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Estimation of Blood Concentration of Drugs after Topical Application from *in Vitro* Skin Permeation Data. II.[1] Approach by Using Diffusion Model and Compartment Model

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In order to estimate the *in vivo* behavior (*e.g.*, blood concentration) of drugs on percutaneous absorption from *in vitro* permeation data using excised skin and pharmacokinetic parameters at intravenous administration, two models, a diffusion model and a compartment model with or without shunt transport, were established and applied to the *in vitro* and *in vivo* skin permeation data of nicorandil in hairless rats. When water was employed as a donor solvent (water treatment), the plasma concentration–time curve, which was calculated by means of the diffusion model, was similar to the observed values without introduction of shunt transport. In the case of propylene glycol treatment, on the other hand, the calculated plasma levels were not necessarily similar to the observed values, but it was found that the calculated values were better fitted to the observed values by introduction of shunt transport in the *in vitro* skin diffusion model. Similar results were obtained with the compartment model. These results suggest that both models may be useful to predict the plasma concentration after application of topical formulations. The characteristics and usefulness of these models were compared with those of the previously reported convolution method.

Keywords——blood concentration; diffusion model; compartment model; skin permeation; nicorandil; shunt transport; convolution method; estimation

Introduction

It is well known that the permeation of drug molecules across the skin occurs by a passive diffusion process according to the activity gradient.[2] Therefore, the pharmacokinetics of percutaneous absorption have generally been discussed in terms of a diffusion model.[3] On the other hand, another kind of approach, which is called the compartment model, has also been presented.[4] Both models have unique characteristics, but no report has appeared comparing their usefulness and shortcomings. In the previous study,[1] we presented a convolution method to estimate the blood (plasma) concentration of drugs after topical application from *in vitro* skin permeation data and pharmacokinetic parameters at intravenous administration. The convolution method was suitable for estimating the *in vivo* behavior within the range of acceptable variation. For predicting long-term blood level, however, the convolution method requires a long-term sampling in the *in vitro* skin permeation experiment on the same schedule as required *in vivo*. Moreover, the method might not be suitable for the comparison of *in vitro* skin permeation behaviors among several drugs, because no parameters characterizing drug transport in the skin are used.

In the present paper, a complete set of *in vitro* skin permeation data for nicorandil was analyzed first by applying the diffusion model and compartment model without shunt transport to obtain parameters of the skin permeation (model I). Secondly, the first half set of

data for the *in vitro* skin permeation (data from 0 to 10 h) was analyzed in a similar manner (model II). As the skin conditions between *in vitro* and *in vivo* experiments may be somewhat different, that is, *in vitro* skin permeation coefficients of drugs increase with time of aqueous immersion (as discussed in detail later), a new model with a zero-order transport rate (shunt) only for the later phase (after t_s) of the *in vitro* permeation was constructed. The whole data set was also analyzed by applying both models with the shunt transport (model III). The resulting parameters were used to estimate the *in vivo* percutaneous absorption of the drug. Finally, the usefulness and shortcoming of the diffusion model and compartment model were compared to those of the convolution method. Previously reported data[1] for skin permeation involving water and propylene glycol (PG) treatments were employed.

Theoretical

Diffusion Model

(a) Analysis of the *in Vitro* Experiment——If the skin is supposed to be a single membrane, a diffusion model for percutaneous drug absorption may be set up as illustrated in Fig. 1(a). It is assumed that shunt transport exists after the time, t_s. The amount of a drug dissolved in the vehicle is assumed to be constant (solubility) when the drug is applied topically as a suspension. The fluid-filled receiver from which samples are withdrawn is assumed to obey the sink condition. Under these assumptions, differential equations describing the drug transfer phenomena in terms of Fig. 1(a) are

$$dX_v/dt = 0 \tag{1}$$

$$dX_s/dt = -DA_1(\partial C_s/\partial x)_{x=0} + DA_1(\partial C_s/\partial x)_{x=L} \tag{2}$$

$$dX_r/dt = -DA_1(\partial C_s/\partial x)_{x=L} \qquad (0 \le t < t_s) \tag{3}$$

$$= -DA_1(\partial C_s/\partial x)_{x=L} + P_s A_1 C_v \qquad (t \ge t_s) \tag{3'}$$

The details of the equations and abbreviations are given in the legend to Fig. 1. The amount of drug in the receiver phase as a function of time can be solved by integrating Eq. 3′ as follows:

$$X_r = -DA_1 \int_0^t (\partial C_s/\partial x)_{x=L} \cdot dt + P_s A_1 C_v (t - t_s) \tag{4}$$

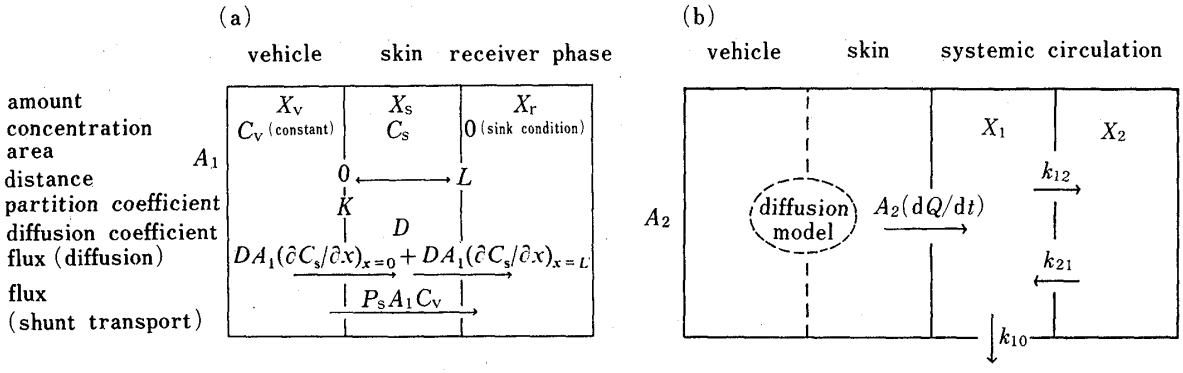

Fig. 1. Schematic Diffusion Model of *in Vitro* (a) and *in Vivo* (b) Percutaneous Drug Absorption

The skin is postulated to be a diffusion membrane with thickness L, diffusion coefficient D and transport rate constant of shunt pathway P_s. The amounts of the drug dissolved in the vehicle, skin and receiver phase are designated as X_v, X_s and X_r, respectively. The concentration of the drug in the vehicle and effective diffusional area are designated as C_v and A_1, respectively. At the vehicle-skin boundary, the drug is partitioned according to the partition coefficient K.

The cumulative amount of drug permeated per unit area, Q, is

$$Q=-D\int_0^t(\partial C_s/\partial x)_{x=L}\cdot dt+P_sC_v(t-t_s) \quad (5)$$

In order to relate Q to the physicochemical process taking place, it is necessary to solve Fick's Second Law of Diffusion (Eq. 6),

$$\partial C_s/\partial t=D(\partial^2 C_s/\partial x^2) \quad (6)$$

for the drug in the skin with appropriate boundary conditions. The latter are as follows (refer to Fig. 1(a)):

$$x=0, \quad C_s=KC_v \quad (t>0)$$

$$x=L, \quad C_s=0 \quad (t>0)$$

The initial condition is described as

$$t=0, \quad C_s=0 \quad (0<x<L)$$

From Eq. 6, boundary conditions and the initial conditions,

$$(\partial C_s/\partial x)_{x=L}=-KC_v\left\{1/L+2/L\sum_{n=1}^{\infty}(-1)^n\cdot\exp(-n^2\pi^2D/L^2\cdot t)\right\} \quad (7)$$

Substituting Eq. 7 into Eq. 5 yields the following general equation for the cumulative amount of drug permeated.

$$Q=DKC_v/L\cdot(t-L^2/6D)-2KC_vL/\pi^2\sum_{n=1}^{\infty}(-1)^n/n^2\cdot\exp(-n^2\pi^2D/L^2\cdot t)+P_sC_v(t-t_s) \quad (8)$$

DKC_v/L, $6D/L^2$ and P_sC_v may be replaced with $P(1)$, $P(2)$ and $P(3)$, respectively. Eq. 8 becomes

$$Q=P(1)\{t-1/P(2)\}-12P(1)/\pi^2P(2)\sum_{n=1}^{\infty}(-1)^n/n^2\cdot\exp(-n^2\pi^2P(2)/6\cdot t)+P(3)(t-t_s) \quad (9)$$

where $P(1)$, $P(2)$ and $P(3)$ can be calculated from the *in vitro* permeation data by curve-fitting using a non-linear least-squares method.[5] The final term in Eq. 9, $P(3)\cdot(t-t_s)$, is used only for $t\geq t_s$ and is supposed to be zero for $0\leq t<t_s$ (refer to Eqs. 3 and 3′).

(b) Prediction of the *in Vivo* Behavior——If the drug elimination process obeys a two-compartment model, the diffusion model for *in vivo* percutaneous drug absorption may be set up as illustrated in Fig. 1(b). In this case, differential equations describing the system in terms of Fig. 1(b) are

$$dX_1/dt=A_2(dQ/dt)-(k_{12}+k_{10})X_1+k_{21}X_2 \quad (10)$$

$$dX_2/dt=k_{12}X_1-k_{21}X_2 \quad (11)$$

where A_2, X_1 and X_2 are the application area and the amounts of drug in central and peripheral compartments, respectively, and k_{10}, k_{12} and k_{21} are the first-order rate constants.

The *in vivo* absorption rate, dQ/dt, can be obtained by differentiating Eq. 9,

$$dQ/dt=P(1)\left\{1+2\sum_{n=1}^{\infty}(-1)^n\cdot\exp(-n^2\pi^2P(2)/6\cdot t)\right\} \quad (12)$$

provided that $P(3)$ is zero, because of the assumption that the shunt exists only *in vitro*.

Substituting Eq. 12 into Eq. 10 yields the following equation showing the blood

concentration of drug, C_p, after topical application.

$$C_p=\frac{P(1)MA_2}{D_o(\mathrm{iv})}\left[\frac{1-e^{-\alpha t}}{\alpha}-\frac{e^{-\alpha t}}{P(2)}-\frac{12}{\pi^2P(2)}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\left\{\frac{\frac{n^2\pi^2P(2)}{6}e^{-n^2\pi^2P(2)\cdot t/6}-\alpha e^{-\alpha t}}{\frac{n^2\pi^2P(2)}{6}-\alpha}\right\}\right]$$
$$+\frac{P(1)NA_2}{D_o(\mathrm{iv})}\left[\frac{1-e^{-\beta t}}{\beta}-\frac{e^{-\beta t}}{P(2)}-\frac{12}{\pi^2P(2)}\sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\left\{\frac{\frac{n^2\pi^2P(2)}{6}e^{-n^2\pi^2P(2)\cdot t/6}-\beta e^{-\beta t}}{\frac{n^2\pi^2P(2)}{6}-\beta}\right\}\right] \quad (13)$$

where M, N, α and β are the pharmacokinetic parameters after intravenous administration at a dose of D_o(iv) as shown in the next equation for drug concentration in plasma, C_p(iv).

$$C_p(\mathrm{iv})=M\cdot e^{-\alpha t}+N\cdot e^{-\beta t} \quad (14)$$

Compartment Model

(a) Analysis of the *in Vitro* Experiment——When the vehicle, skin and receiver phase are regarded as independent compartments, the compartment model for percutaneous drug absorption may be set up as illustrated in Fig. 2(a). It is assumed that shunt transport exists after the time, t_s. The amount of a drug dissolved in the vehicle is assumed to be constant (solubility) when the drug is applied topically as a suspension. The receiver phase is assumed to obey the sink condition. Under these assumptions, differential equations describing the drug transfer phenomena in each compartment as shown in Fig. 2(a) are

$$dX_v/dt=0 \quad (15)$$

$$dX_s/dt=k_iX_v-k_eX_s \quad (\text{where } k_e=k_j+k_d) \quad (16)$$

$$dX_r/dt=k_dX_s \quad (0\le t<t_s) \quad (17)$$

$$=k_dX_s+k_sX_v \quad (t\ge t_s) \quad (17')$$

The details of the equations are given in the legend to Fig. 2. The amount of the drug in the receiver compartment as a function of time becomes

$$X_r=k_ik_dX_v/k_e\{t+\exp(-k_et)/k_e^{-1}/k_e\}+k_sX_v(t-t_s) \quad (18)$$

The cumulative amount of drug permeated per unit area, Q, is

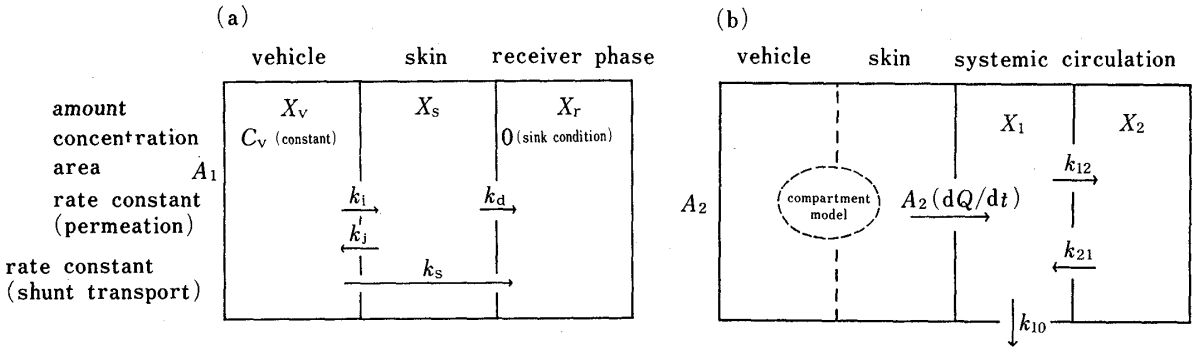

Fig. 2. Schematic Compartment Model of *in Vitro* (a) and *in Vivo* (b) Percutaneous Drug Absorption

X_v, X_s, X_r, C_v and A_1 are the same in the legend to Fig. 1. The first-order rate constants connecting each compartment are designated as k_i, k_j and k_d, and that of the shunt transport is indicated as k_s.

$$Q = k_i k_d X_v / k_e A_1 \{t + \exp(-k_e t)/k_e - 1/k_e\} + k_s X_v / A_1 \cdot (t - t_s) \quad (19)$$

$k_i k_d X_v / k_e A_1$, k_e and $k_s X_v / A_1$ may be replaced with $P(1)$, $P(2)$ and $P(3)$, respectively. Eq. 19 becomes

$$Q = P(1)\{t + \exp(-P(2) \cdot t)/P(2) - 1/P(2)\} + P(3)(t - t_s) \quad (20)$$

where $P(1)$, $P(2)$ and $P(3)$ can be calculated from the *in vitro* permeation data by curve-fitting using the non-linear least-squares method. The final term in Eq. 20, $P(3) \cdot (t - t_s)$, is used only for $t \geq t_s$ and is supposed to be zero for $0 \leq t < t_s$ (refer to Eqs. 17 and 17′).

(b) Prediction of the *in Vivo* Behavior——If the drug elimination process obeys the two-compartment model, the compartment model for the *in vivo* percutaneous drug absorption can be set up as illustrated in Fig. 2(b). In this case, differential equations describing the system in terms of Fig. 2(b) are

$$dX_1/dt = A_2(dQ/dt) - (k_{12} + k_{10})X_1 + k_{21}X_2 \quad (21)$$

$$dX_2/dt = k_{12}X_1 - k_{21}X_2 \quad (22)$$

The *in vivo* absorption rate, dQ/dt, can be obtained by differentiating Eq. 20,

$$\frac{dQ}{dt} = P(1)\{1 - \exp(-P(2) \cdot t)\} \quad (23)$$

provided that $P(3)$ is zero, because of the assumption that the shunt exists only *in vitro*.

Substituting Eq. 23 into Eq. 21 yields the following equation showing the blood concentration of drug after topical application.

$$C_p = \frac{A_2}{D_0(\mathrm{iv})}\left[\frac{MP(1)P(2)}{\alpha\{\alpha - P(2)\}}e^{-\alpha t} + \frac{NP(1)P(2)}{\beta\{\beta - P(2)\}}e^{-\beta t} + \frac{P(1)\{P(2)(M+N) - \alpha N - \beta M\}}{\{\alpha - P(2)\}\{\beta - P(2)\}}e^{-P(2)t} + \frac{P(1)(\alpha N + \beta M)}{\alpha\beta}\right] \quad (24)$$

Results and Discussion

Previously reported data for nicorandil permeation,[1] that is, the cumulative amount of the drug permeated through excised hairless rat skin and the plasma concentration after topical application of gel ointments, were employed as raw data.

In each figure below, the theoretical curve of *in vitro* skin permeation calculated by employing either Eq. 9 (diffusion model) or Eq. 20 (compartment model) is drawn together with the observed values on the left part (a) of each figure, and the plasma concentration–time curve estimated by employing either Eq. 13 (diffusion model) or Eq. 24 (compartment model) is plotted against the observed values on the right (b).

Analysis by Diffusion Model

Values of the steady-state flux and the reciprocal of lag time, which were calculated from the *in vitro* skin permeation profiles, were employed as the initial values of $P(1)$ and $P(2)$, respectively, for curve-fitting by the non-linear least-squares method. All initial and final values of each parameter are listed in Table I.

The analytical results for the *in vitro* and *in vivo* skin permeation in water and PG treatments over 24 h are shown as dotted lines in Figs. 3 and 4 (model D-I). As it was stipulated that shunt transport was not operating, the rate of shunt transport, P_s, in Fig. 1(a) was set at zero at all times. As shown in Figs. 3(a) and 4(a), the *in vitro* skin permeation

TABLE I. Calculated Final Values of Each Parameter in Diffusion (Model D) and Compartment (Model C) Models

Model	Water treatment			PG treatment		
	$P(1)$	$P(2)$	$P(3)$	$P(1)$	$P(2)$	$P(3)$
D-I	18.086 (18.591)	0.243 (0.223)	—	35.380 (33.878)	0.138 (0.154)	—
D-II	10.698 (10.661)	1.285 (1.327)	—	21.839 (17.707)	0.224 (0.316)	—
D-III	10.137 (10.698)	1.709 (1.285)	9.176 (7.388)	20.796 (21.839)	0.230 (0.224)	13.169 (13.541)
C-I	20.201 (18.591)	0.167 (0.223)	—	52.071 (33.878)	0.062 (0.154)	—
C-II	10.851 (10.661)	1.139 (1.327)	—	20.605 (17.707)	0.197 (0.316)	—
C-III	10.195 (10.851)	1.585 (1.139)	9.117 (9.350)	29.079 (20.605)	0.120 (0.197)	9.159 (31.466)

The values in parentheses are the initial values for curve-fitting by the non-linear least-squares method. Initial $P(1)$ is the steady-state flux over 24 h (model I) or 10 h (model II). Initial $P(2)$ in models I and II is the reciprocal of lag time. Initial $P(1)$ and $P(2)$ in model III are the final values obtained from model II. Initial $P(3)$ is obtained by subtracting final $P(1)$ in model II from final $P(1)$ in model I.

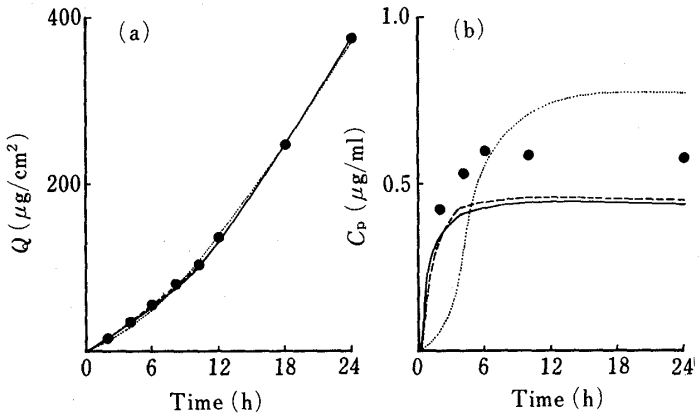

Fig. 3. Comparison between Observed and Calculated (Diffusion Model) Values of Skin Permeation of Nicorandil in Water Treatment

(a) Cumulative amount of permeation *in vitro*. (b) Time course of plasma concentration *in vivo*. (------), model D-I; (– – – –), model D-II; (——), model D-III.

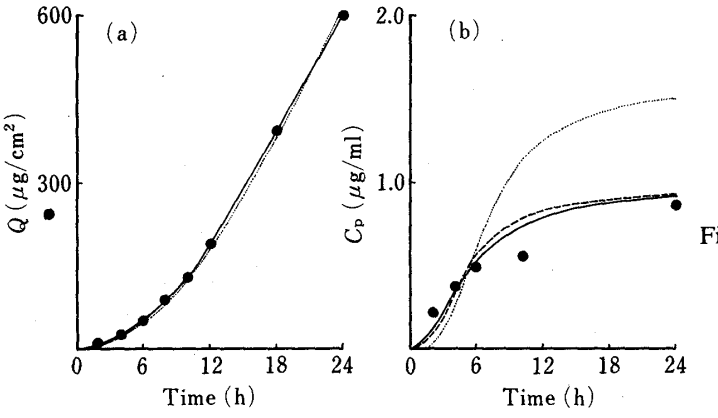

Fig. 4. Comparison between Observed and Calculated (Diffusion Model) Values of Skin Permeation of Nicorandil in PG Treatment

(a) Cumulative amount of permeation *in vitro*. (b) Time course of plasma concentration *in vivo*. (------), model D-I; (– – – –), model D-II; (——), model D-III.

profiles simulated by model D-I were well fitted to the observed values in both water and PG treatments. In water treatment, the calculated plasma steady-state level was similar to the observed value at 24 h, but did not fit all the data points, especially in the early phase (Fig. 3(b)). In PG treatment, on the other hand, the calculated values were not necessarily similar to the observed values in the later phase (Fig. 4(b)).

Next, the first half set of the *in vitro* data (from 0 to 10 h) was employed for the analysis and estimation as shown by the dashed lines in Figs. 3 and 4 (model D-II). The calculated plasma concentration–time curve fitted the observed values better than those in the case of model D-I, not only in water treatment but also in PG treatment.

These results suggest that the skin condition in the first phase of the *in vitro* permeation experiment would be similar to the *in vivo* situation, especially when hydrophilic vehicles such as water or PG were employed as the drug-donor compartment. One reason for the difference of skin condition between the *in vitro* and *in vivo* experiments after application of such vehicles is considered to be hydration effect. Behl *et al.*[6] have reported that hydration-related alteration of the permeation behavior was related to the physicochemical properties of the permeants, that is, the permeation coefficients of butanol and hexanol through hairless mouse skin doubled during a 10 h aqueous immersion (hydration) in contrast to polar permeants such as water and methanol, which were not affected by hydration. Since most drugs for transdermal delivery, including nicorandil, are rather lipophilic, the change in skin permeation coefficients in the *in vitro* experiment would be owing to skin hydration.

Therefore, the next analyses were carried out by using the model with shunt transport only for the later phase ($t_s = 10$ h) in the *in vitro* skin permeation (model D-III) as shown by a solid line in Figs. 3 and 4. The calculation method for the initial value of $P(3)$ is indicated in the footnote to Table I. As shown in Figs. 3(b) and 4(b), the calculated plasma concentration–time curve (solid line) fitted the observed values as well as the results simulated by model D-II (dashed line). It was apparent that introduction of shunt transport together with the passive diffusion process in *in vitro* skin permeation would be useful to describe the percutaneous absorption process of nicorandil.

Analysis by Compartment Model

In the same manner as for the diffusion model, the experimental data were analyzed on the basis of the compartment model, and the results are indicated in Figs. 5 and 6. The calculation method of initial values of $P(1)$, $P(2)$ and $P(3)$ for curve-fitting by the non-linear least-squares method was the same as that in the diffusion model. All initial and final values of each parameter are listed in Table I.

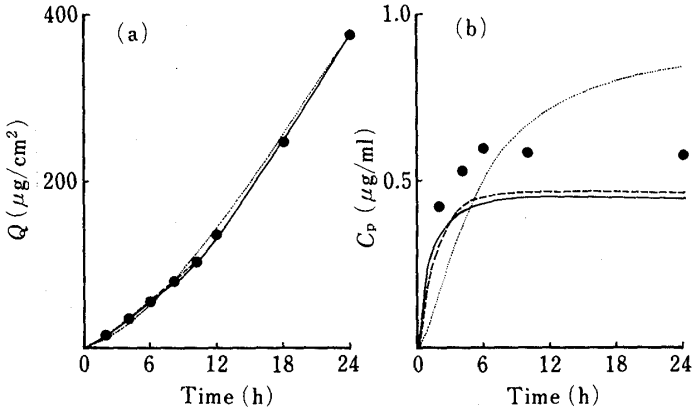

Fig. 5. Comparison between Observed and Calculated (Compartment Model) Values of Skin Permeation of Nicorandil in Water Treatment

(a) Cumulative amount of permeation *in vitro*. (b) Time course of plasma concentration *in vivo*. (------), model C-I; (– – – –), model C-II; (——), model C-III.

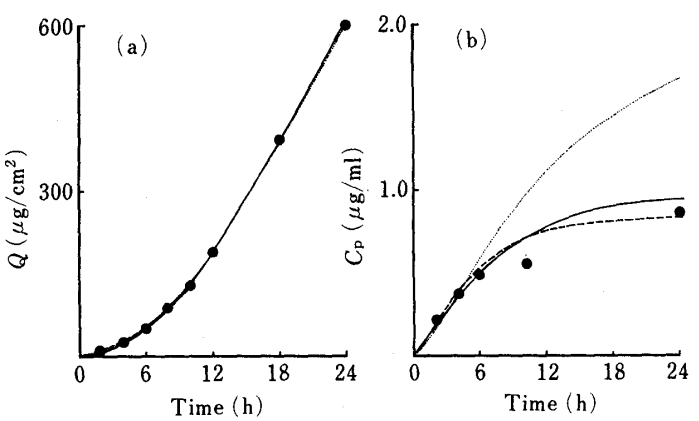

Fig. 6. Comparison between Observed and Calculated (Compartment Model) Values of Skin Permeation of Nicorandil in PG Treatment

(a) Cumulative amount of permeation *in vitro*. (b) Time course of plasma concentration *in vivo*. (------), model C-I; (– – – –), model C-II; (——), model C-III.

In the case of water treatment, the calculated plasma steady-state level was similar to the observed values at 24 h without introduction of shunt transport (dotted line, model C-I), similarly to the diffusion model (model D-I). In the case of PG treatment, it was also evident that the analyses by model C-II employing the *in vitro* data over 10 h (dashed line) and by model C-III with shunt transport for the later phase ($t_s = 10$ h) in the *in vitro* permeation (solid line) would be useful to consider nicorandil permeation in terms of the compartment model.

From these results, the models with shunt transport (model III), based on the assumption that the effect of skin hydration in the *in vitro* experiment increases gradually with time compared to that *in vivo*, would be appropriate rather than the models without the shunt (model I). The results simulated by model II were equivalent to those by model III, and hence the simpler model II (as compared with model III) was also considered to be available in the present analyses.

Characteristics of Diffusion Model and Compartment Model

The diffusion model is a most general theory of the percutaneous absorption process of drugs. In the diffusion model, each parameter influencing the rate of drug permeation, such as diffusion coefficient, D, partition coefficient between vehicle and skin, K, and/or thickness of skin barrier, L, has a clear physicochemical meaning. Therefore, the change of permeation flux may be related to the change of these parameters. However, complex numerical calculations are required in order to estimate the permeability of drugs. In the compartment model, on the other hand, the equations for calculation are simpler than those in the diffusion model, but the physiological significance is less clear, especially concerning the first-order rate constants connecting each compartment.

In the present study, it was found that both models (diffusion and compartment) were available for predicting the *in vivo* behavior from *in vitro* experimental data, that is, prediction of the plasma concentrations over 24 h was possible only from the *in vitro* data over 10 h (model II). Moreover, the model with shunt transport *in vitro* (model III) was also suggested to be useful. Shunt transport in the percutaneous absorption process has generally been thought to be parallel to the penetration through skin appendages such as hair follicles and sweat glands.[7] The present results suggest that an increase in flux owing to hydration (or solvation) is also related to the shunt transport. It is not clear when such hydration would start to influence the skin permeation profile, but the present models introducing shunt transport for the later phase *in vitro* are considered to be useful. Wallece and Barnett had also

TABLE II. Comparison of the Characteristics of the Diffusion Model, Compartment Model and Convolution Method

Method (Model)	Diffusion	Compartment	Convolution
Model dependency	Dependent	Dependent	Independent[a]
Physicochemical meanings of the parameters in relation to skin permeability	Parameters (D, K, L) are meaningful	Parameters (k_i, k_j, k_d) are less meaningful	No parameters are used
Derivation and utilization of the equation	Most difficult	Difficult	Easier
Estimation of the blood concentration beyond the *in vitro* experimental period	Possible	Possible	Impossible
Slight change or modification of model	Possible[b]	Possible[b]	Impossible

a) Useful whatever drugs are metabolized and/or adsorbed in the skin. *b*) For example, introduction of shunt transport.

introduced shunt transport into a compartment model,[4b] but it has not previously been used in a diffusion model in pharmacokinetic analysis.

Prediction of the *in vivo* blood concentration from *in vitro* permeation data using excised human or animal skin has become increasingly important in the development of transdermal therapeutic systems, and pharmacokinetic models such as diffusion and compartment models with or without shunt transport would be available in such cases. However, both methods are model-dependent, and hence it is necessary to construct a reasonable model in cases where the drug is metabolized and/or adsorbed in the skin. Therefore, several improved models based on detailed cutaneous physiology, incorporating metabolism in the skin and partitioning of drugs at the stratum corneum-viable tissue interface, have been reported.[8] On the other hand, novel pharmacokinetic analyses on percutaneous absorption have also been attempted.[9] In particular, a physiological model reported by McDougal *et al.*[9b] might be available for assessment of dermal absorption of chemical vapors and extrapolation to humans by substituting human physiological parameters for the animal values. However, since that model requires the general physiological parameters (*i.e.* blood flows and compartment volumes) and the *in vivo* skin permeation coefficients, it has not been attempted so far to predict the *in vivo* skin permeation behavior from the *in vitro* data after topical application of transdermal therapeutic systems. A complex model for pharmacokinetic analysis generally needs many parameters, and hence construction of simple but well-fitting models is a crucial requirement.

In summary, the characteristics of the two models (diffusion model and compartment model), which were derived from the present results for nicorandil, are compared with those of the convolution method in Table II.

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