and C_0 are the CAR concentration, the 5-FU concentration and the initial concentration of CAR (mM), respectively; and K_d is the decomposition rate constant from CAR to 5-FU (h^{-1}). Equations 2 and 3 were used to estimate the values of the decomposition rate constant of CAR using the nonlinear least-squares regression program MacCurveFit.[12]

Estimation of the Permeability Coefficient of CAR In order to estimate the permeability coefficient of CAR, it was assumed that the influx of CAR into the acceptor compartment through the rat skin occurs in

accordance with the zero-order absorption rate (Flux), and that the decomposition from CAR to 5-FU in the acceptor compartment follows the typical first-order kinetics: $dC_{CAR}/dt = Flux - K_d C_{CAR}$, $dC_{5\text{-}FU}/dt = K_d C_{CAR}$. Based on these assumptions, Flux of CAR could be determined using the total concentration of CAR plus the productive 5-FU in the acceptor compartment. The cumulative total concentration of CAR plus 5-FU in this compartment was plotted against time. Flux of CAR was calculated from the obtained slope value of the linear portion of this plot and the permeability coefficient of CAR was also calculated using Eq. 1.

Statistics Differences in the solubility of DOX, 5-FU and TEG in PBS, PG and PGNDP were evaluated statistically by one-way analysis of variance followed by the Tukey test. The difference in the solubility of CAR in PG and PGNDP was evaluated statistically by Student's *t*-test. Differences of the permeability coefficient and the lag time of DOX, 5-FU, TEG and CAR on PBS and PG were also evaluated by Student's *t*-test. The correlation between *n*-octanol/water partition coefficient and the permeability coefficient of DOX, 5-FU and TEG in PBS, PG and PGNDP was evaluated statistically by Student's *t*-test. The 0.05 level of probability was used as the level of significance.[13]

Results and Discussion

***n*-Octanol/Water Partition Coefficients and Solubility** The obtained values of the *n*-octanol/water partition coefficients and the solubility of DOX, 5-FU, TEG and CAR in PBS, PG and PGNDP are listed in Table 1. The values of the *n*-octanol/water partition coefficient of 5-FU and TEG were almost the same as the literature values (5-FU: 0.106, TEG: 0.331).[14] These values were used to examine the correlation between the *n*-octanol/water partition coefficient and the permeability coefficient of DOX, 5-FU and TEG in PBS, PG and PGNDP. The solubility of DOX in PG and PGNDP was statistically lower than that in PBS. The solubility of 5-FU was unchanged in PBS, PG and PGNDP, while the solubility of TEG in PG and PGNDP was statistically higher than that in PBS. Moreover, the solubility of CAR in PGNDP was statistically higher than that in PG. The values of the solubility of these model drugs were used to calculate the permeability coefficient (Eq. 1). Since CAR is known to decompose into 5-FU in neutral and alkaline solution, the value of the *n*-octanol/water partition coefficient of CAR and the solubility of CAR in PBS were not measured.

Correlation between Permeability Coefficients and *n*-Octanol/Water Partition Coefficients of DOX, 5-FU and TEG on PBS The penetration profiles of DOX, 5-FU and TEG on PBS are shown in Fig. 1. The values of the permeability coefficients and the lag time were obtained by the usual method, (Eq. 1) and the obtained values of the permeability coefficients and the lag time are listed in Table 2. The skin penetrations of DOX, 5-FU and TEG on PBS were increased with the enlargement of the *n*-octanol/water partition coefficients of these model drugs. The

Table 1. Values of *n*-Octanol/Water Partition Coefficient, Lipophilic Index and Solubility of 5-FU Derivatives

	Partition coefficient	Lipophilic index	Solubility (mM)		
			PBS	PG	PGNDP
DOX	0.0337±0.0088	0.110	210.6±42.4	115.2±16.2*	120.3±27.5*
5-FU	0.1123±0.0123	—	84.4±13.3	114.9±21.7	115.4±28.5
TEG	0.3300±0.0134	0.455	83.1± 7.9	124.5± 5.8*	139.2±18.4*
CAR	—	41.90	—	140.7± 3.4	209.5±10.6**

All values are expressed as the mean±S.D. ($n=3$—6). Since the retention time of 5-FU was almost equal to that of the solvent peak, the value of the lipophilic index of 5-FU could not be determined. Significantly different from the result on PBS by one-way analysis of variance followed by the Tukey test: $*p<0.05$. Significantly different from the result on PG by Student's *t*-test: $**p<0.05$.

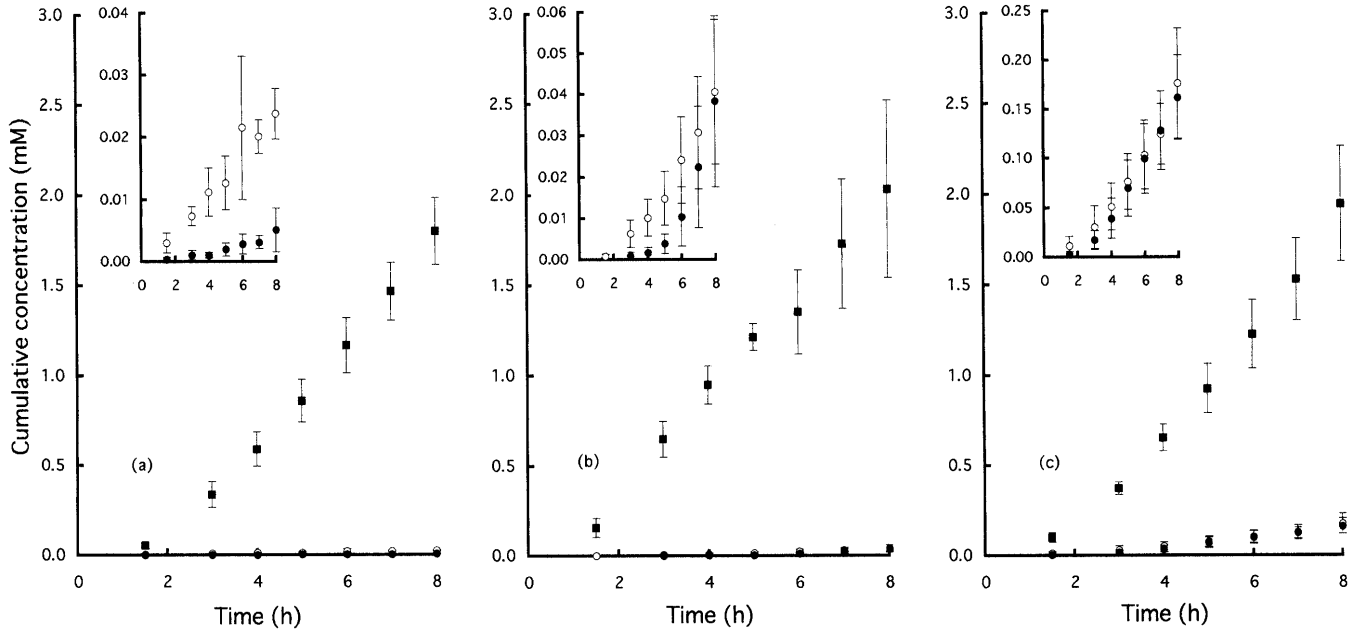

Fig. 1. Penetration Profiles of DOX (a), 5-FU (b) and TEG (c) on PBS (○), PG (●) and PGNDP (■) through Rat Skin

The upper panels represent the same profile data but on a different concentration scale. Each experimental point is shown as the mean±S.D. ($n=3$—5).

Table 2. Values of Permeability Coefficient and Lag Time of 5-FU Derivatives

	PBS	PG	PGNDP
Permeability coefficient (cm/h)			
DOX	1.13×10^{-4}	4.11×10^{-5}	1.37×10^{-2}
	$\pm3.80\times10^{-5}$	$\pm2.65\times10^{-5}$	$\pm1.35\times10^{-3}$
5-FU	4.39×10^{-4}	6.10×10^{-4}	1.46×10^{-2}
	$\pm1.96\times10^{-4}$	$\pm3.32\times10^{-4}$	$\pm3.74\times10^{-3}$
TEG	1.80×10^{-3}	1.24×10^{-3}	1.24×10^{-2}
	$\pm5.08\times10^{-4}$	$\pm3.35\times10^{-4}$	$\pm2.06\times10^{-3}$
CAR	—	1.11×10^{-3}	6.06×10^{-3}
		$\pm2.19\times10^{-4}$	$\pm1.27\times10^{-3}$
Lag time (h)			
DOX	1.01±0.44	2.00±1.21	1.64±0.16
5-FU	1.93±0.34	4.91±0.09*	0.70±0.49
TEG	1.64±0.55	2.05±0.27	1.51±0.21
CAR	—	3.29±0.16	2.50±0.08

All values are expressed as the mean±S.D. ($n=3$—5). Significantly different from the result on PBS by Student's t-test: $*p<0.05$.

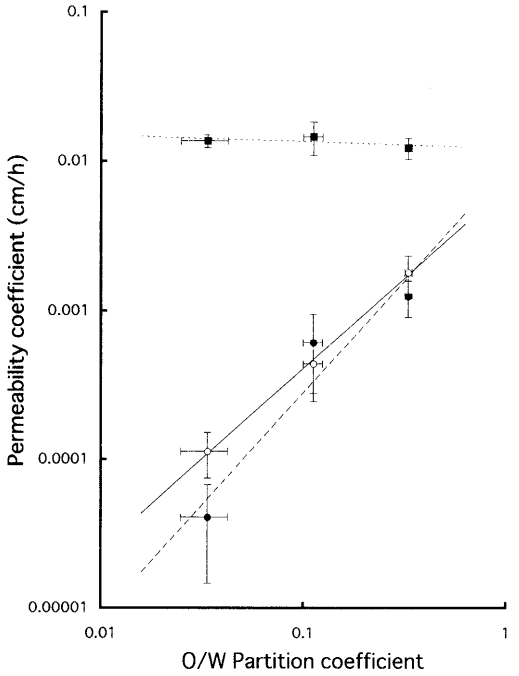

Fig. 2. Logarithmic Relationship between the n-Octanol/Water Partition Coefficient (P_{oct}) and the Permeability Coefficient (K_p) of DOX, 5-FU and TEG on PBS (○), PG (●) and PGNDP (■)

The solid, broken and dotted lines represent the correlation lines on PBS, PG and PGNDP; PBS: $\log K_p=1.213\log P_{oct}-2.176$ ($\gamma=0.9981$, $p<0.05$), PG: $\log K_p=1.507\log P_{oct}-2.044$ ($\gamma=0.9167$, non-sig), PGNDP: $\log K_p=-0.0439\log P_{oct}-1.9111$ ($\gamma=0.3430$, non-sig)

relationship between the n-octanol/water partition coefficient and the permeability coefficient on PBS was examined using both logarithmic plots and is shown in Fig. 2. The permeability coefficients (K_p) of DOX, 5-FU and TEG were significantly correlated with their n-octanol/water partition coefficients (P_{oct}) on PBS ($\log K_p=1.213\log P_{oct}-2.1761$, $\gamma=0.9981$, $p<0.05$). This result indicated that the non-polar stratum corneum lipid lamella in the skin might act as a rate limiting step on the skin penetration of DOX, 5-FU and TEG.

Effect of PG on the Percutaneous Absorption of DOX, 5-FU and TEG The penetration profiles and the obtained values of the parameters of DOX, 5-FU and TEG on PG are also shown in Fig. 1 and Table 2. The value of the permeability coefficient of DOX on PG was lower than that on PBS (but the difference was not significant) and the values of the permeability coefficient of 5-FU and TEG on PG were not significantly different from those on PBS (Table 2). It has been reported that PG is a penetration enhancer[15]; its enhancing effect on the penetration of a drug is reportedly caused by its solvating to keratin and its occupation of the hydrogen binding sites in the stratum corneum, followed by enlargement of the solubility (partition coefficient) of the drug in the stratum corneum.[16] Therefore, reduction of the penetration of DOX on PG was thought possibly to be caused by the decline of the solubility of DOX in PG (Table 1). The lag-time of 5-FU in PG was significantly prolonged compared with that in PBS (Table 2), possibly due to alterations of the penetration behavior of 5-FU on the stratum corneum which was occupied by PG. To learn whether the correlation between the n-octanol/water partition coefficients and the permeability coefficients is present on PG, the correlation was also examined using the two logarithmic plots (Fig. 2). Although there was no significant correlation, the permeability coefficients (K_p) of DOX, 5-FU and TEG were increased with the enlargement of their n-octanol/water partition coefficients (P_{oct}) on PG ($\log K_p=1.507\log P_{oct}-2.0436$, $\gamma=0.9167$).

Effect of NDP on the Percutaneous Absorption of DOX, 5-FU and TEG The penetration profiles and the obtained values of the parameters of DOX, 5-FU and TEG on PGNDP are also shown in Fig. 1 and Table 2. The penetrations of DOX, 5-FU and TEG were greatly increased on PGNDP (Fig. 1). NDP has the heterocyclic ring structure which is a 5-membered ring; Azone also has the heterocyclic ring structure which is a 7-membered ring. It has been reported that the enhancing effect of Azone on the percutaneous absorption of a hydrophilic drug might be caused by its direct partition into the lipid bilayer and the disruption of this bilayer, and then the creation of larger pores for the passive diffusion of the co-administered drug.[17] Therefore, NDP which has smaller heterocyclic rings was thought to allow easier intercalation on the tightly packed lipids of the stratum corneum and greater disruption of these lipids. In fact, it was demonstrated that the enhancing effect of NDP on the skin penetration of hydrophilic drugs including 5-FU is greater than that of Azone.[4] The values of the permeability coefficient of DOX, 5-FU and TEG were increased on PGNDP (Table 2). The effect of NDP on the correlation between the n-octanol/water partition coefficient and the permeability coefficient was determined by examining the correlation using both logarithmic plots (Fig. 2). The enhancing effect of NDP on the permeability coefficients was more effective at higher hydrophilic drugs, and these values were almost the same on PGNDP; the dependency of the permeability coefficients of DOX, 5-FU and TEG on their n-octanol/water partition coefficients disappeared in the presence of NDP ($\log K_p=-0.0439\log P_{oct}-1.9111$, $\gamma=0.343$). These results indicated that the enhancing effect of NDP on the percutaneous absorption of DOX, 5-FU and TEG might be closely related to the

perturbation of stratum corneum lipid lamella.

Decomposition of CAR into 5-FU Since it is recognized that CAR is decomposed into 5-FU in neutral and alkaline solution,[6] the decomposition rate of CAR was determined using PBS solution. The time courses of CAR and the productive 5-FU concentration in the PBS solution are shown in Fig. 3. The solid lines in the figure represent the calculated values obtained by the typical first-order kinetic model (Eqs. 2, 3). The time courses of CAR and the productive 5-FU concentrations in the PBS solution could be described quantitatively by this kinetic model. The values of the initial concentration and the decomposition rate constant of CAR were 0.50 ± 0.0055 mM and $3.17 \pm 0.097\ h^{-1}$, respectively. Alterations in the CAR concentration and the 5-FU concentration were measured using the 2% and 10% rat skin homogenate PBS solution to know whether the CAR decomposition rate is accelerated by the rat skin (data not shown). The decomposition rate constant of CAR was decreased in the rat skin homogenate PBS solution (2%: $2.45 \pm 0.12\ h^{-1}$, 10%: $2.25 \pm 0.054\ h^{-1}$). These results indicated that the decomposition rate of CAR was not accelerated at least by the rat skin, so that decline of the decomposition rate constant of CAR on the rat skin homogenate might be caused by the strong binding of CAR to the skin.

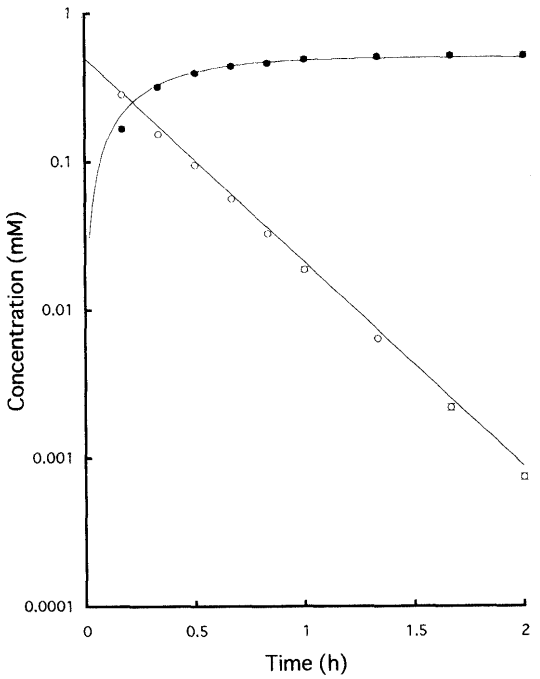

Fig. 3. Time Courses of CAR (○) and 5-FU (●) Concentration in PBS pH 7.4

The solid lines represent the calculated values using the typical first-order kinetic model (Eqs. 2 and 3).

Percutaneous Absorption of CAR Alterations of the CAR concentrations in the donor solutions PG and PGNDP were observed. The concentrations unaltered for at least 24 h (data not shown), indicating that CAR is chemically stable in these solutions. The skin penetration profile of CAR and the time course of the productive 5-FU concentration in the acceptor compartment on PG are shown in Fig. 4. CAR concentrations in the acceptor compartment were not increased and almost the same as the initial level after 5 h; they were much lower than the 5-FU concentrations. In this study, it was assumed that the influx of CAR into the acceptor compartment through the rat skin was due to the zero-order absorption rate (Flux) and that decomposition from CAR to 5-FU in the acceptor compartment followed typical first-order kinetics. The time course of CAR concentration in the acceptor compartment could be expressed as follows: $C_{CAR} = \text{Flux}/K_d(1-\exp(-K_d \cdot t))$. This equation indicated that the CAR concentration became constant in infinity and that steady state CAR concentration was decreased with the enlargement of the decomposition rate constant of CAR. In fact, the decomposition rate of CAR in PBS solution was very rapid. These results caused us to feel that the skin penetration behavior of CAR might be explained by these assumptions. The 5-FU concentration in the acceptor

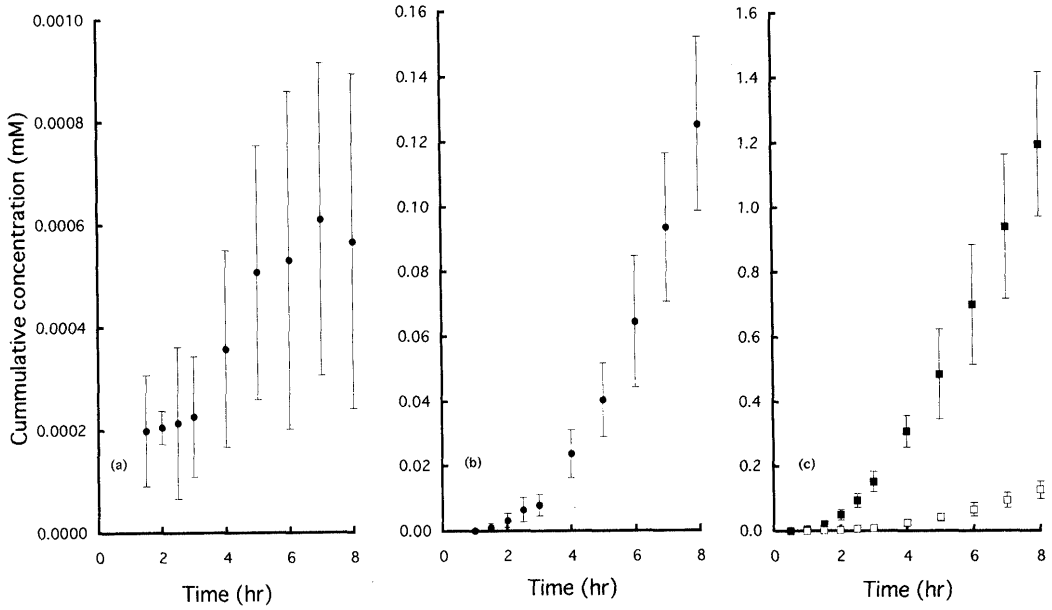

Fig. 4. Time Courses of CAR and Productive 5-FU Concentration and Total Concentration of CAR Plus 5-FU in Acceptor Compartment

Panel (a) and (b) represent the penetration profiles of CAR and the time course of productive 5-FU concentration in acceptor compartment on PG, respectively. Panel (c) represents the time courses of total concentration of CAR plus 5-FU in acceptor compartment on PG (□) and PGNDP (■).

compartment was increased with time (Fig. 4b). The time course of 5-FU concentration in the acceptor compartment could be expressed as follows: $C_{5\text{-FU}} = \text{Flux} \cdot t - C_{\text{CAR}}$. This equation indicated that the Flux value of CAR could be calculated from the slope value of the linear portion of the total concentration of CAR plus 5-FU in the acceptor compartment without determining the decomposition rate constant of CAR. Figure 4c shows the time course of the total concentrations of CAR plus 5-FU in the acceptor compartment on PG and PGNDP. The obtained values of the permeability coefficients and the lag-time of CAR on PG and PGNDP are also listed in Table 2.

The relationship between the lipophilic property and the permeability coefficient of CAR were examined to determine the skin penetration behavior of CAR. Since the *n*-octanol/water partition coefficient of CAR could not be determined, the lipophilic index ($\log k'$) of CAR, TEG and DOX was measured using HPLC. These results are also listed in Table 1. The lipophilic index of CAR was 92 times higher than that of TEG. The obtained value of the permeability coefficient of CAR on PG was almost the same as that of TEG on PG, while that of CAR on PGNDP was less than that of TEG on PGNDP (Table 2). It has been reported that the dermis of the skin might act as a significant additional barrier to the permeation of drugs, especially highly lipophilic drugs.[18] Our results suggested that CAR is a higher lipophilic compound and that the smaller value of its permeability coefficient compared with that of TEG on PGNDP might be due to its strong binding to the rat skin (dermis); the dermis might act as a rate limiting step in the skin penetration of CAR. The permeability coefficient of CAR on PGNDP was about 6 times greater than that on PG, indicating that the stratum corneum might also act as a rate limiting step on the skin penetration of CAR. Thus, it appeared possible that the skin penetration behavior of CAR might be controlled by both the stratum corneum lipid lamella and the dermis.

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