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Physical Model Evaluation of Topical Prodrug Delivery—Simultaneous Transport and Bioconversion of Vidarabine-5′-valerate III: Permeability Differences of Vidarabine and *n*-Pentanol in Components of Hairless Mouse Skin

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Received August 2, 1979, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.* Accepted for publication December 13, 1979. *Present address: Syntex Research, Stanford Industrial Park, Palo Alto, CA 94304.

Abstract □ The permeation behavior of ^{3}H-vidarabine (^{3}H-9-β-D-arabinofuranosyladenine) and ^{14}C-*n*-pentanol through different strata of hairless mouse skin was studied using a diffusion cell at 37° under steady-state conditions. Partition coefficients for the skin components *versus* 0.9% aqueous NaCl solution also were obtained. Various skin preparations including full-thickness skin, cellophane-stripped skin, and dermis membranes of different thicknesses were employed. The dermis membranes were considered to be diffusionally homogeneous, and the product of the permeability coefficient and the thickness was taken as the apparent diffusivity. The apparent diffusivities for both compounds investigated were independent of thickness. Therefore, it was concluded that the molecular diffusivity is constant throughout the dermis. Comparisons of permeability coefficients in various strata of the skin revealed that, while the stratum corneum is the major diffusional barrier, the epidermis appears to be significantly less permeable than the dermis.

Keyphrases □ Vidarabine valerate prodrug—topical dosage forms, permeability differences through various mouse skin strata □ Prodrugs, topical—based on simultaneous transport and bioconversion of vidarabine, permeability differences in various components of mouse skin □ Diffusivity—vidarabine and *n*-pentanol, effect of skin strata and thickness on diffusivity □ Models, physical—based on simultaneous transport and bioconversion, effect of homogeneous distribution on prodrug evaluation

The basic experimental methods for determining key parameters and evaluating the transport and metabolism of a prodrug of vidarabine (9-β-D-arabinofuranosyladenine, I) in hairless mouse skin were discussed previously (1). Among the parameters needed for the mechanistic quantification of the problem are the transport parameters, the permeability coefficients, and the diffusivities in the various components of the skin. These parameters and the metabolizing enzyme constants, k_1 and k_2, permit a quantitative analysis of the prodrug delivery behavior in the skin.

The barrier nature of the various skin components is not fully understood. Several studies concerned the possible differences between the diffusional resistances of the epidermis and the dermis (2, 3). The purposes of this investigation were to study mechanistically the barrier nature of the various components of the hairless mouse skin using membrane preparations of different strata of the skin and to investigate the possible variations in the diffusivity with respect to position in the dermis component.

Studies were conducted on the permeabilities of I in various membrane preparations of the hairless mouse skin

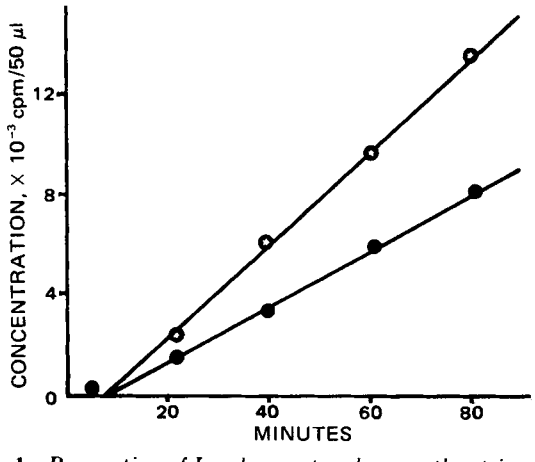

Figure 1—*Permeation of I and* n*-pentanol across the stripped skin. Key:* ●, ^{3}*H-2-I; and* ✪, ^{14}*C-*n*-pentanol. Both permeants were run concurrently, and the* Δ*C values were* 6.68×10^{5} *and* 2.55×10^{5} *cpm/50 µl for I and* n*-pentanol, respectively. The fluxes may be calculated according to flux = (slope) (volume/area), where the volume is 3.0 ml and the area is 1.767 cm^{2}.*

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Table I—Permeability Coefficients of I and *n*-Pentanol for Full-Thickness Skin

Mouse	Permeability Coefficients, × 10^8 cm/sec	
	I	*n*-Pentanol
A	1.87	—
B	2.33	—
C	2.96	—
D	4.76	331
E	—	315
—	—	408[a]

[a] From Ref. 2.

Table II—Permeability Coefficients of I and *n*-Pentanol for Stripped Skin

Mouse	Permeability Coefficients, × 10^6 cm/sec	
	I	*n*-Pentanol
F	2.12	22.6
G	4.72	19.8
H	4.37	17.4
—	—	40.1[a]

[a] From Ref. 3.

from which the permeability coefficients for the stratum corneum, the epidermis, and the dermis were calculated. Experiments with *n*-pentanol were included to determine any possible effects of molecular size.

EXPERIMENTAL

The experimental procedures for the permeation studies were described previously (1). The materials were the same as those used previously (1), except ^{14}C-*n*-pentanol[1] was diluted with saline to give a 1-μCi/μl solution. As described previously (1), full-thickness skin, cellophane[2]-stripped skin, and dermatome-planed dermis preparations were the membranes used.

RESULTS AND DISCUSSION

Figure 1 shows raw data obtained from a typical permeation experiment. The accumulation of I or *n*-pentanol in the receiver chamber increased linearly after a short lag time. The slopes gave the fluxes, F, for the permeants. The permeability coefficients, P, then were calculated from the slope and the concentration differential, ΔC, for each species from:

$$P = \frac{F}{\Delta C} = \frac{\left(\frac{V}{A}\right)\text{slope}}{\Delta C} \qquad \text{(Eq. 1)}$$

where V is the volume of the receiver solution and A is the diffusional area of the membrane. The permeability coefficients of I and *n*-pentanol for the full-thickness skin, stripped skin, and dermis membranes are shown in Tables I–IV. The apparent diffusivity[3], D, which is determined from the lag time, also is listed in Tables III and IV. Experimental reproducibility was satisfactory and consistent with previous experiments (1). Repeated permeation experiments showed an animal-to-animal variation of approximately a factor of two. Also, the stabilities of the various membrane preparations were satisfactory up to ~2–3 days.

Comparison of the permeability coefficients of I in the various membrane preparations (Table V) revealed that stripped skin was ~100 times more permeable than full-thickness skin, while the dermis was >1000 times more permeable. A similar relationship also existed for the permeation of *n*-pentanol, but the permeability differences were much smaller (Table V). This evidence suggests that, while the stratum corneum is the major diffusional barrier, the epidermis may be significantly less permeable than the dermis.

It was noted previously that the epidermis may be significantly less permeable than the dermis and that it may be a biopharmaceutically important barrier. Feldmann and Maibach (2) reported that both the stratum corneum and the Malpighian–basal layers serve as skin barriers for the penetration of hydrocortisone through normal human skin. The anatomy of the skin reveals a significant structural difference between the epidermis[4] and the dermis: the former consists of highly packed, well-aligned epithelial cells, whereas the latter is composed of cells loosely scattered throughout a gel-like interstitial matter. Therefore, epidermis may be less permeable than the dermis for some molecules having low overall skin permeabilities such as those that are simultaneously large and polar.

The diffusivities (both D_{app} and D) of I and *n*-pentanol were relatively constant with respect to position in the dermis membrane. The permeation data on the dermis membranes of different thicknesses (Tables III and IV) show a random scattering around a constant value when D_{app} is plotted *versus* the membrane thickness (Fig. 2).

Table VI presents the calculated permeability coefficients for the stratum corneum and the epidermis. The permeability coefficients for

Table III—Permeation Data for I with Dermis Membranes of Different Thicknesses

Mouse	h, μm	P, × 10^5 cm/sec	D_{app}[a], × 10^6 cm^2/sec	t_l, min	D[b], × 10^6 cm^2/sec
I	277	5.28	1.46	1.47	1.45
J	292	4.34	1.27	2.87	0.83
K	320	4.43	1.42	2.74	1.04
L	336	2.90	0.97	4.73	0.66
M	341	2.79	0.95	6.27	0.52
N	351	4.60	1.61	2.82	1.21
O	371	4.40	1.63	4.17	0.92
P	400	2.59	1.42	—	—
Average	—	—	1.34	—	0.95

[a] $D_{app} = P \times h$. [b] $D = h^2/6t_l$.

Table IV—Permeation Data for *n*-Pentanol with Dermis Membranes of Different Thicknesses

Mouse	h, μm	P, × 10^5 cm/sec	D_{app}[a], × 10^6 cm^2/sec	t_l, min	D[b], × 10^6 cm^2/sec
J	292	10.26	3.00	1.57	1.51
Q	312	6.61	2.06	3.61	0.75
L	336	6.92	2.33	2.86	1.10
N	351	8.91	3.13	2.21	1.55
Average	—	—	2.63	—	1.23

[a] $D_{app} = P \times h$. [b] $D = h^2/6t_l$.

Table V—Permeability Differences of I and *n*-Pentanol in Various Components of Hairless Mouse Skin

Membrane	Average Permeability Coefficients, cm/sec		Ratio[a]	
	I	*n*-Pentanol	I	*n*-Pentanol
Full-thickness skin	2.98×10^{-8}	3.23×10^{-6}	1.0	1.0
Stripped skin	3.74×10^{-6}	1.99×10^{-5}	125.5	6.2
Dermis[b]	3.83×10^{-5}	7.51×10^{-5}	1285.2	23.3

[a] The ratios were referred to a unity full-thickness permeability coefficient. [b] Average permeability coefficients in the dermis were calculated from $P = D_{app}/h$, with the assumption that the average thickness of the intact dermis is 350 μm.

Table VI—Permeability Coefficients of I and *n*-Pentanol in Various Components of Hairless Mouse Skin

Stratum	Permeability Coefficients, cm/sec	
	I	*n*-Pentanol
Stratum corneum[a]	3.00×10^{-8}	3.85×10^{-6}
Epidermis[b]	4.14×10^{-6}	2.71×10^{-5}
Dermis	3.83×10^{-5}	7.51×10^{-5}

[a] Calculated from the data in Table V using Eq. 2. [b] Calculated from the data in Table V using Eq. 3.

[1] California Bionuclear Co., Sun Valley, CA 91352.

[2] Scotch tape, Commercial Tape Division, 3M Corp., St. Paul, MN 55101.

[3] By definition, $D_{app} = P \times h$; therefore, it also can be related to D and the membrane–water partition coefficient, $K_{m/w}$, by $D_{app} = D \times K_{m/w}$ since $P = (D/h)K_{m/w}$.

[4] The epidermis includes the stratum granulosa and the basal layer, *i.e.*, the epidermal layer excluding the stratum corneum.

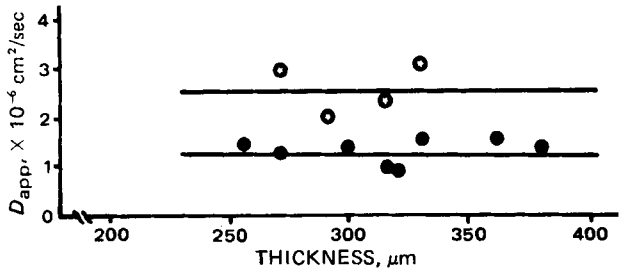

Figure 2—*Plot of the apparent diffusivities* versus *the thickness of the dermis. Key: see Fig. 1.*

the stratum corneum were calculated from the permeation data on whole skin and stripped skin from:

$$\frac{1}{P_{sc}} = \frac{1}{P_{ws}} - \frac{1}{P_{ss}} \qquad \text{(Eq. 2)}$$

where P_{sc}, P_{ws}, and P_{ss} are the permeability coefficients for the stratum corneum, the whole skin, and the stripped skin, respectively. Similarly, the permeability coefficients for the epidermis may be calculated from:

$$\frac{1}{P_e} = \frac{1}{P_{ss}} - \frac{1}{P_d} \qquad \text{(Eq. 3)}$$

where P_e and P_d are the permeability coefficients for the epidermis and dermis, respectively.

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Physical Model Evaluation of Topical Prodrug Delivery—Simultaneous Transport and Bioconversion of Vidarabine-5′-valerate IV: Distribution of Esterase and Deaminase Enzymes in Hairless Mouse Skin

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Received August 2, 1979, from the *College of Pharmacy, University of Michigan, Ann Arbor, MI 48109.* Accepted for publication December 13, 1979. *Present address: Syntex Research, Stanford Industrial Park, Palo Alto, CA 94304.

Abstract □ A semiquantitative assessment of esterase and deaminase distributions in hairless mouse skin was performed *in vitro*. The enzyme activities were quantified using ^{3}H-vidarabine and its 5′-valerate as the substrates. Full-thickness skin of the hairless mouse was cut into two halves, and each half was homogenized in pH 7.4 buffer. Both the supernate and the residue of the homogenate were assayed for esterase and deaminase activities. Results show that the outer half-thickness of the skin contained more esterase but slightly less deaminase than the other half. The characteristics of the esterase and the deaminase reactions also were studied employing the crude enzyme extract; these reactions were essentially irreversible. The deaminase reaction was in the linear region of Michaelis–Menten kinetics for substrate concentrations up to 4.5×10^{-5} *M*.

Keyphrases □ Vidarabine valerate prodrug—topical dosage forms, distribution of adenosine deaminase and esterase enzymes in various mouse skin strata □ Prodrugs, topical—simultaneous transport and bioconversion of vidarabine, distribution of adenosine deaminase and esterase enzymes in mouse skin, pharmacokinetics □ Enzyme distribution studies—location and nature of adenosine deaminase and esterase in mouse skin, pharmacokinetic parameters of deaminase and esterase □ Models, physical—based on simultaneous transport and metabolism, effect of nonhomogeneous distribution on prodrug evaluation

A physical model for the evaluation of a topical prodrug of vidarabine (9-β-D-arabinofuranosyladenine, I) was presented previously (1). The activity of the esterase that converts vidarabine-5′-valerate (III) to I and the activity of the deaminase that converts I to 9-β-D-arabinofuranosylhypoxanthine (II) were potentially capable of influencing the efficacy of the prodrug according to the model. For mathematical simplicity, the model assumed that the enzyme reactions were irreversible and within the linear range of Michaelis–Menten kinetics and that the distributions of both enzymes were homogeneous throughout the viable cutaneous tissue.

Subsequent experiments and data analysis using the model revealed some inconsistencies. Experiments conducted under two different boundary conditions yielded significantly different *in situ* enzyme rate constants. This result raised questions concerning the validity of the homogeneous enzyme distribution assumption employed in the calculations and the need for a more detailed study of the enzyme distribution in the membrane. Thus, the purpose of the present investigation was to study the distributions of the two enzymes in hairless mouse skin as well as the nature of the enzyme reactions.

EXPERIMENTAL

Materials—All materials used were described previously (2), except

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