QSAR Analysis of Skin Permeability of Various Drugs in Man as Compared to *in Vivo* and *in Vitro* Studies in Rodents

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A general mathematical model involving partition coefficient, molecular weight and hydrogen bonding has been formulated for correlating the structures and skin permeability of a wide range of compounds through human skin and through hairless mouse skin. The correlations obtained are dependent not only on the biological system but also on the vehicle used. Without the use of lipophilic vehicle, the ideal lipophilicity for maximum permeability through human skin as measured by log $P_{o(oct/w)}$ ranges from 2.5 to 6 (extrapolated value). When a lipophilic vehicle was used in hairless mouse skin study, the log $P_{o(oct/w)}$ was lowered to around 0.4 ~ 0.6. While increased M.W. always has a negative effect on the permeability, increased H-bond can have either a slight positive or a slight negative effect, depending on the experiments (absorption vs. permeability constant). Cross validations with previously unanalyzed data as well as other biological systems support the usefulness of the general model developed for passive diffusion.

KEY WORDS: QSAR; skin permeability; human skin absorption; hairless mouse skin; lipophilicity; molecular weight; H-bond.

INTRODUCTION

Adequate percutaneous absorption of antiinflammatory drugs can provide direct and immediate relief of pain and inflammation at the application sites.[1-5] Other advantages of transdermal administration of drugs include: by-passing first-pass effect, minimizing inter- and intrapatient variation, providing steady-state plasma level of drug and long-term therapy from a single dose, and allowing easy termination of drug input.[6] Recently excised skin of hairless female mouse has been used as a model for *in vivo* and *in vitro* experiments.[6,7] In these recent studies the lipophilicity as measured by log P (octanol/water) has been shown to be an important factor in determining the skin permeability of various drugs in both humans and in the animal model.[5,6]

As a continuous effort in refining the mathematical models used in correlating drug absorption,[8,9] we wish to report the following general model and subject the recent data to test the usefulness of the model[10-14]:

$$\text{Permeability} = a \cdot e^{-(\log P - \log P_0)^{2b}} \cdot (\text{M.W.})^n \, (10^{H_b})^q \quad (1)$$

$$\log(\text{Permeability}) = -k_1(\log P)^2 + k_2 \log P + n \cdot \log(\text{MW}) + q \cdot H_b + k_3 \quad (2)$$

where the constants a, b, n, q, k_1, k_2, k_3 are functions of the systems (both the animal model and the solvent pair) used as well as the conditions of testing and the type of compounds being absorbed by passive diffusion. Besides permeability, first order absorption constant k or % absorption data are frequently used in the literature. Since % absorption ranges from 0 to 100, log % Abs will always be less than two logarithmic units.

The same problem exists in protein binding studies. The narrow range in the % absorption or % protein bound will mathematically dictate the low r value in regression analysis. For example, a range of 1% to 99% absorption or binding will give only a range of 0 to 1.996 after log transformation. If, on the other hand, one performs a log transformation of (% bound/% free), a wider range of log (1/99) to log (99/1) or -1.996 to $+1.996$ will be obtained. This will result in a significantly higher r value. This has been shown by the human serum protein binding of 79 different penicillins as reported by Bird and Marshall in 1967[15]: $\log(B/F) = 0.488\,\pi - 0.628$ ($n = 79$; $r = 0.924$), where B/F is the ration of bound/free, and π is the Hansch-Fujita lipophilic parameter measured in 1-octanol/water.[14-16]

METHODS

In this study, we have reanalyzed human skin permeability of 17 different non-steroidal anti-inflammatory drugs reported by Yano et al.[5] (see Table I) using stepwise regression (nonweighted least squares).

The maximum hydrogen bond-forming ability (H_b) is calculated based on the following rules: a) the number of hydrogen donors should equal the number of hydrogens which can form hydrogen bonds as donors; b) the number of hydrogen acceptors should equal to the lone electron pairs of a given group. For example, for $-OH$ the number of hydrogen donor should be 1, and there are 2 hydrogen acceptors. Therefore, the H_b for $-OH$ equals to 3, and that of $C=O$ equals to 2.

RESULTS AND DISCUSSION

Human Skin Permeability of NSAIDs

The data for human skin permeability of NSAIDs were presented in Table 1. Equations 3 and 4 were obtained from regression.

		n	r	s	
$\log R =$	$-0.370(\log P)^2 + 1.824 \log P - 1.930$	17	0.861	0.376	(3)
	$\log P_o = 2.46$	$F_{2,14} = 20.07$			
$\log R =$	$-0.374(\log P)^2 + 1.938 \log P + 0.048\,H_b - 0.922 \log MW - 0.427$	17	0.868	0.397	(4)
	$\log P_o = 2.59$	$F_{4,12} = 9.14$			

where R = (% absorbed)/(% unabsorbed). Both equations 3 and 4 are statistically significant according to the F-test. Be-

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Table I. Percent Absorption, log P and Other Physiochemical Parameters of Salicylates and Non-steroidal Antiinflammatory Drugs in Man

Drug	% Absorption[a] (0–4 hrs)	Log R^b Obsol.	Log R^b Calcd[c]	Log P[a]	M.W.	H_b
Salicylic acid	70.8	0.38	0.45	2.25	138.12	8
Methylalicylate	92.9	1.12	0.30	2.46	152.14	5
Ethylsalicylate	58.6	0.15	0.22	2.96	166.17	5
n-propylsalicylate	37.7	−0.22	−0.04	3.46	180.19	5
n-butylsalicylate	17.1	−0.69	−0.49	3.96	194.21	5
Ethylene glycol monosalicylate	87.8	0.86	0.24	1.80	182.16	10
Salicylamide	29.7	−0.37	−0.15	1.28	137.13	8
Salicyluric acid	21.5	−0.56	−0.26	1.13	195.17	12
Alclofenac	74.5	0.47	0.24	2.48	226.66	7
Aspirin	16.9	−0.69	−0.26	1.23	180.15	9
Bufexamac	18.3	−0.65	−0.89	0.77	223.28	9
Diclofenac	9.1	−1.00	−0.96	4.31	296.14	7
Flufenamic acid	3.1	−1.50	−1.80	4.88	281.24	7
Flubiprofen	23.9	−0.50	−0.43	3.81	244.27	5
Ibuprofen	23.0	−0.52	−0.13	3.51	206.27	5
Indomethacin	9.1	−1.00	−1.04	4.42	357.81	10
Ketoprofen	43.9	−0.10	0.15	2.94	254.29	7
Naproxen	(1.3)[d]	(−1.88)[d]	(0.12)	3.18	230.26	7

[a] From ref. 5.
[b] R = % Absorption/(100-% Abs).
[c] Calculated from Eq. 4.
[d] Not used in deriving Eq. 4, it is a statistical outlier.

cause of the limited data, stepwise addition of H_b or log M.W. term is not justified statistically, this is due to the narrow range of the molecular modification. The ideal lipophilicity (Log P_o) of around 2.5 is quite similar to those of previous reports.[11]

From equations 3 and 4 methylsalicylate behaves as a statistical outlier and has deviation of $0.813 > 2s$. When it is eliminated from the regression analysis Eqs. 4 and 5 are obtained. Both the log P_o values and the coefficients are quite comparable to those of Eqs. 3 and 4.

$$\log R = -0.329 (\log P)^2 + 1.612 \log P - 1.769 \quad \log P_o = 2.45 \qquad n = 16,\ r = 0.881,\ s = 0.310,\ F_{2,13} = 22.46 \qquad (5)$$

$$\log R = -0.366 (\log P)^2 + 1.918 \log P + 0.080\ H_b - 0.586 \log MW - 1.520 \quad \log P_o = 2.62 \qquad n = 16,\ r = 0.899,\ s = 0.311,\ F_{4,11} = 11.58 \qquad (6)$$

Human Skin Permeability of Miscellaneous Compounds

A better set of human skin permeability constant data on a wide range of compounds was obtained from the compilation of Scheuplein and Bronaugh[7] (see Table II). Equations 7 and 8 are obtained for all the 23 compounds with r of 0.927 and 0.965, respectively. *p*-Nitrophenol behaves as a statistical outlier in these two equations. When it is eliminated from the regression analysis, similar equations 9 and 10 are obtained with improved r and reduced s.

$$\log k_p = 0.372 \log P - 0.351\ H_b + 1.396 \qquad n = 23,\ r = 0.927,\ s = 0.491,\ F_{2,20} = 60.81 \qquad (7)$$

$$\log k_p = -0.096 (\log P)^2 + 1.027 \log P - 0.201\ H_b - 2.633 \log MW + 5.597 \quad \log P_o = 5.35 \qquad n = 23,\ r = 0.965,\ s = 0.361,\ F_{4,18} = 61.04 \qquad (8)$$

where k_p is permeability constant in cm/hr ($\times 10^3$).

$$\log k_p = 0.366 \log P - 0.379\ H_b + 1.466 \qquad n = 22,\ r = 0.963,\ s = 0.362,\ F_{2,19} = 120.70 \qquad (9)$$

$$\log k_p = -0.07 (\log P)^2 + 0.835 \log P - 0.265\ H_b - 1.844 \log MW + 4.388 \quad \log P_o = 5.96 \qquad n = 22,\ r = 0.978,\ s = 0.295,\ F_{4,17} = 93.63 \qquad (10)$$

The ideal lipophilic character (log P_o) for maximum skin permeation appears to be from 5.35 to 5.96 from Eqs. 8 and 10. Since these values are higher than the highest log P (3.87 for progesterone) used, they are extrapolated values, and may not be as reliable as the statistics would indicate.

Figure 1 shows a plot of log k_p vs log P with a lot of scattering. Figure 2 is a plot of log k_p after correcting for different contributions from log M.W. and H_b terms. Much less scattering is seen in Figure 2, suggesting the significance and validity of the use of log M.W. and H_b terms in the correlation.

Rat and Mouse Skin Permeation of Various Compounds

Recently Lee et al. have reported the *in vitro* hairless

Table II. Permeability Constants through Human Skin and Physicochemical Parameters of Various Compounds

Compound	k_p[a] cm hr^{-1} × (10^3)	log k_p obsd	log k_p calcd[c]	H_b	log $P^b_{oct/w}$	M.W.
Ethyl ether	16	1.2041	1.1087	2	0.89	74.12
2-Butanone	4.0	0.6021	0.5915	2	0.29	72.10
1-Butanol	3.0	0.4771	0.8988	3	0.88	74.12
2-Ethoxy ethanol	0.2	−0.6990	−0.9289	5	−0.35	90.12
2,3-Butanediol	0.05	−1.3010	−1.7849	6	−0.92	90.12
Benzyl alcohol	6.0	0.7782	0.6495	3	1.10	108.13
Phenol	8.2	0.9138	1.0920	3	1.46	94.11
Cresol	18	1.2553	1.2836	3	1.96	108.13
p-Ethyl phenol	35	1.5441	1.5123	3	2.58	122.16
Thymol	53	1.7243	1.6121	3	3.30	150.21
p-Chlorophenol	40	1.6021	1.3489	3	2.39	128.56
Dichlorophenol (2,4)	60	1.7782	1.4171	3	3.06	163.01
2,4,6-Trichlorophenol	59	1.7709	1.4391	3	3.69	197.46
p-Nitrophenol	5.6	0.7482	−0.0442**	8	1.91	139.11
Water	0.5	−0.3010	−0.1150	4	−1.38	18.02
Ethanol	0.8	−0.0969	0.2828	3	−0.31	46.07
Pentanol	6.0	0.7782	1.2407	3	1.56	88.15
Octanol	52	1.7160	1.6333	3	2.97	130.22
Progesterone	1.5	0.1761	0.7606	4	3.87	314.45
Cortexone	0.45	−0.3468	−0.2793	7	2.88	330.45
Cortexolone	0.075	−1.1249	−0.8458	10	3.08	346.45
Cortisone	0.01	−2.0000	−1.8458	12	1.47	360.46
Hydrocortisone	0.003	−2.5229	−2.3536	13	1.61	362.47

[a] From ref. 6.
[b] From ref. 7.
[c] Calculated from Eq. 8.

mouse skin and *in vivo* permeability (in rats) of 16 different drugs with an ethanol/panasate 800 (40/60) as a lipophilic vehicle.[8] From their data (see Table III) the following equations are obtained:

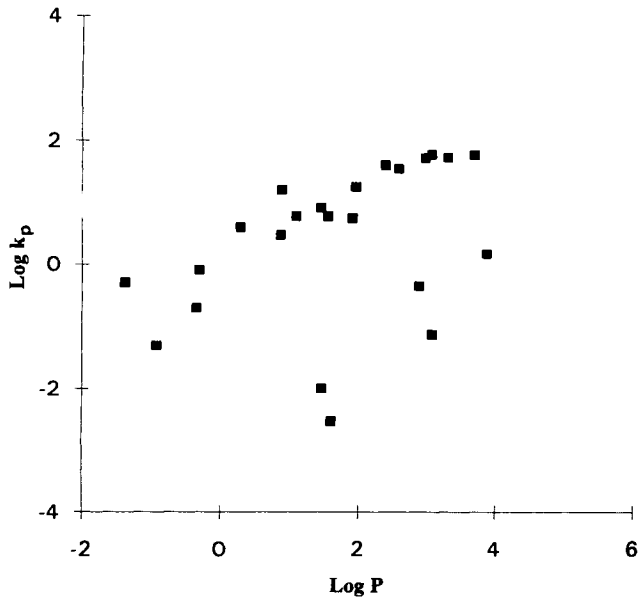

Fig. 1. A plot of log k_p vs log P showing the wide scattering of the data points, [Log k_p = −0.012 (Log P)^2 + 0.374 Log P − 0.183, n = 23, r = 0.404, s = 1.196].

In Vivo Study of Miscellaneous Drugs Using Rats and a Lipophilic Vehicle

	n	r	s	
log R = −0.039 (log P)^2 − 0.054 log P + 0.236	16	0.826	0.226	(11)
log P$_o$ = −0.69	$F_{2,13}$ = 14.00			
log R = −0.039 (logP)^2 + 0.031 log P + 0.037 H_b − 1.754 log MW + 3.905	16	0.926	0.165	(12)
log P$_o$ = 0.40	$F_{4,11}$ = 16.59			

In Vitro Study of Miscellaneous Drugs Using Female Hairless Mouse Skin and a Lipophilic Vehicle

	n	r	s	
log R = −0.051 (log P)^2 − 0.105 log P + 0.004	16	0.938	0.179	(13)
log P$_o$ = −1.03	$F_{2,13}$ = 47.82			
log R = −0.049 (logP)^2 − 0.057 log P + 0.032 H_b − 0.985 log MW + 1.973	16	0.958	0.161	(14)
log P$_o$ = −0.58	$F_{4,11}$ = 30.71			

The addition of H_b and log MW term in Eq. 12 is statistically highly significant as indicated by both the F-test

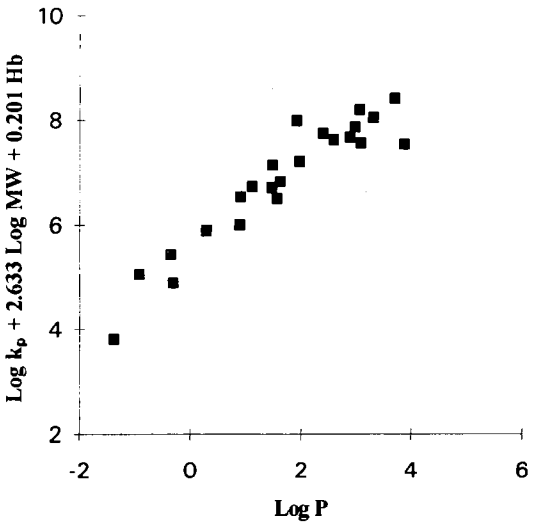

Fig. 2. A plot of log k_p after correcting for differences in log M.W. and H_b vs log P (Eq. 8, n = 23, r = 0.965, s = 0.361). Note the tighter fit of the data points as compared to that of Figure 1.

and the increased r value accompanied by decreased s. The contribution of Log MW to skin permeability has also been shown by Potts and Guy[17] for a series of alkanes. Similar situation exists for the *in vitro* data (Eqs. 13 and 14).

Validation of the Model

In order to verify the utility of the model employed, we have calculated the log k_p values of eight compounds reported previously by Schuplein *et al.*[18,19] in other publications but not included in reference 6 (Table IV). The "predicted" values calculated from Eq. 10 agree very well with the observed values (Eq. 15). This serves as a positive validation of the usefulness and reliability of this general equation. Further more, it has also been cross validated in other systems including *Chara* cells[20] for a series of nonelectrolytes.

$$\text{Log } k_{p(obsd)} = 0.870 \text{ Log } k_{p(calcd)} - 0.106 \qquad \begin{matrix} n & r & s \\ 8 & 0.960 & 0.393 \end{matrix} \qquad F_{1,8} = 70.06 \qquad (15)$$

The log P_o values from Eqs. 11 ~ 14 are much lower than 2.5. This is not only due to the different animal models used (rats vs. hairless mice), but also due to the use of a lipophilic vehicle. It should also be noted that the *in vivo* permeation in 12 hrs is significantly higher than the *in vitro* value for all the drugs studied reflecting not only the species difference between rats and mice but also the facilitation of drug removal away from the skin by blood circulation after permeation through the stratum corneum in the *in vivo* study. This is most prominent for lipophilic drugs like tetracaine, dibucaine and ibuprofen, for which *in vitro* data were greatly below those obtained *in vivo*. Without the use of lipophilic vehicle, the ideal lipophilicity for maximum permeability through human skin as measured by log $P_{o(oct/w)}$ ranges from 2.5 to 6 (extraploate value). When a lipophilic vehicle was used in hairless mouse skin study, the log $P_{o(oct/w)}$ was lowered to around −0.69 to 0.4 (Eqs. 11–14). While increased M.W. always has a negative effect on the permeability, increased H-bond can have either a slight positive or a slight negative effect, depending on the experiments (absorption vs. permeability constant).

Table III. *In Vivo* and *in Vitro* Permeability of Various Drugs from Ethanol/Panasate 800 (40/60) Binary Vehicle

	In Vivo rat skin permeability			*In Vitro* hairless mouse permeability					
		Log R^b			Log R				
Drug	% Per[a] (12 hrs)	obsol	calcd[c]	% Per[a] (12 hrs)	obsol	calcd[d]	Log P	M.W.	H_b
5-Fluorouracil	69.4	0.36	0.43	54.3	0.07	0.15	−0.95	130.08	8
Tegafur	68.2	0.33	0.18	56.8	0.12	0.01	−0.48	200.16	9
Caffeine	60.5	0.19	0.19	47.2	−0.05	−0.02	−0.07	194.19	8
Theophylline	55.9	0.10	0.28	47.4	−0.04	0.04	−0.02	180.17	9
Pentoxifylline	46.1	−0.07	−0.01	32.7	−0.31	−0.18	0.72	278.31	10
Propentofulline	32.0	−0.33	−0.15	18.8	−0.64	−0.40	1.72	306.36	10
Procaine	55.2	0.09	−0.04	41.5	−0.15	−0.38	1.87	236.30	8
Lidocaine	33.1	−0.31	−0.19	19.4	−0.62	−0.58	2.26	234.33	5
Tetracaine	21.6	−0.56	−0.51	5.1	−1.27	−1.09	3.73	264.37	7
Dibucaine	15.3	−0.74	−0.86	4.7	−1.31	−1.47	4.40	343.47	8
Antipyrine	61.0	0.19	0.07	47.7	−0.04	−0.16	0.23	188.22	4
Salicyluric acid	71.4	0.40	0.32	57.5	0.13	−0.03	1.13	195.20	12
Salicylic acid	68.9	0.35	0.32	33.2	−0.30	−0.26	2.25	138.12	8
Alclofenac	59.9	0.17	−0.13	25.4	−0.47	−0.57	2.48	226.66	7
Ketoprofen	28.9	−0.39	−0.30	16.1	−0.72	−0.77	2.94	254.29	7
Ibuprofen	23.3	−0.52	−0.34	7.7	−1.08	−0.95	3.51	206.27	5

[a] From ref. 8.
[b] R = log [% permeation/(100-% permeation)].
[c] Calculated from Eq. 12.
[d] Calculated from Eq. 14.

Table IV. Permeability Constants through Human Epidermis and Physicochemical Parameters of Alcohols and Steroids

Compounds	Log k_p obsd[a]	Log k_p calcd[b]	H_b	Log $P^c_{oct/w}$	Log MW
Methanol	−3.00	−2.86	3	−0.77	1.51
n-Propanol	−2.85	−2.48	3	0.25	1.78
n-Hexanol	−1.89	−1.71	3	2.03	2.01
n-Heptanol	−1.49	−1.52	3	2.57	2.06
Testosterone	−3.40	−2.47	5	3.32	2.46
Corticosterone	−4.22	−4.59	10	1.94	2.54
Aldosterone	−5.52	−5.69	12	1.08	2.56
Estrone	−2.44	−2.64	5	2.76	2.43

[a] From ref. 18 and 19 for which Log $P_{oct/w}$ values are available.
[b] Calculated from Eq. 10.
[c] From ref. 7.

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