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Estimation of Blood Concentration of Drugs after Topical Application from *in Vitro* Skin Permeation Data. I. Prediction by Convolution and Confirmation by Deconvolution

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A new theoretical expression is derived to estimate the rate and amount of the *in vivo* percutaneous absorption of drugs from *in vitro* skin permeation data. Based on two assumptions, that the skin permeation rate of the drug in the *in vivo* experiment is equal to that *in vitro* and that the drug elimination follows linear kinetics, the time course of the blood (total blood, plasma or serum) concentration of the drug after topical application could be expressed as a convolution equation. Based on the same concept, the permeation rates in *in vitro* and *in vivo* experiments are also compared by means of a deconvolution equation.

In the present study, nicorandil was selected as a model drug. The simulated plasma nicorandil level *vs.* time curve from the *in vitro* permeation study was approximately fitted to the *in vivo* experimental data in hairless rats, particularly up to 10 h. This method was valuable to estimate the time course of plasma concentration after topical application within the range of acceptable variation from the results of *in vitro* permeation experiments. Some difference between the observed and calculated plasma levels might be due to differences of the skin condition and so on in the *in vitro* and *in vivo* experiments.

Keywords——theoretical expression; percutaneous absorption; convolution; deconvolution; nicorandil; hairless rat; *in vitro–in vivo*; prediction

Introduction

In vitro permeation experiments using animal or cadaver skins have generally been utilized for estimating the skin permeability of drugs. An advantage of the method is the ready screening of vehicles. It is known that the steady-state blood concentration can be estimated from both the steady-state permeation rate across the excised skin and the total body clearance.[1] However, the estimation method cannot be applied to predict the time course of the blood concentration of drugs after topical application.

The processes of percutaneous absorption and elimination of drugs may be modeled as shown in Fig. 1. The time course of blood concentration may be calculated from the results of *in vitro* skin permeation experiments and the pharmacokinetic parameters estimated after intravenous administration *via* the model.

In the present paper, nicorandil, a potent coronary vasodilator,[2] was selected as a model drug because of its high skin permeability in order to verify our concept.

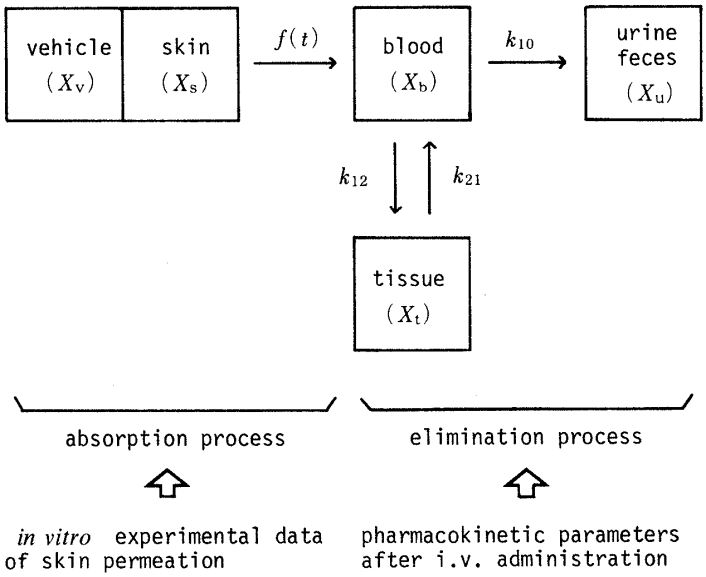

Fig. 1. Schematic Model of Percutaneous Absorption and Elimination of Drugs

Theoretical

Estimation of Blood Concentration from *in Vitro* Experimental Data by the Convolution Method

In the percutaneous absorption model illustrated in Fig. 1, symbols X_v, X_s, X_b, X_t and X_u are the amounts of drug at time t in the vehicle, skin, blood, tissue and urine (+feces), respectively. The sum of their values is equal to the initial dose, X_o.

$$X_v+X_s+X_b+X_t+X_u=X_o \tag{1}$$

When the drug disposition fits a two-compartment model, the following equations are obtained by differentiating Eq. 1:

$$-\left(\frac{dX_v}{dt}+\frac{dX_s}{dt}\right)=\frac{dX_b}{dt}+\frac{dX_t}{dt}+k_{10}\cdot X_b \tag{2}$$

$$\frac{dX_t}{dt}=k_{12}\cdot X_b-k_{21}\cdot X_t \tag{3}$$

where k_{10}, k_{12} and k_{21} are the first-order rate constants (refer to Fig. 1 for details).

The skin permeation rate, $f(t)$, is assumed to be given by:

$$A\cdot f(t)=-\left(\frac{dX_v}{dt}+\frac{dX_s}{dt}\right)=\frac{dX_b}{dt}+\frac{dX_t}{dt}+k_{10}\cdot X_b \tag{4}$$

where A is the application area.

From Eqs. 3 and 4, the time course of the blood concentration of a drug after topical application, $C_{ta}(t)$, can be expressed by the convolution equation:

$$\begin{aligned}C_{ta}(t)&=\int_0^t A\cdot f(\theta)\left\{\frac{C_{iv}(t-\theta)}{D_{iv}}\right\}\cdot d\theta\\&=\int_0^t A\cdot f(\theta)\left[\frac{1}{V_d(\alpha-\beta)}\{(\alpha-k_{21})\cdot e^{-\alpha(t-\theta)}+(k_{21}-\beta)\cdot e^{-\beta(t-\theta)}\}\right]\cdot d\theta\end{aligned} \tag{5}$$

where $C_{iv}(t)$ and D_{iv} are the blood concentrations after intravenous administration and the intravenous dose, respectively. The details of other abbreviations are given in the footnotes in Table I.

The time course of $C_{ta}(t)$ may be calculated from the time course of the *in vitro* skin permeation rate, $f'(t)$, and of the blood concentration after intravenous administration, $C_{iv}(t)$, by the convolution method.

Confirmation by Deconvolution

In a similar manner, a convolution equation is also set up between the *in vivo* skin permeation rate, $f''(t)$, and observed blood concentration after topical application, $C_{ta}'(t)$, as follows:

$$C_{ta}'(t)=\int_0^t A\cdot f''(\theta)\left\{\frac{C_{iv}(t-\theta)}{D_{iv}}\right\}\cdot d\theta \qquad (6)$$

The time course of $f''(t)$ may be calculated from the time courses of the blood concentration after topical ($C_{ta}'(t)$) and intravenous ($C_{iv}(t)$) administrations by the deconvolution method.

Materials and Methods

Materials——Nicorandil, *N*-(2-hydroxyethyl)nicotinamide nitrate, was supplied by Nisshin Flour Milling Co. (Tokyo). Propylene glycol (PG), polyethylene glycol 400 (PEG), isopropanol (IPA), carboxyvinyl polymer (Carbopol 934, B.F.Goodrich, Cleveland, OH, U.S.A.) and hydroxypropyl cellulose (HPC-M, Nippon Soda, Tokyo) were commercial-grade products. Gel ointments were prepared by adding 0.8% (w/w) Carbopol 934 for aqueous gel or PG gel, and by adding 3% (w/w) HPC-M for PEG gel or IPA gel. Excess nicorandil was dissolved in each gel (3%(w/w) in aqueous gel, 15% in IPA gel, 20% in PG or PEG gel).

Animals——Male hairless rats (WBN/kob strain),[3] with an approximate weight of 150 g each, were supplied by Saitama Laboratory Animals (Saitama).

Intravenous Administration Studies——Three doses of 2.5, 5 and 10 mg/kg of nicorandil in physiological saline (2 ml/kg) were injected into the tail vein of unanaesthetized restrained rats. At appropriate times (3, 5, 10, 15 and 30 min, 1, 1.5, 2 and 3 h), blood samples ($\simeq$0.3 ml) were withdrawn from the jugular vein into a heparinized syringe to measure plasma drug concentration.

***In Vitro* Skin Permeation Studies**——For the skin membrane permeation studies, a section of abdominal skin of a hairless rat was excised and mounted between two half diffusion cells, each having 2.0 ml volume and 0.636 cm^2 effective diffusion area.[4] The receiving compartment (dermis side of the skin) of each half cell was filled with 2 ml of saline and the donor compartment (stratum corneum side of the skin) was filled with 2 ml of drug suspension. The diffusion cells were maintained at 37 °C in water bath. The donor and receiver compartments were mixed throughout the experiment with a Teflon stirrer driven by a 150 rpm constant speed motor. At appropriate times, 100 μl samples were withdrawn from the receiver compartment, and 100 μl internal standard solutions (ethyl *p*-hydroxybenzoate 1 μg/ml in methanol) were added to them. After sampling, 100 μl of saline was added to the receiver compartment to keep the volume constant.

***In Vivo* Studies**——Rats were restrained in an unanaesthetized condition, and a glass cylindical cell was glued to the abdomen of each rat with a cyanoacrylate adhesive. Gel ointments (2 g) were applied in a thin layer on the 4.91 cm^2 area demarcated by the cell. The application site was then covered with a Parafilm (American Can Co., Greenwich, CT, U.S.A.) attached to the cell. At appropriate times (2, 4, 6, 10 and 24 h), blood samples ($\simeq$0.5 ml) were withdrawn from the jugular vein into a heparinized syringe to measure drug concentration.

HPLC Conditions——In all studies, the concentration of nicorandil was determined by high performance liquid chromatography (HPLC) (LC-6A, Shimadzu, Kyoto) under the following conditions: column, 4.6 mm × 250 mm stainless steel column packed with Nucleosil 5C_{18} (Macherey Nagel, Germany); mobile phase, water : acetonitrile (6 : 4); detector, ultraviolet (UV) 254 nm. Clean-up of plasma samples was carried out by the method of Kamiyama *et al.*[5] The minimum detectable plasma concentration was 0.02 μg/ml of plasma.

Numerical Calculation——The convolution and deconvolution calculations were carried out by the rectangular method.[6] The $f(t)$ and $C_{ta}'(t)$ values at every hour, except the experimental points, were estimated by interpolation using a cubic-spline function. The computation of the pharmacokinetic parameters and the theoretical values was done on a personal computer (PC-9801, NEC, Tokyo).

Statistics——Pharmacokinetic parameters were analyzed statistically by means of Student's *t*-test.

Results

Pharmacokinetic Parameters after Intravenous Administration

The time courses of the plasma concentration after intravenous administration of

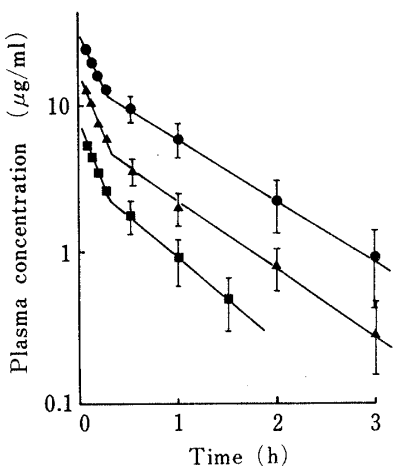

Fig. 2. Plasma Concentrations of Nicorandil after Intravenous Administration in Hairless Rats

Each point represents the mean ± S.D. of 3 animals. ■, 2.5 mg/kg; ▲, 5 mg/kg; ●, 10 mg/kg.

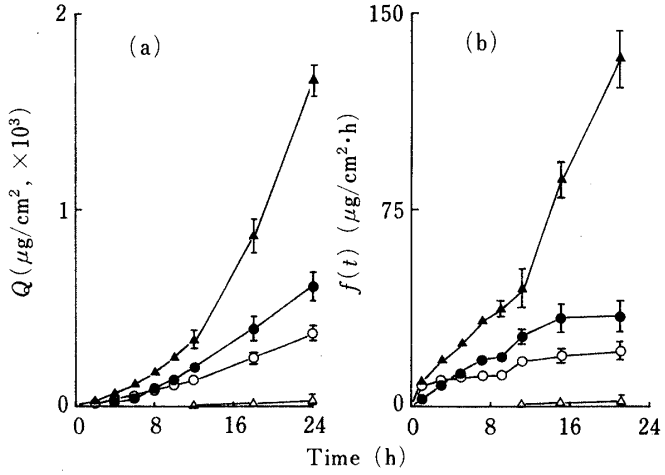

Fig. 3. Effect of Solvents on the Skin Permeation of Nicorandil across the Hairless Rat Skin

(a) Cumulative amount of permeation, Q with water (○), PG (●), PEG (△) and IPA (▲).
(b) Permeation rate, $f(t)$; symbols as in (a).
Each point represent the mean of 3—6 experiments. Vertical bars indicate the standard deviation.

TABLE I. Pharmacokinetic Parameters of Nicorandil after Intravenous Administration

Parameter	Dose (mg/kg)		
	2.5	5	10
V_d (l/kg)	0.393 ± 0.061	0.326 ± 0.049	0.347 ± 0.082
α (h^{-1})	9.763 ± 4.412	8.554 ± 3.499	10.757 ± 0.987
β (h^{-1})	1.323 ± 0.395	0.934 ± 0.222	1.173 ± 0.038
$t_{1/2\beta}$ (h)	0.552 ± 0.083	0.769 ± 0.098	0.592 ± 0.014
k_{12} (h^{-1})	3.168 ± 1.761	3.533 ± 1.542	3.387 ± 0.175
k_{21} (h^{-1})	5.623 ± 2.604	3.556 ± 2.328	6.602 ± 1.432
k_{10} (h^{-1})	2.295 ± 0.584	2.399 ± 0.218	1.942 ± 0.308
CL (l/h/kg)	0.914 ± 0.300	0.788 ± 0.187	0.661 ± 0.052
AUC (μg·h/ml)	2.926 ± 0.875	6.563 ± 1.378	15.179 ± 1.216

Abbreviations: V_d, apparent volume of central compartment; α and β, disposition rate constants; $t_{1/2\beta}$, elimination half-life at β phase; CL, total body clearance; AUC, area under the plasma concentration–time curve. Each value represents the mean ± S.D. of 3 animals.

nicorandil (2.5, 5 and 10 mg/kg) in hairless rats were fitted to biexponential equations (Fig. 2). Table I shows the pharmacokinetic parameters. The area under the plasma concentration–time curve (AUC) was proportionally increased by increasing the dose from 2.5 to 10 mg/kg and the rates of disposition at the β phase were similar within the dosage range in this experiment. Therefore, it was confirmed that the elimination process of nicorandil in hairless rats follows linear kinetics.

Effect of Solvents on the Skin Permeation of Nicorandil

The effect of solvents on the skin permeation of nicorandil across the full-thickness skin was investigated. Figures 3a and 3b show the time courses of the cumulative amount of penetrant permeated per unit area, Q, and of the rate of skin permeation, $f(t)$, respectively. Rank order of the rates of skin permeation at the steady-state was IPA > PG > water > PEG. Increases in the permeability at about 10 h in water may be due to skin hydration.[7]

Relationship between the Observed and Calculated Values for the Plasma Concentration after Topical Application by Convolution

Plasma concentrations of nicorandil were calculated from the skin permeation data as shown in Fig. 3 and the pharmacokinetic parameters after intravenous administration (5 mg/kg) as shown in Table I by using the convolution equation (Eq. 5). The data in rats at a dose of 5 mg/kg were employed as the disposition parameters, since pharmacokinetic parameters containing $t_{1/2\beta}$ were not significantly different between the three doses ($p > 0.05$). The calculated values were compared with the observed values which were obtained after topical application of each gel ointment (Fig. 4). The observed and calculated values agreed well until 10 and 6 h after topical applications of aqueous gel and PG gel, respectively. Both calculated values, however, tended to increase thereafter compared with the observed values. After applications of PEG gel and IPA gel, calculated values were approximately fitted to the observed values over 24 h.

Comparison between the *in Vitro* Skin Permeation Rate and the Value Calculated from the Plasma Concentration after Topical Application by Deconvolution

For the purpose of testing the appropriateness of the present method, the *in vivo* permeation rates were calculated by deconvolution and compared with the *in vitro* experimen-

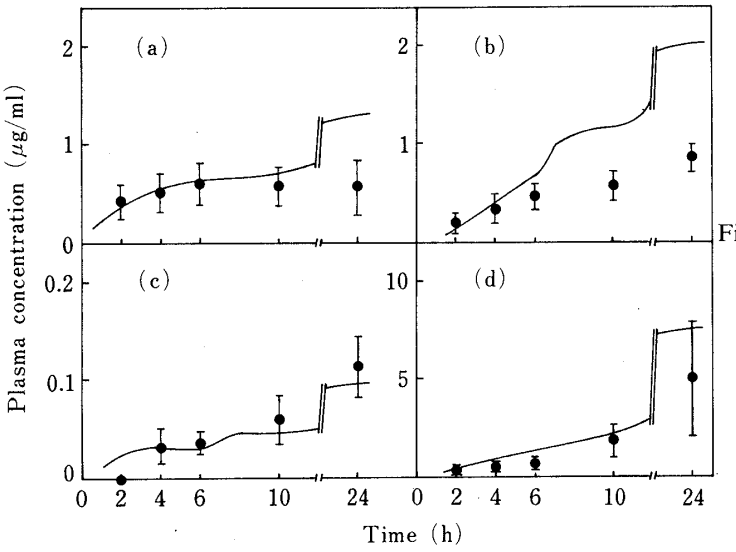

Fig. 4. Relationship between Observed and Calculated Plasma Concentrations of Nicorandil after Topical Application of Gel Ointments in Hairless Rats

(a) Aqueous gel; (b) PG gel; (c) PEG gel; (d) IPA gel.
Each point, $C'_{ta}(t)$, represents the mean ± S.D. of 3—6 animals.
The solid line, $C_{ta}(t)$, was calculated by using Eq. 5.

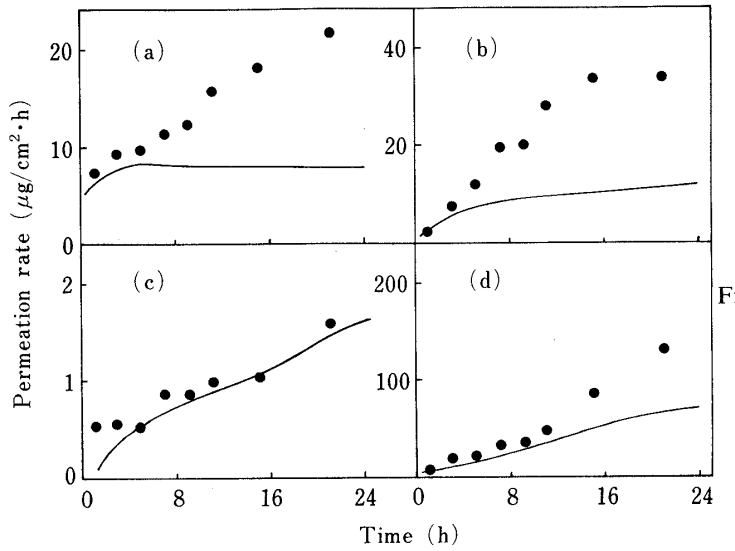

Fig. 5. Comparison between the *in Vitro* Skin Permeation Rate and Calculated Value for Nicorandil

(a) Water; (b) PG; (c) PEG; (d) IPA.
Each point, $f(t)$, represents the mean of 3—4 animals.
The solid line, $f'(t)$, was calculated by using Eq. 6.

tal data (Fig. 5). When PEG was used as a solvent, the *in vivo* permeation rate was almost the same as the *in vitro* rate. However, when water or PG was used, the *in vivo* permeation rate tended to deviate gradually from the *in vitro* experimental data.

Discussion

It is well known that diffusion theory is useful for the analysis of drug transport in ointments.[8] It is also accepted that drug transport in the skin can be explained by diffusion theory.[9] Therefore, the pharmacokinetics of percutaneous absorption have been discussed in terms of a two layer (vehicle and skin) diffusion model.[10] However, complex numerical calculations are required in order to estimate the permeability of drugs, since the skin is not a simple homogeneous membrane. On the other hand, another approach involving compartment models has been reported,[11] but the physiological significance is not entirely clear, especially concerning the penetration rate constant from the vehicle to the skin.

In recent years, many studies using percutaneous absorption enhancers such as Azone[12] have been reported. Although the permeation rate of drugs increases in such cases, the time taken to reach a steady-state level becomes longer than that in the absence of the enhancer.[13] Consequently, it is desirable to develop a method of estimating the rate and amount of percutaneous absorption of drugs, *e.g.*, the time course of blood concentration after topical application.

It was considered that the prediction of the time course of blood concentration would be possible by relating the *in vitro* skin permeation experiments to a linear compartment model as indicated in Fig. 1 under the assumption that the *in vivo* skin permeation rate of the drug is equal to that *in vitro*. As a result, the blood concentration after topical application was expressed by a convolution equation in the particular case when the pharmacokinetics for elimination conformed to a linear model. Advantages of this method are easy mathematical expression without consideration of the diffusivity in the vehicle and skin, and applicability to drugs which are degraded, metabolized and/or adsorbed in the vehicle and skin.

In the present paper, skin permeation of a drug was studied *in vitro* and *in vivo* with four solvents which are widely used in commercial preparations. Nicorandil was selected as a model compound because of its low molecular weight and high solubility in both water and organic solvents such as chloroform. In all experiments, drug suspensions were used in order to keep the thermodynamic activity of the drug in the vehicle constant throughout the experimental period. Gel ointments were prepared with a small amount of polymer in order that the drug release from the vehicle would not become a rate-limiting step. Consequently, curves calculated by the present convolution method were nearly within the range of error of observed values for each gel. Although there was a tendency for the *in vitro* and *in vivo* data to deviate gradually, particularly in the case of PG treatment, the deviation was only approximately 100% even at 24 h. A similar tendency was found by comparing the observed permeation rate in the *in vitro* experiment with calculated value from the *in vivo* data by deconvolution. The reason why the permeation rate in the *in vivo* situation becomes lower than that *in vitro* which the passage of time is obscure, but several factors may be considered as possible reasons for such deviation: that is, the temperature of the gel applied on the skin surface in the *in vivo* experiment might be lower than that in the *in vitro* experiment (37 °C), the solvent composition in the donor compartment might be altered gradually by an influx of water from the receiver compartment in the *in vitro* experiment, and the degree of skin hydration might be different between the *in vitro* and *in vivo* experiments, especially when water treatment is involved.

In conclusion, the present results suggest that the convolution method is suitable for estimating *in vivo* behavior from *in vitro* experiments within the range of acceptable variation.

The results also suggest that the deconvolution method, which is known to be available for the evaluation of absorption or release rates of drugs in the field of pharmacokinetic analysis,[14] is useful not only for comparing *in vitro* and *in vivo* skin permeation data but also for confirmation of the convolution method. Some difference between the *in vitro* and *in vivo* skin permeation rates, apparent in the present results, might be due to differences of the skin condition and other experimental conditions between the *in vitro* and *in vivo* experiments, since the convolution method was based on the assumption that the permeation rate of the drug in the *in vivo* experiment is equal to that *in vitro*. Therefore, other methods which make it possible to treat the difference between the *in vitro* and *in vivo* situations may also be required. Further studies relating to the application of other methods and to the usefulness and shortcomings of the present convolution method will be presented in the following paper.

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