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Prediction of human intestinal permeability using artificial membrane permeability

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Abstract

The purpose of the present study was to examine a correlation between the human intestinal permeability (P_{eff}) and the bio-mimetic artificial membrane permeability corrected by the paracellular pathway model based on the Renkin function ($P_{PAMPA\text{-}PP\text{-}RF}$) and to construct a prediction scheme. The effect of the unstirred water layer was incorporated to the prediction scheme. Eighteen P_{eff} values of passively absorbed drugs were employed for the analysis. The correlation coefficient (CC) between the predicted and observed log P_{eff} was 0.91. P_{eff} of furosemide, hydrochlorothiazide and creatinine were underestimated by $P_{PAMPA\text{-}PP\text{-}RF}$. When these compounds were excluded, CC was 0.97. Without the correction for the paracellular pathway, P_{eff} of small, cationic and hydrophilic compounds were underestimated. Therefore, $P_{PAMPA\text{-}PP\text{-}RF}$ was found to be an adequate in vitro surrogate for P_{eff}.

Keywords: Permeability; Artificial membrane; Paracellular; Unstirred water layer; PAMPA

1. Introduction

In the recent drug discovery and development process, the prediction of in vivo pharmacokinetics (PK) from in vitro data is recognized as a key technique. As an in vivo PK parameter of intestinal absorption, the fraction of a dose absorbed in humans (Fa%) has been intensively investigated for the prediction (Hidalgo, 2001). However, Fa% is not a kinetic parameter and it could not be used to predict the plasma concentration–time profile, which is necessary to simulate the drug–drug interaction and pharmacodynamics, etc. To predict the plasma concentration–time profile, kinetic parameters of oral absorption processes are required. Previously, as a kinetic parameter of intestinal membrane permeation, the effective human intestinal permeability coefficient (P_{eff}), which was measured in vivo in humans, was validated for in vivo PK simulation (Yu et al., 1996; Lennernäs, 1998). However, P_{eff} itself, of course, could not be measured at the drug discovery stage.

The parallel artificial membrane permeation assay (PAMPA) was first introduced by Kansy et al. as a rapid in vitro assay of transcellular permeation (Kansy et al., 1998). PAMPA is an application of the filter supported lipid membrane, and is completely artificial without pores and active transporter systems (Thompson et al., 1980). PAMPA is currently used by many pharmaceutical companies (Kerns, 2001; Wohnsland and Faller, 2001; Veber et al., 2002; Zhu et al., 2002). Recently, we introduced a bio-mimetic version of PAMPA which utilized a lipid composition

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similar to the intestinal brush border membrane (Proulx, 1991; Sugano et al., 2001). In addition, the bio-mimetic PAMPA was combined with the paracellular pathway model based on the Renkin function (bio-mimetic PAMPA-PP-RF system) resulting in the precise prediction of Fa% for drugs absorbed via both the passive transcellular and paracellular pathway (Sugano et al., 2002). However, the P_{eff} predictability of the bio-mimetic PAMPA-PP-RF system remains unknown.

The purpose of the present study was to examine the P_{eff} predictability of the bio-mimetic PAMPA-PP-RF system and to enable the prediction of P_{eff} directly using the bio-mimetic PAMPA-PP-RF system.

2. Materials and methods

2.1. Materials

L-α-Phosphatidylserine (PS), L-α-phosphatidylinositol (PI), and cholesterol (CHO) were purchased from Sigma Chemical (St. Louis, MO). L-α-Phosphatidylcholine (PC) and L-α-phosphatidylethanolamine (PE) were purchased from Nippon Oil & Fats Corporation (Tokyo, Japan). 1,7-Octadiene was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). All generic drugs were purchased from Sigma Chemical (St. Louis, MO) or Wako Pure Chemicals (Tokyo, Japan), except for fluvastatine which was extracted from marketed formulations. The hydrophobic filter plate (PVDF, pore size 0.45 μm) was purchased from Millipore Corporation (Bedford, MA).

2.2. Measurement of apparent artificial membrane permeability (P_{am})

Permeability studies were performed in the same manner as described previously (Kansy et al., 1998; Sugano et al., 2001). In brief, a 96-well microplate (acceptor compartment) was completely filled with pH 6.0 Na-PO_4 buffer containing 5% DMSO. A hydrophobic filter plate was fixed on the buffer filled plate. The filter surface was impregnated with 5 μl lipid solution, which was composed of PC (0.8% w/w)/PE (0.8% w/w)/PS (0.2% w/w)/PI (0.2% w/w)/CHO (1.0% w/w), and 1,7-octadiene (97.0% w/w). A 0.5 mM sample stock solution (100 μl) was added to the filter plate and incubated at 30 °C for 2 or 15 h. The compound concentration in the acceptor compartment was quantified by UV spectroscopy.

2.3. Calculation of the paracellular pathway corrected permeability ($P_{PAMPA\text{-}PP\text{-}RF}$)

$P_{PAMPA\text{-}PP\text{-}RF}$ was calculated using Eq. (1) which utilizes the theory of molecular size-restricted diffusion within a negative electrostatic field of force as described previously (Adson et al., 1994; Sugano et al., 2002).

$$P_{PAMPA\text{-}PP\text{-}RF} = P_{am} + P_{PP\text{-}RF} = P_{am} + A\frac{1}{r}F\left(\frac{r}{R}\right) \times \left(f^0 + \sum^{z(z\neq 0)} f^z \left(\frac{Bz}{1-e^{Bz}}\right)\right) \quad (1)$$

$$F\left(\frac{r}{R}\right) = \left[1-\left(\frac{r}{R}\right)\right]^2 \left[1 - 2.104\left(\frac{r}{R}\right) + 2.09\left(\frac{r}{R}\right)^3 - 0.95\left(\frac{r}{R}\right)^5\right] \quad (2)$$

where P_{am} is the apparent artificial membrane permeability which corresponds the passive transcellular pathway permeability, $P_{PP\text{-}RF}$ is the relative paracellular pathway permeability, r is the molecular radius (Å), z is the molecular charge, f is the fraction of each charged species, $B = ez/k_BT$, e is the unit charge of an ion, 4.8×10^{-10} esu, $|\Delta\Psi|$ is the apparent potential drop across the barrier, k_B is the Boltzmann constant, 1.38×10^{-23} J/K, T is the temperature in Kelvin and R is the apparent pore size of the paracellular pathway (Å). As a molecular sieving function, the Renkin function ($F(r/R)$) was employed. $R = 5.61$ Å, $A = 7.8 \times 10^{-4}$ and $|\Delta\Psi| = 75$ mV ($B = 2.94$) were used in the present study (Sugano et al., 2002). The relative contribution ratio of the paracellular pathway to $P_{PAMPA\text{-}PP\text{-}RF}$ (Para%) was calculated using Eq. (3).

$$\text{Para\%} = \frac{P_{PP\text{-}RF}}{P_{PAMPA\text{-}PP\text{-}RF}} \times 100 \quad (3)$$

2.4. Non-linear regression analysis

Non-linear regression analysis of Eq. (5) (described in Section 3) were performed by the weighted least

square method (Excel 2000, Microsoft, Redmont, WA). Sum of the squares of the difference between calculated and observed P_{eff}, weighted by the reciprocal of the square of observed P_{eff}, was minimized using the Quasi-Newton method to obtain each coefficient.

3. Results and discussion

In the present study, 18 in vivo P_{eff} values were collected from the literature (Winiwarter et al., 1998; Takamatsu et al., 2001; Lennernäs et al., 2002). P_{eff} values were obtained by using a technique based on single-pass perfusion of human jejunum segment between two inflated balloons (Lennernäs, 1998). P_{eff} was previously validated for in vivo PK simulation (Yu et al., 1999). P_{eff} of actively transported compounds were excluded from this study.

$P_{PAMPA\text{-}PP\text{-}RF}$ correlated adequately to P_{eff} (Fig. 1). In the lower permeability range ($P_{eff} < 2 \times 10^{-4}$, $P_{PAMPA\text{-}PP\text{-}RF} < 10 \times 10^{-6}$), a linear relation was found in the logarithmic plot between P_{eff} and $P_{PAMPA\text{-}PP\text{-}RF}$. Therefore, the membrane permeation was assumed to be an exponent of $P_{PAMPA\text{-}PP\text{-}RF}$. In this region, the rate-limiting process was suggested to be the membrane permeation in both in vivo and PAMPA (Lennernäs, 1998; Avdeef, 2001). However, in the higher permeability range ($P_{eff} > 3 \times 10^{-4}$, $P_{PAMPA\text{-}PP\text{-}RF} > 20 \times 10^{-6}$), P_{eff} reached the upper limit. This finding suggested that, in vivo, permeation through the unstirred water layer (UWL), which is adjacent to the membrane, would limit the intestinal permeation in this permeability range. To take the resistance of UWL (R_{UWL}) to the transport into account, the permeability through UWL (P_{UWL}) was represented by the diffusion coefficient (D) and the thickness (δ) of UWL. The diffusion coefficient (D) was assumed to follow the Stokes-Einstein equation for small, spherical molecules:

$$P_{UWL} = \frac{1}{R_{UWL}} = \frac{D}{\delta} = \frac{k_B T}{6\pi\eta\delta}\frac{1}{r} \times 10^8 = \frac{c}{r} \quad (4)$$

where, η was the viscosity of UWL. P_{eff} and effective resistance to the transport (R_{eff}) were represented as:

$$\frac{1}{P_{eff}} = \frac{1}{(aP_{PAMPA\text{-}PP\text{-}RF})^b} + \frac{1}{P_{UWL}} = \frac{1}{(aP_{PAMPA\text{-}PP\text{-}RF})^b} + \frac{r}{c} \quad (5)$$

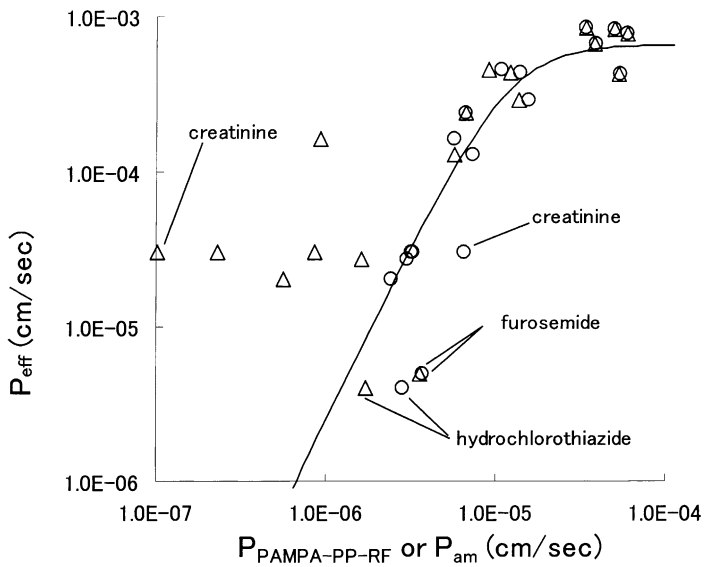

Fig. 1. P_{eff} vs. $P_{PAMPA\text{-}PP\text{-}RF}$ or P_{am}. The circles represent $P_{PAMPA\text{-}PP\text{-}RF}$ and the triangles represent P_{am}. The detection limit P_{am} values were used for creatinine and terbutaline. The curved line is the fitting line of Eq. (5) for a molecular radius (r) = 4.28 Å, which corresponds to ketoprofen. The curved line was obtained excluding creatinine, furosemde and hydrochlorothiazide.

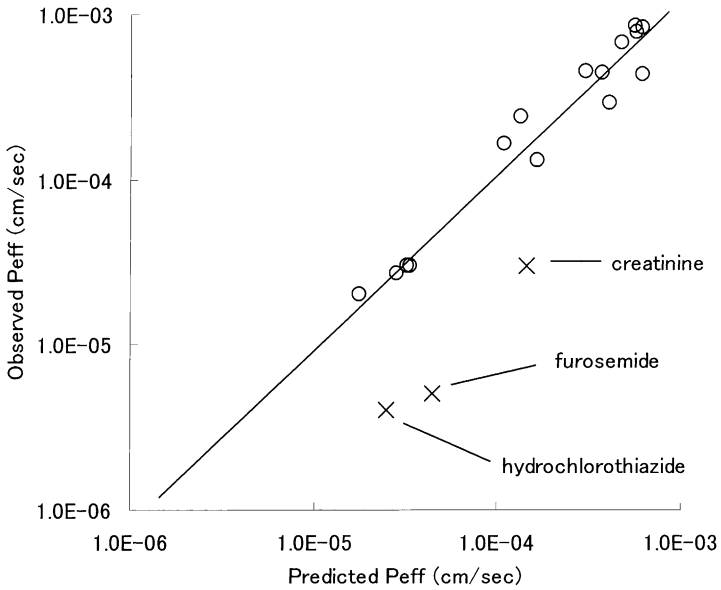

Fig. 2. Predicted vs. observed P_{eff}. P_{eff} was predicted using Eq. (5) without hydrochlorothiazide, furosemide and creatinine.

$$R_{eff} = R_{mem} + R_{UWL} \quad (6)$$

where R_{mem} is the membrane resistance to the transport. By multiple non-linear regression analysis utilizing 18 compounds, a, b, and c were obtained as 7.0×10^3, 3.2 and 2.7×10^{-3}, respectively, and the correlation coefficient (CC) between the predicted and observed $\log P_{eff}$ was 0.91. P_{eff} of hydrochlorothiazide (HZ), furosemide (FS) and creatinine (CR) were underestimated. However, the P_{eff} of HZ, FS and CR were also lower than expected from their Fa% (70, 60 and 80%, respectively) and Caco-2 permeability (0.42 ± 0.03, 0.11 ± 0.01 and 0.77 ± 0.03 ($\times 10^{-6}$ cm/s), respectively) (Karlsson et al., 1999; Yu and Amidon, 1999; Yamashita et al., 2000). Therefore, P_{eff} of HZ, FS and CR could have been under assessed. When HZ, FS and CR were excluded from the regression analysis, a, b, c, CC were obtained as 3.5×10^3, 2.3, 2.6×10^{-3} and 0.97, respectively (Figs. 1 and 2). Without the correction for the paracellular pathway, P_{eff} of small, cationic and hydrophilic compounds were underestimated. In these sorts of compounds, the paracellular pathway permeation would contribute significantly to the intestinal permeability (Table 1, Para%). Above all, bio-mimetic artificial membrane permeability corrected for the paracellular pathway and UWL was found to be adequate for the prediction of P_{eff}.

Previously, a correlation between P_{eff} and PAMPA with the PC/dodecane membrane was examined (Avdeef, 2001, 2002). The slope of the logarithmic plot (b) was reported to be approximately 0.5, 4–5-fold lower than that of the present study. The difference between the previous and present findings could be derived from the correction for the paracellular pathway and the difference in the membrane lipid composition. In the present study, when the paracellular pathway was not corrected, the slope between $\log P_{am}$ and $\log P_{eff}$ became approximately unit or less. In addition, permeability of the basic compound was larger in bio-mimetic PAMPA than in the PC system, due to the negative charge of PS and PI on the membrane (Sugano et al., 2001). PS and PI increase the binding of a basic compound to the membrane (Krämer et al., 1998), and might facilitate the transport of a basic compound through the hydrophobic part of the membrane by formation of an ion pair (Neubert, 1989; Sugano et al., unpublished results).

In the present study, P_{UWL} was obtained as $5.2–8.7 \times 10^{-4}$ cm/s for compounds with $r = 3–5$ Å. Previously, as an UWL limited permeation compound, glucose was employed to measure the UWL

Table 1
Fa%, permeability and molecular properties

Compound	r (Å)[a]	Charge[b]	P_{am}[c] (10^{-6} cm/s)	$P_{PAMPA\text{-}PP\text{-}RF}$ (10^{-6} cm/s)	Para%[f]	P_{eff}[g] (10^{-4} cm/s)
Amiloride	3.91	+	0.93 ± 0.08	5.74	84	1.63 ± 0.51
Antipyrine	3.89	0	9.12 ± 0.70	10.7	15	4.5 ± 2.5
Atenolol	4.42	+	0.56 ± 0.11	2.44	77	0.2 ± 0.2
Carbamazepine	4.16	0	53.0 ± 0.4[d]	54.0	2	4.3 ± 2.7
Cimetidine	4.27	+	0.86 ± 0.16	3.26	74	0.30 ± 0.05
Creatinine	3.21	+	<0.10[e]	<6.56[e]	>98[e]	0.3 ± 0.2
Desipramine	4.47	+	12.2 ± 0.8[d]	13.9	12	4.4 ± 1.8
Fluvastatine	4.99	–	6.62 ± 0.05[d]	6.64	0	2.4 ± 1.8
Furosemide	4.37	–	3.60 ± 0.08	3.71	3	0.05 ± 0.04
Hydrochlorothiazide	4.09	0	1.71 ± 0.06	2.83	40	0.04 ± 0.05
Ketoprofen	4.28	–	33.8 ± 2.1	33.9	0	8.4 ± 3.3
Metoprolol	4.47	+	5.67 ± 0.55	7.35	23	1.3 ± 1.0
Naproxen	4.13	–	49.5 ± 3.2	49.7	0	8.3 ± 4.8
Piroxicam	4.47	–	59.3 ± 7.6[d]	59.4	0	7.8 ± 7.5
Propranolol	4.40	+	13.7 ± 1.4	15.6	12	2.9 ± 2.2
Ranitidine	4.56	+	1.63 ± 0.03	3.01	46	0.27 ± 0.06
Terbutaline	4.18	+	<0.23[e]	<3.20[e]	>93[e]	0.3 ± 0.3
Verapamil	5.32	+	38.4 ± 0.9	38.4	0	6.7 ± 2.9

[a] Molecular radius.
[b] Charge at pH 6.0.
[c] Values from Sugano et al., 2002 or otherwise noted. Values are represented as the mean ± S.D. The assays were performed in triplicate.
[d] Measured in this study.
[e] Less than the detection limit. Detection limit was set at $OD_{ac} = 0.005$. The detection limit value was used as P_{am} to calculate $P_{PAMPA\text{-}PP\text{-}RF}$ and Para%.
[f] Contribution of the paracellular pathway to the predicted total passive transport.
[g] Human intestinal permeability from Winiwarter et al., 1998, Takamatsu et al., 2001 and Lennernäs et al., 2002.

permeability in vivo (Levitt et al., 1990). The intestinal membrane permeability of glucose ($r = 3.74$ Å) was reported to be 10.0 ± 8.2 and $16 \pm 2 \times 10^{-4}$ cm/s, in humans and dogs, respectively (Levitt et al., 1990; Lennernäs, 1998; Winiwarter et al., 1998). The UWL of the gastrointestinal tract was maintained by the mucus layer. In the mucus layer, the diffusion coefficient of glucose was found to be about 2–3-fold that of lipophilic compounds (Larhed et al., 1997). Utilizing these reported values, P_{UWL} for lipophilic compounds was calculated to be $4\text{–}13 \times 10^{-4}$ cm/s for $r = 3\text{–}5$ Å. In the present study, compounds with $P_{eff} > 3 \times 10^{-4}$ were all lipophilic (Winiwarter et al., 1998). Therefore, P_{UWL} obtained in the present study was in agreement with the calculation utilizing reported values. In piroxicam, naproxen, verapamil, ketoprofen and desipramine, greater than 75% of the resistance to the transport was suggested to be derived from the UWL.

Previously, the unstirred water layer permeability in PAMPA was reported to be approximately $20\text{–}50 \times 10^{-6}$ for compounds with $r = 4\text{–}5$ Å (Avdeef, 2002). In the present bio-mimetic PAMPA system, the unstirred water layer permeability was found to be 61×10^{-6} for ketoprofen (data not shown). These values were approximately 10-fold smaller than P_{UWL} in humans. However, overall P_{am} was approximately 1/8–1/40 of P_{eff}. Therefore, the relative contribution of the UWL resistance to P_{am} would be less than to P_{eff}. In vivo, the membrane surface is approximately 30-fold expanded by the villous structure, whereas UWL is not expanded because it locates on top of the villi (Levitt et al., 1990). In the calculation of P_{eff}, the villous structure was not considered (Lennernäs, 1998). Therefore, the intrinsic membrane permeability is smaller than P_{eff} for membrane controlled permeation, whereas the intrinsic UWL permeability is close to P_{eff} for UWL limited permeation.

In the present study, the slope between $\log P_{eff}$ and $\log P_{PAMPA\text{-}PP\text{-}RF}$ was 2.3–3.2-fold larger than unit. Although a wide variability of P_{eff} was observed in the human jejunum, it was unlikely that the

observed increment of the slope was related to the variability. Therefore, this would reflect the morphological and chemical difference between the human intestinal membrane and the PAMPA membrane. The human intestinal membrane has a fractal-like structure, i.e. the ridges oriented circumferentially around the lumen, villi and microvilli. In addition, although the structure of the PAMPA membrane remains unknown, it might not be a single plane bilayer because it was constructed on the sponge-like filter support (Avdeef, 2001). The difference in morphology results in generating the permeability dependent concentration gradient in the inter-villous channel, in addition to the difference in the surface area (Winne, 1978; Oliver et al., 1998). In addition to the morphological difference, the difference in the lipid composition could be another reason of the difference in the absolute value of permeability. The PAMPA membrane contains organic solvents and lacks some lipids, i.e. sphingomyelin (Proulx, 1991; Xiang et al., 1992). More detailed studies are necessary to clarify the effect of the morphological and the chemical difference on the absolute value of permeability.

In conclusion, the bio-mimetic PAMPA-PP-RF system predicted the P_{eff} adequately. With the predicted P_{eff} value, it may be possible to simulate the in vivo plasma concentration–time profile. In addition, the results of this study provided a basis that, as a permeability assay in the early drug discovery stage, the bio-mimetic $P_{PAMPA\text{-}PP\text{-}RF}$ system can be used for the biopharmaceutics classification system (Amidon et al., 1995; Obata et al., 2002). This may result in the discovery of promising clinical candidates.

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