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Prediction of oral drug absorption in humans by theoretical passive absorption model☆

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Abstract

The purpose of the present study was to examine the oral drug absorption predictability of the theoretical passive absorption model (TPAM). As chemical descriptors of drugs, the octanol/buffer distribution coefficient at pH 6.0 (D_{ow}), intrinsic octanol–water partition coefficient (P_{ow}), pK_a, and molecular weight (MW) were calculated from the chemical structure. Total passive intestinal membrane permeation consists of transcellular, paracellular and unstirred water layer (UWL) permeation. Transcellular permeation was modeled based on the pH-partition hypothesis with correction for cationic species permeation, and the independent variables were D_{ow}, P_{ow}, and pK_a. Paracellular permeation was modeled as a size-restricted diffusion within a negative electrostatic field-of-force, and the independent variables were MW and pK_a. UWL permeation was modeled as diffusion across a water layer, and the independent variable was MW. Cationic species permeation in the transcellular permeation model and the effect of a negative electric field-of-force in the paracellular permeation model were the extensions to the previous TPAM. The coefficients of the paracellular and UWL permeation models were taken from the literature. A data set of 258 compounds with observed values of Fa% (the fraction of a dose absorbed in humans) taken from the literature was employed to optimize four fitting coefficients in the transcellular permeation model. The TPAM predicted Fa%, with root mean square errors of 15–21% and a correlation coefficient (CC) of 0.78–0.88. In addition, the TPAM predicted the effective human intestinal membrane permeability with a CC of 0.67–0.77, as well as the contribution of paracellular permeation. The TPAM was found to predict oral absorption from the chemical structure of drugs with adequate predictability for usage in drug discovery.

Keywords: Oral absorption; Lipophilicity; pK_a; Octanol; In silico; Permeability

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1. Introduction

In the recent drug discovery and development process, in silico prediction of absorption, metabolism, distribution, and excretion (ADME) is recognized as a key technique (van de Waterbeemd and Gifford, 2003). Among ADME properties, oral absorption has been most intensively investigated for in silico prediction. As an oral absorption parameter, the fraction of a dose absorbed in humans (Fa%), the effective intestinal membrane permeability in humans (P_{eff}), Caco-2 permeability, etc., have been studied as targets for in silico prediction (Wessel et al., 1998; Winiwarter et al., 1998; Zhao et al., 2001; Yamashita et al., 2002). Oral absorption from a solid dosage is determined by the dissolution rate, the solubility, and the intestinal membrane permeability (Yu and Amidon, 1999). Intestinal membrane permeation consists of transcellular, paracellular, and unstirred water layer (UWL) permeation. Most of the previous in silico prediction studies scrambled these absorption processes, and the contribution of each process cannot be predicted. In addition, the previous in silico methods often used descriptors that are not easy to translate into better drug design.

Previously, the theoretical passive absorption model (TPAM) had been proposed for describing passive intestinal membrane permeation (Camenisch et al., 1996, 1998). The TPAM consists of three partial models, i.e., the transcellular, paracellular and UWL permeation models. The TPAM is beneficial for qualitatively comprehending the membrane permeation from the viewpoint of both the physiology of the intestine and the chemical structure of drugs. However, the predictability of the TPAM for the oral absorption in humans has not been examined. The purpose of the present study was to quantitatively examine the oral drug absorption predictability of the TPAM.

2. Calculation

2.1. Transcellular permeation model

Passive transcellular permeation is diffusion across a lipid bilayer. Therefore, the permeability depends on the lipophilicity of the permeant. In the previous TPAM, the passive transcellular permeability (P_{trans}) was expressed by the 1-octanol/buffer distribution coefficient (D_{ow}), with the help of so-called Collander equations (Collander, 1950, 1951; Camenisch et al., 1998).

$$P_{trans} = a \cdot D_{ow}^{\alpha} \quad (1)$$

To reflect the pH at the intestinal epithelial membrane surface, the D_{ow} at pH 6.0 was employed (Maxwell et al., 1968). The pragmatic reason for using the 1-octanol/buffer system is its high publicity in the drug discovery process (Kerns and Di, 2003). Furthermore, various computational prediction systems have been developed for 1-octanol/buffer system (van de Waterbeemd and Gifford, 2003). Because the intrinsic octanol/water partition coefficient of ionized species is negligibly small, Eq. (1) represents the permeation of non-ionized species (pH-partition hypothesis) (Hogben et al., 1959). However, recently, the permeability of basic compounds was found to be larger than expected from the D_{ow} (Sugano et al., 2001). It was suggested that cationic species of basic compounds can permeate the negatively charged membrane with the aid of anionic lipids in the membrane, depending on the lipophilicity of the cationic species (Neubert et al., 1988; Ozaki et al., 2000; Sugano et al., 2001, 2004). The intestinal epithelial membrane contains anionic lipids (Proulx, 1991; Lipka et al., 1991). Therefore, Eq. (1) was extended for the permeability of mono-cationic species of basic compounds. The lipophilicity of the cationic species may be scaled by the octanol–water partition coefficient (P_{ow}) of neutral species, with the help of Collander equations (Collander, 1950, 1951). Eq. (1) was extended to:

$$P_{trans} = a \cdot D_{ow}^{\alpha} + b \cdot f_{+1} \cdot P_{ow}^{\beta} \quad (2)$$

where f_{+1} is the fraction of mono-cationic species. The f_{+1} was calculated from the pK_a. Coefficients a, b, α, and β are fitting parameters to be optimized in the present study.

2.2. Paracellular permeation model

Paracellular permeation is diffusion through the negatively charged tight junction between the intestinal epithelial cells, and was modeled by a size-restricted diffusion within a negative electrostatic field-of-force (Adson et al., 1994, 1995; Sugano et al., 2002, 2003). Small and cationic species can easily permeate the paracellular pathway, whereas large and anionic

species permeates little. As a molecular-sieving function, the Renkin function ($F(B)$, Eq. (4)) was employed. In addition, an electric field-of-force function ($E(Z)$, Eq. (6)) was employed, as an extension to the previous TPAM (Camenisch et al., 1996, 1998). The paracellular permeability (P_{para}) is expressed as:

$$P_{para} = A \cdot \frac{1}{MW^{1/3}} \cdot F(B) \left(f_0 + \sum^{z(z\neq 0)} f_z \cdot E(z) \right) \quad (3)$$

$$F(B) = (1-B)^2(1 - 2.104 \cdot B + 2.09 \cdot B^3 - 0.95 \cdot B^5) \quad (4)$$

$$B = \frac{MW^{1/3}}{R_{MW}} \quad (5)$$

$$E(Z) = \frac{C \cdot z}{1 - e^{-C \cdot z}} \quad (6)$$

where z is the molecular charge, f_z the fraction of each charged species, MW the molecular weight, and R_{MW} is the apparent pore size of the paracellular pathway based on a MW scale. Previously, molecular volume was employed as a parameter of molecular size. However, in the present study, MW was employed, because MW is more public in the drug discovery process, and easier to calculate. The replacement of molecular volume to MW did not affect the predictability of the P_{para} model (data not shown). R_{MW}, A, and C were previously optimized utilizing Fa% and artificial membrane permeability data, as previously reported (Sugano et al., 2002). $R_{MW} = 8.46$, $A = 2.41 \times 10^{-2}$, and $C = 2.39$ were used in the present study.

2.3. Unstirred water layer permeation model

The UWL is adjacent to the intestinal epithelial membrane. UWL permeation was modeled as a simple diffusion process in a water layer. The UWL permeability (P_{UWL}) is reciprocal to $MW^{1/3}$ (Larhed et al., 1997). Previously, the P_{eff} of glucose, the permeation of which is rate-limited by the UWL, was reported to be 10×10^{-4} cm/s (Lennernäs, 1998). MW of glucose is 180. Therefore, the P_{UWL} of each drug is expressed as:

$$P_{UWL} = 10 \times 10^{-4} \left(\frac{180}{MW} \right)^{1/3} \quad (7)$$

2.4. Total passive intestinal membrane permeability and Fa%

It may be assumed that total resistance to permeation (R_{tot}) is the sum of the resistances of the membrane (R_m) and UWL (R_{UWL}) on the membrane:

$$R_{tot} = R_m + R_{UWL} \quad (8)$$

Resistance is the inverse of permeability. Therefore, the total passive permeability across the intestinal membrane (P_{tot}) is expressed by the membrane permeability (P_m) and P_{UWL} (Camenisch et al., 1996; Pade and Stavchansky, 1997).

$$\frac{1}{P_{tot}} = \frac{1}{P_m} + \frac{1}{P_{UWL}} \quad (9)$$

P_m is the sum of P_{trans} and P_{para}. Therefore, P_{tot} is converted to:

$$\frac{1}{P_{tot}} = \frac{1}{P_{trans} + P_{para}} + \frac{1}{P_{UWL}} \quad (10)$$

The contributions of the transcellular pathway permeability of neutral species ($Trans_n$%), mono-cationic species ($Trans_c$%), and the paracellular pathway permeability (Para%) to P_m are expressed as:

$$Trans_n\% = \frac{a \cdot D_{ow}^{\alpha}}{P_m} \times 100 \quad (11)$$

$$Trans_c\% = \frac{b \cdot f_{+1} \cdot P_{ow}^{\beta}}{P_m} \times 100 \quad (12)$$

$$Para\% = \frac{P_{para}}{P_m} \times 100 \quad (13)$$

The contribution of the UWL to the total resistance is expressed as:

$$R_{UWL}\% = \frac{R_{UWL}}{R_{tot}} \times 100 = \left(1 - \frac{P_{tot}}{P_m}\right) \times 100 \quad (14)$$

When we employ a plug-flow model as an absorption model from the intestinal tube (Yu and Amidon, 1999), the calculated Fa% is expressed as:

$$Fa\%_{calc} = (1 - \exp(-Gz \cdot P_{tot})) \times 100 \quad (15)$$

where Gz is the lump constant of available intestinal surface area and transit time. In the present study, $Gz = 1.39 \times 10^4$ was employed to arrange the

scale identical between P_{tot} and P_{eff} (cm/s) (Yu and Amidon, 1999).

2.5. Human intestinal absorption data and physicochemical parameters

Three hundred forty three observed Fa% values ($Fa\%_{obs}$) were collected from literature compilations, and stored in the in-house database (Noel, 1979; Dressman et al., 1985; Taylor et al., 1985; Artursson and Karlsson, 1991; Chong et al., 1996; Walter et al., 1996; Palm et al., 1997; Yee, 1997; Chiou and Barve, 1998; Chiou et al., 2000; McEvoy, 1998; Yazdanian et al., 1998; Balon et al., 1999; Irvine et al., 1999; Wessel et al., 1998; Winiwarter et al., 1998; Karlsson et al., 1999; Wohnsland and Faller, 2001; Zhao et al., 2001). Most of these literatures were in silico–in vitro correlation or in vitro–in vivo correlation studies. Therefore, we assumed that Fa% data from these literatures compilations were also suitable for the purpose of the present study. In addition, drugs which undergo active transport (both influx and efflux), intestinal metabolism, and solubility-limited absorption were excluded from the analysis (Adair and McElnay, 1987; Adam and Timmler, 1982; Avdeef, 2001; Behrens et al., 2001; Chiou et al., 2001; Chong et al., 1996; Dantzig et al., 1992; Eneroth et al., 2001; Fraga Fuentes et al., 1997; Groen et al., 1988; Hashida, 1995; Hochman et al., 2001; Holdiness, 1984; Kim et al., 1998; Koup et al., 1988; Liang et al., 2000;Matsuda et al., 1998a,b; McEvoy, 1998; Nicolaos et al., 2003; Orlowski et al., 1998; Overdiek and Merkus, 1986; Poschet et al., 1996; Pradhan and Majumdar, 1986; Schanker et al., 1963; Seelig, 1998; Shu et al., 2001; Smith et al., 2001; Takanaga et al., 1994; Tamai and Tsuji, 1996; Thwaites et al., 1994; Tsuji et al., 1982; Wakasugi et al., 1998; Walter et al., 1996; Welker et al., 1998; Wenzel et al., 1996; Williams and Harding, 1984; Yee, 1997). Drugs with $\log D_{ow} > 4$ were excluded because low solubility was expected (Yalkowsky and Valvani, 1980; Avdeef, 2003). Quaternary ammonium compounds and polymers were also excluded because Pallas 3.1 (CompuDrug, Hungary) could not calculate $\log P_{ow}$ adequately.

Typical $Fa\%_{obs}$ values and physicochemical parameters are shown in Table 1. The D_{ow} at pH 6.0, P_{ow} and pK_a were calculated from the chemical structure of drugs by Pallas 3.1. When the drug contained ortho-carbonyl phenol fragment, 0.55 per fragment was added to $\log P_{ow}$ and $\log D_{ow}$ (Pallas was found to underestimate these drug's $\log P_{ow}$ at an average of 0.55 (data not shown)).

2.6. Optimization of coefficients

Coefficients a, b, α, and β in the transcellular permeation model (Eq. (2)) were optimized by fitting Eq. (15) to $Fa\%_{obs}$ values using the least square method. Eqs. (2)–(7) were converted to P_{tot} by inserting into Eq. (10), and Eq. (10) was converted to Fa% by inserting into Eq. (15). The sum of squares of the difference between $Fa\%_{calc}$ and $Fa\%_{obs}$ ($Fa\%_{diff}$) was minimized using the Quasi-Newton method (EXCEL 2000, Microsoft, Redmont, WA). After preliminary optimization of the prediction scheme, large outliers are additionally surveyed by literature and excluded if they were reported to undergo active transport, intestinal metabolism, and solubility-limited absorption.

3. Results and discussion

Previously, Wenlock et al. (2003) reported that the mean $\log D_{ow}$ (pH 7.4) of the marketed oral drugs was 1.0 and the standard deviation was 3.4. Distribution of $\log D_{ow}$ (pH 7.4) and electrical charge of drugs collected in this study ($N = 343$) is shown in Fig. 1. The mean $\log D_{ow}$ (pH 7.4) was -0.41 and the standard deviation was 3.3. After excluding efflux substrates, intestinal metabolism substrates and low solubility drugs, the mean $\log D_{ow}$ (pH 7.4) was -0.62 and the

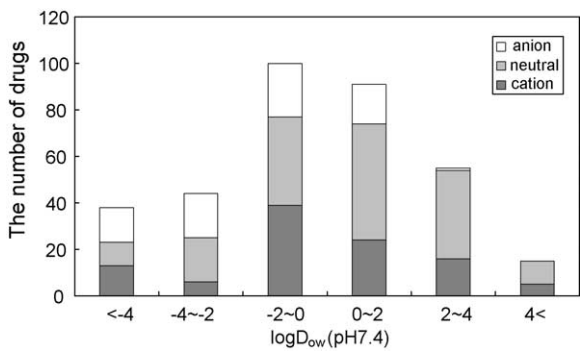

Fig. 1. Distribution of calculated $\log D_{ow}$ (pH 7.4) and electrical charge of drugs used in this study.

Table 1
Fa%, physicochemical properties, and predicted oral absorption parameters

No.	Compound	MW	pK_a [a,b]	log P_{ow} [a]	log D_{ow} [a]	$Fa\%_{obs}$ [c]	log P_{eff} [c]	$Fa\%_{calc}$ [e]	log P_{tot} [e]	$Trans_n\%$ [e]	$Trans_c\%$ [e]	Para% [e]	$R_{UWL}\%$ [e]
1	Amiloride	230	5.26 (b)	−1.03	−1.10	50	−3.79	70	−4.06	65	3	32	9
2	Antipyrine	188		1.79	1.79	97	−3.35	100	−3.26	97	0	3	55
3	Atenolol	266	10.08 (b)	0.44	−2.10	50	−4.70	72	−4.04	22	34	44	10
4	Carbamazepine	236		2.28	2.28	[d]	−3.37	100	−3.20	99	0	1	68
5	Cimetidine	241	6.71 (b)	−0.09	−0.87	64	−4.52	84	−3.88	51	16	33	15
6	Creatinine	113		−1.82	−1.82	80	−4.52	84	−3.88	20	0	80	11
7	Desipramine	266	10.63 (b)	4.00	0.67	100	−3.36	99	−3.48	70	22	8	38
8	Fluvastatine	411	4.32 (a)	4.71	3.03	100	−3.62	100	−3.19	100	0	0	85
9	Furosemide	331	4.06 (a)	2.20	0.25	61	−5.30	93	−3.72	99	0	1	23
10	Hydrochlorothiazide	298		−0.36	−0.36	67	−5.40	82	−3.91	92	0	8	14
11	Ketoprofen	254	3.49 (a)	3.67	1.18	100	−3.08	99	−3.43	99	0	1	42
12	Metoprolol	267	10.08 (b)	1.97	−0.90	95	−3.89	87	−3.83	43	33	24	17
13	Naproxen	230	4.06 (a)	3.26	1.32	99	−3.08	100	−3.39	99	0	1	44
14	Piroxicam	331	4.66 (b)	0.45	0.43	100	−3.11	95	−3.65	96	1	3	27
15	Propranolol	259	10.08 (b)	3.00	−0.08	90	−3.54	96	−3.64	57	27	15	26
16	Ranitidine	314	9.04 (b)	0.79	−1.71	50	−4.57	71	−4.05	34	39	27	11
17	Terbutaline	225	12.01 (b)	1.07	−1.63	62	−4.52	83	−3.90	25	29	46	14
18	Acyclovir	225		−2.08	−2.09	20	[d]	47	−4.34	47	0	53	5
19	Ceftriaxone	555	2.33 (a)	−1.53	−4.68	1	[d]	2	−5.79	98	0	2	0
20	Cefuroxime	424	2.12 (a), 6.10 (b)	−1.09	−3.29	5	[d]	11	−5.08	78	0	22	1
21	Oxacillin	401	2.52 (a)	1.56	−1.69	33	[d]	37	−4.48	97	0	3	4
22	Timolol	316	10.5 (b)	0.51	−2.06	90	[d]	66	−4.11	27	42	31	9

[a] Calculated by Pallas 3.1.
[b] The pK_a values of the acid ($pK_a < 7.3$) and base ($pK_a > 4.6$) are indicated ((a): acid and (b): base).
[c] Obtained from the literature listed in the text.
[d] Not reported.
[e] Calculated with the coefficients set V in Table 2.

Table 2
Fitting coefficients and Fa% predictability statistics

Set	N	a ($\times 10^{-4}$)	α	b ($\times 10^{-4}$)	β	RMSE	CC
I	258	1.4	0.32	0.23	0.19	19	0.81
II[a]	258	1.3	0.34	0.43	0.14	19	0.80
III[b]	258	1.6	0.29			20	0.80
IV[a,b]	258	1.8	0.29			21	0.78
V	242	1.9	0.44	0.30	0.15	15	0.88
VI[a]	242	1.8	0.46	0.50	0.13	16	0.87
VII[b]	242	2.1	0.38			16	0.87
VIII[a,b]	242	2.3	0.35			18	0.84

[a] Without the extension for electric field-of-force in paracellular pathway model.
[b] Without the extension for cationic species permeation in transcellular pathway model.

standard deviation was 2.8 ($N = 258$). The drugs used in this study were biased to low lipophilicity, comparing with the marketed oral drugs. Because $\log D_{ow}$ of dissociable molecule is lower than that of neutral species, the percentage of neutral compounds would increase at high $\log D_{ow}$.

Coefficients a, b, α, and β were optimized using 258 $Fa\%_{obs}$ values (Table 2, the coefficients set I–IV). Sixteen large outliers ($Fa\%_{diff} > 2 \times$ root mean square error (RSME)) were identified. Eight of these outliers were due to the pK_a and P_{ow} calculation error (data not shown). Other outliers were amphotericin B ($Fa\%_{obs}$, $Fa\%_{calc}$: 5%, 44% (same order in the following parentheses)), carfecillin (100%, 59%), cymarin (47%, 97%), dantrolene (35%, 98%), mercaptethanesulfonic acid (77%, 27%), metaproterenol (44%, 83%), miglitol (100%, 59%), and nizatidine (100%, 61%). Reasons for these outliers could not be clarified by a literature survey. Optimization of a, b, α, and β without large outliers was also performed (Table 2, the coefficients set V–VIII).

The relationship between $Fa\%_{obs}$ and $Fa\%_{calc}$ with the coefficients set V is shown in Fig. 2A. In addition, the relationship between $Fa\%_{obs}$ and P_{tot} is shown in Fig. 2B. Correlation coefficients (CC) and RMSE, which are predictability indicators, are summarized in Table 2. The TPAM calculated Fa% with adequate correlation (CC = 0.78–0.88, RMSE = 15–21). Therefore, the TPAM was suggested to be applicable for drug discovery. Without the extensions to the previous TPAM, i.e., the negative electric field-of-force in the paracellular permeation model and the cationic species permeation in the transcellular permeation model, predictability was slightly lowered (Table 2).

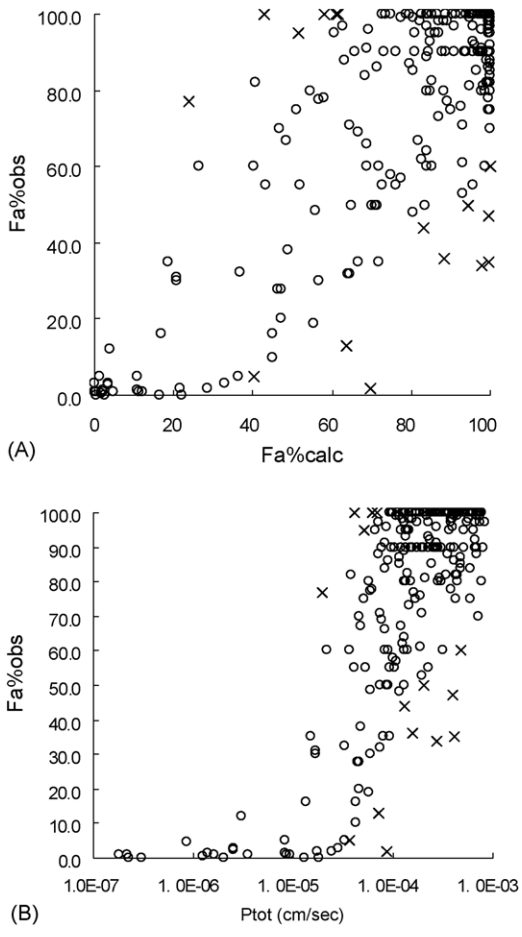

Fig. 2. Relationship between calculated oral absorption data and observed Fa%. (A) $Fa\%_{obs}$ vs. $Fa\%_{calc}$. (B) $Fa\%_{obs}$ vs. P_{tot}. $Fa\%_{calc}$ and P_{tot} were calculated with the coefficients set V in Table 2. Cross indicates large outliers excluded from the analysis (see text).

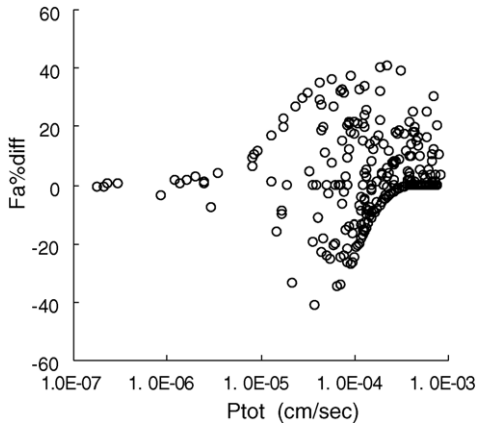

Fig. 3. Relationship between P_{tot} and $Fa\%_{diff}$. $Fa\%_{diff}$ was calculated with the coefficients set V in Table 2.

The relationship between P_{tot} and $Fa\%_{diff}$ with coefficients set V is shown in Fig. 3. The $Fa\%_{diff}$ was largest around $\log P_{tot} = -4.5$, probably because the Fa%–$\log P_{tot}$ relationship was sigmoidal, and had a large slope around this $\log P_{tot}$ value (Fig. 2B). From Fig. 3, we can estimate the prediction probability. Prediction probability is important information for decision-making in drug discovery, however it was not often considered in the previous in silico studies.

The relationship between P_{eff} and P_{tot} with coefficients set V was also investigated (Fig. 4). P_{eff} values were measured using a technique based on single-pass perfusion of a human jejunum segment between two inflated balloons (Lennernäs, 1998). Eighteen in vivo P_{eff} values were collected from the literature (Winiwarter et al., 1998; Takamatsu et al., 2001; Lennernäs et al., 2002). P_{eff} of actively transported compounds were excluded. P_{tot} correlated to P_{eff} with CC = 0.67 (set V). P_{eff} of furosemide and hydrochlorothiazide were underestimated. However, their P_{eff} were also lower than expected from their Fa% (61% and 67%) and Caco-2 permeability (0.11 ± 0.01 and 0.42 ± 0.03 ($\times 10^{-6}$ cm/s)) (Wessel et al., 1998; Yamashita et al., 2000), suggesting that their P_{eff} could have been under assessed. Without furosemide and hydrochlorothiazide, P_{tot} correlated to P_{eff} with CC = 0.77.

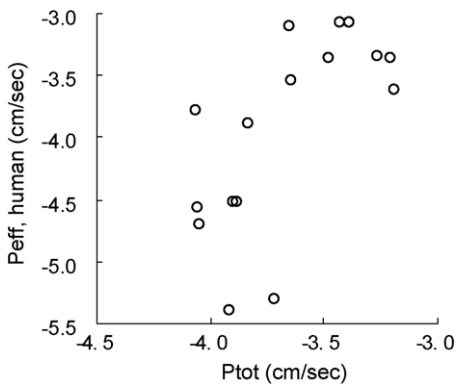

Fig. 4. Relationship between P_{tot} and P_{eff}. P_{tot} was calculated with the coefficients set V in Table 2.

Relative contributions of paracellular permeation (Para%) and transcellular permeation ($Trans_n\%$ and $Trans_c\%$) were calculated with coefficients set V (Table 1). Previously, in Caco-2 at pH 5.4, the relative contributions of paracellular permeation for cimetidine, furosemide, naproxen, and propranolol were reported to be 31%, 1%, 0%, and 3%, respectively (Pade and Stavchansky, 1997). In the present study, they were predicted as 33%, 1%, 1%, and 15%, respectively. Therefore, it is suggested that the TPAM adequately predicted the main permeation pathway. Relative contribution of mono cationic species permeation ($Trans_c\%$) in metoprolol and timolol were 33% and 42%, respectively (Table 1). Previously, the permeability of basic compounds across bio-mimetic artificial membrane was found to be larger than expected from the D_{ow}, suggesting that contribution of the permeation of cationic species is significant (Sugano et al., 2001, 2004). Relative contribution of the UWL to the total resistance was also calculated (Table 1). The UWL of the gastrointestinal tract was maintained by mucus layer. In case of fluvastatine, because of its high lipophilicity ($\log D_{ow} = 3.03$), the transcellular pathway permeability could be high ($\log P_{trans} = -2.38$). However, the UWL resistance was suggested to be more effective on total resistance to permeation ($R_{UWL}\% = 85\%$) than the membrane resistance. Consequently, the total permeability could be settled in the upper limit ($\log P_{tot} = -3.19$, $\log P_{eff} = -3.62$).

Prediction of the permeation mechanism, which was not incorporated in the previously reported in silico methods, is an advantage of the TPAM approach. Permeation mechanism is important information for rational drug design. In addition, the TPAM approach has several other pragmatic advantages. The prediction scheme is explicit and corresponds to physiological structure of intestinal membrane. In the present TPAM

study, simple molecular descriptors, which are public and available to most drug discovery scientists were utilized. The possibility of over-learning is low, because only four fitting coefficients in the transcellular permeation model were optimized by fitting with hundreds of $Fa\%_{obs}$ values. The coefficients in the paracellular and UWL permeation models had been derived and checked by physiological and/or model experiments. These features of TPAM may increase the accountability for users in the drug discovery stage.

In conclusion, for the use in drug discovery, the TPAM has adequate predictability and provides information about permeation pathway for drug discovery scientists. To improve the predictability, the effect of villosity of the intestinal membrane and pH variation along the absorption process can be incorporated to the TPAM (Said et al., 1986; Winne, 1989). Also, additional molecular descriptors might be required for the transcellular permeation model to correct the difference between octanol and the phospholipid bilayer (Zhao et al., 2001). The parallel artificial membrane permeability assay, which is an assay for passive transcellular permeation, may contribute to increasing the predictability of the transcellular permeation model (Kansy et al., 1998; Sugano et al., 2001; Avdeef, 2003). To predict the oral absorption of low solubility compounds, solubility/dissolution processes and the gastro-intestinal transit process are additionally required (Yu and Amidon, 1999). In addition, effects of transporters and intestinal metabolisms can be incorporated to the TPAM. These further extensions to the TPAM may complicate the prediction scheme. However, the prediction scheme may still possess divisibility and provide mechanistic insights. Therefore, the advantages of the TPAM approach may not be compromised by these extensions.

References

Adair, C.G., McElnay, J.C., 1987. The effect of dietary amino acids on the gastrointestinal absorption of melphalan and chlorambucil. Cancer Chemother. Pharmacol. 19, 343–346.

Adam, D., Timmler, R., 1982. Pharmacokinetics of ceftizoxime with and without probenecid. Arzneimittel-Forschung 32, 416–419.

Adson, A., Ruab, T.J., Burton, P.S., Barsuhn, C.L., Hilgers, A.R., Audus, K.L., Ho, N.F.H., 1994. Quantitative approaches to delineate paracellular diffusion in cultured epithelial cell monolayers. J. Pharm. Sci. 83, 1529–1530.

Adson, A., Burton, P.S., Ruab, T.J., Barsuhn, C.L., Audus, L., Ho, N.F.H., 1995. Passive diffusion of weak organic electolytes across Caco-2 cell monolayers: uncoupling the contributions of hydrodynamic, transcellular and paracellular barriers. Pharm. Res. 84, 1197–1203.

Artursson, P., Karlsson, J., 1991. Correlation between oral absorption in humans and apparent drug permeability coefficients in human intestinal epithelial Caco-2 cells. Biochem. Biophys. Res. Comm. 175, 880–885.

Avdeef, A., 2001. Physicochemical profiling (solubility, permeability and charge state). Curr. Top. Med. Chem. 1, 277–351.

Avdeef, A., 2003. Absorption and drug development solubility. In: Permeability and Charge State. Wiley–Interscience, NJ.

Balon, K., Riebesehl, B.U., Muller, B.W., 1999. Drug liposome partitioning as a tool for the prediction of human passive intestinal absorption. Pharm. Res. 16, 882–888.

Behrens, I., Stenberg, P., Artursson, P., Kissel, T., 2001. Transport of lipophilic drug molecules in a new mucus-secreting cell culture model based on HT29-MTX cells. Pharm. Res. 18, 1138–1145.

Camenisch, G., Folkers, G., van de Waterbeemd, H., 1996. Review of theoretical passive drug absorption models: historical background, recent developments and limitations. Pharm. Acta Helvetiae 71, 309–327.

Camenisch, G., Folkers, G., van de Waterbeemd, H., 1998. Shapes of membrane permeability-lipophilicity curves: extension of theoretical models with an aqueous pore pathway. Eur. J. Pharm. Sci. 6, 321–329.

Chiou, W.L., Barve, A., 1998. Linear correlation of the fraction of oral dose absorbed of 64 drugs between humans and rats. Pharm. Res. 15, 1792–1795.

Chiou, W.L., Jeong, H.Y., Chung, S.M., Wu, T.C., 2000. Evaluation of using dog as an animal model to study the fraction of oral dose absorbed of 43 drugs in humans. Pharm. Res. 17, 135–140.

Chiou, W.L., Chung, S.M., Wu, T.C., Ma, C., 2001. A comprehensive account on the role of efflux transporters in the gastrointestinal absorption of 13 commonly used substrate drugs in humans. Int. J. Clin. Pharmacol. Ther. 39, 93–101.

Chong, S., Dando, S.A., Soucek, K.M., Morrison, R.A., 1996. In vitro permeability through Caco-2 cells is not quantitatively predictive of in vivo absorption for peptide-like drugs absorbed via the dipeptide transporter system. Pharm. Res. 13, 120–123.

Collander, R., 1950. The distribution of organic compounds between iso-butanol and water. Acta Chem. Scand. 4, 1085–1098.

Collander, R., 1951. The partition of organic compounds between higher alcohol and water. Acta Chem. Scand. 5, 774–780.

Dantzig, A.H., Tabas, L.B., Bergin, L., 1992. Cefaclor uptake by the proton-dependent dipeptide transport carrier of human intestinal Caco-2 cells and comparison to cephalexin uptake. Biochem. Biophys. Acta 1112, 167–173.

Dressman, J.B., Amidon, G.L., Fleisher, D., 1985. Absorption potential: estimating the fraction absorbed for orally administered compounds. J. Pharm. Sci. 74, 588–589.

Eneroth, A., Astrom, E., Hoogstraate, J., Schrenk, D., Conrad, S., Kauffmann, H.-M., Gjellan, K., 2001. Evaluation of a vincristine resistant Caco-2 cell line for use in a calcein AM extrusion

screening assay for P-glycoprotein interaction. Eur. J. Pharm. Sci. 12, 205–214.
Fraga Fuentes, M.D., Garcia Diaz, B., de Juana Velasco, P., Bermejo Vicedo, M.T., 1997. Influence of foods on the absorption of antimicrobial agents. Nutr. Hosp. 12, 277–288.
Groen, K., Warrander, A., Miles, G.S., Booth, B.S., Mulder, G.J., 1988. Sulphation and glucuronidation of xamoterol in the dog: dose dependence and site of sulphation. Xenobiotica 18, 511–518.
Hashida, M. (Ed.), 1995. Keikou Touyo Seizai no Sekkei to Hyouka. Yakugyo Jiho-sha, Tokyo.
Hochman, J.H., Chiba, M., Yamazaki, M., Tang, C., Lin, J.H., 2001. P-glycoprotein-mediated efflux of indinavir metabolites in Caco-2 cells expressing cytochrome P450 3A4. J. Pharmacol. Exp. Ther. 298, 323–330.
Hogben, C.A.M., Tacco, D.J., Brodie, B.B., Schanker, L.S., 1959. On the mechanism of intestinal absorption of drugs. J. Pharmacol. Exp. Ther. 269, 275–282.
Holdiness, M.R., 1984. Clinical pharmacokinetics of the antituberculosis drugs. Clin. Pharmacokinet. 9, 511–544.
Irvine, J.D., Takahashi, L., Lockhart, K., Cheong, J., Tolan, J.W., Selick, H.E., Grove, J.R., 1999. MDCK (Madin-Darby canine kidney) cells: a tool for membrane permeability screening. J. Pharm. Sci. 88, 28–33.
Kansy, M., Senner, F., Gubernator, K., 1998. Physicochemical high throughput screening: parallel artificial membrane permeation assay in the description of passive absorption processes. J. Med. Chem. 41, 1007–1010.
Karlsson, J., Ungell, A.l., Grasjo, J., Artursson, P., 1999. Paracellular drug transport across intestinal epithelia: influence of charge and induced water flux. Eur. J. Pharm. Sci. 9, 47–56.
Kerns, E.H., Di, L., 2003. Pharmaceutical profiling in drug discovery. Drug Discov. Today 8, 316–323.
Kim, A.E., Dintaman, J.M., Waddell, D.S., Silverman, J.A., 1998. Saquinavir, an HIV protease inhibitor, is transported by P-glycoprotein. J. Pharmacol. Exp. Ther. 286, 1439–1445.
Koup, J.R., Dubach, U.C., Brandt, R., Wyss, R., Stoeckel, K., 1988. Pharmacokinetics of cefetamet (Ro 15-8074) and cefetamet pivoxil (Ro 15-8075) after intravenous and oral doses in humans. Antimicrob. Agents Chemother. 32, 573–579.
Larhed, A.W., Artursson, P., Grasjo, J., Bjork, E., 1997. Diffusion of drugs in native and purified gastrointestinal mucus. J. Pharm. Sci. 86, 660–665.
Lennernäs, H., 1998. Human intestinal permeability. J. Pharm. Sci. 87, 403–410.
Lennernäs, H., Knutson, L., Knutson, T., Hussain, A., Lesko, L., Salmonson, T., Amidon, G.L., 2002. The effect of amiloride on the in vivo effective permeability of amoxicillin in human jejunum: experience from a regional perfusion technique. Eur. J. Pharm. Sci. 15, 271–277.
Liang, E., Proudfoot, J., Yazdanian, M., 2000. Mechanisms of transport and structure-permeability relationship of sulfasalazine and its analogs in Caco-2 cell monolayers. Pharm. Res. 17, 1168–1174.
Lipka, G., Op den Kamp, J.A., Hauser, H., 1991. Lipid asymmetry in rabbit small intestinal brush border membrane as probed by an intrinsic phospholipid exchange protein. Biochemistry 30, 11828–11836.
Matsuda, K., Yuasa, H., Watanabe, J., 1998a. Dose dependency in the gastrointestinal absorption of cefatrizine: correlation between in vivo and in situ. Biol. Pharm. Bull. 21, 604–609.
Matsuda, K., Yuasa, H., Watanabe, J., 1998b. Fractional absorption of L-carnitine after oral administration in rats: evaluation of absorption site and dose dependency. Biol. Pharm. Bull. 21, 752–755.
Maxwell, J.D., Watson, W.C., Watt, J.K., Ferguson, A., 1968. Radiotelemetering studies of jejunal pH before and after vagotomy and pyloroplasty. Gut 9, 612–616.
McEvoy, G.K. (Ed.), 1998. AHFS Drug Information. American Society of Health-system Pharmacist, Bethesda, MD.
Neubert, R., Albrecht, G., Taube, C., Weiss, M., Furst, W., 1988. Influence of ion-pair-formation on the pharmacokinetic properties of drugs. Part 3. Influence of hexylsalicylic acid on the pharmacokinetics of pholedrine. Die Pharmazie 43, 632–633.
Nicolaos, G., Crauste-Manciet, S., Farinotti, R., Brossard, D., 2003. Improvement of cefpodoxime proxetil oral absorption in rats by an oil-in-water submicron emulsion. Int. J. Pharm. 263, 165–171.
Noel, M., 1979. Kinetic study of normal and sustained release dosage forms of metformin in normal subjects. Res. Clin. Forums 1, 33–44.
Orlowski, S., Valente, D., Garrigos, M., Ezan, E., 1998. Bromocriptine Modulates P-Glycoprotein Function. Biochem. Biophys. Res. Commun. 244, 481–488.
Overdiek, H.W., Merkus, F.W., 1986. Influence of food on the bioavailability of spironolactone. Clin. Pharmacol. Ther. 40, 531–536.
Ozaki, S., DeWald, D.B., Shope, J.C., Chen, J., Prestwich, G.D., 2000. Intracellular delivery of phosphoinositides and inositol phosphates using polyamine carriers. Proc. Natl. Acad. Sci. U.S.A. 97, 11286–11291.
Pade, V., Stavchansky, V., 1997. Estimation of the relative contribution of the transcellular and paracellular pathway to the transport of passively absorbed drugs in the Caco-2 cell culture model. Pharm. Res. 14, 1210–1215.
Palm, K., Stenberg, P., Luthman, K., Artursson, P., 1997. Polar molecular surface properties predict the intestinal absorption of drugs in humans. Pharm. Res. 14, 568–571.
Poschet, J.F., Hammond, S.M., Fairclough, P.D., 1996. Characterisation of penicillin-G uptake in rabbit small-intestinal brush-border membrane vesicles. Biochem. Biophys. Acta 1278, 233–240.
Pradhan, A., Majumdar, M.K., 1986. Metabolism of some drugs by intestinal lactobacilli and their toxicological considerations. Acta Pharmacol. Toxicol. 58, 11–15.
Proulx, P., 1991. Structure-function relationships in intestinal brush border membranes. Biochem. Biophys. Acta 1071, 255–271.
Said, H.M., Blair, J.A., Lucas, M.L., Hilburn, M.E., 1986. Intestinal surface acid microclimate in vitro and in vivo in the rat. J. Lab. Clin. Med. 107, 420–424.
Schanker, L.S., Jeffrey, J.J., Tocco, D.J., 1963. Interaction of purines with the pyrimidine transport process of the small intestine. Biochem. Pharmacol. 12, 1047–1053.
Seelig, A., 1998. A general pattern for substrate recognition by P-glycoprotein. Eur. J. Biochem. 251, 252–261.

Shu, C., Shen, H., Hopfer, U., Smith, D.E., 2001. Mechanism of intestinal absorption and renal reabsorption of an orally active ace inhibitor: uptake and transport of fosinopril in cell cultures. Drug Metab. Dispos. 29, 1307–1315.

Smith, P.F., DiCenzo, R., Morse, G.D., 2001. Clinical pharmacokinetics of non-nucleoside reverse transcriptase inhibitors. Clin. Pharmacokinet. 40, 893–905.

Sugano, K., Hamada, H., Machida, M., Ushio, H., 2001. High throuput prediction of oral absorption: improvement of the composition of the lipid solution used in parallel artificial membrane permeation assay. J. Biomol. Screen. 6, 189–196.

Sugano, K., Takata, N., Machida, M., Saitoh, K., Terada, K., 2002. Prediction of passive intestinal absorption using bio-mimetic artificial membrane permeation assay and the paracellular pathway model. Int. J. Pharm. 241, 241–251.

Sugano, K., Nabuchi, Y., Machida, M., Aso, Y., 2003. Prediction of human intestinal permeability using artificial membrane permeability. Int. J. Pharm. 257, 245–251.

Sugano, K., Nabuchi, Y., Machida, M., Aso, Y., 2004. Permeation characteristics of a hydrophilic basic compound across a biomimetic artificial membrane. Int. J. Pharm. 275, 271–278.

Takamatsu, N., Kim, O.N., Welage, L.S., Idkaidek, N.M., Hayashi, Y., Barnett, J., Yamamoto, R., Lipka, E., Lennernäs, H., Hussain, A., Lesko, L., Amidon, G.L., 2001. Human jejunal permeability of two polar drugs: cimetidine and ranitidine. Pharm. Res. 18, 742–744.

Takanaga, H., Tamai, I., Tsuji, A., 1994. pH-dependent and carrier-mediated transport of salicylic acid across Caco-2 cells. J. Pharm. Pharmacol. 46, 567–570.

Tamai, I., Tsuji, A., 1996. Carrier-mediated approaches for oral drug delivery. Adv. Drug Deliv. Rev. 20, 5–32.

Taylor, D.C., Pownall, R., Burke, W., 1985. The absorption of beta-adrenoceptor antagonists in rat in situ small intestine; the effect of lipophilicity. J. Pharm. Pharmacol. 37, 280–283.

Thwaites, D.T., Hirst, B.H., Simmons, N.L., 1994. Substrate specificity of the di/tripeptide transporter in human intestinal epithelia (Caco-2): identification of substrates that undergo H(+)-coupled absorption. Br. J. Pharmacol. 113, 1050–1056.

Tsuji, A., Miyamoto, E., Terasaki, T., Yamana, T., 1982. Carbenicillin prodrugs: kinetics of intestinal absorption competing degradation of the alpha-esters of carbenicillin and prediction of prodrug absorbability from quantitative structure-absorption rate relationship. J. Pharm. Sci. 71, 403–406.

van de Waterbeemd, H., Gifford, E., 2003. ADMET in silico modelling: towards prediction paradise? Nat. Rev. Drug Discov. 2, 192–204.

Wakasugi, H., Yano, I., Ito, T., Hashida, T., Futami, T., Nohara, R., Sasayama, S., Inui, K., 1998. Effect of clarithromycin on renal excretion of digoxin: interaction with P-glycoprotein. Clin. Pharmacol. Ther. 64, 123–128.

Walter, E., Janich, S., Roessler, B.J., Hilfinger, J.M., Amidon, G.L., 1996. HT29-MTX/Caco-2 cocultures as an in vitro model for the intestinal epithelium: in vitro–in vivo correlation with permeability data from rats and humans. J. Pharm. Sci. 85, 1070–1076.

Welker, H.A., Wiltshire, H., Bullingham, R., 1998. Clinical pharmacokinetics of mibefradil. Clin. Pharmacokinet. 35, 405–423.

Wenlock, M.C., Austin, R.P., Barton, P., Davis, A.M., Leeson, P.D., 2003. A comparison of physiochemical property profiles of development and marketed oral drugs. J. Med. Chem. 46, 1250–1256.

Wenzel, U., Gebert, I., Weintraut, H., Weber, W.M., Clauss, W., Daniel, H., 1996. Transport characteristics of differently charged cephalosporin antibiotics in oocytes expressing the cloned intestinal peptide transporter PepT1 and in human intestinal Caco-2 cells. J. Pharmacol. Exp. Ther. 277, 831–839.

Wessel, M.D., Jurs, P.C., Tolan, J.W., Muskal, S.M., 1998. Prediction of human intestinal absorption of drug compounds from molecular structure. J. Chem. Inf. Comput. Sci. 38, 726–735.

Williams, P.E., Harding, S.M., 1984. The absolute bioavailability of oral cefuroxime axetil in male and female volunteers after fasting and after food. J. Antimicrob. Chemother. 13, 191–196.

Winiwarter, S., Bonham, N.M., Ax, F., Hallberg, A., Lennernäs, H., Karlen, A., 1998. Correlation of human jejunal permeability (in vivo) of drugs with experimentally and theoretically derived parameters. A multivariate data analysis approach. J. Med. Chem. 41, 4939–4949.

Winne, D., 1989. Effect of villosity and distension on the absorptive and secretory flux in the small intestine. J. Theor. Biol. 139, 155–186.

Wohnsland, F., Faller, B., 2001. High-throughput permeability pH profile and high-throughput alkane/water log P with artificial membranes. J. Med. Chem. 44, 923–930.

Yalkowsky, S.H., Valvani, S.C., 1980. Solubility and partitioning. I. solubility of non-electrolytes in water. J. Pharm. Sci. 8, 912–922.

Yamashita, S., Furubayashi, T., Kataoka, M., Sakane, T., Sezaki, H., Tokuda, H., 2000. Optimized conditions for prediction of intestinal drug permeability using Caco-2 cells. Eur. J. Pharm. Sci. 10, 195–204.

Yamashita, F., Wanchana, S., Hashida, M., 2002. Quantitative structure/property relationship analysis of Caco-2 permeability using a genetic algorithm-based partial least squares method. J. Pharm. Sci. 91, 2230–2239.

Yazdanian, M., Glynn, S.L., Wright, J.L., Hawi, A., 1998. Correlating partitioning and Caco-2 cell permeability of structurally diverse small molecular weight compounds. Pharm. Res. 15, 1490–1494.

Yee, S., 1997. In vitro permeability across Caco-2 cells (colonic) can predict in vivo (small intestinal) absorption in man-fact or myth. Pharm. Res. 14, 763–766.

Yu, L.X., Amidon, G.L., 1999. A compartmental absorption and transit model for estimating oral drug absorption. Int. J. Pharm. 186, 119–125.

Zhao, Y.H., Le, J., Abraham, M.H., Hersey, A., Eddershaw, P.J., Luscombe, C.N., Boutina, D., Beck, G., Sherborne, B., Cooper, I., Platts, J.A., 2001. Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure-activity relationship (QSAR) with the Abraham descriptors. J. Pharm. Sci. 90, 749–784.