Penetration Kinetics of 2',3'-Dideoxyinosine in Dermis Is Described by the Distributed Model

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The present study evaluated the kinetics of drug penetration in the dermis. A rat was given a dermal dose of 2',3'-dideoxyinosine (ddI). At 6 hr, the skin tissue was excised, immediately frozen and sectioned, and the decline of drug concentration as a function of tissue depth was determined. The tissue concentration-depth profile showed a semilogarithmic decline, as would be expected in a distributed tissue kinetic model which incorporates diffusion and capillary membrane transport. The goodness of fit of the profiles by the simple diffusion and the distributed models were compared using four statistical criteria, i.e., coefficient of determination, Akaike Information criterion, Schwartz criterion and Imbimbo criterion. These analyses showed that the decline of tissue concentration versus tissue depth in the dermis was better described by the distributed model than by the diffusion model in all 7 animals. To examine the effect of blood perfusion on the tissue concentration-depth profiles, some of the tissues were frozen after 1 and 2 hr storage at room temperature. In contrast to the adjacent tissues frozen immediately, the concentration-depth profiles in tissues frozen after a 1-2 hr delay were described equally well by distributed and diffusion models. A comparison of the concentration-depth profiles in the tissues processed immediately or after a delay showed a 7 fold more shallow slope and a 60% lower concentration at the epidermis-dermis interface after storage. However, storage did not alter the total amount of drug in the entire dermis. Drug degradation during storage was further ruled out by the insignificant ddI degradation in 10% skin homogenate (a half-life of ~70 hr). These results indicate that under *in vitro* conditions, where there is no blood flow to remove the drug, the kinetics of drug penetration in the dermis are described by simple diffusion in accordance with the concentration gradient. In summary, these data indicate the importance of capillary blood flow on drug penetration profiles in the dermis, and that concentration-depth profiles in the dermis is described by the distributed model.

KEY WORDS: percutaneous penetration kinetics; 2',3'-dideoxyinosine; distributed model.

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Abbreviations used: ddI, 2',3'-dideoxyinosine; HPLC, high pressure liquid chromatography; $w_{1/2}$, tissue depth over which the concentration decreases by 50%; C_0, concentration at the epidermis-dermis interface.

INTRODUCTION

The skin consists of three main regions: a lipophilic stratum corneum, a hydrophilic epidermal layer and the dermal layer which constitutes the bulk of the skin. The stratum corneum is about 15-20 cell layers thick and mainly composed of dead keratinized cells. This layer is considered to be a significant barrier to drug penetration, the barrier function being dependent upon the thickness of the intact layers, diffusion pathways through the follicles and sweat glands, lipid content and degree of hydration (1). The underlying epidermal layer undergoes continuous differentiation to produce the stratum corneum and is believed to be a barrier layer only for extremely lipophilic drugs (1). The dermis supports the cutaneous vasculature and is the region where the absorption of drugs by blood and lymphatic vessels occurs (2). The process of percutaneous absorption is considered to be a summation of the sequential transport processes through the three layers of the skin. The current belief is that percutaneous absorption depends upon the physicochemical characteristics of the drug and vehicle, and the properties of the various layers of the skin (3). The drug penetration through the avascular stratum corneum and epidermis is often considered to be by simple diffusion in accordance with the concentration gradient, resulting in a linear concentration decline with respect to tissue depth (4).

Several studies suggest the importance of drug transport via the blood and lymph capillaries in the dermis. Benowitz et al. observed a significantly reduced systemic absorption of transdermal nicotine. After vasoconstriction of the dermal capillaries was induced by a concomitant intravenous dose of the same drug. This reduction of systemic absorption, indicated by a slower rise in plasma and nicotine concentrations, a delayed peak in plasma concentrations, a lower peak concentrations, showed a blood flow rate limited percutaneous absorption for nicotine (5). Distribution of caffeine in the avascular stratum corneum and epidermis was unaltered under *in vivo* and *in vitro* conditions (6). However, under *in vitro* conditions the accumulation of caffeine and 8-methoxypsoralen in the dermis was higher, which is likely due to the absence of drug removal by perfusing blood (6,7). While these studies suggest the role of drug removal by the perfusing blood, the kinetics of drug absorption in the dermis have not been defined.

Dedrick and coworkers proposed a distributed model that describes the decline of drug concentration with depth in capillary perfused tissues as a function of diffusion and capillary membrane transport. The decline in concentration is log-linear with tissue depth (8-12). This model was proposed to extrapolate the drug removal by capillary blood flow in the intestinal wall during peritoneal dialysis with anticancer drugs, but underwent only preliminary verification because of the technical difficulties associated with studying drug penetration in the adjacent tissues. Recently, our laboratories have verified the application of the distributed model to the bladder penetration of mitomycin C in dogs and in man. Our data show that the distributed model gave a better description of the concentration-depth profile in the capillary

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perfused bladder wall than the simple diffusion model (13-15). In the present study, the kinetics of drug absorption in the rat dermis were analyzed using the simple diffusion and the distributed models. The model drug was a weak acid, 2',3'-dideoxyinosine (ddI), which has shown a significant transdermal absorption in rats in our previous study (16).

MATERIALS AND METHODS

Chemicals. All high pressure liquid chromatographic (HPLC) solvents and reagents were of analytical grade and were purchased from Fisher Scientific Co. (Cincinnati, OH). ddI was a gift from the National Institutes of Health (Bethesda, MD). The internal standard ftorafur (N^1-(2-tetrahydrofuranyl)-5-fluorouracil) was a gift from Mead Johnson Research Laboratory (Evansville, IN). The purities of ddI and ftorafur were evaluated by HPLC and were ≥99%.

Equipment. The HPLC equipment was as described previously (16). Frozen tissues were sectioned using a cryotome (Reichert/American Optical, Buffalo, NY).

Animal Protocol. Female Fischer rats (Charles River Breeding Laboratories, Kingston, NJ) were used. A 4 × 2 cm^2 area on the dorsal interscapular surface was clipped free of fur. A dermal dose of ddI (10 mg/0.5 ml physiological saline) was applied. Six hr after application, the animals were euthanized and the treatment area was excised and placed on a flat stainless steel plate cooled on dry ice. The tissue was frozen immediately (within 1 min after excision) by pouring liquid nitrogen on the tissue segments.

Tissue Preparation. After rinsing the skin surface with methanol to remove the residual dose, the frozen tissues were trimmed to 1 × 1 cm^2 pieces with a scalpel blade, and glued onto the cryotome object holder with Tissue-Tek cryoadhesive (Miles Lab, Naperville, IL), such that the stratum corneum was exposed for sectioning. The cryoadhesive material was applied in moderation to avoid contamination of the sectioned tissue segments with the material. The object holder was oriented to bring the stratum corneum in exact alignment to the cutting edge. Sections of 50 μm thickness were cut parallel to the skin surface, and their weights were determined. In two experiments, the tissue was divided into two parts. One part was frozen immediately and one part was stored at room temperature for 1 or 2 hr, to simulate *in vitro* conditions where the capillary transport system becomes nonfunctional. The tissues were then frozen and processed.

Drug Analysis. Plasma samples were analyzed by HPLC as described previously (17). Tissue samples were processed as follows. The weighed tissue sections (5-8 mg) were mixed with 1200 μl acetonitrile. The mixtures were transferred to 50 ml nalgene tubes. After adding the internal standard ftorafur (6.4 μg/0.8 ml), the samples were homogenized for 60 sec with a rotor/stator type biohomogenizer (Biospec Products Inc., Bartlesville, OK). Complete homogenization of the tissue was found to be critical for drug extraction and analysis. After centrifugation, 1 ml of the clear supernatant was transferred, and evaporated to dryness under nitrogen. The residues were reconstituted with physiological saline and analyzed by HPLC. The extraction efficiency was >98%. Freezing by liquid nitrogen did not affect the extraction efficiency. A reversed phase μBondapak C_{18} column (Waters Associates) was used with an aqueous mobile phase consisting of 4.4% acetonitrile in 200 mM phosphate buffer, pH 5.0. All analyses were performed at ambient temperature. The retention volumes for ddI and ftorafur were 20 and 22 ml, respectively.

ddI Metabolism in Skin. Metabolism of ddI (~55 μg/ml) in skin homogenate (10% w/v) was used to estimate the extent of enzymatic ddI degradation. Nonenzymatic degradation was determined in skin homogenate preboiled at 100°C for 1 hr and in physiological saline. The mixtures were incubated in a shaking water bath at 37°C for 6 hr. The pH was maintained at 6.9. Two experiments with duplicate samples were performed; one experiment was with phosphate (111 mM), and one was without. Serial samples were taken over 24 hr incubation, and ftorafur (1.6 μg/0.4 ml) was added.

Data Analysis. The tissue concentration-depth profiles were analyzed by two models, i.e. distributed and simple diffusion models. We previously discussed the application of the distributed model to describe the decline of drug concentration with respect to tissue depth in capillary-perfused tissues (13-15). The model assumes that (a) metabolism is negligible, (b) capillaries are uniformly distributed, and (c) capillary transport is a passive process. The distributed model is described by the following equations.

$$\frac{C_x - C_b}{C_0 - C_b} = e^{-\frac{0.693x}{w_{1/2}}} \text{ where } w_{1/2} = 0.693\sqrt{\frac{D(pa+q)}{paq}} \quad (1)$$

where C_0 is the concentration at the epidermal-dermal junction, C_b is the drug concentration in the perfusing blood, C_x is the concentration at distance x into the capillary tissue, p and a are the permeability coefficients and surface area per unit tissue volume of the capillaries, respectively, D is the diffusivity of the drug through the extracellular region, q is the capillary blood flow rate per unit tissue volume, and half-width ($w_{1/2}$) is defined as the thickness of the tissue over which the concentration declines by one-half (10). The units for D, p, a, q and $w_{1/2}$ are cm^2/min, cm/min, cm^2/cm^3, 1/min, and cm, respectively. Rearrangement of equation 1 gives equation 2.

$$C_x = (C_0 - C_b)\, e^{-\frac{0.693x}{w_{1/2}}} + C_b \quad (2)$$

A simple diffusion process is described by equation 3.

$$C_x = C_0 - k_0 x \quad (3)$$

where k_0 is the zero order rate constant to describe the decline of tissue concentration with depth. Note that the distributed model describes a log-linear decline in the concentration-depth profile, whereas the simple diffusion model describes a linear decline (13, 18, 19).

The basalis or the epidermal-dermal junction is undulating in structure and therefore it is difficult to determine an exact depth for this junction. The distance from the rat skin surface to the microcirculation is reported to be 150-200 μm (20). In our experiments, we considered the epidermal-dermal junction to be at a depth of 150 μm. C_0 was calculated

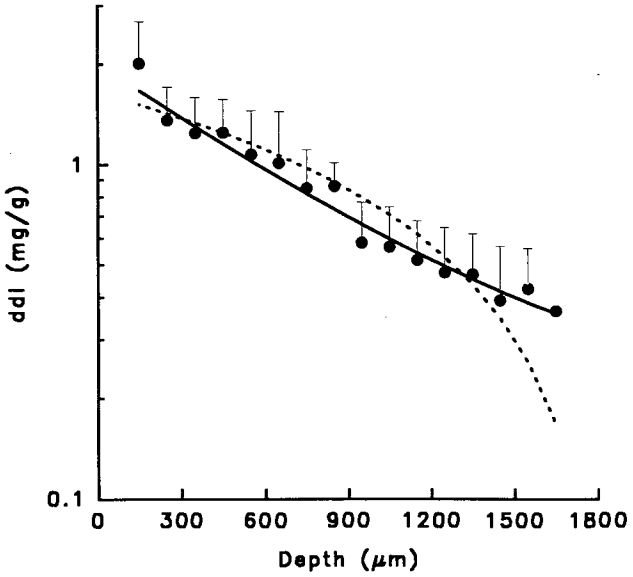

Figure 1. ddI concentration-depth profile in dermis. Rats were given a dermal dose of ddI (10 mg per 0.5 ml physiological saline). Six hours after dosing, the application area was excised and sectioned. Data shown are the mean and one S.D. (n = 7). The tissue concentrations were plotted against the midpoint tissue depths. Solid line represents best fit line for exponential concentration decline (distributed model). Broken line represents best fit line for linear concentration decline (simple diffusion model).

by extrapolation of the computer fitted line to a depth of 150 μm. The average concentration within the tissue was obtained by dividing the area under the tissue concentration-depth profile by the tissue depth.

Tissue concentrations were plotted against the midpoint depths of the respective sections. The tissue concentration versus depth profiles were analyzed using equations 2 and 3. The best fit curves were determined by using PCNONLIN 4.0 (Statistical Consultants Inc., Lexington, KY). The statistical criteria used to compare the goodness of fits were: coefficients of determination (r^2) (21), Akaike Information Criterion (AIC) (22), Schwartz Criterion (SC) (23) and Imbimbo Criterion (I_p) (24). The values of r^2 would give an estimate of the extent of correlation between the experimental and model predicted values. AIC, SC and I_p give a quantitative measure of the balance between the goodness of fit, measured by the sum of squared residuals, and the complexity of the mathematical model.

RESULTS

Concentration-Depth Profile in Dermis. Figure 1 shows the average ddI concentration versus tissue depth profiles in 7 rats. There was an exponential decrease in the tissue concentration from about 2 mg/g at 150 μm (epidermal-dermal interface) to about 0.4 mg/g at 1600 μm. This indicated an 80% decline in ddI concentration in the dermis layer. The distributed model gave a better fit of the concentration-depth profile as indicated by the higher r^2 and lower AIC, SC and I_p values (Table 1). Analysis using the distributed kinetic model gave a $w_{1/2}$ value of 386 ± 118 μm (mean ± S.D.; range, 187-530 μm), a C_0 value of 2.46 ± 0.95 mg/g (range, 1.45-4.25 mg/g), a C_b value of 0.29 ± 0.26 (range, 0.0002-0.633) and an average tissue concentration of 0.88 ± 0.23 mg/g (range, 0.63-1.27 mg/g).

Effect of Time Delay in Tissue Freezing. The tissues frozen after a 1 or 2 hr storage at room temperature showed an altered concentration-depth profile compared with the adjacent tissues frozen immediately (Table 2). In tissues frozen after 1 and 2 hr delay, the decline in the tissue concentration with tissue depth was described equally well by either the diffusion or the distributed models, as shown by nearly identical r^2 and AIC, SC and Ip values (data not shown). In comparison, the profiles in the adjacent tissues frozen immediately showed a better fit by the distributed model. Furthermore, the profiles in tissues with delayed freezing showed a 7 fold longer $w_{1/2}$ and a 60% lower C_0, whereas there was little change in the average tissue concentration over the entire tissue depth of 1600 μm indicating that the total tissue concentration remained constant.

Metabolism in Skin. In vitro degradation of ddI in skin homogenates showed that less than 5 and 10% of ddI were degraded in 2 and 24 hr, respectively. Similar data were found in physiological saline and in preboiled skin homogenates. Addition of inorganic phosphate did not enhance the degradation. These data indicate that ddI was not metabolized nor degraded in the skin under *in vitro* conditions.

DISCUSSION

The first objective of the present study was to evaluate

Table 1. The Goodness of Fit of Tissue Concentration-Depth Profiles by the Distributed Model (Equation 2) and the Diffusion Model (Equation 3) was Compared Using Four Statistical Criteria. A Lower Value of Akaike Information Criterion (AIC), Schwartz Criterion (SC) or the Imbimbo Criterion (I_p) and a Higher Value of the Coefficient of Determination (r^2) Indicate a Better Fit

	r^2		AIC		SC		I_p	
Rats	Distributed	Diffusion	Distributed	Diffusion	Distributed	Diffusion	Distributed	Diffusion
1	0.80	0.66	−11.4	11.49	−13.1	10.3	0.15	0.25
2	0.95	0.86	−29.6	−16.5	−31.5	−17.9	0.09	0.13
3	0.84	0.81	11.4	12.7	9.62	11.7	0.21	0.34
4	0.88	0.68	1.34	12.8	−0.62	11.5	0.19	0.25
5	0.85	0.76	−12.7	−7.76	−14.5	−8.99	0.13	0.15
6	0.92	0.83	−28.2	−18.8	−28.1	−20.0	0.08	0.13
7	0.91	0.80	−4.91	7.53	−6.67	6.35	0.18	0.22

Table 2. The Data from Skin Sections Processed With and Without Delay were Analyzed Using Diffusion Model and Distributed Model, Respectively. The Average Tissue Concentrations were Estimated by Dividing the Area Under the Concentration-Depth Profile by the Total Depth. Changes in the Tissue Pharmacokinetic Parameters Due to the Delay in Freezing are Shown as % Deviations from the Respective Adjacent Tissues that were Frozen Immediately (<1 min Delay). The Tissues with a 1 and 2 hr Delay were Obtained from Rats 5 and 1, Respectively. The Respective $w_{1/2}$ and C_0 Values in Tissues Processed Without Delay were 384 μm and 2.06 μg/g for Rat 5, and 284 μm and 2.02 μg/g for Rat 1

Delay in processing	C_0	$w_{1/2}$	Average concentration
1 hr	−50%	+523%	−8%
2 hr	−67%	+899%	−4%
Average	−58%	+711%	−6%

the kinetics of drug penetration in the dermis and to compare two kinetic models, i.e., the distributed model which takes into account both passive diffusion and drug removal by the perfusing blood, and the simple diffusion model which ignores the capillary transport process. The results showed a semi-logarithmic decline in ddI concentration as a function of tissue depth in the dermis. This kinetic profile was better described by the distributed model than by the simple diffusion model, indicating the important role of the drug transport by the blood capillaries. A better understanding of the kinetics of drug absorption in the dermis may allow calculation of the input rate into the systemic circulation and, therefore, the prediction of *in vivo* drug concentrations after a dermal dose.

The second objective of the study was to examine the differences in kinetics under *in vivo* (viable skin with intact vasculature) and *in vitro* (excised skin) conditions. Under *in vivo* conditions, drug molecules in the dermis layer are continuously removed by the capillary drainage. Under *in vitro* conditions, the drug penetrates the dermis by passive diffusion in accordance with the concentration gradient. The present study directly compared adjacent tissues, obtained from the same animal, that were either frozen immediately (within 1 min after excision) or after a 1 to 2 hr delay. The delay simulated *in vitro* conditions where the vascular system was disrupted and nonfunctional, and where the drug continued to be transported by diffusion. The changes in tissue-depth profiles after storage suggest a change in the transport process under *in vitro* conditions. Compared to tissues frozen immediately, tissues frozen with a 1-2 hr delay showed a 7 fold longer $w_{1/2}$ and a 60% lower C_0. The insignificant drug degradation in 10% w/v skin homogenate and the little change in the total amount of drug in the dermis confirm that the changes in tissue pharmacokinetics were not due to drug metabolism in the skin. These results suggest that the changes were due to the passive diffusion that continued to operate under *in vitro* conditions resulting in a flattening of the concentration decline with depth and a lower concentration at the epidermis-dermis interface. Collectively, these data indicate the important role of capillary transport in the dermis in the determination of tissue concentration-depth profile, and the substantial differences in this profile when there is no blood flow in the dermis as in *in vitro* situations.

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