Skin Disposition of Drugs after Topical Application in Hairless Rats

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Drug fraction transported from a topical formulation on skin to subcutaneous tissues or muscles is dependent on the physicochemical properties of the entrapped drug. Cutaneous disposition of model drugs, antipyrine (ANP), lidocaine (LC) and piroxicam (PXC) as well as flurbiprofen (FP) was thus evaluated in hairless rats in which an agar gel disc was subcutaneously inserted into the abdominal region as a drug receptor and a drug donor cell was placed above it. Time courses of plasma level and agar gel amount were measured after topical application of 50% ANP, 3% LC, 1% PXC and 1% FP in hydroxypropylcellulose gel. Percutaneous absorption clearance of unionized form, CL^*_{ab} was proportional to true octanol/water distribution coefficient and the order of FP>PXC>LC>ANP, suggesting that skin permeation of the drug was determined mainly by its distribution from the formulation to the skin barrier. PXC, however, had a relatively low flux compared to the other three drugs, probably due to its high molecular weight and melting point. Migration clearance of unionized form from systemic circulation to the subcutaneous agar gel, CL^*_g was also influenced by the lipophilicity of drugs. On the other hand, fraction from the formulation to the systemic circulation was in the order of PXC>FP>ANP>LC. This fraction was much higher than the direct migration fraction from the formulation to the subcutaneous agar gel. Factors determining for these fractions are still unclear. A drug having a low lipophilicity and a low protein binding, however, had a tendency to have a great targeting ability to the subcutaneous agar gel. In addition, most of the drug in the agar gel was contributed by the direct flow from formulation, not from the systemic circulation. The present *in situ* experimental method is a useful tool to evaluate skin disposition of drugs. Detailed understanding of the skin disposition of drugs from several formulations will enable the finding of a good drug and formulation candidates.

Key words percutaneous absorption; skin disposition; topical application; pharmacokinetics; *in situ* experiment; rat

There are two categories of topical formulations; one has systemic pharmacological action and the other has local action in the application skin site and the deeper subcutaneous tissues and muscles.[1)] The former represents an effect after the drug is taken up into the vascular system at the upper dermis, and is exemplified in the transdermal delivery systems (TDSs) containing scopolamine, nitroglycerin or estradiol. Plasma concentration of drugs is determined mainly by their permeation rates through the stratum corneum, the uppermost layer of skin for the TDSs. Long and/or high accumulation of drugs in skin is not beneficial for the formulations because it increases side effects on the local tissues. On the other hand, the latter topical formulations are applied to treat diseases in the cutaneous and subcutaneous tissues. The pharmacological action is obtained by retaining a therapeutic agent in the local tissues. Maintaining and targeting of a drug in the local tissues is beneficial, whereas a high systemic bioavailability is not important for topical formulations with action on the deeper tissues, such as a plaster or a cataplasma containing a non-steroidal antiinflammatory drug (NSAID).[2—4)] Thus, clear understanding of the cutaneous disposition of drugs is necessary to design such a topical formulation.

Several *in vitro* and *in vivo* experimental methods have been developed to study drug disposition in skin.[5—8)] The *in vitro* skin permeation technique has been broadly utilized when the rate limiting step of overall skin permeation of a drug is diffused across the dead stratum corneum layer, because the method is simple to apply and physicochemical permeation parameters are easy to reproduce. The technique, however, gives little information on the drug disposition or metabolism in the cutaneous and subcutaneous tissues. *In vivo* experiments are also not easy, especially for periodically determining the tissue levels of a drug, although they enable the measurement of tissue levels. We attempted to design an *in situ* experimental method to evaluate cutaneous disposition of a topically-applied drug.[9,10)] Flurbiprofen (FP), an NSAID, was used as a model drug. A disc-shaped agar gel was implanted in the abdominal region of hairless rats as a drug receptor and the drug levels in plasma and the cutaneous gel were followed after intravenous (i.v.) injection and topical application. FP was first penetrated into skin and permeated through the stratum corneum to reach viable epidermis and dermis, where some of the drug was taken up into the cutaneous blood vessels and some was diffused into the subcutaneous agar gel. FP found in the subcutaneous gel arrived through two migration processes: direct diffusion from the formulation, and redistribution from the cutaneous vessels through the systemic circulation. Thus, local action by the topical formulations was thought to be due to the amount of drug in the target tissues (*e.g.*, subcutaneous tissues and muscles) migrated directly from the formulation and from the systemic circulation. Drug fraction from the topical formulations directly to the subcutaneous tissues and that from the systemic circulation to the subcutaneous tissues must be dependent upon the physicochemical properties of the topically applied drug. It is important to understand the relation between the cutaneous disposition and physicochemical properties of a drug. Cutaneous disposition of several drugs was studied and discussed from a physicochemical point of view by several researchers.[11,12)] The effect of physicochemical properties of drugs on the skin profiles, however, is not yet known due to complex disposition of drugs in skin. We therefore measured the cutaneous disposition of three other

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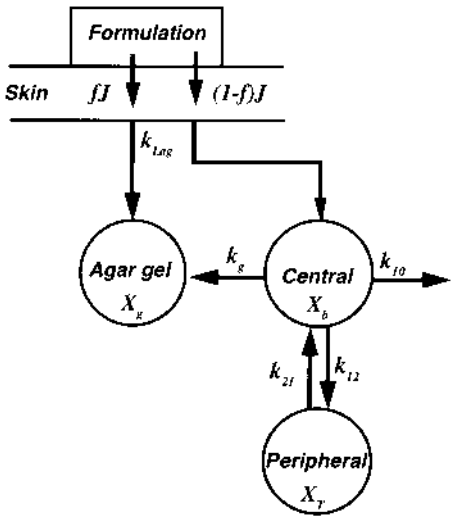

Fig. 1. Pharmacokinetic Model for Each Drug after Topical Application

drugs, antipyrine (ANP), lidocaine (LC) and piroxicam (PXC), having different physicochemical properties using our simple *in situ* method in hairless rats, and the results were compared to those of FP.

Theoretical

Figure 1 illustrates a pharmacokinetic model showing the cutaneous disposition of each drug in agar gel-inserted rats after topical application. In this model, a sink condition was assumed for each drug, so that the drug migration from the agar gel to the central compartment was ignored. A general linear two-compartment model was applied for the elimination pharmacokinetics of a drug.

Thus, plasma concentration, Cb_{iv} and cumulative amount in agar gel of drugs, Xg_{iv} at time t after i.v. injection can be represented as follows:

$$Cb_{iv}=\frac{X_0(k_{21}-\alpha)}{Vd(\beta-\alpha)}e^{-\alpha t}+\frac{X_0(k_{21}-\beta)}{Vd(\alpha-\beta)}e^{-\beta t}=Ae^{-\alpha t}+Be^{-\beta t} \quad (1)$$

$$Xg_{iv}=\frac{k_g X_0 k_{21}}{\alpha\beta}+\frac{k_g X_0(k_{21}-\alpha)}{\alpha(\alpha-\beta)}e^{-\alpha t}+\frac{k_g X_0(k_{21}-\beta)}{\beta(\beta-\alpha)}e^{-\beta t} \quad (2)$$

where $\alpha+\beta=k_{10}+k_g+k_{12}+k_{21}$, $\alpha\beta=(k_{10}+k_g+k_{12})\cdot k_{21}-k_{12}k_{21}$, X_0 is dose and k_{12}, k_{21}, k_{10}, and k_g are first-order rate constants (Fig. 1). Similarly plasma concentration and the cumulative amount in agar gel of drugs, Cb_{td} and Xg_{td}, after topical application can be represented as:

$$Cb_{td}=\frac{(1-f)Jk_{21}}{Vd\alpha\beta}+\frac{(1-f)J(k_{Lag}-k_{21})}{Vd(\alpha-k_{Lag})(\beta-k_{Lag})}e^{-k_{Lag}t}+\frac{(1-f)Jk_{Lag}(\alpha-k_{21})}{Vd\alpha(k_{Lag}-\alpha)(\beta-\alpha)}e^{-\alpha t}+\frac{(1-f)Jk_{Lag}(\beta-k_{21})}{Vd\beta(k_{Lag}-\beta)(\alpha-\beta)}e^{-\beta t} \quad (3)$$

$$Xg_{td}=\left(fJ+\frac{(1-f)Jk_gk_{21})}{\alpha\beta}\right)t+\left\{\frac{fJ}{k_{Lag}}+\frac{(1-f)Jk_g(k_{21}-k_{Lag})}{k_{Lag}(\alpha-k_{Lag})(\beta-k_{Lag})}\right\}(e^{-k_{Lag}t}-1)+\frac{(1-f)Jk_gk_{Lag}(k_{21}-\alpha)}{\alpha^2(k_{Lag}-\alpha)(\beta-\alpha)}(e^{-\alpha t}-1)+\frac{(1-f)Jk_gk_{Lag}(k_{21}-\beta)}{\beta^2(k_{Lag}-\beta)(\alpha-\beta)}(e^{-\beta t}-1) \quad (4)$$

where J is skin permeation rate from the formulation directly to the subcutaneous agar gel and to the central compartment, and f or $1-f$ ($0\leq f<1$) describes transfer ratio to the agar gel or central compartment, respectively. The k_{Lag} is the first order rate constant to describe the lag time, where $J=J_{ss}(1-e^{-k_{Lag}t})$ (J_{ss}: steady state skin permeation rate).

Materials and Methods

Materials ANP was purchased from Tokyo Chemical Pharmaceutical Co., Ltd. (Tokyo, Japan). LC and PXC were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). FP was supplied by Kaken Pharmaceutical Co., Ltd. (Urayasu, Chiba, Japan). Hydroxypropylcellulose (HPC-H) was supplied by Nihon Soda Co., Ltd. (Tokyo). Agar was purchased from Funakoshi Co., Ltd. (Tokyo). Other chemicals and solvents were of reagent grade and were used without further purification.

Determination of Drug Solubility in Phosphate Buffered Saline (PBS) (pH7.4) and Distilled Water Solubility of each drug was measured in 1/30 M PBS (pH7.4) and distilled water at 37 °C. The obtained apparent solubility was denoted as S'_{PBS} and S'_w, respectively.

Determination of *n*-Octanol/PBS and *n*-Octanol/Distilled Water Distribution Coefficients *n*-Octanol was saturated at 37 °C with pH7.4 PBS or distilled water for at least 24 h before the experiment. An aliquot of test drug was dissolved in *n*-octanol-saturated PBS or water. The obtained solution was mixed with an equal volume of PBS or water-saturated *n*-octanol at 37 °C until equilibrium. Aqueous phase was then separated by centrifugation to analyze the drug concentration by HPLC. Apparent *n*-octanol/PBS or *n*-octanol/water distribution coefficients ($K'_{o/PBS}$, $K'_{o/w}$) were determined. The true distribution coefficient (K) was also estimated by the resulting $K'_{o/PBS}$ and Ka (acid dissociation constant) for each drug.

Agar Gel Preparation[9,10] Agar was dissolved in pH7.4 PBS at 80 °C to obtain a 2.5% solution. The resulting agar solution was poured into a polystyrene tissue culture dish (Iwaki Glass, Tokyo) and the top was sealed with a polyethylene sheet (0.1 mm in thickness) as a drug-impermeable backing. The solution was stored at 4 °C to constitute a solid gel. The resulting agar gel was a disc shape 3.85 cm in diameter, 0.5 cm in thickness and weighing about 6 g.

Preparation of Formulation Each drug and HPC-H were dispersed in an adequate amount of pH7.4 PBS at 80 °C. The solution was stored at ambient temperature to constitute a gel. Final concentrations of ANP, LC, PXC and FP were 50, 3, 1 and 1%, respectively.

***In situ* Experimental Method** Male hairless rats (WBN/ILA-Ht) weighing 220—300 g, supplied either by Life Science Research Center, Josai University (Sakado, Saitama, Japan) or Ishikawa Experimental Animal Laboratory (Fukaya, Saitama) were used in all animal experiments. One agar gel was surgically inserted between the abdominal subcutaneous tissue and the muscle layer, as described earlier.[9,10] A glass diffusion cell (effective diffusion area, 3.14 cm^2) was then attached to the skin just above the implanted agar gel using a cyanoacrylate adhesive (Aron Alpha®, Konishi, Osaka, Japan).

Intravenous Injection Studies A bolus i.v. injection study was conducted in rats with the implanted agar gel and a drug-free formulation on skin. Each drug, dissolved in PBS (0.8 or 1.5 mg/ml), was injected as a bolus into the jugular vein (2.0 or 4.0 mg/kg). At predetermined times, blood samples (0.25 ml) were collected from the contralateral jugular vein over a period of 10 h. The agar gel was removed at 0.5, 1, 2, 4, 6, 8 or 10 h after injection to measure the drug content. A fresh agar gel disc was inserted again at the sampling times.

Topical Application Studies The topical formulation containing a drug was placed in a glass cell (1.0 g/3.14 cm^2). A blood sample (0.25 ml) was withdrawn every two hours over a period of 10 h. The agar gel was also replaced with a new one at each blood sampling to maintain a sink condition for the drug against the formulation.

Analytical Methods The blood samples were centrifuged to obtain plasma. Plasma samples were mixed with two volumes of acetonitrile containing an internal standard. The agar gels were homogenized (13000 rpm, 3 min) in 10 ml of an internal standard solution with an Ace homogenizer (Nihon Seiki, Tokyo). After centrifugation (4 °C, 15000×***g***, 5 min), each supernatant was injected into an HPLC which was composed of a pump (LC-10AS, Shimadzu, Kyoto, Japan), a UV detector (SPD-10A, Shimadzu), an integrator (C-R5A, Shimadzu), a system controller (SCL-10A, Shimadzu), an auto injector (SIL-$10A_{XL}$, Shimadzu), and a reverse phase column (Inertsil ODS, 4.6 mm×250 mm, GL Sciences Inc., Tokyo). The flow rate was 1.0 ml/min. Table 1 lists the mobile phase, UV wavelength and internal standard for each drug.

Table 1. Analytical Conditions

Drug	Mobile phase	Wavelength (nm)	Internal standard
ANP	Acetonitrile : water (15 : 85)	242	Methyl *p*-hydroxybenzoate
LC	Acetonitrile : 0.1% phosphoric acid (50 : 50)+5mM sodium dodecylsulfate	210	Amyl *p*-hydroxybenzoate
PXC	Acetonitrile : 0.1% phosphoric acid (35 : 65)	240	Isopropyl *p*-hydroxybenzoate
FP	Acetonitrile : 0.1% phosphoric acid (50 : 50)	245	Isopropyl *p*-hydroxybenzoate

Table 2. Physicochemical Parameters and Protein-Unbound Ratio in Plasma for Each Drug

Drug	M.W. (g/mol)	mp (°C)	p*K*a	log $K'_{o/w}$	log $K'_{o/PBS}$	log K	S'_w (μg/ml)	S'_{PBS} (μg/ml)	*fu*
ANP	188.23	105	1.5	0.30 (±0.02)	0.37 (±0.01)	0.37	6.27×10^6 ($\pm13.2\times10^3$)	5.86×10^6 ($\pm21.2\times10^3$)	0.98
LC	234.34	65	8.0	2.38 (±0.04)	1.38 (±0.05)	2.08	3471 (±45)	4006 (±63)	0.30
PXC	331.35	201	1.8, 5.1	0.19 (±0.02)	1.14 (±0.04)	3.44	18.85 (±0.16)	738.8 (±5.4)	0.01
FP	244.26	113	3.7	2.72 (±0.09)	0.90 (±0.02)	4.57	8.59 (±0.74)	3810 (±65)	0.01

Each value in parenthesis represents S.E. of at least three experiments.

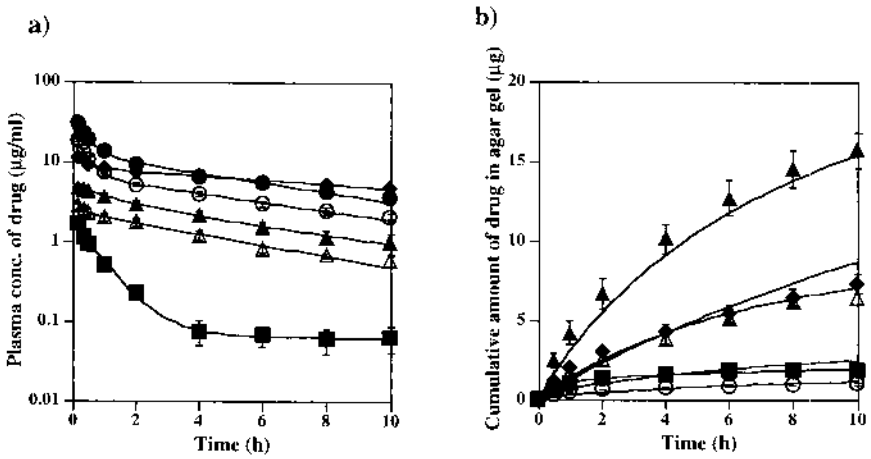

Fig. 2. Time Courses of Plasma Concentration (a) and Amount in Agar Gel (b) of Each Drug after i.v. Injection

Key: closed symbol, 4 mg/kg; open symbol, 2 mg/kg; ● ○, flurbiprofen; ▲ △, antipyrine; ■, lidocaine; ◆, piroxicam. The line was obtained by curve-fitting to Eqs. 1 and 2, and each point represents the mean±S.E. of at least three experiments.

Estimation of Pharmacokinetic Parameters Pharmacokinetic parameters of each drug were estimated using time courses of plasma and agar gel levels of the drugs and a nonlinear least-squares method (algorithm: Damping-Gauss Newton). Plasma concentration after i.v. injection was first analyzed to obtain A, B, α and β, using Eq. 1. The parameters, f, J, k_{Lag} and k_g were obtained by simultaneous curve-fitting of the time courses of cumulative amounts in the agar gel after i.v. and topical applications and plasma concentration after topical application (to Eqs. 2, 3 and 4), by fixing the A, B, α and β values. In the case of FP and ANP, 2mg-data were used for the curve fitting.

Table 3. Pharmacokinetic Parameters for Each Drug after i.v. Injection

	ANP		LC	PXC	FP	
Dose (mg/kg)	2.0	4.0	4.0	4.0	2.0	4.0
k_{12} (h^{-1})	1.33	0.15	0.98	0.79	1.70	1.52
k_{21} (h^{-1})	3.03	0.53	0.06	1.65	0.87	0.94
$k_{10}+k_g$ (h^{-1})	0.23	0.17	0.19	0.088	0.42	0.40
V_1 (l/kg)	0.574	0.799	2.284	0.308	0.080	0.098
V_2 (l/kg)	0.250	0.226	40.144	0.148	0.157	0.159

Results

Table 2 shows molecular weight (M.W.), melting point (mp), pKa, apparent *n*-octanol/distilled water and *n*-octanol/PBS distribution coefficient ($K'_{o/w}$, $K'_{o/PBS}$), true *n*-octanol/water distribution coefficient (=distribution coefficient of unionized drug) (K), solubility in distilled water and pH7.4 PBS (S'_w, S'_{PBS}), and plasma protein-unbound fraction (*fu*) of each drug. Under physiological condition (pH7.4), ANP exists mostly as an unionized form and PXC and FP exist mostly as an ionized form. LC exists as both forms (unionized : ionized=1 : 4). The K value is an index for lipophilicity of unionized form that was in the order of FP> PXC>LC>ANP. The *fu* value of FP and PXC is low and that for the others is high.

Figures 2a and b show the time courses of plasma concentration and the cumulative amount in agar gel, respectively, of PXC, LC and ANP after i.v. injection (4 mg/kg). The result of FP is presented for comparison. A general linear two-compartment model was applied for every drug disposition (Fig. 2a). Pharmacokinetic parameters of each drug obtained

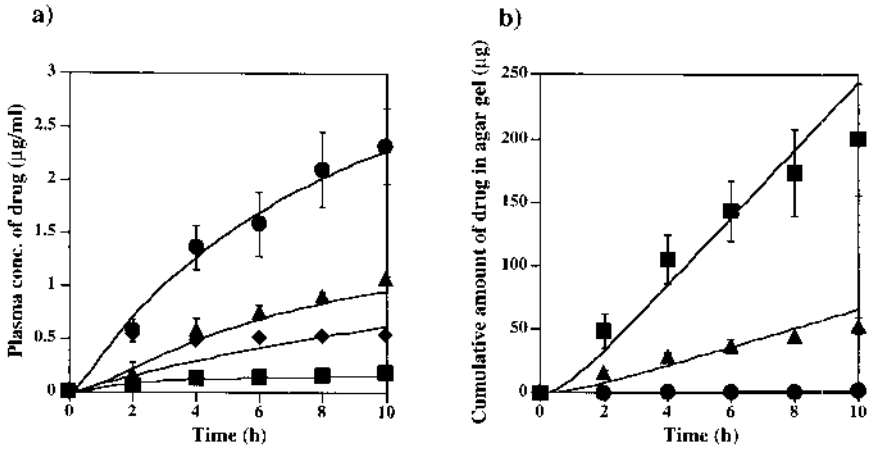

Fig. 3. Time Courses of Plasma Concentration (a) and Amount in Agar Gel (b) of Each Drug after Topical Application

Key: ●, flurbiprofen; ▲, antipyrine; ■, piroxicam; ◆, lidocaine. The line was obtained by curve-fitting to Eqs. 3 and 4, and each point represents the mean±S.E. of at least three experiments.

Table 4. Pharmacokinetic Parameters for Each Drug after Topical Application

	ANP	LC	PXC	FP
J (μg/h)	49.66 (62.27)	113.68	10.57	25.77
$CL_{ab}\times10^{-3}$ (ml/h)	0.1	28.4	14.31	6.76
CL^*_{ab} (ml/h)	0.0001	0.1413	2.8686	31.6388
f(%)	14	23	0.24	0.71
$k_g\times10^{-3}$ (h^{-1})	3.9	1.5	1.4	1.3
CL_g (ml/h/kg)	2.23	3.48	0.43	0.1
CL^*_g(ml/h/kg)	2.23	17.33	85.48	467.84
CL_g/fu (ml/h/kg)	2.28	11.60	42.63	10
CL^*_g/fu (ml/h/kg)	2.28	57.78	8548.4	46783.5
k_{Lag} (h^{-1})	1.04	1.22	1.46	1.25

Table 5. Drug Disposition in Skin after Topical Application

(a) % fraction of distributed drug into skin from the formulation

	ANP	LC	PXC	FP
To blood	86.0	76.6	99.8	99.3
To agar gel	14.0	23.4	0.2	0.7

(b) % fraction of drug in agar gel

	ANP	LC	PXC	FP
From blood	4.8	0.4	61.9	17.2
From formulation	95.2	99.6	39.1	82.8

using a nonlinear least-squares method are summarized in Table 3. The fitting line in Fig. 2a was close to the observed values. The cumulative amount in agar gel of each drug was in the order of ANP>PXC>FP>LC. The effects of the surgical procedure and agar gel implantation on the pharmacokinetics of each drug in rats were negligible because the total body clearance in rats with and without the agar gel was almost the same.[9] The cumulative amount in agar gel was proportional to the area under plasma concentration–time curves (AUC) at each sampling time point (data not shown).

Figures 3a and b show the time courses of plasma concentration and the cumulative amount in agar gel of each drug after topical application. Solid lines in Fig. 3a and b are fitting curves to Eqs. 3 and 4, respectively. The fitting lines were close to the observed values, suggesting that the present pharmacokinetic model is valid.

Table 4 summarizes the pharmacokinetic parameters (J, f, k_g and k_{Lag}) together with clearances after topical application. J in parenthesis of ANP indicates the value by assuming the drug was applied as a suspension in the HPC gel formulation. Absorption clearance (CL_{ab}) was obtained by dividing the steady-state flux (J_{ss}) by S'_{PBS} (applied conc. only for ANP). Absorption clearance of unionized drug (CL^*_{ab}) (=permeability coefficient of unionized drug×application area) was determined by dividing the steady-state flux (J_{ss}) by the applied free drug concentration. The CL^*_{ab} was in the order of FP>PXC>LC>ANP. The direct transfer ratio from the formulation to the subcutaneous agar gel (f) was higher for LC and ANP and lower for FP and PXC. A product of k_g and V_1 denoted as CL_g. CL^*_g means transfer clearance from systemic circulation to agar gel for unionized form. Values of CL^*_g and CL^*_g/fu (transfer clearance from systemic circulation to agar gel for protein-unbound and unionized form) were in the order FP>PXC>LC>ANP.

Table 5 shows the drug transfer ratio from the formulation to agar gel and systemic circulation using the obtained results after i.v. injection and topical application. These transfer ratios were calculated using the fitting parameters shown. These results indicate that over 75% of the drug amount in skin absorbed from the formulation was transferred to the systemic circulation. This transfer ratio was in the order PXC>FP>ANP>LC. Furthermore, the direct transfer ratio to subcutaneous agar gel was higher for LC and ANP and lower for FP and PXC. The amount in agar gel after topical application includes directly distributed drug from the epidermis and redistributed drug from systemic circulation. Each contribution was also calculated using the obtained pharmacokinetic parameters. The calculated results are shown in Table 5b. The transferred FP, ANP and LC in agar gel was less than 20% from systemic circulation and more than 80% directly from formulation. Consequently, most of the drug in agar gel came from the formulation. In contrast, only PXC had an especially higher transfer ratio from systemic circulation to agar gel, *i.e.* only 39.1% in agar gel was transferred directly from formulation.

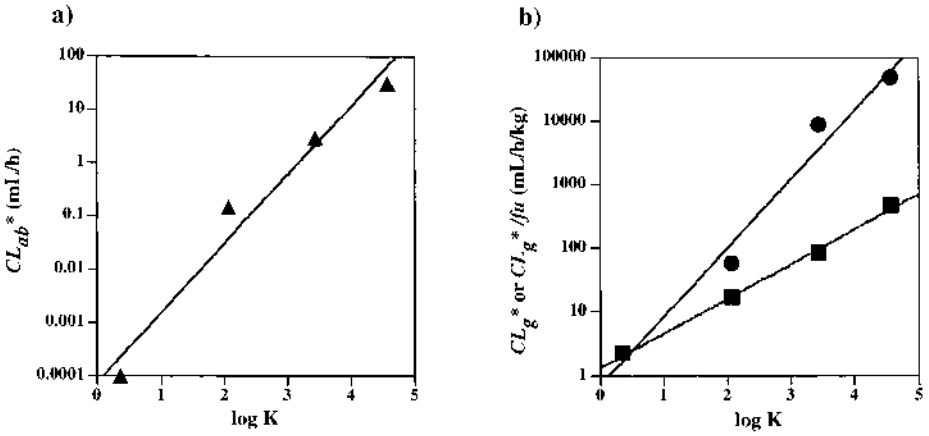

Fig. 4. Relationship between Absorption Clearance of Unionized Form (a) or Migration Clearance from Systemic Circulation to Subcutaneous Agar Gel (b) and True *n*-Octanol/Water Distribution Coefficient

Key: ●, CL_g^*; ■, CL_g^*/fu. Each point represents the mean value of at least three experiments.

Discussion

Time courses of plasma concentration and subcutaneous agar gel amount were followed after i.v. injection or topical application of drugs in hairless rats having an agar gel disc in the abdominal region, to evaluate systemic and cutaneous drug disposition. Elimination processes were expressed by linear 2-compartment model for ANP, LC and PXC as well as FP. No significant difference was found between the total clearances in rats with and without the subcutaneous agar gel for each drug. We can thus disregard the effect of agar gel disc-implantation on the elimination kinetics of the drugs. A fairly good relationship was found between the *AUC* and the cumulative amount in the agar gel of each drug, suggesting that reabsorption from the agar gel to the cutaneous blood could be ignored and that the agar gel could be assumed to be a sink, at least for the present experimental period. Previous data for the zero-order i.v. infusion of FP showed a good relationship between the *AUC* and the cumulative amount in the agar gel for each time period with and without change in the infusion rate, indicating no time- or dose-independent pharmacokinetics.[9)]

Next, the effect of physicochemical properties of drugs on the cutaneous disposition is discussed. Let us consider the percutaneous absorption process of drugs. In general, skin permeation rate J is determined by concentration gradient and diffusion coefficient of a drug across the stratum corneum, the biggest skin barrier. The concentration gradient is affected by the amount of drug applied on the skin surface. This was determined by a product of saturated concentration in the vehicle and distribution coefficient of drugs except ANP, because other drugs were saturated in the HPC gels. mp may be an index for the drug solubility in vehicle, and M.W. has great influence on the diffusivity. In the present experiments, a drug having a high mp and a large M.W. (PXC) showed a relatively low J value. More drugs should be evaluated to ensure the relation due to a narrow distribution of M.W. of the present four model drugs (188—331 Da). Figure 4a shows a good relationship between CL_{ab}^* and K, indicating that the percutaneous drug absorption was determined primarily by the drug distribution from the vehicle to the skin barrier. Similar results were reported using *in vitro* and *in vivo* experiments.[13—15)]

Redistribution from the systemic circulation to the subcutaneous agar gel was evaluated using a concept of clearance as follows. Drug migration from blood to cutaneous or subcutaneous tissues is generally affected by many factors such as distribution coefficient from blood to the tissues, diffusion properties through the tissues and the cutaneous epithelial membranes, protein binding in blood and tissues, and the cutaneous blood flow. Figure 4b illustrates the relation between CL_g^* or CL_g^*/fu and K. Although both lines are close to the data points, a better relationship was obtained between CL_g^* and K, suggesting that redistribution from the cutaneous vessels to the agar gel through the systemic circulation should be influenced mainly by the distribution property of unionized drugs. Blood flow and drug diffusivity in the cutaneous tissues may also be important, so that detailed experiments should be carried out to totally understand the drug disposition phenomena in skin.

Let us turn to a parameter, f, the drug fraction from topical formulation directly to the subcutaneous agar gel. No clear physicochemical parameters related to f were obtained. Solubility in blood, resistance across the transepithelial permeation, distribution into skin and diffusivity across skin of drugs may be factors correlating with f. A tendency was found that a drug with low lipophilicity and low protein binding had a high f value. Detailed experiments, however, are necessary to determine a physicochemical parameter which well correlates with f.

Finally, drug migrations into the systemic circulation and the cutaneous and subcutaneous tissues are considered. Migration rate from the formulation to the systemic circulation, which is related to $(1-f)J$, was in the order of PXC>FP>ANP>LC and much greater than that to agar gel disc for all the drugs. The amount of drugs in the agar gel was mainly contributed from direct migration from the formulation, except PXC, and a minor contribution was redistribution from the cutaneous blood vessels. This clearly shows that a higher effective targeting of drugs into the cutaneous and subcutaneous tissues can be obtained by topical application than by systemic administration. Drug migration from the systemic circulation to the agar gel was decreased in the order of PXC>FP>ANP>LC, and the rate was closely related to the k_g value. A low targeting ability of PXC into the cutaneous

and subcutaneous tissues from topical formulation may be due to a high protein binding which decreases drug diffusivity in the cutaneous tissues. A higher M.W. of PXC than FP may be another reason.

Conclusion

The present *in situ* experimental method was utilized to evaluate and differentiate drug transports from topical formulation either to the cutaneous and subcutaneous tissue or to the systemic circulation. Such evaluation of different properties of compounds enables clear understanding of each process of drug migration in skin.

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