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Report

A Method to Predict the Percutaneous Permeability of Various Compounds: Shed Snake Skin as a Model Membrane

Tomoo Itoh,[1] Ravi Magavi,[1] Roger L. Casady,[1] Toshiaki Nishihata,[1,2] and J. Howard Rytting[1,3]

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Penetration of various compounds through shed snake skin was measured *in vitro* to examine the effect of lipophilicity and molecular size of a compound on permeability through this model membrane. The permeabilities were found to be controlled by the lipophilicity and the molecular size of the permeant. The smaller and the more lipophilic the compound, the greater the permeability. Equations have been developed to predict the permeability from the molecular weight and the distribution coefficient of a compound. Further, the lipophilicity of shed snake skin is similar to that of human skin and the response of shed snake skin to the molecular size of a permeant is more similar to human skin than to hairless mouse skin. Considering the similarities between shed snake skin and human stratum corneum in terms of structure, composition, and permeability characteristics, the same considerations may apply to permeability through human stratum corneum.

KEY WORDS: skin penetration; shed snake skin; *Elaphe obsoleta;* black rat snake; distribution coefficient; molecular weight; permeability.

INTRODUCTION

The use of human skin for *in vitro* penetration studies is limited because human skin is often difficult to obtain, expensive, difficult to store, and variable in permeation properties. Excised animal skins also have variable properties depending on preparation methods and animal species and are usually more permeable than human skin (1–4), partly because of the greater number of hair follicles. Shed snake skin appears to be a useful alternative to animal skin in assessing the potential for transdermal drug delivery. Shed snake skin is nonliving, pure stratum corneum with no hair follicles. The structure of the shed snake skin and its advantages over other model membranes were discussed in our previous report (5). The permeability of several compounds through shed snake skin was found to be similar to, but often slightly less than, that through human skin (5), which may make shed snake skin a better model membrane than other animal skins because most animal skins are much more permeable than human skin.

In the present study, shed snake skin of *Elaphe obsoleta* (black rat snake) was used as a model membrane to examine the effects of lipophilicity and molecular size of a compound on its permeability.

[1] Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66045.

[2] Present address: Upjohn Tsukuba Research Labs., 23 Wadai, Tsukuba-shi, Ibaraki-ken 305, Japan.

[3] To whom correspondence should be addressed.

MATERIALS AND METHODS

Ibuprofen, ketoprofen, naproxen, indomethacin, hydrocortisone, 11alpha-hydroxyprogesterone (11a-HPG), deoxycorticosterone (DCC), progesterone, methylparaben, ethylparaben, propylparaben, butylparaben, and polyethylene glycol 400 (PEG 400) were purchased from Sigma Inc. (St. Louis, MO) and used as received. Hydroxypropyl cellulose (high-viscosity type, HPC-H) was obtained from Nihon Soda (Tokyo). Ethanol (95%) was obtained from Aldrich Chemicals (Milwaukee, WI) together with 1-octanol.

Distribution Coefficient Study

1-Octanol and pH 7.2 phosphate buffer (0.1 *M*) were saturated with each other prior to use. Hydrocortisone was dissolved in presaturated 1-octanol and pH 7.2 buffer at a concentration of ca. 15 μg/ml, respectively. Five milliliters of 1-octanol solution with hydrocortisone was mixed with 5 ml of hydrocortisone-free presaturated pH 7.2 buffer for 30 min with a vortex mixer. The mixture was then centrifuged for 10 min at 3000 rpm and the hydrocortisone concentration in each phase was determined spectrophotometrically. The distribution coefficient was calculated as the ratio of the drug concentration in the 1-octanol phase to that in the aqueous phase. The distribution coefficient was also determined by mixing 5 ml of the drug containing pH 7.2 buffer with 5 ml of drug-free presaturated 1-octanol followed by the same procedure in order to verify that the mixing time was sufficient.

For progesterone, DCC, 11a-HPG, and corticosterone, the drug was dissolved in presaturated 1-octanol at the con-

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centration of 1–6 mg/ml and the distribution coefficient was measured following the same procedure as described above. The concentration of the drug in the aqueous phase was determined with HPLC.

For ibuprofen, ketoprofen, and indomethacin, pH 3.0 phosphate buffer (0.01 *M*) was used instead of pH 7.2 buffer to measure the distribution of neutral species. These compounds were dissolved in 1-octanol presaturated with pH 3.0 buffer at the concentration of 1–2 mg/ml and the distribution coefficient was measured following the same procedure. The drug concentration in the aqueous phase was determined with HPLC.

Solubility Measurement

Hydrocortisone, corticosterone, 11a-HPG, DCC, or progesterone (100–300 mg) was stirred in 15 ml of pH 7.2 isotonic buffer at 37°C for 40–48 hr. The solution was centrifuged and the supernatant filtered with a Millipore FH filter (0.5 μm). The drug concentration in the filtrate was determined spectrophotometrically, except for progesterone. For ibuprofen, ketoprofen, naproxen, and indomethacin, the solubility in pH 3.0 phosphate buffer (0.01 *M*) was measured following the same procedure. For progesterone and indomethacin, the concentration in the filtrate was determined by HPLC.

Preparation of Thin-Film Patches

A viscous solution consisting of 0.3 g drug, 0.4 g PEG 400, and 0.8 g HPC-H in 20 g ethanol was spread on a waxed paper with a bar-coaster to approximately 1 mm in thickness, followed by drying at 40°C for 1 hr. The film sheet thus obtained was punched into a 12 mm diameter patch and was used for *in vitro* penetration studies. The compounds used in the thin-film formulation were ibuprofen, ketoprofen, indomethacin, and progesterone and the drug content in a 12-mm-diameter patch was approximately 1 mg for each compound.

In Vitro Penetration Study

Shed snake skins of *Elaphe obsoleta* (black rat snake) were used as a model membrane for *in vitro* penetration studies. To measure the penetration of parabens and ibuprofen from the solution through shed snake skin, an automated diffusion cell system previously reported (6) was used and the UV absorbance of the receptor solution was monitored every 5 min. As a donor solution, 0.1–0.2 mg/ml solution in pH 7.2 isotonic buffer was used for parabens and the saturated solution in pH 3.0 phosphate buffer was used for ibuprofen.

For hydrocortisone, corticosterone, 11a-HPG, and DCC, a saturated solution in pH 7.2 isotonic buffer was used as the donor phase and the penetration was measured with a Franz-type diffusion cell as reported previously (6). The sampled receptor solution was analyzed with HPLC.

The penetration of ketoprofen and naproxen through shed snake skin was measured using a Franz-type diffusion cell with the saturated solution in pH 3.0 buffer as a donor solution. Again, the sampled receptor solution was analyzed with HPLC.

The penetration of indomethacin and progesterone was measured from a thin-film patch formulation instead of from a solution because of their very low aqueous solubilities. The thin-film patch was applied on a shed snake skin, covered with a dialysis membrane, and then mounted on a Franz-type diffusion cell. Approximately 0.2 ml of pH 3.0 buffer was added to the indomethacin patch in the donor phase in order to hydrate the patch for drug release and the same amount of pH 7.2 isotonic buffer was added to the progesterone patch. The sampled receptor solution was analyzed with HPLC. The penetration of ibuprofen and ketoprofen was also measured from a thin-film patch formulation in order to compare the permeability obtained from solution with that from a thin-film patch formulation.

As a receptor solution, pH 7.2 isotonic buffer was used for all the compounds and all the penetration studies were conducted at 37°C.

HPLC Conditions

A Perkin–Elmer HPLC system with a RP-18 column (Brownlee Labs., U.S.A.) was used at a flow rate of 0.8 ml/min. The mobile phase composition and the UV detection for each compound were as follows: CH_3CN, pH 3.0 phosphate buffer(0.01 *M*) = 65:35 at 220 nm for ibuprofen; CH_3CN, pH 3.0 buffer = 53:47 at 230 nm for naproxen; CH_3OH, pH 3.0 buffer = 68:32 at 263 nm for ketoprofen; CH_3CN, pH 3.0 buffer = 60:40 at 260 nm for indomethacin; CH_3CN, H_2O = 35:65 at 242 nm for hydrocortisone; CH_3CN, H_2O = 40:60 for corticosterone; CH_3CN, H_2O = 50:50 at 248 nm for 11a-HPG; CH_3CN, H_2O = 56:44 at 241 nm for DCC; and CH_3CN, H_2O = 65:35 at 241 nm for progesterone.

RESULTS AND DISCUSSION

The molecular weight and the ln(distribution coefficient) for the compounds used in the present study are listed in Table I. The distribution coefficients in the parentheses are literature values and were used for the data analysis of the parabens. Also listed in Table I is the solubility for each compound and these values were used to calculate the permeability from the penetration data.

The amount of penetration through shed snake skin from solution is shown in Fig. 1 for butylparaben and ibuprofen. Although the data were collected every 5 min, the penetration is shown for every 30 min in Fig. 1. Other parabens showed similar penetration profiles but the penetration rate was smaller for less lipophilic parabens. The penetration of ibuprofen and butylparaben was faster than that of the other compounds used in the present study and little or no lag time was observed for these two compounds. Permeability was calculated according to Eq. (1) from the initial linear portion of the penetration curve with less than 5–10% of penetration.

$$P = \frac{dQ}{dt}\frac{1}{A}\frac{1}{C_d} \tag{1}$$

where P = the permeability, dQ/dt = the slope of the straight portion of the penetration curve, A = the surface area (1.8 cm^2 for the diffusion cells used in the present study), and C_d = the drug concentration in donor phase. The

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

	MW	ln(*DC*)	Solubility[a]
Ibuprofen	206.3	8.95[b]	58.3 ± 0.8[c]
Naproxen	230.3	7.14[b]	36.1 ± 0.7[c]
Ketoprofen	254.3	7.06[b]	176 ± 5.0[c]
Indomethacin	357.8	9.64[b]	1.71 ± 0.01[c]
Progesterone	314.5	9.12[d] (8.91)[e]	9.10 ± 0.96[f]
DCC	330.5	6.46[d] (6.68)[e]	74.3 ± 0.4[f]
11a-HPG	330.5	5.89[d] (5.43)[e]	176 ± 2.3[f]
Corticosterone	346.5	4.63[d] (4.47)[e]	296 ± 17[f]
Hydrocortisone	362.5	3.51[d] (3.57)[e]	379 ± 7.0[f]
Methylparaben	152.2	— (3.82)[e]	—
Ethylparaben	166.0	— (5.04)[e]	—
Propylparaben	180.2	— (6.24)[e]	—
Butylparaben	194.2	— (7.46)[e]	—

[a] Solubility as μg/ml, mean ± SD, n = 3–4.
[b] Distribution coefficient between 1-octanol/pH 3.0 buffer.
[c] Solubility in pH 3.0 buffer.
[d] Distribution coefficient between 1-octanol/pH 7.2 buffer.
[e] Distribution coefficient between 1-octanol/water reported by Valvani *et al.* (11).
[f] Solubility in pH 7.2 isotonic buffer.

Table II. The Observed Permeability (P) and ln(P) of Various Compounds (Mean ± SD, n = 3–7) Together with the Calculated ln(P) According to Eqs. (2) and (3)

	Permeability ($\times 10^3$ cm/hr)	ln(P)	Calculated ln(P) Eq. (2)	Calculated ln(P) Eq. (3)
Ibuprofen	88.1 ± 9.9	−2.43	−1.99	−1.99
Naproxen	14.8 ± 1.7	−4.21	−4.27	−4.40
Ketoprofen	6.22 ± 0.49	−5.08	−4.71	−4.86
Indomethacin	97.8 ± 24.4	−2.33	−3.45	−3.28
Progesterone	15.9 ± 2.9	−4.14	−3.39	−3.38
DCC	3.98 ± 0.35	−5.53	−6.46	−6.49
11a-HPG	0.826 ± 0.086	−7.10	−7.09	−7.15
Corticosterone	0.127 ± 0.016	−8.97	−8.65	−8.70
Hydrocortisone	0.0228 ± 0.0132	−10.7	−10.1	−10.1
Methylparaben	2.67 ± 0.30	−5.93	−6.69	−6.53
Ethylparaben	4.97 ± 0.96	−5.30	−5.59	−5.50
Propylparaben	8.75 ± 1.02	−4.74	−4.51	−4.48
Butylparaben	22.6 ± 0.48	−3.79	−3.41	−3.41

permeabilities thus calculated are listed in Table II for ibuprofen and parabens.

The amount of penetration for ketoprofen and naproxen through shed snake skin from the solution is shown in Fig. 2. These compounds showed short lag times of 1–3 hr and the penetration rate was significantly lower than for ibuprofen or butylparaben. Also, the donor pH was unchanged after 24 hr, although the pH's of the donor and receptor solutions were different. The permeabilities were again calculated from the linear portion of the penetration curve, that is, from the 4- to 12-hr data, and the results are summarized in Table II.

The penetration profiles for 11a-HPG and corticosterone are shown in Fig. 3. These compounds showed approximately 6–8 hr of lag time and the penetration rate was significantly lower than for ketoprofen or naproxen. Permeabilities were calculated from the straight portion of the penetration profiles (24–72 hr) and the permeabilities thus obtained are again listed in Table II. Deoxycorticosterone (DCC) and hydrocortisone showed similar penetration profiles and their permeabilities are also listed in Table II.

Because of the very low aqueous solubility of progesterone and indomethacin, as shown in Table I, it was not possible to measure the penetration of these compounds from solution. Instead, these compounds were formulated into a thin-film patch formulation and this patch formulation was used to measure the penetration. The advantage of using a thin-film patch is that the drug concentration in the donor phase is maintained at saturation and therefore the steady-state flux is maintained over a prolonged period of time. To verify the utility of using a thin-film patch formulation, the penetration of ibuprofen and ketoprofen was measured from both the saturated solution and the thin-film patch. The permeabilities were calculated from the penetration–time profiles according to Eq. (1) assuming the saturation concentration in the donor phase. This assumption was made because of the following three observations: (i) the drug release from the thin-film patch was much faster than the penetration through shed snake skin (ca. 80% release within 3 hr); (ii) the drug release was found to follow the Higuchi equation for a variety of compounds; and (iii) the drug content in the thin-film patch was far larger than that needed to ensure satura-

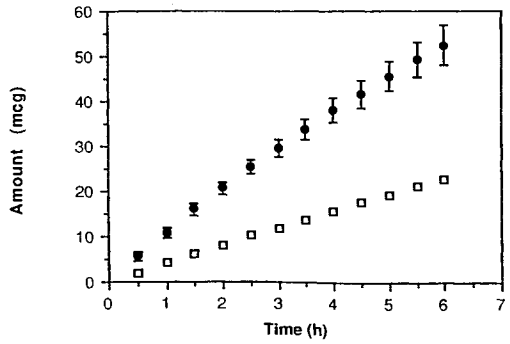

Fig. 1. The amount of ibuprofen (●) and butylparaben (□) penetrating through shed snake skin from the solution measured with an automated diffusion cell system. Mean ± SD; n = 3–5.

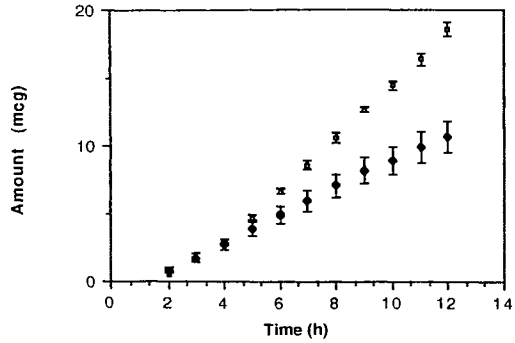

Fig. 2. The amount of ketoprofen (□) and naproxen (♦) penetrating through shed snake skin from the solution measured with a Franz type diffusion cell. Mean ± SE; n = 4–5.

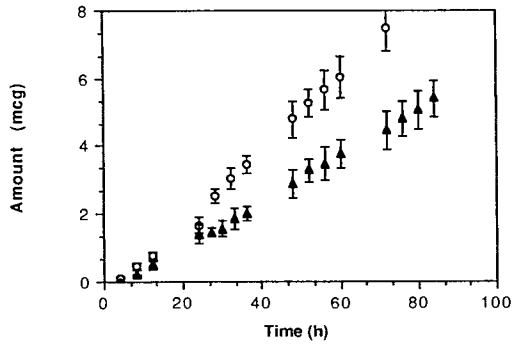

Fig. 3. The amount of penetration from the solution through shed snake skin for 11a-HPG (○) and corticosterone (▲). Mean ± SD; n = 3–4.

tion in the amount of buffer added to the donor phase. The permeability thus obtained from the thin-film patch was 195 ± 25 × 10^{-3} cm/hr for ibuprofen and 11.6 ± 1.4 × 10^{-3} cm/hr for ketoprofen (mean ± SD; n = 6). These permeability values were about twice as large as those obtained from solution (Table II). This may be due to the drug molecules being dispersed in the polymer matrix of the film formulation to form a molecular dispersion. This formulation may form a supersaturated solution upon hydration, leading to greater penetration than that observed from the saturated solution. Another possibility is that the PEG 400 incorporated in the thin-film formulation may increase the solubilities of the drugs or enhance their penetration. In either case, the permeabilities obtained from the thin-film patch formulation were larger than those from the solution for ibuprofen and ketoprofen. Therefore, the permeabilities obtained for indomethacin and progesterone from the thin-film patch, which are listed in Table II, may be an overestimate when compared to solution.

The ln(P) values listed in Table II were plotted against the ln(DC) for each compound in Fig. 4 to examine the effect of lipophilicity of a compound on the permeability. Although there appears to be a good correlation between ln(P) and ln(DC) for the group of parabens and for the group of other compounds, respectively, the slope for the paraben group is significantly smaller than that for the group of other compounds. It should be noted that for the paraben group the distribution coefficient increases slightly as the molecular weight increases, but that for the group of other compounds, except for indomethacin and progesterone, the distribution coefficient increases as the molecular weight decreases. This may account for the difference in the slope between the paraben group and the rest of the compounds, that is, the simultaneous decrease in molecular weight while increasing the distribution coefficient in the latter group of compounds may lead to a larger slope in the ln(P) vs. ln(DC) plot. Also, the permeability of methylparaben is much larger than hydrocortisone permeability even though the distribution coefficients of these compounds are similar. However, the molecular weight of hydrocortisone is more than twice as large as that of methylparaben.

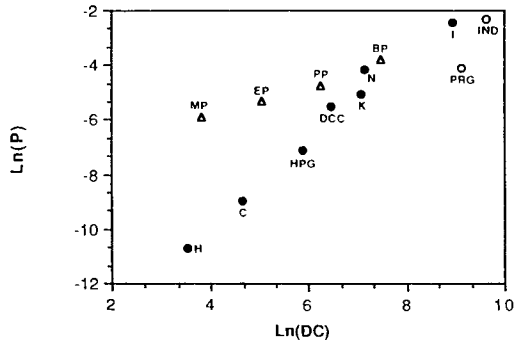

Fig. 4. ln(P) vs ln(DC) plot for the compounds used in the present study. I, ibuprofen; N, naproxen; K, ketoprofen; IND, indomethacin; PRG, progesterone; DCC, deoxycorticosterone; HPG, 11alpha-hydroxyprogesterone; C, corticosterone; H, hydrocrotisone; MP, methylparaben; EP, ethylparaben; PP, propylparaben; BP, butylparaben.

Since the importance of the permeant molecular size to the penetration through various biological membranes has recently been pointed out by several researchers (7–9), the present data were fitted with the following equations.

$$\ln(P) = \alpha \ln(DC) + \beta\, MW + \gamma \quad (2)$$

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

where P = the permeability (cm/hr), DC = the distribution coefficient, MW = the molecular weight, and α, α', β, β', γ, and γ' are constants. Equations (2) and (3) assume a dependency of ln(P) on the molecular weight and ln(MW), respectively. By a least-squares fit of the data in Fig. 4, the following values were obtained for the constants; α = 1.07, β = −0.0145, and γ = −8.57 with a correlation coefficient of 0.967 for Eq. (2) and α' = 1.11, β' = −3.72, and γ' = 7.95 with a correlation coefficient of 0.972 for Eq. (3). The coefficient for the ln(P)–ln(DC) relationship (α or α') is larger than that reported for human skin [0.6 for the slope of the log(P) vs log(DC) plot by Roberts *et al.* (10)]. However, the molecular weight difference was not taken into account in their study and the slope of the ln(P) vs ln(DC) plot in our study is 0.58 for parabens, which are similar in molecular weight to the compounds used by Roberts *et al.* This suggests that the membrane lipophilicity may be similar between shed snake skin and human skin. On the other hand, the coefficient for the ln(P)–MW relationship ($|\beta|$ = 0.0145) is slightly smaller than that reported for human skin [0.0216 reported by Kasting *et al.* (7)] and the coefficient for the ln(P)–ln(MW) relationship ($|\beta'|$ = 3.72) is about 3.5 times larger than that reported for hairless mouse skin [1.05 reported by Guy *et al.* (9)]. This indicates that shed snake skin is more similar to human skin than to hairless mouse skin with respect to its response to the permeant molecular size and that hairless mouse skin may be more permeable than human skin or shed snake skin. In any of these model membranes, however, the molecular weight dependency is much larger than that predicted by the Stokes–Einstein equation for diffusion in a liquid medium. Therefore, the diffusion process in the skin probably differs from that in a liquid medium and the molecular size of a permeant as well as the permeant lipophilicity plays an important role in determining the permeability through the skin.

Permeabilities were calculated according to Eqs. (2) and (3) using the α, α', β, β', γ, and γ' values, the distribution coefficient and molecular weight of a compound, and the

calculated ln(P) values are listed in Table II for each compound. The calculated ln(P) according to Eq. (2) was also plotted against the observed ln(P) in Fig. 5, which was very similar to the plot between the observed ln(P) and the calculated ln(P) from Eq. (3). As is shown in Table II and Fig. 5, there is a good agreement between the calculated ln(P) and the observed ln(P), suggesting that the permeability through shed snake skin may be predicted from the distribution coefficient and the molecular weight of a compound.

In the present study, it was found that the permeability of a compound through shed snake skin is controlled by both the lipophilicity and the molecular size of a permeant and that the molecular size dependency is much larger than that for diffusion in a liquid medium. Also, equations were developed to predict the permeability of a compound through shed snake skin from the distribution coefficient and the molecular weight of a compound. Considering the similarities between shed snake skin and human stratum corneum in terms of structure, composition, and permeability characteristics, it is possible that the permeabilities of compounds through human stratum corneum may be estimated from the shed snake skin data once the correlation is established between human skin and shed snake skin.

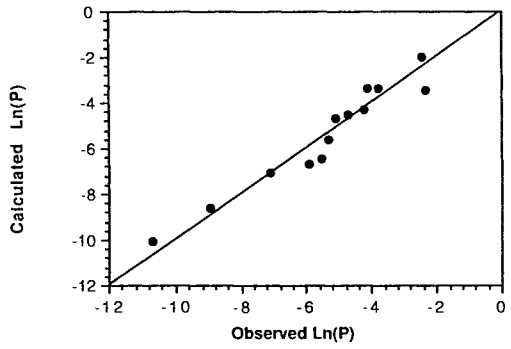

Fig. 5. The observed ln(P) vs calculated ln(P) according to Eq. (2). The straight line is for the perfect correlation between the observed and the calculated ln(P).

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