A Method for Predicting Steady-state Rate of Skin Penetration In Vivo

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A simple in vivo method was proposed for predicting the steady-state rate of penetration of drugs across the stratum corneum. Both the diffusion coefficient and the partition coefficient in the stratum corneum can be determined by the amounts of drug in the stratum corneum at two time intervals under transient conditions after transdermal drug application. The amount of drug entering the stratum corneum is determined by 20 strippings with an adhesive tape. The steady-state rate of penetration was then calculated for the thickness of the stratum corneum and the concentration of the donor solution. The steady-state rates of penetration of ascorbic acid and estradiol across hairless mouse skin were evaluated from this in vivo approach and compared with those obtained from in vitro penetration experiment using excised hairless mouse skin. The data confirmed that the proposed in vivo method can predict the steady-state rate of penetration of these drugs across the stratum corneum in normal skin. *J Invest Dermatol 92:105–108, 1989*

It is well established that the principal resistance to drug transport in transdermal drug delivery resides in the diffusion process through the stratum corneum [1]. Drug molecules released from the delivery system first partition toward the surface of the stratum corneum and then permeate into its lower layer by passive diffusion. Because of the barrier function of the stratum corneum, the diffusion coefficient in the stratum corneum is usually extremely low ($10^{-10} - 10^{-12}$ cm^2/s) for most drugs [1,2]. Due to such slow diffusion, a skin penetration study requires a long duration, usually 24 to 48 h, in order to observe the steady-state rate of penetration of a drug in in vitro experiments. Under in vivo conditions, on the other hand, the amount of drug excreted from the body must be monitored for several days after the transdermal application to evaluate the fraction of absorption [3]. Recently, Dupuis et al [4] reported a simple method to predict the stratum corneum reservoir function in vivo. They found a linear relationship between the amount of drug present in the stratum corneum at the end of application (30 min) and the total amount permeated in 4 d. However, at this stage of research on transdermal drug delivery, no in vivo approach has emerged for predicting the steady-state rate of penetration under in vivo conditions.

Typically, in transdermal drug delivery, the drug molecule requires 1 h or more to traverse the entire thickness of the stratum corneum; the drug concentration near the boundary between the stratum corneum and viable skin remains zero or at a very low level. Under such conditions, the amount of drug entering the stratum corneum is controlled by the physicochemical properties of the drug and of the stratum corneum.

In the present study, we propose a simple and quick method based on the transient diffusion theory for predicting the steady state rate of penetration of a drug in vivo after transdermal drug administration. Based on the amounts of drug entering the stratum corneum during two time intervals (t_1 and t_2) within 1 h after the application, the diffusion and partition coefficients were determined. The steady-state rate of penetration was then evaluated for a given donor concentration. The steady-state rate of penetration predicted was compared with that obtained from an in vitro skin permeation experiment using an excised hairless mouse skin. Ascorbic acid and estradiol were used as model drugs because these drugs are stable and bind negligibly in hairless mouse skin.

Theory Assuming that the skin consists of the stratum corneum and the viable epidermis (Fig 1) and neither binding nor enzymatic reaction takes place in the skin, the concentration profile of the drug is given as the solution of the following governing equation equation 1 [5] subject to the boundary and initial conditions equations 2–5:

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x}\left(D\frac{\partial C}{\partial x}\right) \tag{1}$$

$$C_1 = K_1 C_2 \quad \text{at } x = h \tag{2}$$

$$C_H = 0 \quad \text{at } x = H \text{ (sink)} \tag{3}$$

$$C = KC_d = C_0 \text{ (constant)} \quad \text{at } x = 0 \ (t > 0) \tag{4}$$

$$C = 0 \quad 0 \le x \le H \quad (t = 0), \tag{5}$$

where the diffusion coefficient D is a function of the distance x from the surface of the skin and is assumed to be constant in each skin layer; D_1 in the stratum corneum and D_2 ($\gg D_1$) in the viable epidermis.

Equation 1 was solved by the Method of Lines procedure [6]. An IBM PC/AT computer was employed to numerically integrate the differential equations. Microsoft FORTRAN77 compiler (v.4) was used in this study. The transient profiles of drug concentration in the stratum corneum were calculated with two different diffusion coefficients and are plotted in Fig 2 as a parameter of time. This

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Abbreviations:
DPM: disintegrations per minute
h: stratum corneum thickness
H: total skin thickness
J: steady-state flux
k: ratio of stripping times
K: partition coefficient
Q: cumulative amount of drug penetrated
t: time
x: distance from skin surface

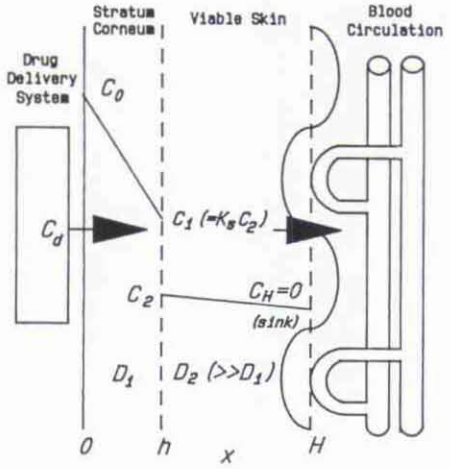

Figure 1. Steady-state concentration profile in the two-layer skin. Neither binding nor metabolism is assumed in the skin.

figure simulates the drug concentration in the human stratum corneum (about 20 μm thick). The concentration changes discontinuously on the boundary between the stratum corneum and viable epidermis (x = h) due to partitioning, although this is not shown in Fig 2. The concentration on the lower surface of the stratum corneum increases gradually with time until the steady-state profile is developed. Because the diffusion across the stratum corneum is very slow, as can be expected from the value of the diffusion coefficient, the concentration on the lower boundary of the stratum corneum remains negligible within the initial 1-h period after the transdermal administration of drug. Figure 2 indicates that the drug molecules do not reach the lower surface 1 h after the onset of the transdermal drug application. Under such transient conditions, the boundary condition (equation 2) is simplified to $C_1 = 0$ (at x = h), and the total amount of drug which has entered the stratum corneum during the period of time t is given by [5]

$$\frac{m}{m_0} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2\pi^2} \exp\{-D(2n+1)^2\pi^2 t/h^2\}, \qquad (6)$$

where m_0 is the total amount of drug entered in the stratum corneum after infinite time.

The ratio of the amount of the drug at two different time intervals, t_1 and t_2 (= kt_1, $k > 1$), is then

$$\frac{m_1}{m_2} = \frac{1 - \sum_{n=0}^{\infty} 8 \exp\{-D(2n+1)^2\pi^2 t_1/h^2\}/\{(2n+1)^2\pi^2\}}{1 - \sum_{n=0}^{\infty} 8 \exp\{-kD(2n+1)^2\pi^2 t_1/h^2\}/\{(2n+1)^2\pi^2\}}. \qquad (7)$$

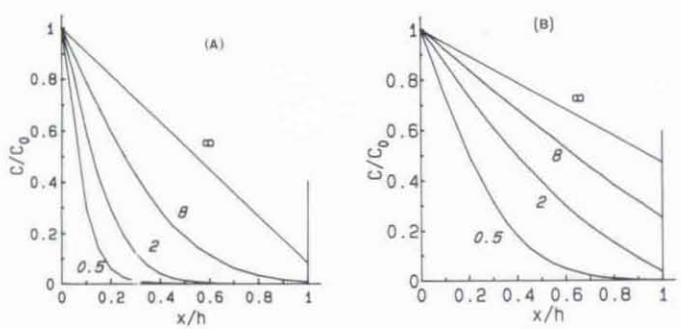

Figure 2. Transient profiles of drug concentration in the stratum corneum calculated from equation 1 subject to equations 2 – 5. The numbers with the lines are the values of time (*h.*) after the drug application on the surface of the stratum corneum. h = 20 μm, H = 200 μm, $K_s = 10$, $D_2/D_1 = 10^3$. *A*: $D_1 = 10^{-11}$ cm^2/s. *B*: $D_1 = 10^{-10}$ cm^2/s.

The relationship between the dimensionless quantity Dt_1/h^2 and m_1/m_2 is plotted as a parameter of k (= t_2/t_1) in Fig 3. The ratio m_1/m_2 approaches the square root of 1/k and is independent of the diffusion coefficient when Dt_1/h^2 is small ($Dt_1/h^2 < 0.02$). Beyond this short time period, the ratio m_1/m_2 increases appreciably as the dimensionless quantity Dt_1/h^2 increases. Figure 3 suggests that the diffusion coefficient in the stratum corneum can be evaluated from the ratio m_1/m_2 if the time intervals t_1 and t_2 are suitably selected. Because the concentration on the boundary between the stratum corneum and viable skin depends largely on the thickness of the stratum corneum, the time intervals t_1 and t_2 must be carefully determined. For normal human stratum corneum (about 20 μm thick), 20 – 30 min and 60 – 90 min may be used as the time intervals t_1 and t_2, respectively. For hairless mouse skin (about 10 μm thick), however, 10 min and 30 min may be used for most drugs.

The total amount of drug which enters the stratum corneum by time t may be determined by stripping using adhesive tape [4,7]. From the profile of the amount of drug as a function of the number of strippings, we can extrapolate the quantity of drug on the surface of stratum corneum. The ratio of the surface concentration thus determined to that of the donor vehicle is defined as the partition coefficient K.

Once the diffusion coefficient of the drug in the stratum corneum D and the stratum corneum/vehicle partition coefficient are determined from the present in vivo approach, the steady-state flux of penetration across the stratum corneum can be calculated by

$$J = \frac{dQ}{dt} = \frac{DKC_d}{h}. \qquad (8)$$

Assuming negligible resistance to drug transport across the viable skin, the time-lag, which is defined as the time intercept of the steady state penetration profile, is given by

$$t_d = \frac{h^2}{6D}. \qquad (9)$$

From equations 8 and 9, we can predict both the steady-state rate of penetration and the approximate time-course of the cumulative amount of drug penetrated under in vivo conditions.

MATERIALS AND EXPERIMENTAL METHOD

Ascorbic acid-^{14}C, 10.0 mCi/mM, was obtained from E. I. du Pont (Wilmington, DE). Estradiol-^{14}C, 56 mCi/mM was obtained from Amersham (U.K.). About 30 – 40 nmol of radiolabeled ascorbic acid and estradiol were applied in 50 μl of 50% glycerin solution and 40% PEG 400 solution, respectively, on the abdominal site of a hairless mouse (Jackson Lab, 5 – 8 weeks age, 18 ± 2 g weight, stratum corneum thickness about 10 μm). Prior to administration, the animals were anesthetized. Each drug was applied to a 3.14 cm^2 area of the abdomen by using an open cell fixed with silicone glue.

After 10 or 30 min of application, the excess substance on the treated area was quickly washed twice with methanol, then rinsed twice with distilled water and dried with cotton wool. The entire washing process was carried out within 1 min. At the end of wash-

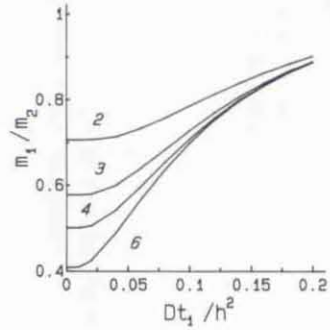

Figure 3. Effect of Dt_1/h^2 on m_1/m_2 (equation 7). The numbers with the curves are the values of k (= t_2/t_1).

ing, the stratum corneum was removed by 20 strippings using adhesive tape (Scotch, 3M810). The radioactivity in each stripping was then measured by a scintillation counter.

The concentration profile as a function of the number of strippings was approximated by a nonlinear exponential equation using the Modified Marquardt method for extrapolating the surface concentration. The amount of drug that entered the stratum corneum during the time intervals (10 or 30 min) was determined by the sum of the radioactivity in the 20 strippings. The diffusion coefficient was then calculated from equation 7 using the Newton-Raphson method. The partition coefficient K was determined by

$$K = \frac{\text{Surface radioactivity per unit volume of stratum corneum}}{\text{Radioactivity per unit volume of donor solution}}. \quad (10)$$

After determining both the diffusion and partition coefficients, the steady state rate of penetration was computed from equation 8 for a given donor (or vehicle) concentration C_d.

An in vitro permeation experiment was also carried out using a hydrodynamically well-calibrated in vitro skin permeation system [8]. The details of the experimental procedure were described previously [9]. The in vitro penetration profile was compared with the rate predicted from the present in vivo method.

RESULTS AND DISCUSSION

The concentration profiles (radioactivity) in the stratum corneum of the hairless mouse after 10 min (t_1) and 30 min (t_2) application have been plotted as a function of the number of strippings in Fig 4. A sharp decrease in quantities of drug was observed with each successive stripping, and after 10 strippings the drug concentration remains very low. It is evident that the total amount of the drug entering the stratum corneum within 30 min is greater than that within 10 min. From the total radioactivity at each time interval, the ratio of the amounts m_1/m_2 for ascorbic acid and estradiol was determined to be 0.595 and 0.749, respectively. Knowing the ratio m_1/m_2, the diffusion coefficients for ascorbic acid and estradiol were calculated from equation (7) and bound to be 6.2×10^{-11} cm²/s and 1.8×10^{-10} cm²/s, respectively. These diffusion coefficients were found to satisfy the condition of $Dt/h^2 > 0.02$ for both drugs. From the extrapolation of the profiles, the surface quantities were determined as 2.1×10^4 DPM/cm²/μm-thick for ascorbic acid and 2.8×10^3 DPM/cm²/μm-thick for estradiol, respectively. By assuming a uniform distribution of the donor solution over the applied area (3.14 cm²), the skin partition coefficients were then calculated to be 1.05 for ascorbic acid and 1.23 for estradiol, respectively.

The penetration profiles determined by the present in vivo approach were compared with the in vitro data in Fig 5. This figure indicates that the present in vivo method predicts well the in vitro penetration profiles. The steady-state rates of penetration obtained by the different approaches are compared in Table I. It is found that the steady-state penetration rates based on the present in vivo method are close to but slightly lower than those obtained from the in vitro permeation experiments. This is probably due to skin hydration during the long-term in vitro permeation experiment using excised hairless mouse skin. The in vitro penetration profile of ascorbic acid deviates increasingly from that predicted after about 12 h. This is due to the skin damage by the acidic donor solution (pH 2.6) in addition to the skin hydration. In general, however, the good agreement between the in vivo and in vitro penetration rates indicates that the present method is useful for predicting the in vivo steady-state rate of penetration across the stratum corneum of normal skin for ascorbic acid, and estradiol and could also be useful for testing other drugs and formulations. However, if the drug is metabolized and/or binds in the skin during the penetration, the present approach may not be applied directly.

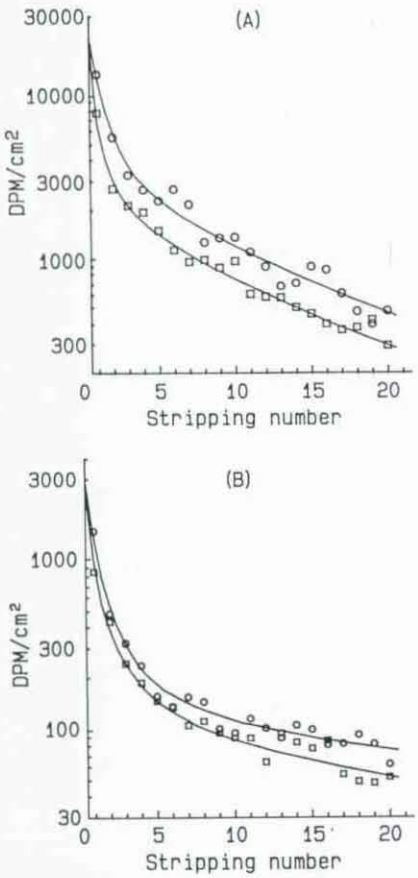

Figure 4. Profiles of the quantity of drug in the stratum corneum of hairless mouse skin as a function of the number of stripping. *Open circle:* 30 min; *open square:* 10 min. Average values of triplicate experiment. *A:* ascorbic acid; *B:* estradiol.

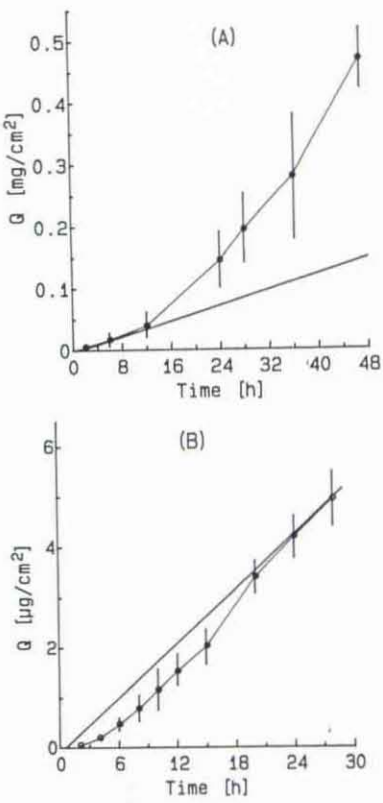

Figure 5. Comparison of the in vivo and in vitro penetration profiles. *Open circle:* in vitro penetration experiment; *solid line:* present in vivo method. *A:* ascorbic acid; *B:* estradiol.

Table I. Comparison of the Steady-state Rate of Penetration of Ascorbic Acid and Estradiol under In Vivo and In Vitro Conditions

	Steady State Rate of Permeation	
Drug	In vitro ($\mu g/cm^2$-h)	In vivo ($\mu g/cm^2$-h)
Ascorbic acid	3.43 ± 0.74 (2–12 h) 9.75 ± 3.83 (12–36 h)	2.9
Estradiol	0.207 ± 0.043 (4–28 h)	0.18

Shaefer and Zesch [10] reported that the amount of the material sticking to adhesive tape during consecutive strippings decreased continuously in the human stratum corneum. Based on their experimental data [10], about 20% of the human stratum corneum was removed by the first stripping. This fraction is obviously influenced not only by the type of adhesive tape and the pressure applied, but also by the skin species. Previously, we found that the enhancement factor in the steady-state rate of penetration across hairless mouse skin due to continuous stripping using the same technique as employed in this study was explained by assuming that an equal amount (1 μm thick) of stratum corneum was removed by each stripping [11]. The barrier function for drug transport across the stratum corneum of this animal model was also found to be removed almost completely after 10 to 15 strippings [11]. In the present study, therefore, we assumed that the major part of the stratum corneum of the hairless mouse skin was removed by the initial 10 strippings, and the material adhering in each stripping was identical (1 μm thick). This assumption can be applied only to the hairless mouse skin. For human stratum corneum, however, the detailed relationship between the amount of the stripped material and the number of strippings must be established in order to evaluate the partition coefficient from equation 10.

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