Effects of Transdermal Penetration Enhancers on the Permeability of Shed Snakeskin

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The effects of Azone and lauryl alcohol on the permeability of shed snakeskin were examined. Permeability of a variety of compounds through shed snakeskin was increased after Azone or lauryl alcohol pretreatment but the magnitude of the enhancement varied depending on the lipophilicity and the molecular size of the permeant. It was found that the shed snakeskin became more permeable after Azone or lauryl alcohol pretreatment, with a greater permeability increase for more hydrophilic and larger-molecular size permeants. As has been shown for untreated shed snakeskins, both the lipophilicity and the molecular size of the permeants are important in skin penetration and in determining the effects of transdermal penetration enhancers.

KEY WORDS: shed snakeskin; *Elaphe obsoleta;* transdermal; penetration enhancer; Azone; lauryl alcohol.

INTRODUCTION

Transdermal administration of drugs is currently receiving a great deal of interest in pharmaceutical research. However, the poor permeability of most drug molecules through the skin has led to the use of a variety of transdermal penetration enhancers. Some of the enhancers are oily, water-immiscible liquids, e.g., Azone (1,2), oleic acid (3,4), and lauryl alcohol (5), and others are water-miscible organic solvents, e.g., ethanol (6–8), PEG 400 (9), and propylene glycol (10), often used as cosolvents in some formulations. Although the mechanism of action of penetration enhancers has been studied and some models proposed (4,10,11), the exact mechanism is not fully understood.

Shed snakeskin of *Elaphe obsoleta* (black rat snake) has been used as a model membrane for transdermal research. It has been shown that there are similarities between human skin and the shed snakeskin of this species in terms of structure/composition, permeability of several compounds, and the functional group contribution to the permeability (12).

In order to examine the effects of transdermal penetration enhancers on the permeability of shed snakeskin, Azone and lauryl alcohol were used as model enhancers in the present study. Since it has been shown that both the lipophilicity and the molecular size of the permeant are important in controlling the permeability through shed snakeskin (13), several compounds with various lipophilicities and molecular weights were used as model permeants to examine the effect of the enhancers.

MATERIALS AND METHODS

Methylparaben, ethylparaben, propylparaben, butylparaben, 5-fluorouracil (5-FU), ibuprofen, ketoprofen, hydrocortisone, deoxycorticosterone (DCC), indomethacin, *m*-cresol, phenol, and lauryl alcohol were purchased from Sigma Inc. (St. Louis, MO) and used as received. *m*-Hydroxybenzyl alcohol (m-HBAl) was purchased from Aldrich Chemical (Milwaukee, WI) and Azone was supplied by Nelson Research Laboratories (CA). All the other chemicals were reagent grade and were used as received.

In Vitro Penetration Study

Shed snakeskins of *Elaphe obsoleta* (black rat snake) were used as the model membrane for *in vitro* penetration studies as reported previously (12,13). For the control study, shed snakeskin was hydrated at 40°C for 30 min and was mounted on a diffusion cell. For the pretreatment study, 100 μl of Azone or lauryl alcohol was applied on the exterior side of the dorsal portion of the shed snakeskin 4 hr prior to the penetration study. Any remaining enhancer on the skin was blotted with a Kimwipe after 4 hr of pretreatment and the shed skin specimen was mounted on a diffusion cell. For the 5-FU study, shed snakeskin was also treated with Azone for 24 hr or treated with Azone for 4 hr, followed by hydration in a shallow pan of deionized water at 40°C for 30 min, and then the penetration was measured in order to examine the effects of the pretreatment time and of hydration on the pretreated skin.

For the control study, the penetration of *m*-cresol, phenol, m-HBAl, ibuprofen, and parabens from aqueous solutions through untreated shed snakeskin was measured at 37°C with an automated diffusion cell system as previously described (14). The effective area of the cells was 1.8 cm^2. The donor volume was 2 ml and the receptor volume was about 10 ml. The donor concentrations ranged from 4 to 8 mg/ml for *m*-cresol, phenol, and m-HBAl and 0.1–1.0 mg/ml for parabens in pH 7.2 isotonic phosphate buffer. For ibuprofen (pK_a = 5.2), a saturated solution (centrifuged to remove excess solid) in pH 3.0 phosphate buffer (0.01 *M*) was used as the donor solution in order to measure the penetration of the neutral species. The receptor solution was pH 7.2 isotonic phosphate buffer for all the compounds. The UV absorbance of the receptor solution was monitored at 5-min intervals at 272 nm for *m*-cresol and m-HBAl, 268 nm for phenol, 255 nm for parabens, and 220 nm for ibuprofen.

The penetration at 5-FU and hydrocortisone from aqueous solution through untreated skin was measured at 37°C with a Franz-type diffusion cell as previously reported (12–14). The donor solution for 5-FU was about 4 mg/ml in pH 7.2 isotonic buffer, and that for hydrocortisone was a saturated solution in the same buffer. The receptor solution (pH

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7.2 isotonic buffer) was sampled at appropriate time intervals and the drug concentration was determined by HPLC.

For indomethacin, a thin film patch formulation consisting of hydroxypropyl cellulose, PEG 400, and indomethacin (1 mg/12-mm-diameter patch) was used to measure the penetration as previously reported (13) because of its very low aqueous solubility at pH 3.0. Approximately 0.2 ml of pH 3.0 phosphate buffer (0.01 *M*) was added to the film formulation in order to maintain a saturated solution in the donor phase. The receptor solution (pH 7.2 buffer) was sampled and analyzed by HPLC.

For the pretreatment study, the penetration of each compound except for indomethacin from aqueous solution through pretreated shed snakeskin was measured with an automated diffusion-cell system as previously described (14). Indomethacin penetration through pretreated skin was measured with a Franz-type diffusion cell with HPLC analysis. The penetration of hydrocortisone was measured using both an automated system and a Franz-type cell with HPLC analysis. The donor solutions and the UV monitoring of the receptor solution for each compound were the same as those used in the control study. In addition, the UV absorbance of the receptor solution (pH 7.2 buffer) was monitored at 5-min intervals at 248 and 266 nm for hydrocortisone and 5-FU in the automated system, respectively.

The penetration of ketoprofen and deoxycorticosterone (DCC) from the aqueous solution through pretreated skin was also measured using an automated diffusion-cell system. A saturated solution in pH 7.2 buffer was used as a donor solution for DCC and a saturated solution in pH 3.0 buffer was used as a donor for ketoprofen. The UV absorption of the receptor solution (pH 7.2 buffer) was monitored at 5-min intervals at 248 and 263 nm for DCC and ketoprofen, respectively.

HPLC Conditions

5-FU was analyzed with a μ-Bondapak C18 column (Waters Associates, MA) using 0.05 *M* KH_2PO_4–H_3PO_4 buffer (pH 3.0) as the mobile phase. The flow rate was 1.0 ml/min, with UV detection at 266 nm. Hydrocortisone and indomethacin were analyzed with a RP-18 column (Brownlee Laboratories, CA) at a flow rate of 0.8 ml/min. The mobile-phase composition and the UV detection for these compounds were CH_3CN:pH 3.0 phosphate buffer (0.01 *M*), 60:40, at 260 nm for indomethacin and CH_3CN:H_2O, 35:65, at 242 nm for hydrocortisone.

RESULTS AND DISCUSSION

The molecular weight and ln(distribution coefficient) for the compounds used in the present study are listed in Table I. Also listed in Table I is the solubility of each compound. These values were used to calculate the permeability of each compound from the penetration data. The solubilities of 5-FU and the parabens were not measured because unsaturated solutions with known concentrations were used as donor solutions. The penetration profiles for 5-FU from aqueous solution through untreated, Azone-treated, and lauryl alcohol-treated shed snakeskins are shown in Fig. 1. The permeability of each compound was calculated from the initial straight portion of the penetration curve according to Eq. (1), with less than 5% of the drug penetrating through the skin.

Table I. Molecular Weight (MW), ln(Distribution Coefficient) [ln(DC)], and Aqueous Solubility of the Compounds Used in the Present Study

	MW	ln(DC)	Solubility (μg/ml)[a]
Ibuprofen	206.3	8.95[b]	58.3 ± 0.8[c]
Ketoprofen	254.3	7.06[b]	193 ± 2.1[c]
Indomethacin	357.8	9.64[b]	1.71 ± 0.01[c]
Hydrocortisone	362.5	3.51[d]	379 ± 7.0[e]
DCC	330.5	6.46[d]	176 ± 2.3[e]
5-FU	130.1	−2.15[d]	—
Methylparaben	152.2	3.82[f]	—
Ethylpaarben	166.2	5.04[f]	—
Propylparaben	180.2	6.24[f]	—
Butylparaben	194.2	7.46[f]	—

[a] Mean ± SD; n = 3–4.
[b] Distribution coefficient between 1-octanol/pH 3.0 buffer (12).
[c] Solubility in pH 3.0 buffer (12).
[d] Distribution coefficient between 1-octanol/pH 7.2 buffer (12).
[e] Solubility in pH 7.2 isotonic buffer (12).
[f] Distribution coefficient between 1-octanol/water reported by Valvani *et al.* (18).

$$P = \frac{dQ}{dt}\frac{1}{A}\frac{1}{C_d} \quad (1)$$

where P is the permeability, dQ/dt is the slope of the straight portion of the penetration curve, A is the surface area (1.8 cm^2 for the diffusion cells used in the present study), and C_d is the drug concentration in the donor phase. The permeabilities thus calculated are listed in Tables II–IV.

The apparent free energies of transfer of the methylene group and the hydroxyl group were calculated from the permeability data according to Eq. 2 (12).

$$\Delta(\Delta G)_X = -RT\ln(P_{RX}/P_{RH}) \quad (2)$$

where $\Delta(\Delta G)_x$ is the apparent free energy of transfer of the functional group X from the aqueous phase to the untreated,

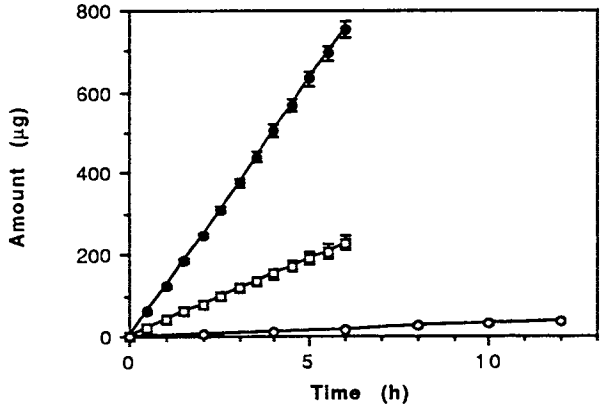

Fig. 1. The amount of 5-FU penetrating from an aqueous solution through untreated (○), Azone-treated (●), and lauryl alcohol-treated (□) shed snakeskins.

Table II. Permeabilities ($\times 10^2$ cm/hr)[a] of *m*-Cresol, Phenol, and m-HBAl from Aqueous Solution Through Untreated, Azone-Treated, and Lauryl Alcohol-Treated Shed Snakeskins and the Apparent Free Energy of Transfer (cal/mol) of the Methylene and Hydroxyl Groups from the Aqueous Phase to the Shed Snakeskin with Each Treatment at 37°C

	Untreated	Azone	Lauryl alcohol
m-Cresol	2.10 ± 0.19	9.25 ± 1.66	15.0 ± 1.4
Phenol	0.936 ± 1.02	9.91 ± 1.79	13.9 ± 0.7
m-HBAl	—	3.24 ± 0.28	2.76 ± 0.40
$\Delta(\Delta G)_{-CH_2-}$	−499 ± 118	42 ± 156	−48 ± 65
	−435 ± 17[b]		
$\Delta(\Delta G)_{-OH}$	2390 ± 190[b]	642 ± 121	1046 ± 90

[a] Mean ± SD; $n = 4$.
[b] Previously reported (11).

Azone-treated, or lauryl alcohol-treated shed snakeskin, R is the gas constant, T is the absolute temperature, P_{RX} is the permeability of the test compound, and P_{RH} is the permeability of the reference compound. The free energy of transfer was calculated with RX = m-cresol and RH = phenol for the methylene group and with RX = m-HBAl and RH = m-cresol for the hydroxyl group. The results are listed in Table II. The free energy of transfer of the methylene group for the untreated skin obtained in the present study was similar to that previously obtained from the paraben permeability data (12). However, when the shed snakeskin was pretreated with Azone or lauryl alcohol, the free energy of transfer of the methylene group increased significantly, from −450 cal/mol to almost 0 cal/mol.

The permeability values for 5-FU, ibuprofen, hydrocortisone, and indomethacin through untreated, Azone-treated, and lauryl alcohol-treated shed snakeskins are listed in Table III. Although the permeability of each compound increased after pretreatment, the magnitude of the enhancement (P_{trt}/P_{unt} in Tables III and IV) appeared to vary depending on the lipophilicity and the molecular size of the compound. The permeability of ibuprofen, which is a small and lipophilic molecule, increased by a factor of only 2–3 with pretreatment. On the other hand, the permeability of 5-FU, which is a small and hydrophilic molecule, increased by a factor of 10–60 with pretreatment. The permeability of indomethacin, which is a large and lipophilic molecule, increased also by a factor of about 20 after pretreatment. Finally, the permeability of hydrocortisone, which is a large and hydrophilic molecule, increased by a factor of 70–350. There seems to be a general tendency that the permeability of a more hydrophilic and larger-molecular size compound is more enhanced after pretreatment compared to that of smaller and less hydrophilic compounds. Moreover, the permeability of hydrophilic compounds (e.g., 5-FU and hydrocortisone) was greater after Azone pretreatment than after lauryl alcohol pretreatment.

Since the permeability of compounds has been reported to be controlled by both the lipophilicity and the molecular size of the permeant for untreated shed snakeskins (13), hairless mouse skin (15), and human skin (16), the permeability data for the parabens, ibuprofen, ketoprofen, hydrocortisone, and DCC were fitted with the following two equations as described previously (13).

$$\ln(P) = \alpha \ln(DC) + \beta\ MW + \gamma \quad (3)$$

$$\ln(P) = \alpha' \ln(DC) + \beta' \ln(MW) + \gamma' \quad (4)$$

where P is the permeability, DC is the distribution coefficient, MW is the molecular weight, and α, α', β, β', γ, and

Table III. Permeabilities ($\times 10^3$ cm/hr; Mean ± SD; n = 3–4) of Various Compounds Through Untreated, Azone-Treated, and Lauryl Alcohol-Treated Shed Snakeskins and the Ratio of the Permeability Through Treated Skin to That Through Untreated Skin (P_{trt}/P_{unt})

	Untreated	Azone	Lauryl alcohol
5-FU[a]	0.190 ± 0.035[b]	11.1 ± 3.5	1.93 ± 0.20
5-FU[a]	0.397 ± 0.126[b]	17.7 ± 0.9	5.23 ± 0.58
Ibuprofen[a]	61.5 ± 5.1	90.1 ± 10.0	124 ± 24
Ibuprofen[a]	122 ± 22	200 ± 9.0	377 ± 54
Hydrocortisone[a]	0.064 ± 0.033[b]	7.18 ± 2.66[b]	4.80 ± 1.00[b]
Hydrocortisone[a]	0.045 ± 0.015[b]	15.7 ± 1.6	7.03 ± 1.55
Indomethacin	75.5 ± 29.0[b]	1670 ± 420[b]	1500 ± 200[b]
P_{trt}/P_{unt}			
5-FU[a]	1.00	58.4	10.1
5-FU[a]	1.00	44.6	13.2
Ibuprofen[a]	1.00	1.47	2.02
Ibuprofen[a]	1.00	1.64	3.09
Hydrocortisone[a]	1.00	112	74.9
Hydrocortisone[a]	1.00	347	155
Indomethacin	1.00	22.1	19.9

[a] Permeabilities and P_{trt}/P_{unt} were determined with shed snakeskins from two snakes.
[b] Penetration was measured using a Franz-type diffusion cell.

Table IV. Permeabilities ($\times 10^3$ cm/hr)[a] of Parabens, Ketoprofen, and DCC from Aqueous Solutions Through Azone- and Lauryl Alcohol-Treated Shed Snakeskins

	Azone	Lauryl alcohol
Methylparaben	58.5 ± 3.2	82.4 ± 12.5
P_{trt}/P_{unt}[b]	11.4	16.0
Ethylparaben	—	89.8 ± 2.7
Propylparaben	58.3 ± 6.4	111 ± 3.4
Butylparaben	—	130 ± 13.8
Ketoprofen	120 ± 14	239 ± 26
DCC	49.7 ± 5.3	65.5 ± 5.7

[a] Mean ± SD; n = 3–4.

[b] Permeability value of 5.15 ± 0.64 × 10^{-3} cm/hr (mean ± SD; n = 4) was used for the methylparaben permeability through untreated skin.

γ' are constants. Equations (3) and (4) assume a dependency of ln(P) on the molecular weight and ln(MW), respectively. The values for the constants were obtained by a least-squares fit of the data in Tables III and IV and the results are listed in Table V.

The α and α' values for the Azone- or lauryl alcohol-treated skins were smaller than those for the untreated skin as shown in Table V. Since α and α' are the coefficients for the relationship between ln(P) and the permeant distribution coefficient [Eqs. (3) and (4)], the smaller values after pretreatment indicate that shed snakeskins are less discriminating to differences in partition coefficient after Azone or lauryl alcohol treatment compared to the untreated skin. This agrees with the free energy study in Table II and the permeability study in Table III. Because the shed snakeskin became less discriminating after pretreatment, permeabilities of hydrophilic molecules were enhanced more by pretreatment.

On the other hand, smaller β and β' values in absolute magnitude ($|\beta|$ and $|\beta'|$) were obtained after Azone or lauryl alcohol pretreatment compared to those for untreated skin. The β and β' are the coefficients for the relationship between ln(P) and the permeant molecular weight and the smaller β and β' values indicate less resistance to the diffusion of molecules through the skin. Therefore, the shed snakeskin appears to be more permeable after pretreatment and resistance to the diffusion of larger molecules is reduced. This may be the reason that the permeability of hydrocortisone was enhanced more than that of methylparaben after Azone or lauryl alcohol pretreatment (P_{trt}/P_{unt} in Tables III and IV), although the distribution coefficients of these compounds are similar (Table I).

Permeabilities were calculated according to Eqs. (3) and (4) using the α, α', β, β', γ, and γ' values, the distribution coefficient, and the molecular weight of a compound. The calculated ln(P) according to Eq. (3) was plotted against the observed ln(P) in Fig. 2 for the lauryl alcohol-treated shed snakeskin. As shown in Fig. 2, there is good agreement between the calculated ln(P) and the observed ln(P), suggesting that the permeability through enhancer treated shed snakeskins may be predicted from the distribution coefficient and the molecular weight of a compound as has been shown for untreated shed snakeskins (13). Both the distribution coefficient and the molecular size are important in predicting permeability under these conditions. For example, if one does not take into consideration the molecular size of the permeants, the differences in P_{trt}/P_{unt} between methylparaben and hydrocortisone are difficult to explain since they have similar distribution coefficients.

5-FU permeability was measured both after 4-hr Azone pretreatment followed by 30-min hydration and after 24-hr Azone pretreatment. The 5-FU permeability after 4-hr pretreatment followed by 30-min hydration was 13.7 ± 3.1 × 10^{-3} cm/hr (mean ± SD, n = 4), which was similar to that after 4-hr pretreatment without hydration (Table III). This observation suggests that the effect of Azone is irreversible or that Azone stays in the skin even after hydration to increase the permeability. Considering the low aqueous solubility of Azone, it is very likely that this compound is not washed away by hydration. On the other hand, the 5-FU permeability after 24-hr Azone pretreatment was 24.3 ± 6.0 × 10^{-3} cm/hr (mean ± SD, n = 3), which was slightly greater than that for the 4-hr pretreatment. This suggests that enhancement may vary slightly depending on the pretreatment time of the skin as well as the enhancer concentration applied as reported elsewhere (17).

The present study has confirmed that both Azone and

Table V. The Values for the Constants in Eqs. (3) and (4) for Untreated, Azone-Treated, and Lauryl Alcohol-Treated Shed Snakeskins

	Untreated	Azone	Lauryl alcohol
α	1.07	0.325	0.459
β	−0.0145	−0.00435	−0.00756
γ	−8.57	−3.63	−3.44
Correlation coefficient	0.967	0.950	0.937
α'	1.11	0.343	0.491
β'	−3.72	−1.04	−1.81
γ'	7.95	0.866	4.38
Correlation coefficient	0.972	0.948	0.934

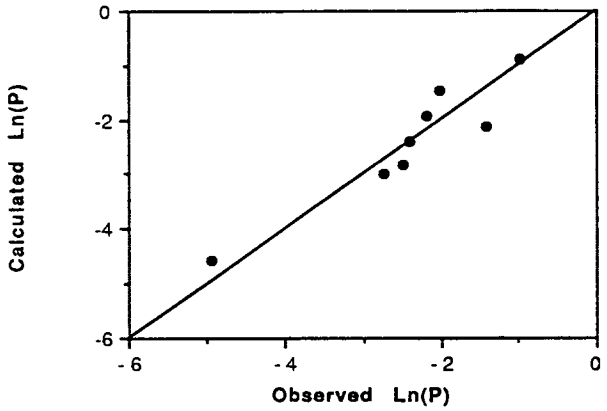

Fig. 2. Observed ln(P) vs calculated ln(P) according to Eq. (3) for lauryl alcohol-treated shed snakeskins. The straight line represents perfect correlation between the observed and the calculated ln(P).

lauryl alcohol increase the permeability of shed snakeskin as reported for other animal and human skins. However, the magnitude of the permeability increase varied depending on the lipophilicity and the molecular size of the permeant, with the permeability of a hydrophilic compound with a large molecular size being increased more by pretreatment than other compounds. As has been shown for Azone and lauryl alcohol as model enhancers in the present study, the lipophilicity and the molecular size of the permeant should be taken into account in examining the effects of transdermal penetration enhancers.

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