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Estimation of a concentration profile of acyclovir in the skin after topical administration

Fumiyoshi Yamashita, Yasuo Koyama, Hitoshi Sezaki [1] and Mitsuru Hashida

Faculty of Pharmaceutical Sciences, Kyoto University, Yoshidashimoadachi-cho, Sakyo-ku, Kyoto 606-01 (Japan)

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Summary

The Laplace transformed equations for the time courses of drug concentration in the skin and net absorption were derived from Fick's diffusion law assuming a two-layer skin model. Based on these equations, a new method for estimating drug concentration in the skin directly from the absorption profile was developed by the use of a fast inverse Laplace transform (FILT) algorithm. The absorption profiles of topically administered acyclovir in rats obtained through deconvolution of urinary excretion time courses against i.v. injection data were analyzed according to this method. The amount of acyclovir excreted into urine was only 0.42% of the applied dose at 8 h after topical application with polyethylene glycol ointment, suggesting low skin permeability, while most of acyclovir injected intravenously was excreted within 4 h. However, the analysis revealed that a considerable level (57 $\mu g/cm^3$) higher than the minimum inhibitory concentration (MIC) was maintained in the viable tissue under the steady-state conditions.

Introduction

In general, the in vivo evaluation of transdermal drug delivery is carried out by monitoring the plasma concentration or urinary excretion of drug after topical application. However, the drug concentration in the skin which determines the topical therapeutic and side effects of an applied drug is not easy to estimate from these experiments. Drug molecules applied to the skin surface passively diffuse into the deeper area and finally are washed away by the blood microcirculation, forming a concentration gradient in the skin during the course of these processes. Therefore, the rate of absorption of drug is intimately related with its concentration gradient in the skin. Tojo et al. (1989) experimentally determined a concentration profile of drug in the stratum corneum and predicted in vivo steady-state flux based on transient diffusion theory. However, it is also important to estimate drug concentration in viable tissues located in succession to the stratum corneum for predicting therapeutic efficacy of

Correspondence to: M. Hashida, Faculty of Pharmaceutical Sciences, Kyoto University, Yoshidashimoadachi-cho, Sakyo-ku, Kyoto 606-01, Japan.

[1] *Present address:* Faculty of Pharmaceutical Sciences, Setsunan University, Nagaotoge-cho, Hirakata, 573-01, Japan.

topically applied drugs. In order to achieve this purpose, the absorption profile must be comprehensively analyzed based on a general skin model constructed to cover the total process of drug absorption after topical application.

We have developed an analytical method for the in vitro skin penetration of drugs based on diffusion models in which the skin was considered to comprise a single layer (Hashida et al., 1988), two homogeneous layers (Okamoto et al., 1989), and two layers with polar and nonpolar routes in the first stratum corneum (Yamashita et al., 1990). The above approaches allow us to discuss the relationship between drug concentration in the skin and penetration. In the present article, we describe a method for estimating the drug concentration in the skin from the data on urinary excretion following topical administration, on the basis of a two-layer skin model. Acyclovir, which would be expected to show therapeutic efficacy against cutaneous herpes simplex virus infection (Schaeffer et al., 1978), was selected as the model drug and was administered to rats as an ointment, its skin concentration being estimated using this method.

Experimental

Materials

Acyclovir was kindly supplied by Nippon Wellcome K. K. (Osaka, Japan). Radiolabeled [^{3}H]-acyclovir was obtained from Daiichi Pure Chemicals Co. Ltd (Tokyo, Japan). Unlabeled and [^{3}H]acyclovir were dissolved in methanol-water (7:3) solution and recrystallized under reduced pressure to give a final specific activity of 18.5 MBq/g. The acyclovir ointment was prepared by suspending recrystallized [^{3}H]acyclovir in polyethylene glycol base at a concentration of 5% w/w. For intravenous administration, 0.9% w/v NaCl solution dissolving 0.0185 MBq/ml [^{3}H] acyclovir and 0.45 mg/ml unlabeled acyclovir was prepared.

In vivo percutaneous absorption experiment

The abdominal hair of a male Wistar rat weighing about 200 g was carefully clipped. After 24 h, urethane was intraperitoneally administered to the rat at a dose of 1 g/kg to induce continuous anesthesia during the experiment and the urinary bladder was cannulated with vinyl tubing (i.d. 0.50 mm, o.d. 0.90 mm, Dural Plastics & Engineering, Dural, Australia). After the surface had been wiped with wet paper, 50 mg of the ointment was rubbed onto the abdominal surface with the help of a thin acrylic plate having a 3.14 cm^2 hole and urine samples were periodically collected for 8 h. Before each sampling time, the urinary bladder was washed twice with 0.2 ml of 0.9% w/v NaCl solutions through the tubing (Nishida et al., 1991). At the end of the experiment, the site of the skin exposed to the ointment was excised and its surface was washed with about 30 ml of water. The radioactivities in the urine, skin samples and washings were measured using a liquid scintillation counter (LSC-5000, Beckman, Tokyo, Japan). Tape-stripping was repeated 15 times until the skin surface glittered and then the ointment was applied in the same way.

Intravenous administration experiment

Under urethane anesthesia, the bladder was cannulated and 0.2 ml of the acyclovir solution was rapidly injected into the femoral vein. Using the pharmacokinetic parameters for i.v. injection, the urinary excretion profiles of acyclovir after topical application of acyclovir were deconvoluted according to the method of Kiwada et al. (1977).

Data analysis

The skin was assumed to be composed of the stratum corneum and the lower layer. Fick's second law of diffusion was expressed according to the following equations for the stratum corneum and the lower viable epidermis and dermis layer, respectively:

$$\partial C_s/\partial t = D_s\left(\partial^2 C_s/\partial x^2\right) \quad (1)$$

$$\partial C_d/\partial t = D_d\left(\partial^2 C_d/\partial x^2\right) \quad (2)$$

where C_s and C_d are the drug concentrations in the stratum corneum and the lower layer, respectively, and x denotes distance. A penetrant dis-

tributes from the vehicle to the surface of stratum corneum with a partition coefficient of K_s and diffuses in the stratum corneum with a diffusion coefficient of D_s. At the boundary between the stratum corneum and the lower layer, the penetrant distributes itself between them according to a partition coefficient of K_d/K_s, in which K_d expresses the partition coefficient of a penetrant between the vehicle and the lower layer, and diffuses in the lower layer with a diffusion coefficient of D_d. Assuming that the drug concentration on the skin surface is constant (C_0; infinite dosing) and that at the edge of the lower layer contact with the blood flow is zero (sink condition), the boundary conditions are as follows:

$$K_s C_0 = C_s \qquad (x = -L_s) \qquad (3)$$

$$(K_d/K_s) C_s = C_d \qquad (x = 0) \qquad (4)$$

$$D_s(\partial C_s/\partial x) = D_d(\partial C_d/\partial x) \qquad (x = 0) \qquad (5)$$

$$C_d = 0 \qquad (x = L_d) \qquad (6)$$

and the initial conditions are given by:

$$C_s = C_d = 0 \quad (t = 0) \qquad (7)$$

These differential equations were solved by means of the Laplace transform (Crank, 1975). According to this model, the Laplace-transformed equations for the drug concentration in both layers in the intact skin ($C_{s,int}(x)$ and $C_{d,int}(x)$) and the amount of drug absorbed into the systemic circulation (Q_{int}) are expressed, respectively, as;

$$C_{s,int}(x) = K_s C_0 \{K_s D_s V_s d_s \sinh d_d \cosh d_s(x/L_s) - K_d D_d V_d d_d \cosh d_s \sinh d_s(x/L_s)\}/s/k(s) \qquad (8)$$

$$C_{d,int}(x) = K_s K_d D_d V_d d_d C_0 \sinh d_d(1 - x/L_d)\}/s/k(s) \qquad (9)$$

$$Q_{int} = K_s K_d D_s D_d V_s V_d d_s d_d C_0/s^2/k(s) \qquad (10)$$

where s is the Laplace operator with respect to time, V denotes the volume of each layer, and;

$$d_s = L_s(s/D_s)^{1/2} \qquad (11)$$

$$d_d = L_d(s/D_d)^{1/2} \qquad (12)$$

$$k(s) = K_s D_s V_s d_s \sinh d_d \cosh d_s + K_d D_d V_d d_d \cosh d_d \sinh d_s \qquad (13)$$

On the other hand, by assuming the tape-stripped skin as a homogeneous plane membrane, the Laplace-transformed equations for drug concentration in the lower viable tissues ($C_{d,str}(x)$) and amount of drug absorbed (Q_{str}) are derived as follows:

$$C_{d,str}(x) = K_d C_0 \sinh d_d(1 - x/L_d)/s/\sinh d_d \qquad (14)$$

$$Q_{str} = K_d D_d V_d d_d C_0/s^2/\sinh d_d \qquad (15)$$

When the unknown parameters are defined as

$$P_1 = D_s/L_s^2 \qquad (16)$$

$$P_2 = D_d/L_d^2 \qquad (17)$$

$$P_3 = K_s D_s V_s C_0/L_s^2 \qquad (18)$$

$$P_4 = K_d D_d V_d C_0/L_d^2 \qquad (19)$$

Eqns 8–15 can be rearranged as follows:

$$C_{s,int}(x) = P_3/P_1/V_s \cdot \{P_3/d_d \cdot \sinh d_d \cosh d_s(x/L_s) - P_4/d_s \cdot \cosh d_d \sinh d_s(x/L_s)\}/s/k'(s) \qquad (20)$$

$$C_{d,int}(x) = P_3/P_1/V_s \cdot P_4/d_s \cdot \sinh d_d(1 - x/L_d)/s/k'(s) \qquad (21)$$

$$Q_{int} = P_3 \cdot P_4/s^2/k'(s) \quad (22)$$

$$d_s = (s/P_1)^{1/2} \quad (23)$$

$$d_d = (s/P_2)^{1/2} \quad (24)$$

$$k'(s) = P_3/d_d \cdot \sinh d_d \cosh d_s + P_4/d_s \cdot \cosh d_d \sinh d_s \quad (25)$$

$$C_{d,str}(x) = P_4/P_2/V_d \cdot \sinh d_d(1 - x/L_d)/s/\sinh d_d \quad (26)$$

$$Q_{str} = P_4/P_2 \cdot d_d/s^2/\sinh d_d \quad (27)$$

Thus, drug concentration in the skin can be directly estimated using hybrid parameters (P_1–P_4) obtained from the analysis of penetration profiles without the determined absolute value of C_0.

On the other hand, the urinary excretion rate of drug after topical application (dX_u/dt) can be expressed as follows by convolution of the absorption and excretion rates after intravenous administration ($(dX_u/dt)_{iv}$) through Laplace transforms:

$$\mathcal{L}(dX_u/dt) = \mathcal{L}(dQ/dt) \cdot \mathcal{L}\{(dX_u/dt)_{iv}\} \quad (28)$$

If the urinary excretion rate after intravenous administration is assumed to be expressed by a two-exponential function ($A \cdot e^{-at} + B \cdot e^{-bt}$), the cumulative amount of drug excreted into urine after topical application is expressed from Eqns 22 or 27 and 28 as follows:

$$X_{u,int} = P_3 \cdot P_4/s^2/k'(s) \cdot \{A/(s+a) + B/(s+b)\} \quad (29)$$

$$X_{u,str} = P_4/P_2 \cdot d_d/s^2/\sinh d_d \cdot \{A/(s+a) + B/(s+b)\} \quad (30)$$

In this study, the parameters P_1–P_4 were calculated by fitting Eqns. 29 and 30 to the corresponding data using a nonlinear regression program combined with the FILT algorithm (MULTI(FILT)) as reported previously (Okamoto et al., 1989; Yano et al., 1989).

Results

In order to determine the pharmacokinetic parameters for in vivo disposition of [^{3}H]acyclovir, the i.v. injection experiment was performed and the urinary excretion rate vs time profile obtained is shown in Fig. 1. Radioactivities were rapidly excreted in urine and the amount recovered in urine until 4 h was 88% of the injected dose. The excretion rate profile is expressed as follows:

$$dX_u/dt = 166 \cdot e^{-2.61 \cdot t} + 17.4 \cdot e^{-0.666 \cdot t} \quad (31)$$

where dX_u/dt expresses the urinary excretion rate of radioactivities after i.v. administration of [^{3}H]acyclovir (% of dose/h). The i.v. administration of 5-fold and one-fifth dose of acyclovir (450 and 18 μg) gave almost equal excretion patterns suggesting that the in vivo disposition of acyclovir is linear (data not shown). The amount of radioactivities excreted in the form of metabolites was also confirmed to be negligible.

Fig. 2 shows the cumulative urinary excretion (A) and absorption profile obtained by deconvolution (B) of [^{3}H]acyclovir through intact and

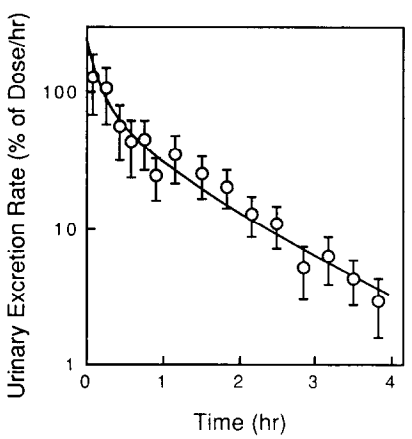

Fig. 1. The urinary excretion profile of [^{3}H]acyclovir injected into the femoral vein of rat. Each point represents the mean ± S.D. of three experiments.

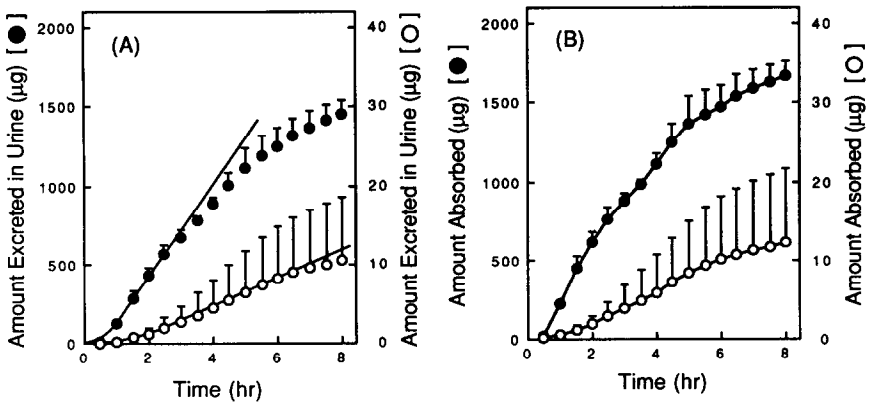

Fig. 2. The urinary excretion (A) and calculated absorption profile by deconvolution (B) of [^{3}H]acyclovir administered with polyethylene glycol ointment to intact (○) and tape-stripped (●) rat skin. Each point represents the mean ± S.D. of three experiments.

tape-stripped rat skin. The plasma concentration of ^{3}H radioactivity absorbed through intact skin was below the limit of detection. Table 1 summarizes the amounts of [^{3}H]acyclovir recovered in the ointment, skin, and urine at the end of in vivo absorption experiment. In intact skin, the amount of [^{3}H]acyclovir excreted in urine until 8 h was 0.42% of the applied dose. By removing the stratum corneum, the extent of excretion increased dramatically to 58% of the dose until 8 h. The cumulative amount of [^{3}H]acyclovir excreted into urine increased linearly until 6 h after application in the intact skin and until 3 h in the tape-stripped skin. Therefore, the data within these time periods including the lag phase were used for data analyses.

Table 2 summarizes the penetration parameters obtained via the present analysis and Fig. 3

TABLE 1

Amounts of [^{3}H]acyclovir recovered at the end of 8 h in the vivo absorption experiment [a,b]

Condition	Amount recovered (μg)		
	Ointment	Skin	Urine
Intact	2660 ± 750	35.1 ± 8.3 (1.96)	10.5 ± 8.06
Stripped	655 ± 91	88.8 ± 17.7 (341)	1450 ± 92.3

[a] The experimental data represent means ± S.D.

[b] Values in parentheses were calculated using the parameters summarized in Table 2.

TABLE 2

Parameters for in vivo skin permeation of [^{3}H]acyclovir obtained by the analysis of the urinary excretion profiles

	D/L^2 (h^{-1})	$KDVC_0/L^2$ (μg/h)
Stratum corneum	6.97 ± 1.05	2.08 ± 0.25
Viable tissue	0.571 ± 0.102	390 ± 12

Parameters are expressed as mean ± computer calculated S.D.

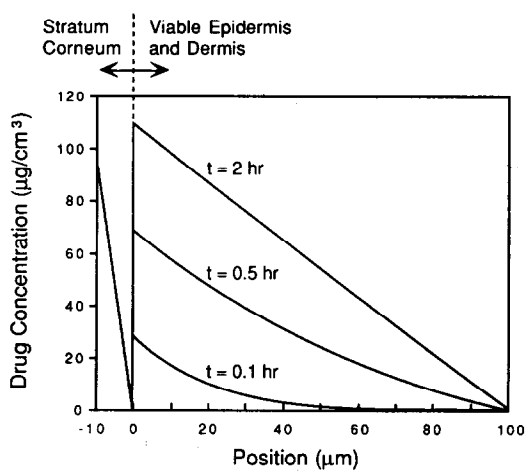

Fig. 3. Simulation of concentration gradient profiles of acyclovir in the skin at different time periods. Each curve was calculated using the parameters summarized in Table 1 assuming the thickness of the stratum corneum and the lower layer to be 10 and 100 μm, respectively.

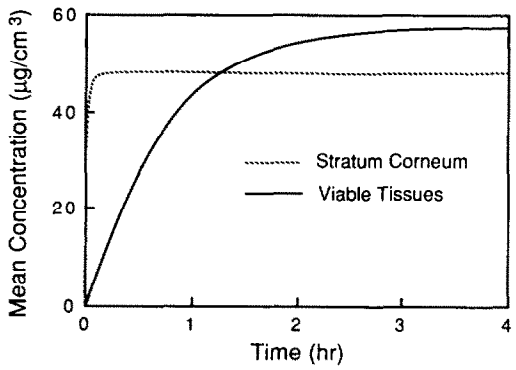

Fig. 4. Simulation of time courses of the mean concentration of acyclovir in the stratum corneum and the lower viable tissues. Each curve was calculated using the parameters summarized in Table 1 assuming the thickness of the stratum corneum and the lower layer to be 10 and 100 μm, respectively.

illustrates the estimated time course of acyclovir concentration in the intact skin simulated using these parameters. For estimating the real concentration, the thicknesses of the stratum corneum and the underlying viable epidermis and dermis layer are assumed to be 10 and 100 μm, respectively. The concentration gradient of acyclovir in the stratum corneum is much steeper than that in the viable tissue. The time course of mean concentration of acyclovir in each layer at steady state was also calculated by integration of the drug concentration (Fig. 4). The estimated amount of acyclovir in each layer under steady-state conditions shown in Table 1 was lower than the experimental value in the intact skin, while the estimated value was greater in the tape-stripped skin.

Discussion

Topical drug absorption occurs by passive diffusion and therefore the drug concentration in the skin is intimately related to the absorption rate. This enables us to estimate drug concentration directly from the diffusion and partition parameters of a drug calculated from an absorption profile based on Fick's second law (Hashida et al., 1988; Okamoto et al., 1989). In this approach, no information about the absolute drug concentration in the vehicle is necessary and thus the analysis can be applied even for complex drug delivery systems provided the concentration at the skin surface is maintained constant. By using the FILT algorithm, the real time course of drug concentration at each position in the skin can be predicted through curve fitting of a penetration profile.

In the present analysis, the absorption process must be extracted from the plasma concentration or urinary excretion data after topical administration of the drug. Deconvolution is useful for this purpose, but divergence of the results frequently occurs during the calculation and renders further analysis difficult. In order to minimize the error during numerical calculation, therefore, we have combined equations and fitting of the equations (Eqns 29 and 30) was performed directly to the urinary excretion data.

In this study, topical administration of acyclovir with polyethylene glycol ointment was analyzed in which only the steady-state permeability had been already evaluated (Freeman et al., 1986).

As shown in Fig. 2, penetration of acyclovir through the intact skin was very slow due to its high hydrophilicity. In this profile, the decrease in urinary excretion rate after 6 h should be due to the failure of renal function under long-term anesthesia, since infusion of a diuretic such as mannitol was not performed. Removal of the stratum corneum markedly enhanced the skin permeation of acyclovir and decreased the lag time, indicating that the stratum corneum acts as a major barrier for skin penetration of acyclovir. Because of this extremely rapid absorption, the penetration rate decreased at a relatively earlier stage where the infinite dosing condition was no longer obeyed. Since 26% of the applied dose of acyclovir remained even after 8 h of being suspended in ointment, the slow dissolution process is considered to lead to the decrease in acyclovir concentration in the vehicle.

The steep concentration gradient in the stratum corneum shown in Fig. 3 was attributed to its strong diffusion resistance. In this simulation, the thicknesses of the stratum corneum and viable epidermis plus dermis layer were assumed to be 10 and 100 μm. Although the thickness of the rat stratum corneum has been reported to be 13.8

μm (Bronaugh et al., 1983), the value varies depending on the methods of measurement (Bronaugh et al., 1982) and our histological observation gave an approximate value of 10 μm.

On the other hand, the distance from the surface to microcirculation has been reported to be 150–200 μm for humans (Scheuplein, 1967) and that of rats is considered to be shorter, since the thickness of rat epidermis is two-thirds of that of human epidermis (Bronaugh et al., 1982). The value of 100 μm appears reasonable, since the thickness of the whole epidermis of rats was reported to be 30.4 μm (Bronaugh et al., 1983) and the capillary in the dermis lies in close apposition to the underside of the epidermis.

In Table 1, the amounts of acyclovir in the skin calculated by integrating the drug concentration with these values are compared with the experimental results. Underestimation of the amount of acyclovir in the intact skin might be due to the difficulty in washout of the ointment from the skin surface. In contrast, the infinite dosing condition was no longer obeyed at the end of the experiment with the tape-stripped skin, which resulted in a considerable decrease in the amount in the skin from the value estimated under the steady-state condition. Since the amount of drug in skin applied in aqueous solution or aqueous polyethylene glycol 400 solution had been successfully predicted via the analysis based on the two-layer skin model (Tojo et al., 1987; Okamoto et al., 1989), the deviation of the estimated values was considered to be due to above-mentioned experimental conditions.

The present results revealed that the concentration of acyclovir was higher than its ID_{50} of 0.1 μM (Schaeffer et al., 1978) in viable tissues susceptible to herpes simplex virus infection. Reports on clinical trials of acyclovir with polyethylene glycol ointment for herpes simplex virus infection show that applied acyclovir accelerates the loss of virus from lesions in immunocompromised patients (Spruance and Crumpacker, 1982; Whitley et al., 1984). Although the concentration of acyclovir in the viable tissues estimated from the present analysis is higher than its ID_{50} by two orders, insufficient therapeutic effect was also reported in humans treated with acyclovir ointment (Spruance et al., 1984). Therefore, enhancement of skin permeation of acyclovir may be required for more effective therapy in humans through the use of penetration enhancers (Gonsho et al., 1990; Okamoto et al., 1990), the selection of vehicle (Freeman et al., 1986), and prodrug approach (Chikhale and Bodor 1991).

As shown in the present results, the tissue concentration of the drug at the administration site is maintained at a high level even when the amount of drug absorbed into the systemic circulation appears negligible. For evaluating the efficacy or comprehensive bioavailability of a transdermal formulation aiming at a local effect, therefore, pharmacokinetic analysis should be conducted not only for the plasma concentration or urinary excretion of drugs but also for the local concentration at the administration site. The method presented in this report provides us with some useful information about the design and evaluation of effective and safe transdermal delivery systems.

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