The "Latent Membrane Permeability" Concept: QSPR Analysis of Inter/Intralaboratorically Variable Caco-2 Permeability

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Received September 26, 2001

Caco-2 cell monolayers grown on a filter support are the most widely used systems for predicting intestinal absorption. However, inter- and intralaboratory variability in Caco-2 permeability makes it difficult to analyze any QSPR relationship for a large number of compounds. We proposed the "latent membrane permeability" concept, assuming that all Caco-2 permeability data sets share a hidden, common relationship between their membrane permeability and physicochemical properties. An iterative calculation method was developed to handle this conceptual approach and applied to the analysis of Caco-2 permeability data sets from different sources. A thorough statistical analysis revealed that the "latent membrane permeability" concept be reasonable.

INTRODUCTION

Of all the possible routes of administration, the oral one is generally preferred for reasons of ease and patient compliance. In order for an orally administered drug to reach the systemic circulation, it must overcome several physicochemical and biological barriers: namely, dissolution, membrane transport, and degradation. It has been reported that poor absorption and related poor pharmacokinetics is one of the main reasons for failure of a candidate compound during the drug development process.[1] As a consequence, many researchers have begun to pay closer attention to the early introduction of ADME screening during the drug discovery process.[2−4]

The use of in vitro cell culture models has made it possible to examine a large number of samples and reduce the quantities of the compounds required, resulting in greatly increased throughput and lower cost as compared with conventional whole animal studies. Caco-2 cells grown on a filter support are the most widely used systems for predicting intestinal absorption.[5−7] These cells, although derived from a human colon adenocarcinoma, possess many of the morphological and functional characteristics of normal intestinal epithelial cells. The permeability of the cell monolayers is known to be reasonably well correlated with human absorption.[8−11] However, it has been pointed out that considerable inter- and intralaboratory variability exists in Caco-2 permeability measurements.[6,7] Artursson et al.[6] compared four calibration curves between fraction absorbed in human and Caco-2 permeability, demonstrating that the curves were shifted relative to one another by approximately 0.25−1.75 log apparent permeability units. This inter- and intralaboratory variability makes it difficult to combine Caco-2 permeability data from different sources to form one large data set.

Several investigators have explored quantitative structure−property relationships (QSPR) involving Caco-2 permeability.[12−16] In their studies, various types of molecular descriptors have been introduced to the QSPR modeling including size and hydrogen-bonding descriptors,[12] ClogP,[13] (dynamic) polar surface area,[12,14,15] and Molsurf-derived descriptors.[16] In all cases, QSPR models predicted Caco-2 permeability with a reasonable degree of accuracy. However, these models might not be practical because the size of permeability data sets is rather small. When the data are limited, statistical models often fail due to an over-fitting problem, resulting in a limitation on their use. Inter- and intralaboratory variability associated with Caco-2 permeability measurements remains a problem to be solved before a widely applicable QSPR model can be constructed.

In the present study, we propose the "latent membrane permeability" concept for simultaneously dealing with Caco-2 permeability data from different sources. This concept is based on an assumption that all Caco-2 permeability data sets share a hidden, common relationship between the membrane permeability and physicochemical properties of the compounds. Assuming the existence of a latent score representing the membrane permeability intrinsic to a compound (f), the apparent permeability coefficient in Caco-2 cell monolayers (P_{app}) was defined as

$$\log P_{app,k} = \beta_k \times f + \epsilon_k \quad (1)$$

where the variability in P_{app} from different sources is characterized by β_k and ϵ_k. The score f was named the "latent membrane permeability" which is assumed to be a function of molecular descriptors. Here, Caco-2 permeability data sets were obtained from seven different sources[8−10,17−20] and analyzed to prove the validity of this data handling method.

METHODS

The latent score representing membrane permeability (f) was assumed to be a function of the molecular descriptors

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10.1021/ci010317y CCC: $22.00
Published on Web 02/19/2002

x_j ($j = 1-p$): that is

$$f = b_1x_1 + b_2x_2 + \cdots + b_px_p \quad (2)$$

where the Euclidian norm of a coefficient vector ($\mathbf{b} = (b_1, b_2, ..., b_p)^T$) was unity ($||\mathbf{b}|| = 1$).

The vector **b** and the specific values for each data set (β_k and ϵ_k, see eq 1) were determined by an iterative calculation method:

For each data set:

(1) $\beta_k = 1$

(2) $\mathbf{y}_k = (y_{1,k}, y_{2,k}, ...)^T/\beta_k$ where y corresponds to log P_{app}.

(3) calculate the sum of the squares or products of deviations:

$$S_{ii',k} = \sum_m (x_{im,k} - \bar{x}_{i,k})(x_{i'm,k} - \bar{x}_{i',k})$$

For total:

(4) solve the normal equation:

$$\begin{pmatrix} S_{c11} & \cdots & S_{c1p} \\ \vdots & \vdots & \vdots \\ S_{cp1} & \cdots & S_{cpp} \end{pmatrix} \begin{pmatrix} b_1 \\ \vdots \\ b_p \end{pmatrix} = \begin{pmatrix} S_{c1y} \\ \vdots \\ S_{cpy} \end{pmatrix}$$

where

$$S_{cii'} = \sum_k S_{ii'}$$

(5) normalize **b** ($\mathbf{b} = \mathbf{b}/||\mathbf{b}||$)

For each data set:

(6) $\mathbf{f}_k = \mathbf{X}_k\mathbf{b}$ where

$$\mathbf{X}_k = \begin{pmatrix} x_{11,k} & \cdots & x_{p1,k} \\ \vdots & \vdots & \vdots \\ x_{1n,k} & \cdots & x_{pn,k} \end{pmatrix}$$

$$(7)\ \beta_k = \beta_k \times \frac{\sum_m (y_{m,k} - \bar{y}_{m,k})(f_{m,k} - \bar{f}_{m,k})}{\sum_m (f_{m,k} - \bar{f}_{m,k})^2}$$

$$(8)\ \epsilon_k = \beta_k \left(\bar{y}_k - \bar{t}_k \times \frac{\sum_m (y_{m,k} - \bar{y}_{m,k})(f_{m,k} - \bar{f}_{m,k})}{\sum_m (f_{m,k} - \bar{f}_{m,k})^2} \right)$$

Check convergence:

(9) compare the **b** value with the one from the preceding iteration ($\mathbf{b}^{old}$). If they are equal ($1 - (\mathbf{b}^{new})^T\mathbf{b}^{old} < 10^{-12}$), stop the iterative calculation. Otherwise, go to step (2) using a new β_k.

Thus, the "latent membrane permeability" concept means that the coefficient vectors in the regression equations derived from different data sets are parallel to one another.

RESULTS AND DISCUSSION

Data Sets of Caco-2 Permeability. The Caco-2 permeability data for 81 compounds were taken from different sources.[8−10,17−20] Here, the following compounds were deleted from the original databases: compounds that are actively transported and large-molecular-weight compounds (MW > 1000). The structures of the compounds were built with Chem 3D Pro Ver. 5.0 software (CambridgeSoft Co., Cambridge, MA) and modeled in their neutral forms. Geometry optimization was performed initially by molecular mechanics (MM2) force field and subsequently using the AM1 Hamiltonian of a semiempirical MO PACkage (MOPAC97). Molecular descriptors of the optimum geometry of each compound were then calculated. These descriptors included polarizability (α), dipole moment (μ), sum of the charges of nitrogen and oxygen atoms (sum(N,O)), and hydrogen atoms bonding to nitrogen and oxygen atoms (sum(H)).

Table 1 summarizes the apparent permeability coefficients and molecular descriptors for the compounds investigated. When correlation coefficients (r) between any two of the molecular descriptors were calculated, the average and the highest value were 0.374 and 0.605, respectively. Thus, no major multicollinearity problem in multiple linear regression analysis is to be expected.

Conventional Regression Analysis for Caco-2 Permeability Data Sets. Multiple linear regression analysis was conducted individually for data sets A−E, that include relatively larger data (Table 2). The average of the multiple correlation coefficients (R) for five data sets was 0.849. Regarding data set A, Waterbeemd et al.[12] obtained several models with R values of 0.513−0.892, using molecular size and hydrogen bonding descriptor as explanatory variables. Among the models tested, the best gave the standard error (SE) of 0.504. For data set C, Ren and Lien[13] obtained a regression model of which the R and SE were 0.797 and 0.465, respectively. The present model was comparable with these models, suggesting that these descriptors be reasonable predictors of the Caco-2 permeability.

When sum(N,O) was divided into two descriptors, namely sum(N) and sum(O), the standard errors for prediction were 0.513, 0.350, 0.483, 0.681, and 0.230 for data sets A−E, respectively. Since no significant improvement was observed, it was confirmed that sum(N,O) be sufficient.

The similarities in a vector of standardized regression coefficients between any two of the regression models (Table 2) were summarized in Table 3. Here, the standardized regression coefficient means a product of the regression coefficient and the standard deviation of the variable. The cosine similarity measure was used for the evaluation, being defined as

$$cosine\_similarity = \frac{\mathbf{v}_i \cdot \mathbf{v}_j}{|\mathbf{v}_i||\mathbf{v}_j|} = \frac{\sum_p e_{p,\mathbf{v}_i} e_{p,\mathbf{v}_j}}{\sqrt{\sum_p (e_{p,\mathbf{v}_i})^2 \sum_p (e_{p,\mathbf{v}_j})^2}} \quad (3)$$

where **v** and e_p represent a vector and its element, respectively. The cosine similarities between any two of the data sets ranged from 0.462 to 0.940. It was shown that the regression models did not necessarily resemble one another. However, the following analysis indicates that lack of similarity would be due to an over-fitting to the data sets.

Simultaneous Analysis of Multiple Data Sets Based on the "Latent Membrane Permeability" Concept. Five

Table 1. Apparent Permeability Coefficients in Caco-2 Cell Monolayer and Molecular Descriptors for 81 Structurally Diverse Compounds

	log P_{app} (cm/s)[a]										
compounds	A	B	C	D	E	F	G	μ[b]	α[c]	sum(N,O)[d]	sum(H)[e]
acebutolol			−6.29		−5.35			6.28	173.9	−1.794	0.590
acebutolol ester[f]					−4.11			3.48	205.1	−2.049	0.377
acetylsalicylic acid	−5.62	−4.51	−5.04					1.45	85.1	−1.211	0.241
acyclovir		−5.70	−6.60					5.96	117.7	−2.119	0.857
alprenolol	−4.39		−4.60		−4.12	−4.39		1.99	131.1	−0.870	0.372
alprenolol ester[f]					−3.97			2.96	165.1	−1.095	0.156
aminopyrine			−4.44					4.38	133.6	−0.818	0.000
antipyrine				−4.31				4.39	107.3	−0.624	0.000
atenolol	−6.70		−6.28	−5.94		−6.69		4.13	133.9	−1.635	0.795
azithromycin		−5.98						4.86	330.0	−4.100	1.057
betaxolol					−4.31			1.14	157.0	−1.140	0.371
betaxolol ester[f]					−4.02			2.46	190.8	−1.437	0.164
bremazocine			−5.10					2.43	162.3	−0.712	0.414
caffeine		−4.30	−4.51	−4.07				3.78	100.7	−1.550	0.000
chloramphenicol		−4.69						6.00	132.9	−1.377	0.693
chlorothiazide			−6.72					4.71	140.5	−5.316	0.674
chlorpromazine			−4.70					2.67	180.2	−0.492	0.000
cimetidine		−5.51	−5.86					9.74	141.3	−1.336	0.671
clonidine		−4.52	−4.66					0.62	111.4	−0.741	0.399
corticosterone	−4.26		−4.67					1.48	161.5	−1.191	0.412
desipramine		−4.67	−4.61					2.31	157.6	−0.548	0.148
dexamethasone	−4.90	−4.63	−4.91					1.96	175.2	−1.490	0.625
diazepam		−4.15	−4.48					3.05	161.3	−0.778	0.000
doxorubicin		−6.80						4.02	259.5	−3.182	1.441
erythromycin		−5.43						9.47	314.3	−4.104	1.063
estradiol			−4.77					2.67	139.1	−0.564	0.411
felodipine	−4.64							4.73	179.7	−1.441	0.208
fluconazole		−4.53						1.95	138.6	−1.288	0.218
ganciclovir			−6.42				−5.57	4.59	128.7	−2.466	1.091
griseofulvin			−4.44					3.16	172.3	−1.297	0.000
H216/44						−6.04		3.41	237.3	−2.794	0.784
hydrochlorothiazide			−6.29					9.15	136.8	−5.362	0.884
hydrocortisone	−4.67		−4.85	−4.91			−4.45	2.85	164.9	−1.509	0.619
ibuprophen		−4.28						1.84	104.0	−0.677	0.241
imipramine		−4.85						1.25	164.6	−0.503	0.000
indomethacin			−4.69					1.45	196.0	−1.396	0.241
labetalol			−5.03					5.03	176.0	−1.733	1.094
mannitol	−6.75	−6.19	−6.42	−5.93			−5.49	4.48	62.8	−1.936	1.266
meloxicam			−4.71					4.58	199.4	−3.463	0.551
methanol							−3.88	1.59	10.9	−0.321	0.194
methylscopolamine			−6.16					11.05	171.6	−1.089	0.209
metoprolol	−4.57		−4.63	−4.74		−4.57		0.64	136.4	−1.121	0.368
nadolol			−5.41					3.25	148.4	−1.466	0.767
naloxone		−4.55						4.78	162.7	−1.229	0.415
naproxen							−4.13	1.25	130.4	−0.885	0.241
nevirapine			−4.52					2.60	157.2	−1.191	0.244
nicotine			−4.71					3.02	86.0	−0.403	0.000
olsalazine	−6.96							1.93	177.8	−2.024	1.032
oxprenolol					−4.18			2.13	129.5	−1.145	0.394
oxprenolol ester[f]					−4.01			0.99	161.6	−1.371	0.155
PEG400				−6.32				2.71	174.4	−2.939	0.402
phencyclidine			−4.61					0.93	131.6	−0.300	0.000
phenytoin			−4.57					3.42	139.0	−1.433	0.526
pindolol			−4.78					1.60	132.6	−1.062	0.610
pirenzepine			−6.36					5.62	195.1	−1.874	0.244
piroxicam			−4.45					4.03	190.0	−3.509	0.545
practolol	−6.05					−6.05		4.49	140.4	−1.529	0.586
prazocin		−4.36						3.36	226.9	−2.140	0.398
progesterone			−4.63				−4.10	2.67	148.2	−0.573	0.000
propranolol	−4.38	−4.56	−4.66	−4.46	−4.08	−4.38		2.04	144.3	−0.871	0.371
propranolol ester[f]					−3.98			3.72	177.3	−1.131	0.155
quinidine		−4.69						1.96	180.6	−0.876	0.207
ranitidine			−6.31					5.61	156.6	−0.918	0.392
salicylic acid	−4.92		−4.66					1.17	67.5	−0.955	0.510
scopolamine			−4.93					1.25	140.6	−1.428	0.207
sucrose			−5.77	−6.15				3.80	136.0	−3.323	1.672
sulfasalazine	−6.89		−6.52					5.64	240.9	−3.717	0.765
sumatriptan		−5.52						3.11	160.7	−2.992	0.448
telmisartan			−4.82					6.25	335.2	−1.401	0.245
tenidap		−4.29						4.67	146.3	−1.184	0.000
terbutaline	−6.42		−6.33	−5.98				3.96	109.9	−1.125	0.804
testosterone	−4.29	−4.14	−4.60	−4.35				4.14	134.3	−0.606	0.197

Table 1 (Continued)

compounds	log P_{app} (cm/s)[a] A	B	C	D	E	F	G	μ[b]	α[c]	sum(N,O)[d]	sum(H)[e]
timolol			−4.89		−4.35			1.53	153.4	−1.922	0.361
timolol ester[f]					−4.10			4.45	186.4	−2.150	0.156
trovafloxacin		−4.52						6.73	223.5	−1.924	0.539
uracil			−5.37					4.19	54.6	−1.394	0.532
urea			−5.34					3.18	24.2	−1.153	0.810
valproic acid		−4.32						1.80	76.2	−0.681	0.239
warfarin	−4.42		−4.68					1.87	167.9	−1.012	0.240
zidovudine			−5.16					2.05	130.9	−2.329	0.475
ziprasidone		−4.91						2.79	235.6	−1.453	0.257

[a] Apparent permeability coefficients (P_{app}). Data sets A−G were obtained from refs 8, 9, 17, 18, 19, 20, and 10, respectively. [b] Dipole moment. [c] Polarizability. [d] Sum of charges of nitrogen and oxygen atoms. [e] Sum of charges of hydrogen atoms bonding to nitrogen or oxygen atoms. [f] O-Cyclopropane carboxylic acid ester.

Table 2. Multiple Linear Regression Analysis of Caco-2 Permeability Data Sets

data set	constant[a]	regression coefficient[b] μ	$\alpha \times 10^2$	sum(N,O)	sum(H)	R[c]	SE[d]	F[e]	n[f]
A	−4.029	−0.1102	0.6434	0.7353	−1.555	0.893	0.543	11.76	17
B	−4.443	0.0484	0.1975	0.2559	−1.237	0.845	0.411	13.09	26
C	−4.387	−0.1689	0.2034	0.0900	−0.762	0.784	0.482	17.95	50
D	−3.324	−0.0914	−0.4030	0.3438	−0.871	0.830	0.622	3.31	11
E	−3.566	−0.1025	0.1989	0.1326	−1.679	0.888	0.217	6.54	12

[a] Constants in linear regression equation. [b] Coefficients in linear regression equation. [c] Multiple correlation coefficient. [d] Standard error of regression. [e] Variance ratio. [f] Sample number.

Table 3. Cosine Similarity Measures between Vectors of Standard Regression Coefficients in Multiple Linear Regression Models

	cosine similarity measure A	B	C	D	E
A	1				
B	0.854	1			
C	0.722	0.462	1		
D	0.800	0.821	0.660	1	
E	0.802	0.705	0.940	0.816	1

Table 4. Linear Correlation of Caco-2 Permeability between Data Sets[a]

	correlation coefficient (r) A	B	C	D	E
A	1				
B	0.886 (5)	1			
C	0.971 (14)	0.951 (11)	1		
D	0.984 (7)	0.979 (4)	0.939 (9)	1	
E	− (2)	− (1)	0.997 (4)	− (1)	1

[a] Values in parentheses are the number of the common compounds between each two of the data sets.

Caco-2 permeability data sets were analyzed based on the "latent membrane permeability" concept using the iterative calculation method described in the Methods section. This analysis is a kind of linear scaling approaches. As shown in Table 4, the correlation coefficients between each two of the data sets were high (r values of more than 0.886), and therefore it would be reasonable the Caco-2 permeability data sets are parallel to one another.

When the iterative calculation was conducted using data sets A−E, the following equation regarding "latent membrane permeability" was obtained:

$$f = -0.09119 \times \mu + 1.270 \times 10^{-3} \times \alpha + 0.1652 \times sum(N,O) - 0.9820 \times sum(H) \quad (4)$$

Table 5. Specific Values for the Calculation of Caco-2 Permeability from "Latent Membrane Permeability"

data set	log $P_{app} = \beta_k \times f + \epsilon_k$ β_k	ϵ_k
A	2.041	−3.584
B	0.852	−4.188
C	0.959	−4.371
D	1.034	−4.182
E	1.311	−3.465

Table 5 summarizes the coefficients for the calculation of "latent membrane permeability" and the specific values for the estimation of Caco-2 permeability. Comparison in the specific values suggests that data sets B, C, and D might be similar in their permeability characteristics. Figure 1 illustrates the relationship between observed and calculated Caco-2 permeability. The correlation coefficients (r) were 0.879, 0.758, 0.750, 0.782, and 0.864 for data sets A−E, respectively. These values were satisfactory as compared to the results of individual analysis. Thus, Caco-2 permeability data from different sources were fairly well explained by the "latent membrane permeability" model.

To confirm the validity of the "latent membrane permeability" concept, the analysis of variance for the residual sum of squares was carried out. The null hypothesis was that the coefficient vectors of individual regression equations were parallel to one another. When five data sets was analyzed simultaneously using the "latent membrane permeability" concept, the residual sum of squares (SSE) was 24.45 when there were 103 degrees of freedom. On the other hand, when the regression analyses were conducted individually, the residual sum of squares (SSE) was 3.54, 3.55, 10.47, 2.32, and 0.33 for data sets A−E, respectively, and the total SSE was 20.12 with 91 degrees of freedom. To check the

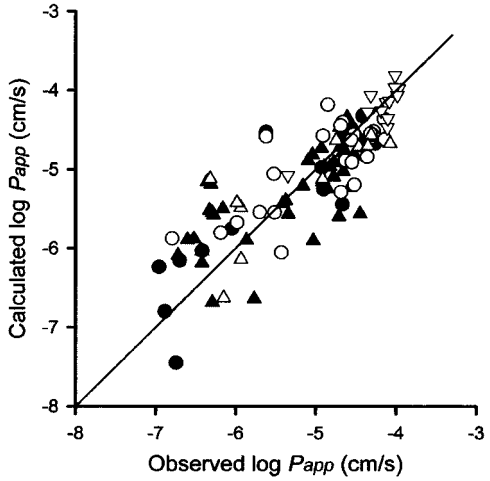

Figure 1. Relationship between observed and calculated Caco-2 permeability. Theoretical Caco-2 permeability was calculated, using the specific values listed in Table 5 and the "latent membrane permeability" estimated in eq 4. Keys: data set A(●), B(○), C(▲), D(△), E(▽).

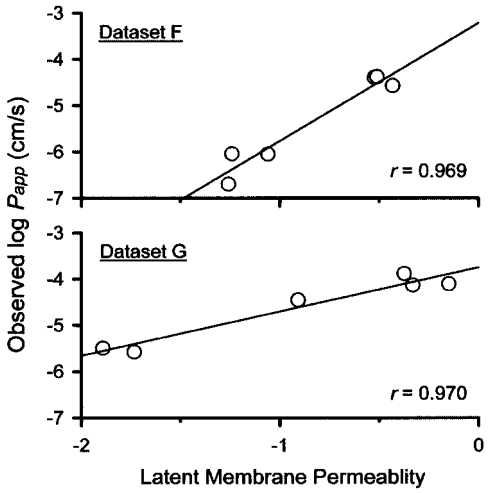

Figure 2. Analysis using small data sets (data sets F and G) on relationship between observed Caco-2 permeability and "latent membrane permeability". The "latent membrane permeability" was estimated from eq 4 that was obtained from data sets A−E.

difference in SSE between the two approaches, the F value was calculated:

$$F_{12,91} = \frac{(24.45 - 20.12)/(103 - 91)}{20.12/91} = 1.59 \quad (P = 0.109) \quad (5)$$

As a result, the null hypothesis could not be rejected; in other words, the "latent membrane permeability" concept is reasonable.

For compounds in data sets F and G, "latent membrane permeability" was estimated using eq 4. Figure 2 shows the relationship between observed Caco-2 permeability and "latent membrane permeability". The correlation coefficients (r) were 0.969 and 0.970 for data sets F and G, respectively. Thus, eq 4 was applicable to different data sets, demonstrating the validity of the "latent membrane permeability" concept.

It often happens that several data sets cannot be combined due to differences in experimental conditions, even when

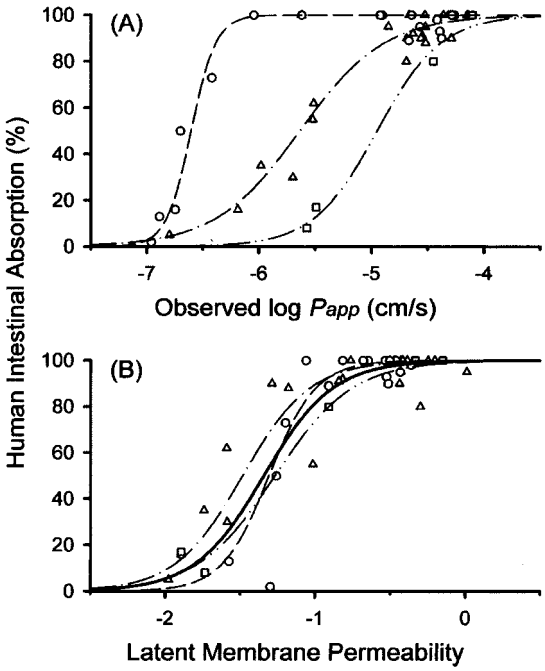

Figure 3. Relationship of observed Caco-2 permeability (A) or "latent membrane permeability" (B) with human intestinal absorption. The data of human intestinal absorption was obtained from the sources corresponding to data set A (○, − − −), B (△, − • −), and G (□, − • • −). Sigmoidal curves were obtained by fitting a logistic function to each data set. The thick, solid curve in Figure 3(B) expresses a fitting curve toward the data compiled from the three data sets.

they are basically equivalent. One of the simple ways of solving this problem is normalization of the objective variables prior to assembling several data sets into a larger data set. The approach proposed in this study is similar to the linear scaling. Unlike the simple normalization method, however, our approach does not assume that all the data sets tested have the same average. Since a collection of compounds is usually different from data set to data set, the averages might be different. Therefore, our approach is a reasonable one. A major drawback of our approach is that the absolute values are lost. However, we believe that the scaled quantity "latent membrane permeability" is a sufficient criterion in a drug discovery setting, as long as a relative evaluation between compounds is allowed. In this sense, the QSPR modeling of "latent membrane permeability" will be of great use.

It would be interesting to examine the relationship between "latent membrane permeability" and human intestinal absorption. As reported previously,[8−11] human intestinal absorption expresses a sigmoidal shape with Caco-2 permeability; however, the position and slope of the transition can be significantly different (Figure 3A). When the Caco-2 data sets were scaled with their own specific values obtained by the present analysis, all the sigmoidal curves could be reasonably well integrated (Figure 3B). Taking together with that Caco-2 permeability data sampled from different sources are parallel to one another (Table 4), this result supports the validity of our linear scaling approach. Although nonlinear approaches may give a better-fit model, it requires a larger degree of freedom. As long as the present data are concerned, nonlinear approaches would not be necessary.

CONCLUSIONS

In the present study, we proposed an approach for the analysis of Caco-2 permeability from different sources simultaneously, assuming that all the data sets share a hidden, common relationship between their membrane permeability and physicochemical properties. By performing a thorough statistical analysis, we have confirmed that the "latent membrane permeability" concept is a reasonable one. This analysis gave a QSPR model for Caco-2 permeability with reasonable accuracy; to get a better model, however, we need to examine the selection of molecular descriptors. Besides Caco-2 permeability data, this approach will be applicable to QSAR/QSPR analysis of various biological data that are equivalent but evaluated by different methods. Thus, the proposed approach will make an important contribution to statistical analysis methods used in drug discovery and development.

ACKNOWLEDGMENT

This research was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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CI010317Y